Polymer 50 (2009) 1778–1789

Contents lists available at ScienceDirect

Polymer

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

# Morphology modulation of polymeric assemblies by guest drug molecules: TEM study and compatibility evaluation

Jianxiang Zhang [a,b,c,*], Shuhui Li [a], Xiaodong Li [d], Xiaohui Li [a,*], Kangjie Zhu [b]

[a] Institute of Materia Medica, College of Pharmacy, Third Military Medical University, Chongqing 400038, China
[b] Institute of Polymer Science, and Key Laboratory of Macromolecule Synthesis and Functionalization (Ministry of Education), Zhejiang University, Hangzhou 310027, China
[c] Department of Biologic and Materials Sciences, University of Michigan, Ann Arbor, MI 48109, USA
[d] Affiliated Stomatology Hospital, College of Medicine, Zhejiang University, Hangzhou 310068, China

ARTICLE INFO

*Article history:*
Received 24 October 2008
Received in revised form
9 January 2009
Accepted 7 February 2009
Available online 13 February 2009

*Keywords:*
Drug delivery
Self-assembly
Nanoparticles

ABSTRACT

Amphiphilic copolymers with poly(N-isopropylacrylamide) and ethyl tryptophan, ethyl 4-aminobenzoate, or ethyl glycinate as side groups were synthesized. Assemblies based on these copolymers were employed as model systems to investigate the morphology transformation upon loading of various hydrophobic drugs. TEM observation suggested that the loading of non-steroidal anti-inflammatory drugs, including ibuprofen, ketoprofen, indomethacin, naproxen and mefenamic acid, can trigger a significant morphological transformation of assemblies based on copolymers with low substitution of hydrophobic group. On the other hand, the introduction of steroidal anti-inflammatory drugs (medroxyprogesterone acetate, prednisone acetate and dexamethasone) and aliphatic acids (caprylic acid, tetradecanoic acid and stearic acid) has no significant effect on the morphology of assemblies derived from the same copolymers, although they do have some effect on the morphology of assemblies based on copolymers with high content of hydrophobic group. In addition, the morphology of assemblies is well correlated with drug loading efficiency. An occurrence of morphology transformation means a higher drug loading, and vice versa. Various physicochemical parameters including partition coefficient, molecular volume and solubility parameters were calculated according to group contribution method. Analysis of these data pointed to the fact that a combination of molecular volume and solubility parameters can be used as a measure to judge whether one molecule is 'active' or 'neutral'. This rule can also be applied to evaluate the compatibility between candidate drugs and nanocarriers based on these copolymers.



## 1. Introduction

During the past few decades, polymeric aggregates assembled by amphiphilic copolymers in aqueous solutions have been widely studied as delivery vehicles for cosmetics, dyes, small molecule drugs, macromolecular bioactive compounds, diagnostic agents and imaging probes [1–6]. These polymeric assemblies exhibit multiple morphologies, including spherical micelles, cylindrical micelles, worm-like micelles, multicompartment micelles, toroidal assemblies, vesicles, nanofibers, helical superstructure and macroscopic tubes [7–14]. Whereas spherical micelles have been frequently studied as promising nanocarriers for drug delivery and gene therapy, little attention has been paid to the effects of shape, which may be due to, in part, the absence of appropriate model vehicles. More recently, research by Discher's group suggested that cylinder micelles, known as filomicelles by authors, exhibited significantly longer circulation time compared with their spherical counterparts after intravenous injection in rodents [15]. These filomicelles can orient and stretch in a flowing stream in a manner that is ideal for flow-intensive drug delivery applications. Consequently, the vehicle morphology plays a strong role not only in transport and trafficking, but also perhaps in application. As well known, the shape of polymeric assemblies can be controlled or modulated through polymer architecture design, preparation procedure (e.g., dialysis or emulsion method and solvent mixtures), and solution conditions such as pH manipulation, salt concentration and temperature [8–12,16–19]. However, knowledge on the

* Corresponding authors at: College of Pharmacy, Third Military Medical University, Chongqing 400038, China. Tel.: +86 23 68753397 81; fax: +86 23 68753397 82.
*E-mail addresses:* jxzhang1980@gmail.com (J. Zhang), lpsh008@yahoo.com.cn (X. Li).

0032-3861/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.polymer.2009.02.004

polymer self-assembly in the presence of small molecules is still limited. Most recently, the influence of an organic diamine on the morphology of assemblies based on a linear poly(acrylic acid)-*block*-poly(methyl acrylate)-*block*-polystyrene (PAA-*b*-PMA-*b*-PS) triblock copolymer has been addressed by Cui et al [20]. By introducing 2,2′-(ethylenedioxy) diethylamine, different nanoscale complex structures were generated by this block copolymer. Nevertheless, as for a delivery system, it is important to elucidate the effect of introducing guest molecules on the morphology of polymeric assemblies. To the best of our knowledge, few studies have been performed to address this issue so far. Our previous study indicated that the loading of a model drug will lead to the morphology transformation of polymeric assemblies based on graft amphiphilic copolymers [21]. In this study, a series of hydrophobic drugs with various chemical structures were selected as guest molecules, to investigate their effect on the morphology of assemblies based on various amphiphilic copolymers with different hydrophobic groups.

## 2. Materials and methods

### 2.1. Materials

Hexachlorocyclotriphosphazene (Strem Chemicals) was purified by recrystallization from hexane and subsequent sublimation at 80–90 °C. *N*-Isopropylacrylamide (NIPAm) obtained from Acros Organics was recrystallized twice from hexane before use. 2,2′-Azobisisobutyronitrile (AIBN) was purified by recrystallization in a benzene/hexane mixture and in ethanol, respectively. Ethyl glycinate (EtGly) and ethyl tryptophan (EtTrp) were synthesized according to literature procedure [22]. 2-Aminoethanethiol hydrochloride (AET·HCl), ethyl 4-aminobenzoate (EAB) and aluminum chloride (99%) obtained from Acros Organics were used as received. Mefenamic acid (MA), ibuprofen (IBU), ketoprofen (KET), naproxen (NAP) and indomethacin (IND) were kindly supplied by Juhua Group Pharmaceutical Factory (Zhejiang, China). Dexamethasone (DMS), medroxyprogesterone 17-acetate (MPG) and prednisone acetate (PNS) were purchased from Zhejiang Xianju Pharmacy Ltd (Zhejiang, China). Caprylic acid (CA), tetradecanoic acid (TA) and stearic acid (SA) were obtained from Acros Organics. All the other reagents were commercially available and used without further purification.

### 2.2. Polymer synthesis and characterization

Oligo-poly(*N*-isopropylacrylamide) (PNIPAm) with a terminal amino group was synthesized by free-radical polymerization using AIBN and AET·HCl as initiator and chain transfer reagent respectively [23]. Poly(dichlorophosphazene) was synthesized by thermal ring-opening polymerization, 5.0 wt.% aluminum chloride was used as catalyst [24]. Copolymer with PNIPAm as hydrophilic segment and EtGly as hydrophobic group (PNIPAm/EtGly-PPP) was synthesized as described previously [25]. Copolymers bearing PNIPAm and EAB as side groups (PNIPAm/EAB-PPP) were obtained according to literature procedure [26]. On the other hand, amphiphilic copolymers containing PNIPAm and EtTrp as flanking units (PNIPAm/EtTrp-PPP) were synthesized as reported in our previous work [18].

The molecular weights of both oligo-PNIPAm and amphiphilic copolymers were determined by a gel permeation chromatography (GPC) equipped with a Waters 515 HPLC Pump and a Waters 2410 refractive index detector. THF was used as solvent with a flow rate of 1.5 ml/min at 40 °C and narrowly dispersed polystyrene as calibration standards. In addition, the molecular weight of PNIPAm was also quantified by elemental analysis and titration in nonaqueous solvent. Copolymer composition of PNIPAm/EtGly-PPP was calculated based on S element analysis. As for PNIPAm/EAB-PPP, the molar ratio of PNIPAm to EAB was calculated according to the UV absorbance of EAB group at 284 nm. In the case of PNIPAm/EtTrp-PPP, copolymer composition was also confirmed by UV–Visible Spectrophotometer (TU-1800PC, Beijing Purkinje General Instrument Co., Ltd, Beijing, China). Absorbance at 283 nm was used to calculate the molar ratio of EtTrp to PNIPAm with the calibration curve constructed by concentration dependent absorbance of EtTrp at 283 nm in the same solvent. The critical micelle concentration (CMC) of copolymers was determined by fluorescence technique using pyrene as probe [27]. Fluorescence measurements were conducted using an F-4500 fluorescence spectrophotometer (Hitachi High-Technologies Co., Tokyo, Japan). All the physicochemical properties of polymers mentioned in this study are presented in Table 1.

### 2.3. Preparation of polymeric assemblies with various model compounds

Polymeric assemblies containing various model drugs including MA, IBU, KET, NAP, IND, DMS, MPG and PNS, were prepared by a dialysis method. Briefly, mixture of small molecule compound and copolymer with a certain weight ratio was co-dissolved in N,N-dimethylacetamide (DMAc) with a final polymer concentration of 10 mg/ml. This solution was placed into the dialysis tubing (MWCO 12 kDa) for dialysis against distilled water for 48 h at 12 °C. The outer aqueous solution was exchanged per 30 min for the first 2 h, and then each 2 h for the remaining period of time. After filtered through a 0.8 μm microporous membrane, the dialysis solution was

**Table 1**
Physicochemical properties of polymers.

| Polymers | Molar ratio | | $M_w$ | $M_n$ | CMC/(g/L)[f] |
|---|---|---|---|---|---|
| | Hydrophobic group | PNIPAm | | | |
| PNIPAm[c] | | | | 2000,[a] 1800[b] | |
| PNIPAm/EtGly-PPP | 0.33 | 1.67 | 26 000 | 13 000 | 0.018 |
| PNIPAm[d] | | | | 1900,[a] 1400[b] | |
| PNIPAm/EAB-PPP-1 | 0.20 | 1.80 | 43 000 | 22 000 | 0.037 |
| PNIPAm/EAB-PPP-2 | 1.00 | 1.00 | 38 000 | 19 000 | 0.028 |
| PNIPAm[e] | | | | 1600[a] | |
| PNIPAm/EtTrp-PPP-1 | 0.79 | 1.21 | 41 000 | 32 000 | 0.102 |
| PNIPAm/EtTrp-PPP-2 | 1.13 | 0.87 | 39 000 | 31 000 | 0.058 |
| PNIPAm/EtTrp-PPP-3 | 1.19 | 0.81 | 21 400 | 12 100 | 0.030 |

[a] Obtained by GPC.
[b] Determined by elemental analysis.
[c] PNIPAm was used to synthesize PNIPAm/EtGly-PPP.
[d] PNIPAm was used to synthesize PNIPAm/EAB-PPP.
[e] PNIPAm was used to synthesize PNIPAm/EtTrp-PPP.
[f] Determined by fluorescence probe technique.

lyophilized to obtain the resultant polymeric aggregates loaded with model drug. Exactly the same procedure was adopted to prepare assemblies containing aliphatic acids.

### 2.4. Determination of drug content in polymeric assemblies

Reversed-phase HPLC (Agilent 1100 HPLC system) was performed to quantify the weight content of model drug in the resultant polymeric assemblies. A 5 μm ODS $C_{18}$ 250 × 4.6 mm column was adopted. Mobile phase consisting of 6 μM phosphoric acid–acetonitrile (45:55, v/v) was adopted for MA, IBU, KET, NAP and IND, and the column temperature was 40 °C. The flow rate was 2.0, 1.5, 1.5, 1.5 and 2.0 ml/min, while detection wavelength was 245, 215, 245, 245 and 245 nm for MA, IBU, KET, NAP and IND respectively. On the other hand, mobile phase was composed of methanol and water with a volume ratio of 70:30, and the flow rate was 1.0, 1.5 and 1.0 ml/min for DMS, MPG and PNS respectively. The column temperature was 25 °C, and the effluent was detected at 240 nm.

### 2.5. Determination of the content of aliphatic acid in polymeric assemblies based on PNIPAm/EtTrp-PPP-1

The content of aliphatic acid in polymeric assemblies was determined by an indirect method. Briefly, the weight of PNIPAm/EtTrp-PPP-1 in aliphatic acid containing polymeric assemblies was quantified by UV measurement at 288 nm, and then the weight of aliphatic acid was obtained by simple subtraction.

### 2.6. Morphology observation by transmission electron microscopy (TEM)

TEM images were obtained using a JEM 1200EX operating at an acceleration voltage of 60 kV. Copper EM grids were precoated with a thin film of Formvar. Samples were prepared at 12 °C by dipping a TEM grid into the aqueous solution of polymer assemblies, and extra solution was blotted with filter paper. After water was evaporated at room temperature for appropriate time, samples were observed directly without any staining.

### 2.7. Particle size determination

Dynamic light scattering (DLS) measurement for the assemblies in aqueous solution was performed with a Malvern Zetasizer Nano ZS instrument at 25 °C. The results are listed in Table S1.

### 2.8. Calculation of octanol–water partition coefficient (log P)

Method established by Moriguchi et al. was adopted to calculate log *P* of various compounds [28].

### 2.9. Calculation of molar volume and total solubility parameters by the group contribution method

The solubility parameter ($\delta$) and molar volume ($V$) of various hydrophobic compounds and hydrophobic groups of amphiphilic copolymers were calculated by the method reported by Fedors [29].

$$\delta = \left(\frac{\Delta E_v}{V}\right)^{1/2}$$

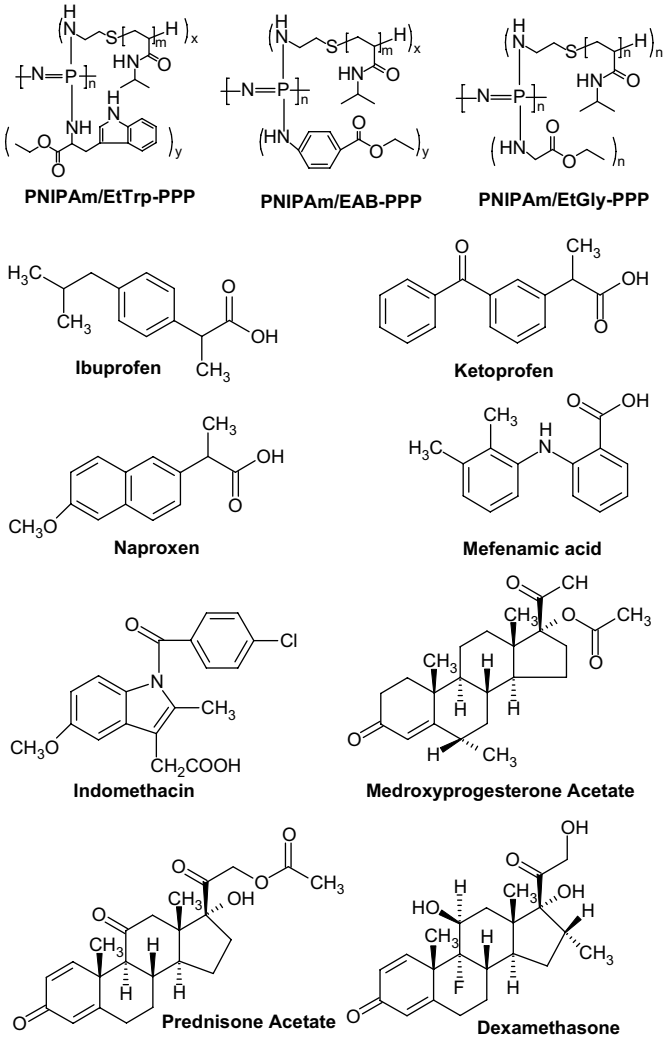
$$\Delta E_v = \sum_i \Delta e_i$$

$$V = \sum_i \Delta v_i$$

Where $\Delta E_v$ is the energy of vaporization at a given temperature and $V$ is the corresponding molar volume, $\Delta e_i$ and $\Delta v_i$ are the additive atomic and group contribution for the energy of vaporization and molar volume respectively.

### 2.10. Calculation of partial and total solubility parameters by group contribution method

The partial solubility parameters $\delta_d$, $\delta_p$ and $\delta_h$, corresponding to contributions from Van der waals dispersion forces, dipole–dipole interactions, and hydrogen bonding respectively, were calculated by the method developed by Van Krevelen [30].




**Scheme 1.** The chemical structure of copolymers and various drugs employed in this study.

$$\delta_d = \frac{\sum F_{di}}{V}$$

$$\delta_p = \frac{\sqrt{\sum F_{pi}^2}}{V}$$

$$\delta_h = \sqrt{\frac{\sum E_{hi}}{V}}$$

$$\delta = \left(\delta_d^2 + \delta_p^2 + \delta_h^2\right)^{1/2}$$

Where $F_{di}$, $F_{pi}$ and $E_{hi}$ are the specific functional group contributions of dispersion forces ($F_{di}$), polar forces ($F_{pi}$), and hydrogen bonding ($E_{hi}$). The molar volume ($V$) of various drugs and the hydrophobic groups of amphiphilic copolymers were obtained by the Fedors' method as described above [29]. The total solubility parameter is then the sum of the contributions from each of these forces.

## 3. Results

### 3.1. Drug loading and TEM images of assemblies based on PNIPAm/EtTrp-PPPs and various model drugs

Amphiphilic polyphosphazenes with PNIPAm and EtTrp as side groups were synthesized by a sequential two-step substitution reaction of poly(dichlorophosphazene) with EtTrp and PNIPAm [19]. The chemical structure of PNIPAm/EtTrp-PPP is illustrated in Scheme 1. Three copolymers with various PNIPAm/EtTrp molar ratios were employed in this study, and their physicochemical characteristics are presented in Table 1. With the aim to address the effect of drug loading on the morphology of assemblies, eight lipophilic drugs with various structures were selected as models (Scheme 1). Among these model drugs, IBU, KET, NAP and MA are non-steroidal anti-inflammatory drugs with carboxylic group, while MPG, PNS and DMS are considered as highly hydrophobic steroidal drugs.

PNIPAm/EtTrp-PPPs based assemblies containing various model drugs were prepared by dialysis method using DMAc as a common solvent for both polymer and drug. TEM images of assemblies derived from PNIPAm/EtTrp-PPP-1 and various drugs are shown in Fig. S1B–J. Independent of the significant difference in the chemical structure of model drugs, network-like assemblies were observed. This morphology is similar to that of copolymer itself assembled in an aqueous solution as shown in Fig. S1A. This result suggests that lower drug loading has no significant effect on the morphology of resultant assemblies. To further investigate the influence of drug content, assemblies of PNIPAm/EtTrp-PPP-1 containing various contents of IND, IBU and MPG were prepared. For IND and IBU, drug loading increased as the feed ratio increased, while no significant change was observed in the case of MPG. Fig. 1 shows the TEM images of PNIPAm/EtTrp-PPP-1 assemblies with various IND loading. Interestingly, obvious morphology changes were observed. With 6.1% IND, the assemblies still exhibited network morphology to some extent (Fig. 1A). However, vesicles were observed as IND loading increased to 14.7% (Fig. 1B and C). Further increasing IND content resulted in the formation of nanospheres (Fig. 1D). For assemblies prepared with extremely high IND feed (47.4%), although the real IND loading was relatively lower (31.6%) compared with that (37.2%) prepared with 41.2% IND feed, totally nano-spherical particles were observed (Fig. 1E). As presented in Fig. 2, a similar drug-loading effect was observed for IBU when its loading was enhanced. For example, morphologies ranging from network to vesicle and nanosphere were found as IBU loading increased from 5.4% to 18.7% and 28.9%. On the other hand, MPG loading almost did not change as its feed increased from 9.1% to 54.6%. In consistent with the lower drug loading, no significant morphology change was observed as shown in Fig. S2.

Similar experiments were performed for assemblies based on PNIPAm/EtTrp-PPP-2. With the same drug feed, drug loading was slightly increased for this copolymer in comparison with that of PNIPAm/EtTrp-PPP-1. Fig. S3 illustrates the TEM images of assembles based on PNIPAm/EtTrp-PPP-2 and various drugs. Assemblies of PNIPAm/EtTrp-PPP-2 alone exhibited network-like structure as well observed in our previous study [18,21]. For assemblies containing DMS and MPG, their morphology looked like that of copolymer alone (Fig. S3A–C). However, in the case of assemblies containing IBU, KET and NAP, dispersed particles were observed (Fig. S3D–F). In addition, significant morphological changes were also observed for assemblies containing various contents of IND (Fig. 3). As IND loading increased from 7.2% to 30.4%, assemblies were transformed from network to high-genus vesicles and even to nanospheres (Fig. 3A–D). For assemblies with 28.2% IND prepared with 47.4% IND feed, almost all of them were nanospheres (Fig. 3E). On the other hand, morphology change did not occur for assemblies containing MPG that were prepared with initial feed ranging from 9.1% to 33.3% and 54.6% (Fig. S4). This result was similar to that observed for PNIPAm/EtTrp-PPP-1.

As far as assemblies based on PNIPAm/EtTrp-PPP-3 was concerned, morphological changes were observed in the presence of various drugs independent of their structure and loading amount as shown in Fig. 4. Network-like assemblies, assembled by PNIPAm/EtTrp-PPP-3 itself in aqueous solution, were transformed into dispersed nanoparticles even when lower content of DMS (0.7%) or MPG (1.3%) was introduced (Fig. 4A–C). Totally dispersed nanoparticles were also obtained as 4.1% IBU was loaded (Fig. 4D). In addition, both vesicle-like assemblies and nanoparticles were achieved with the loading of 1.8% KET, 2.8% MA or 1.2% NAP (Fig. 4E–G). As for IND, assemblies of vesicles and well-defined nanospheres were achieved as IND loading increased from 6.5% to 29.5% (Fig. 5).

In combination of these results regarding assemblies based on PNIPAm/EtTrp-PPPs and various drugs, it seems that drugs with carboxylic group are prone to leading to the morphological changes of drug-loaded assemblies, which is especially true when the drug loading is higher. To demonstrate whether all the hydrophobic compounds with carboxylic group will result to this effect, we selected three hydrophobic aliphatic acids, i.e. CA, TA and SA as guest molecules to prepare assemblies based on PNIPAm/EtTrp-PPP-1. Compared with IBU and IND, the real loading of CA, TA or SA was relatively lower despite the dramatic increase in initial feed. TEM images of assemblies prepared with various contents of CA are presented in Fig. S5. Obviously, almost no changes in the morphology of assemblies containing CA can be observed, and exactly the same result was obtained for assemblies containing TA and SA as shown in Figs. S6 and S7. Interestingly, for the samples prepared with the SA feed of 9.1% or 23.1%, even SA crystals can be observed when no filtration was performed after dialysis process (Fig. S7E and F). This suggests that the interaction between SA molecules is stronger than that between SA and copolymer, which might be responsible for the relatively lower loading of this type of hydrophobic compounds.

### 3.2. Drug loading and TEM images of assemblies based on PNIPAm/EAB-PPPs and various model drugs

To investigate the effect of copolymer structure on the morphology transformation of assemblies mediated by small molecules, amphiphilic polyphosphazenes with PNIPAm and EAB as flanking groups (PNIPAm/EAB-PPPs) were also synthesized

(Scheme 1). The physicochemical properties of polymers are also listed in Table 1. Drug containing assemblies based on PNIPAm/EAB-PPPs were prepared by dialysis method as well. For a certain drug feed, drug loading was significantly higher for drugs like IBU, KET, NAP and IND compared with drugs such as DMS, MPG and PNS. This result is similar to that observed for assemblies derived from PNIPAm/EtTrp-PPPs. In the case of morphology, assemblies formed by PNIPAm/EAB-PPP-1 alone exhibited a network-like structure as presented in Fig. S8A. For assemblies with 10.6% IBU, 7.9% KET, 6.8% NAP, 8.8% IND, 0.4% DMS, 0.3% MPG or 0.5% PNS, network-like structure was also observed (Fig. S8B–H). On the other hand, for IND containing assemblies based on PNIPAm/EAB-PPP-1, separated nanoparticles and nanospheres were achieved when IND content was increased to 15.6% and 29.8% (Fig. 6A and B). For PNIPAm/EAB-PPP-2 assemblies without drug, both network and spherical structure were observed (Fig. 6C). As 16.5% IND was loaded, only nanospheres were obtained (Fig. 6D).

### 3.3. Drug loading and TEM images of assemblies based on PNIPAm/EtGly-PPP and various model drugs

In addition to copolymers with EtTrp and EAB as hydrophobic groups, amphiphilic copolymer with PNIPAm and EtGly as side groups (PNIPAm/EtGly-PPP) was also synthesized (Scheme 1 and

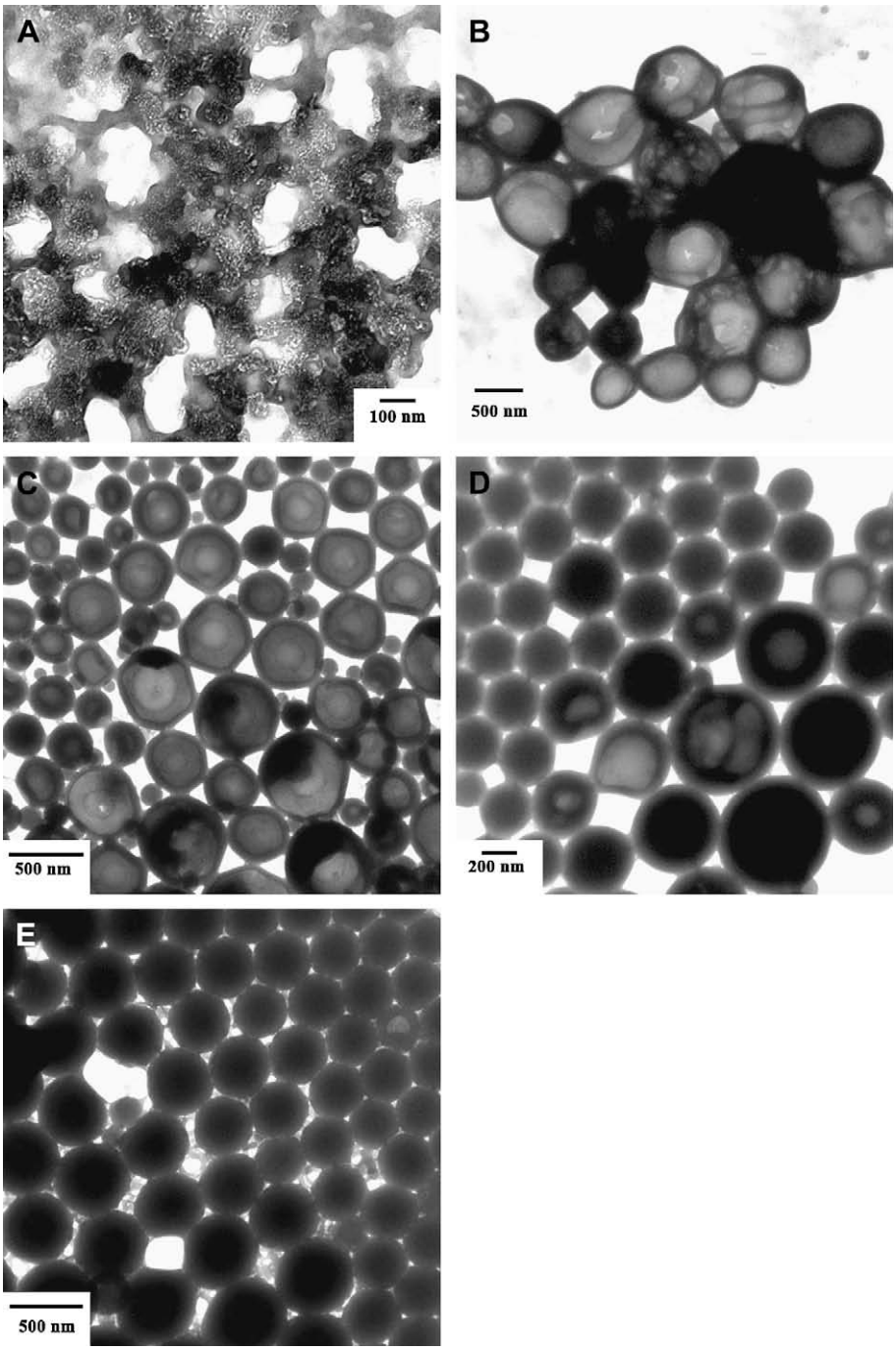



**Fig. 1.** TEM images of assemblies derived from PNIPAm/EtTrp-PPP-1 containing IND (A) 6.1%, (B) 14.7%, (C) 24.1%, (D) 37.2% and (E) 31.6%; the corresponding theoretical IND loading was 9.1%, 23.1%, 33.3%, 41.2% and 47.4% respectively.

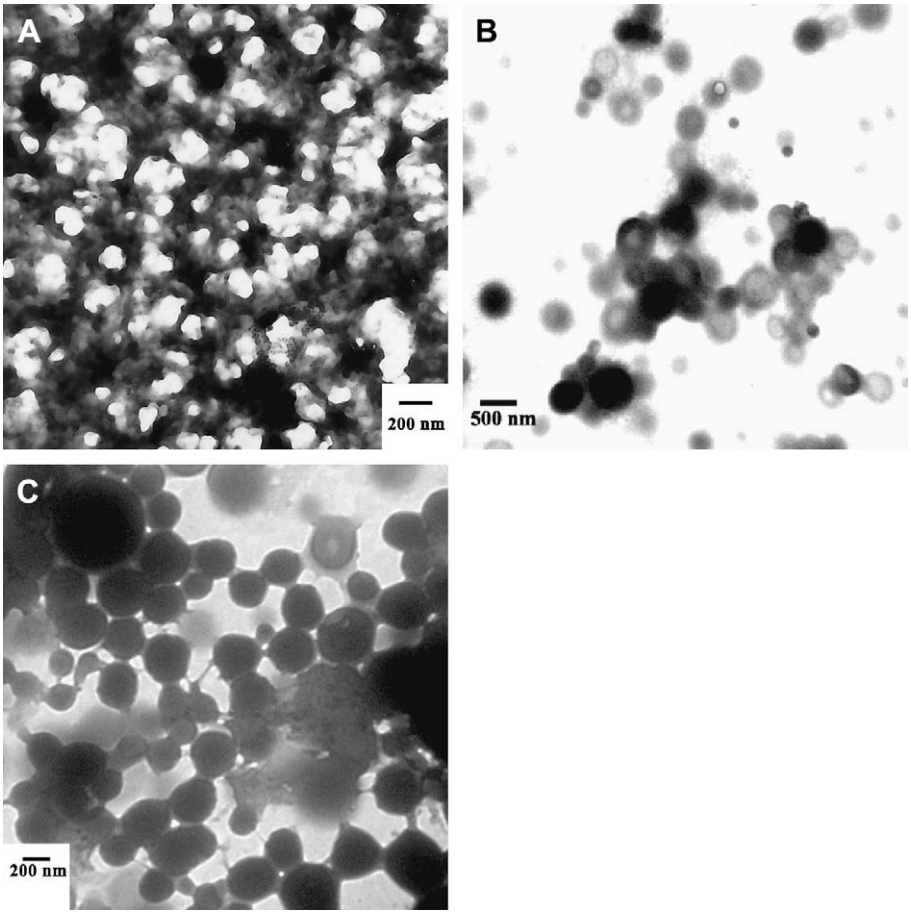



**Fig. 2.** TEM images of assemblies based on PNIPAm/EtTrp-PPP-1 containing IBU: (A) 5.4%, (B) 18.7% and (C) 28.9%.

Table 1). IND containing assemblies based on PNIPAm/EtGly-PPP were produced by dialysis technique. The encapsulation results suggested that IND loading increased as initial feed increased. Morphology study using TEM indicated the structure transformation from network-like to vesicle and nano-spherical architecture when IND content was enhanced (Fig. 7) [31].

### 3.4. Calculation of octanol–water partition coefficient

As a parameter for the hydrophobicity, log *P*, that is the logarithm of partition coefficient between octanol and water, has been frequently used in quantitative structure–activity relationship studies. In current study, log *P* of various small molecules was calculated by the method established by Moriguchi et al.[28], to compare the hydrophobicity of model drugs. In addition, log *P* values of EtTrp, EAB and EtGly were also calculated. Log *P* values thus obtained are listed in Table S2. The values of log *P* difference ($\Delta\log P$) between various hydrophobic compounds and hydrophobic groups are presented in the same table. It can be found that all drugs and aliphatic acids have larger log *P* value compared with that of the hydrophobic groups including EtTrp, EAB and EtGly. For steroidal drugs, with the exception of MPG, DMS and PNS have significantly smaller log *P* than non-steroidal drugs.

### 3.5. Calculation of molar volume

The molar volume ($V$) of various compounds and hydrophobic groups of copolymers was calculated according to the atomic and group contributions developed by Fedors [29]. Table S3 shows the values of $V$ and molar volume difference ($\Delta V$) between various compounds and hydrophobic groups. It can be seen that all the model compounds have larger $V$ compared with those of hydrophobic groups. In addition, the molar volume of steroidal drugs such as DMS, MPG and PNS is significantly larger than that of non-steroidal drugs including MA, IBU, KET, NAP and IND. Among the aliphatic acids, CA has the smallest molar volume, which is also smaller than those of the hydrophobic groups of copolymers, while the $V$ values of TA and SA are significantly larger than those of EtTrp, EAB and EtGly.

### 3.6. Calculation of partial and total solubility parameters

Solubility parameter ($\delta$) has been widely used to evaluate the compatibility between various drugs in solid dispersions or polymer–drug interaction in polymer based delivery systems [32,33]. In this study, two methods were adopted to calculate $\delta$. The first method was established by Fedors, which is frequently used to calculate total solubility [29]. The second method developed by Van Krevelen can be used to calculate both partial and total solubility parameters [30]. The values of $\delta$ obtained according to the first method are summarized in Table S4.

Both partial and total solubility parameters were calculated based on the method by Van Krevelen [30], and the results are listed in Table S5. The difference of both partial and total solubility parameters between various compounds and hydrophobic groups of amphiphilic copolymers is summarized in Tables 2 and S5.

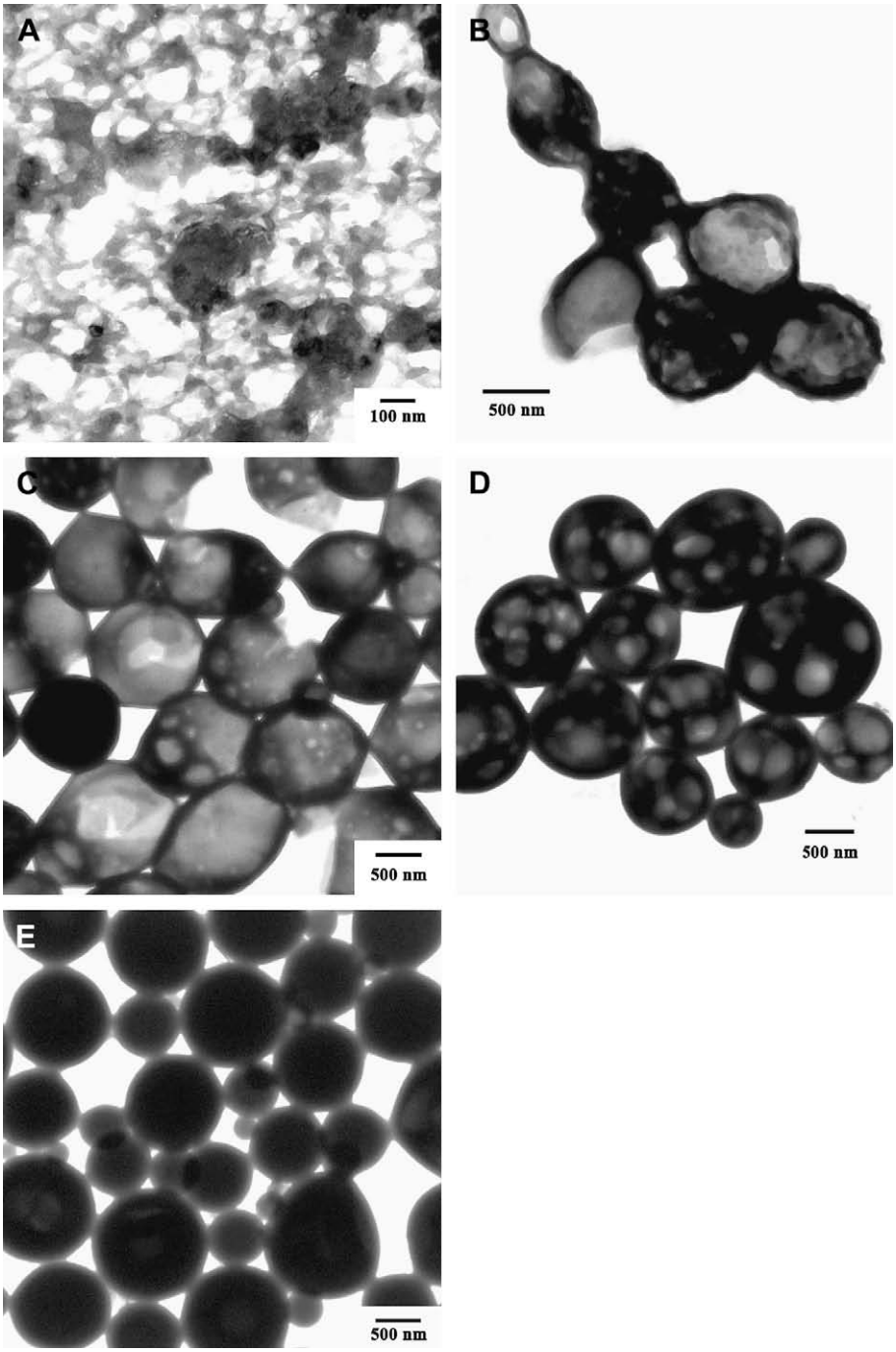



**Fig. 3.** TEM images of assemblies derived from PNIPAm/EtTrp-PPP-2 containing IND (A) 7.2%, (B) 14.2%, (C) 27.3%, (D) 30.4% and (E) 28.2%; the corresponding theoretical IND loading was 9.1%, 23.1%, 33.3%, 41.2% and 47.4% respectively.

## 4. Discussion

While network-like polymeric aggregates were assembled by amphiphilic polyphosphazenes with PNIPAm and hydrophobic groups as substitutes when the hydrophobic content was relatively lower, morphology transformation was indeed observed in the presence of various hydrophobic small molecules. However, depending on specific drug–polymer pairs, difference was observed. Results obtained based on PNIPAm/EtTrp-PPPs and various drugs suggested that non-steroidal drugs including IBU, KET, NAP, MA and IND can easily lead to morphology change as far as their content was above a certain value (critical content), and this effect was independent of copolymer composition. For steroidal drugs like DMS, MPG and PNS, structure changes were only observed for copolymer with higher EtTrp content. A similar phenomenon was observed for assemblies based on copolymers with EAB and EtGly as hydrophobic groups. Accordingly, the more hydrophobic the copolymer is, the lower the critical content will be needed for morphology change. In addition, the presence of carboxylic group seemed to be a prerequisite for structure variation of assemblies based on copolymers with lower content of hydrophobic group. However, experiments using three aliphatic acids indicated that other hydrophobic groups in a hydrophobic small molecule displayed an important role on triggering the

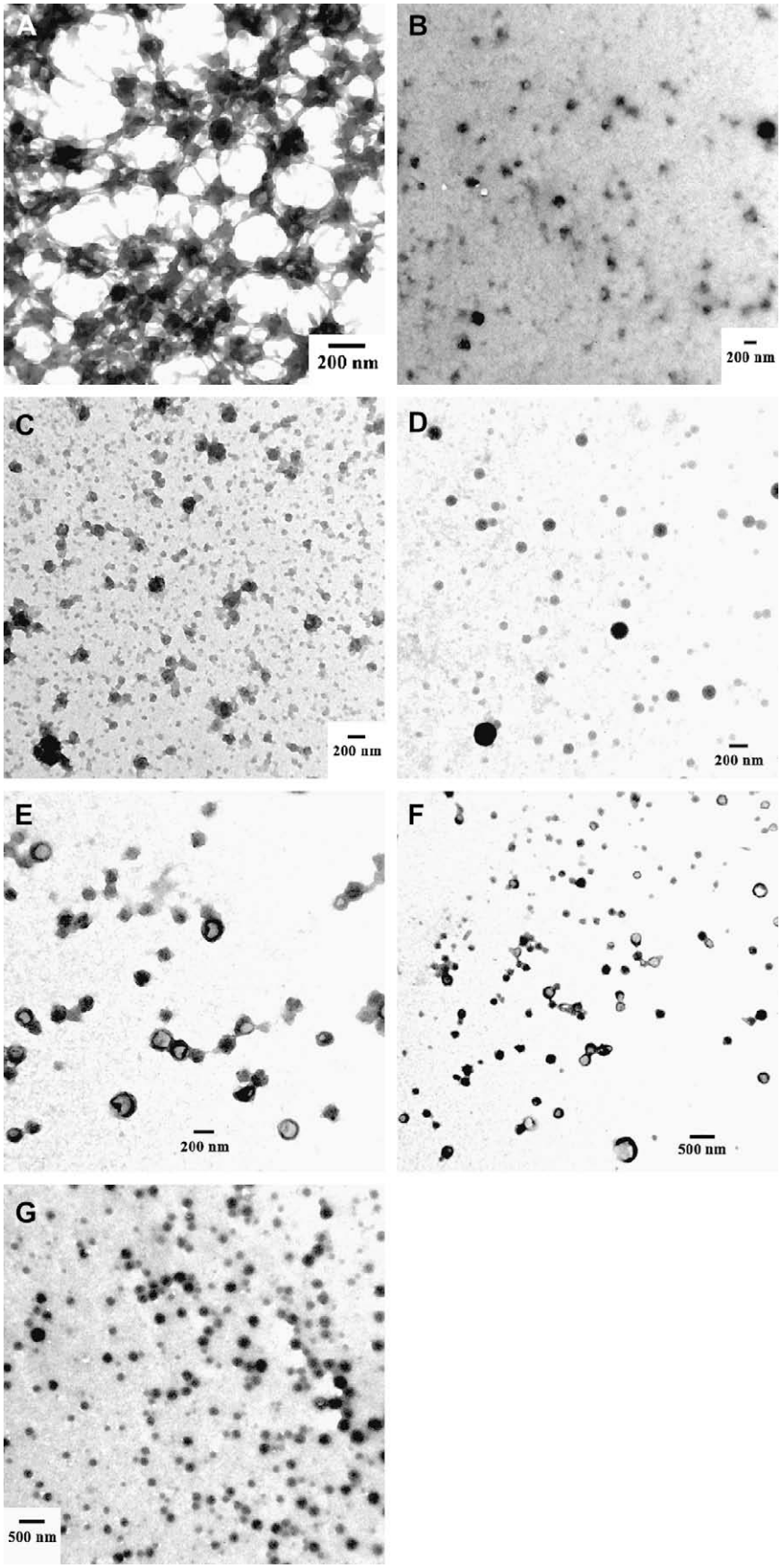



**Fig. 4.** Assemblies based on PNIPAm/EtTrp-PPP-3 loaded with various drugs: (A) control, without drug; (B) 0.7% DMS, (C) 1.3% MPG, (D) 4.1% IBU, (E) 1.8% KET, (F) 2.8% MA, and (G) 1.2% NAP. The theoretical drug loading was 5% for all these drugs.

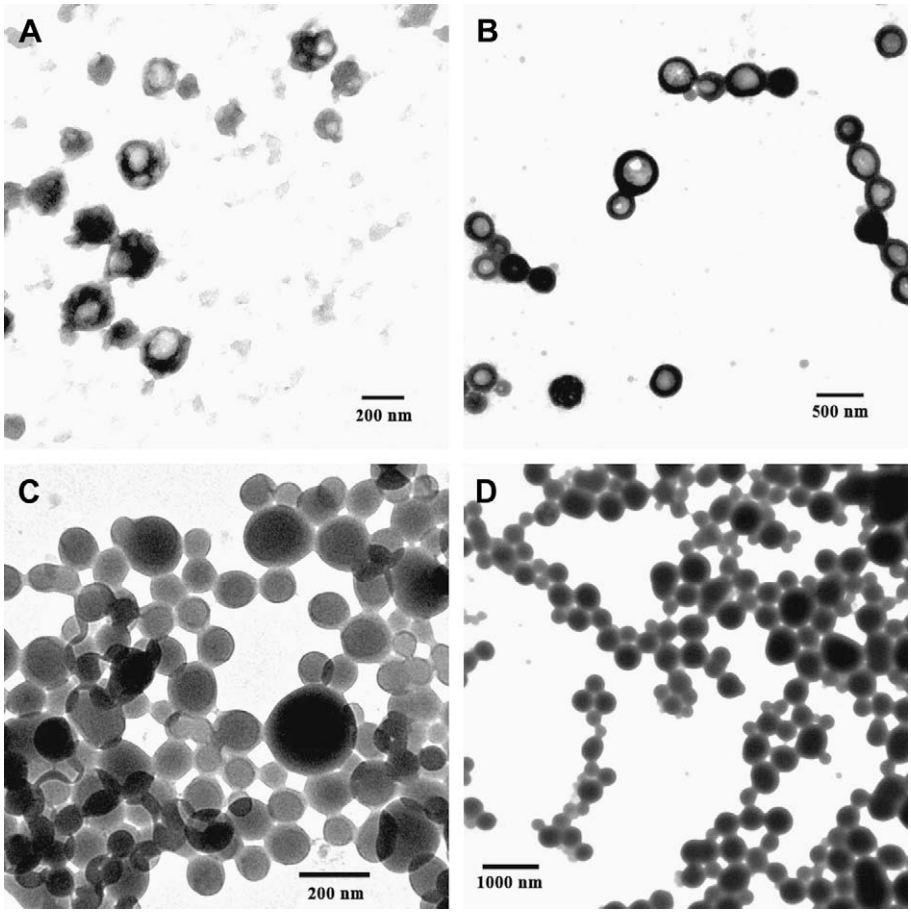



**Fig. 5.** TEM images of assemblies derived from PNIPAm/EtTrp-PPP-3 containing IND: (A) 6.5%, (B) 12.9%, (C) 29.5%, and (D) 20.4%; the corresponding theoretical IND loading was 9.1%, 23.1%, 41.2% and 47.4% respectively.

morphology transformation of assemblies. For simplifying description in the following discussion, small molecules that can lead to morphology evolution are defined as 'active molecule', while other molecules are called 'neutral molecule'.

To correlate the morphology evolution with the physicochemical characteristics of hydrophobic compounds and copolymers, some parameters including log *P*, molar molecular volume, and partial/total solubility parameters were calculated. Since the hydrophobic cores of assemblies based on amphiphilic polyphosphazenes with PNIPAm and hydrophobic groups as co-substitutes were mainly composed of hydrophobic groups, we only calculated the parameters of EtTrp, EAB and EtGly for copolymers, which were used to compare with those of small molecules. As a general evaluation, log *P* was used to compare the relative hydrophobicity of various guest compounds with EtTrp, EAB and EtGly. However, as listed in Table S2, comparison of log *P* values of all these compounds provided no useful information. This suggests that the degree of hydrophobicity itself has no significant effect on the morphology evolution of polymeric assemblies, that is, hydrophobicity is not a good parameter to evaluate whether a molecule is an active/neutral molecule or not. Comparison of the data of *V* and $\Delta V$ presented in Table S3 pointed to the fact that steroidal drugs (DMS, MPG and PNS) have significantly larger value compared with those of MA, IBU, KET, NAP and IND. Interestingly, the latter are active molecules. Accordingly, it seems that molar volume might be used as one parameter to predict the active or neutral nature of a molecule. However, exception was observed for CA. Despite its small *V*, no morphology change was observed for assemblies containing CA as shown in Fig. S5. In addition, no morphology change was found for assemblies based on PNIPAm/EtTrp-PPP-1 when ethyl tryptophan was loaded (Fig. S1J). Obviously, molar volume should be used as a measure in combination with other parameters.

Solubility parameter might be the most frequently used parameter to predict the compatibility between polymer and solvents or other small molecules [30]. For example, Liu et al. used solubility parameter to evaluate the compatibility between an anticancer agent ellipticine and various polymers, and the results obtained can be used to select the most suitable polymer to develop a micelle-type carrier for this drug [34]. The thermodynamic criteria for the compatibility of two substances are based on the free energy of mixing $\Delta G_M$. Two compounds are mutually miscible if $\Delta G_M$ is negative. The $\Delta G_M$ is defined by equation:

$$\Delta G_M = \Delta H_M - T\Delta S_M$$

where $\Delta H_M$ is the mixing enthalpy and $\Delta S_M$ is the mixing entropy. As $\Delta S_M$ is generally positive, there is a certain limiting positive value of $\Delta H_M$ below which miscibility is possible. According to the theory developed by Hildebrand, $\Delta H_M$ can be calculated by the following equation

$$\Delta H_M = \varphi_1\varphi_2(\delta_1 - \delta_2)^2$$

where $\varphi_1$ and $\varphi_2$ are the volume fractions of compounds 1 and 2. This suggests that maximal miscibility will be achieved when $\delta_1 = \delta_2$, which results in zero mixing enthalpy ($\Delta H_M = 0$). One may

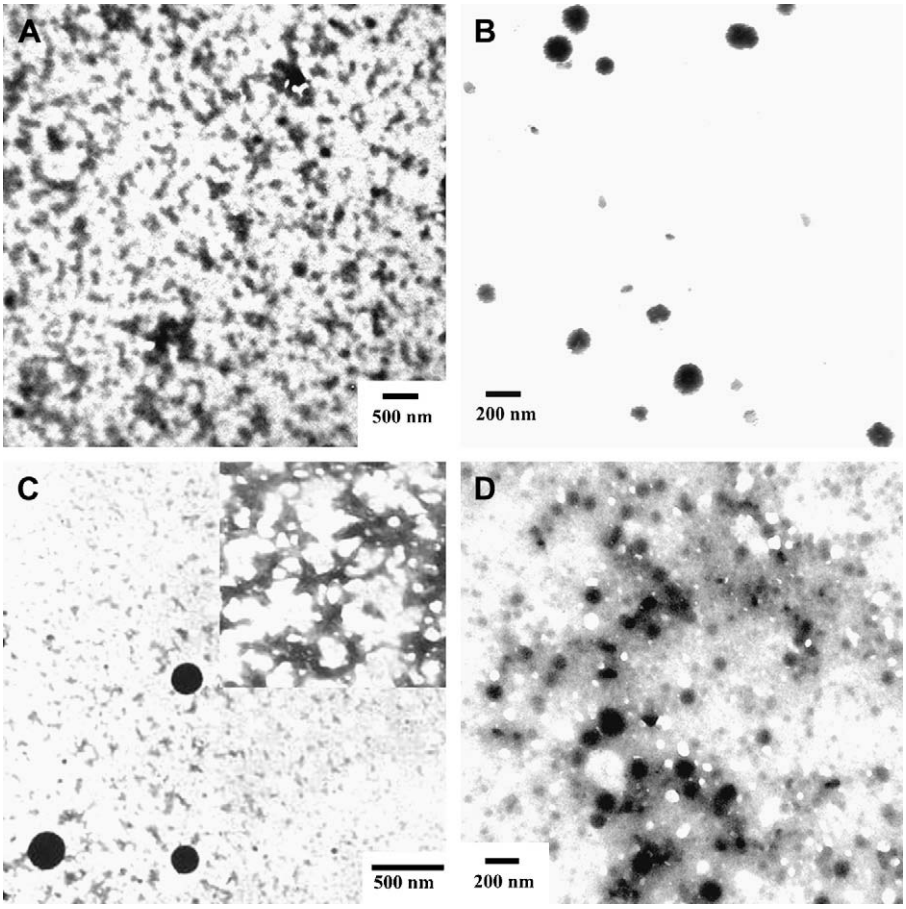



**Fig. 6.** TEM images of assemblies based on: (A) PNIPAm/EAB-PPP-1 with 15.6% IND; (B) PNIPAm/EAB-PPP-1 with 29.8% IND; (C) PNIPAm/EAB-PPP-2, without drug; and (D) PNIPAm/EAB-PPP-2 with 16.5% IND.

conclude that as a requirement for the compatibility of a polymer and a drug, their difference in solubility parameter has to be small, as small as possible [30].

As listed in Table S4, comparison of solubility parameters of various compounds and hydrophobic groups of amphiphilic copolymers suggested that to a certain degree total solubility parameter can serve as a parameter to predict whether a molecule is active or neutral. For copolymers with EtTrp as hydrophobic group, $\Delta\delta$ calculation suggested that MA, KET and NAP are compatible molecules, and they are also active molecules as evidenced by TEM studies. In addition, for neutral molecules, such as DMS, MPG, CA, TA, and SA, they are also incompatible molecules as suggested by the significant difference in $\Delta\delta$. Exception, however, was observed for PNS, IBU and IND. Although both IBU

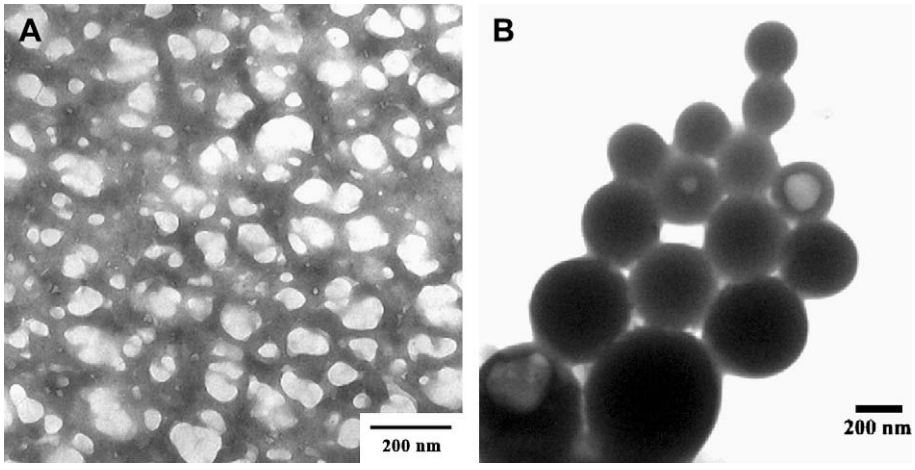



**Fig. 7.** TEM images of assemblies based on PNIPAm/EtGly-PPP with IND: (A) control, without IND; and (B) 25.3%.

**Table 2**
The difference between the partial and total solubility parameters for various model drugs and the hydrophobic groups of copolymers.

| Compounds | $\Delta\delta_d$ ($J^{1/2}/cm^{3/2}$) | | | $\Delta\delta_p$ ($J^{1/2}/cm^{3/2}$) | | | $\Delta\delta_h$ ($J^{1/2}/cm^{3/2}$) | | | $\Delta\delta$ ($J^{1/2}/cm^{3/2}$) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | EtTrp | EAB | EtGly | EtTrp | EAB | EtGly | EtTrp | EAB | EtGly | EtTrp | EAB | EtGly |
| DMS | 0.9 | −6.7 | 3.9 | 1.9 | 0.9 | −0.7 | 6.7 | 6.6 | 4.9 | 4.4 | −2.7 | 5.6 |
| MPG | −1.4 | −9.0 | 1.6 | 0.2 | −0.7 | −2.4 | −3.1 | −3.2 | −4.9 | −2.3 | −9.5 | −1.1 |
| PNS | −0.05 | −7.6 | 3.0 | 1.8 | 0.8 | −0.8 | 1.8 | 1.7 | −0.02 | 1.0 | −6.1 | 2.3 |
| MA | 1.8 | −5.8 | 4.8 | −0.8 | −1.7 | −3.4 | −0.5 | −0.6 | −2.3 | 1.3 | −5.9 | 2.5 |
| IBU | −2.3 | −9.9 | 0.7 | −1.2 | −2.2 | −3.8 | −1.8 | −1.9 | −3.5 | −2.9 | −10.1 | −1.7 |
| KET | 0.4 | −7.2 | 3.4 | 1.1 | 0.2 | −1.5 | −1.1 | −1.2 | −2.9 | 0.2 | −7.0 | 1.4 |
| NAP | −0.8 | −8.3 | 2.2 | −0.2 | −1.1 | −2.8 | −0.3 | −0.4 | −2.2 | −0.9 | −8.0 | 0.4 |
| IND | 4.2 | −3.4 | 7.2 | 3.1 | 2.2 | 0.5 | 0.9 | 0.9 | −0.8 | 4.7 | −2.4 | 6.0 |
| CA | −3.9 | −11.5 | −0.9 | −0.8 | −1.7 | −3.4 | −1.0 | −1.1 | −2.8 | −4.1 | −11.2 | −2.8 |
| TA | −3.7 | −11.3 | −0.7 | −1.8 | −2.7 | −4.4 | −2.7 | −2.7 | −4.4 | −4.6 | −11.8 | −3.4 |
| SA | −3.6 | −11.2 | −0.6 | −2.1 | −3.1 | −4.7 | −3.3 | −3.4 | −5.1 | −4.8 | −12.0 | −3.6 |
| EtTrp | — | — | — | — | — | — | — | — | — | — | — | — |
| EAB | — | — | — | — | — | — | — | — | — | — | — | — |
| EtGly | — | — | — | — | — | — | — | — | — | — | — | — |

and IND are active molecules, the calculated values of $\Delta\delta$ indicated that they are incompatible molecules, and an opposite phenomenon was found for PNS. Interestingly, exactly the same result was found for EAB containing copolymers. Total solubility parameter calculated based on partial solubility parameters ($\Delta\delta_d$, $\Delta\delta_p$ and $\Delta\delta_{vh}$) has been considered to be more reliable to predict the compatibility of various compounds [30,34]. Consequently, the differences of partial and total solubility parameters between various drugs and hydrophobic groups of amphiphilic copolymers were also compared (Table 2). Whereas $\Delta\delta_d$ and $\Delta\delta_p$ cannot provide a good correlation, $\Delta\delta_h$ seems to be a relatively good predictor of active or neutral molecule despite the exception of PNS and CA for all the copolymers. As for $\Delta\delta$ based on partial solubility parameters, inconsistency was found for PNS, IBU and IND for EtTrp containing copolymers, and no good correlation can be found for EAB containing copolymers, while for copolymers with EtGly as hydrophobic group, MPG, MA and IND are exceptions. Unlike assemblies based on amphiphilic block copolymers, the cores are not solely composed of hydrophobic groups or segments for assemblies based on amphiphilic graft copolymers. This might be one possible reason for the exceptions observed. In addition, the calculation methods for some physicochemical parameters such as molar volume and partial solubility parameters need further modification to obtain more reliable results.

In summary, we can conclude that the combination of molecular volume, partial solubility parameter $\delta_h$ and total solubility parameter may provide useful information regarding the potential effect of small guest molecules on the morphology of assemblies based on amphiphilic copolymers with PNIPAm as hydrophilic segment. As we addressed in previous studies [18,26], for assemblies based on amphiphilic polyphosphazenes with PNIPAm and hydrophobic groups as side substitutes, the morphology can be changed from network-like structure to vesicles and nanospheres when the content of hydrophobic group increased. As for assemblies containing hydrophobic compounds, the morphology transformation should be due to the presence of non-covalent interactions such as hydrogen-bonding and hydrophobic interactions between copolymer and small molecules. These non-specific interactions might enhance the hydrophobicity of polymer–drug systems. For instance, the hydrogen bonding interactions between PNIPAm and IND have been evidenced by FT-IR [21]. However, the results obtained in this study suggested that a synergistic effect of both hydrogen bonding and hydrophobic interactions should be responsible for the observed morphology transformation, and more detailed studies are necessary to make a clear conclusion.

## 5. Conclusions

Assemblies based on amphiphilic copolymers with PNIPAm and various hydrophobic groups as side groups were employed as model systems, to study the effect of small guest molecules on the morphological transformation by using an array of drugs with various chemical structures. TEM observation suggested that the loading of non-steroidal anti-inflammatory drugs, including IBU, KET, IND, NAP and MA, can trigger a significant morphology transformation of assemblies based on copolymers with low content of hydrophobic group. On the other hand, the introduction of steroidal anti-inflammatory drugs (MPG, PNS and DMS) and aliphatic acids (CA, TA and SA) has no significant influence on the morphology of assemblies derived from copolymers with low content of hydrophobic group, although they do display some effect on the morphology of assemblies based on copolymers with high content of hydrophobic group. In addition, the morphological transformation is well correlated with drug loading efficiency. The occurrence of morphology transformation means a higher drug loading, and vice versa. The first type of compounds is named as active molecules, otherwise the molecules are considered to be neutral. To correlate the chemical structure of a molecule with its potentially active or neutral nature, various physicochemical parameters including partition coefficient, molecular volume and solubility parameter were calculated according to the group contribution method. The results suggest that a combination of molecular volume and solubility parameter (especially partial solubility parameter due to hydrogen bonding) can be used as a measure to judge whether one molecule is active or neutral. This rule can also be applied to evaluate the compatibility between candidate drug and nanocarriers based on these copolymers, since the compatibility is the prerequisite to achieve efficient drug loading. In addition, current study provides some insight on designing and engineering nanostructured assemblies based on macromolecules and small molecules.

## Acknowledgements

Financial supports from National Natural Science Foundation of China (Nos. 30672641 and 30470465) and High & New Technology 863 Project of the National Ministry of Science and Technology (No. 2007AA10Z356) are gratefully acknowledged.

## Appendix. Supporting information

The calculated values of some physicochemical parameters and some TEM images of assemblies containing various hydrophobic

compounds, this materials is available free of charge via the Internet or from the author. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.polymer.2009.02.004

## References


[1] Riess G. Micellization of block copolymers. Prog Polym Sci 2003;28(7):1107–70.
[2] Hadjichristidis N, Iatrou H, Pitsikalis M, Pispas S, Avgeropoulos A. Linear and non-linear triblock terpolymers. synthesis, self-assembly in selective solvents and in bulk. Prog Polym Sci 2005;30(7):725–82.
[3] Harada A, Kataoka K. Supramolecular assemblies of block copolymers in aqueous media as nanocontainers relevant to biological applications. Prog Polym Sci 2006;31(11):949–82.
[4] Discher DE, Ortiz V, Srinivas G, Klein ML, Kim Y, David CA, et al. Emerging applications of polymersomes in delivery: from molecular dynamics to shrinkage of tumors. Prog Polym Sci 2007;32(8–9):838–57.
[5] Torchilin VP. Multifunctional nanocarriers. Adv Drug Deliv Rev 2006;58(14):1532–55.
[6] van Nostrum CF. Polymeric micelles to deliver photosensitizers for photodynamic therapy. Adv Drug Deliv Rev 2004;56(1):9–16.
[7] Wang XS, Guerin G, Wang H, Wang YS, Manners I, Winnik MA. Cylindrical block copolymer micelles and co-micelles of controlled length and architecture. Science 2007;317(5838):644–7.
[8] Pochan DJ, Chen ZY, Cui HG, Hales K, Qi K, Wooley KL. Toroidal triblock copolymer assemblies. Science 2004;306(5693):94–7.
[9] Li ZB, Kesselman E, Talmon Y, Hillmyer MA, Lodge TP. Multicompartment micelles from ABC miktoarm stars in water. Science 2004;306(5693):98–101.
[10] Jain S, Bates FS. On the origins of morphological complexity in block copolymer surfactants. Science 2003;300(5618):460–4.
[11] Discher BM, Won YY, Ege DS, Lee JCM, Bates FS, Discher DE, et al. Polymersomes: tough vesicles made from diblock copolymers. Science 1999;284(5417):1143–6.
[12] Zhang LF, Eisenberg A. Multiple morphologies of "crew-cut" aggregates of polystyrene-b-poly(acrylic acid) block copolymers. Science 1995;268(5218):1728–31.
[13] Yan DY, Zhou YF, Hou J. Supramolecular self-assembly of macroscopic tubes. Science 2004;303(5654):65–7.
[14] Cornelissen JJLM, Fischer M, Sommerdijk NAJM, Nolte RJM. Helical superstructures from charged poly(styrene)–poly(isocyanodipeptide) block copolymers. Science 1998;280(5368):1427–30.
[15] Geng Y, Dalhaimer P, Cai SS, Tsai R, Tewari M, Minko T, et al. Shape effects of filaments versus spherical particles in flow and drug delivery. Nat Nanotechnol 2007;2(4):249–55.
[16] Raez J, Manners I, Winnik MA. Nanotubes from the self-assembly of asymmetric crystalline-coil poly(ferrocenylsilane-siloxane) block copolymers. J Am Chem Soc 2002;124(35):10381–95.
[17] Zheng RH, Liu GJ, Yan XH. Polymer nano- and microspheres with bumpy and chain-segregated surfaces. J Am Chem Soc 2005;127(44):15358–9.
[18] Zhang JX, Qiu LY, Zhu KJ. Solvent controlled multi-morphological self-assembly of amphiphilic graft copolymers. Macromol Rapid Commun 2005;26(21):1716–23.
[19] Zhang JX, Qiu LY, Li XD, Jin Y, Zhu KJ. Versatile preparation of fluorescent particles based on polyphosphazenes: from micro- to nanoscale. Small 2007;3(12):2081–93.
[20] Cui HG, Chen ZY, Zhong S, Wooley KL, Pochan DJ. Block copolymer assembly via kinetic control. Science 2007;317(5838):647–50.
[21] Zhang JX, Li XD, Yan MQ, Qiu LY, Jin Y, Zhu KJ. Hydrogen bonding-induced transformation of network aggregates into vesicles-a potential method for the preparation of composite vesicles. Macromol Rapid Commun 2007;28(6):710–7.
[22] Dymicky M, Mellon EF, Naghski J. A general, highly efficient azeotropic method of esterification of amino acids. Anal Biochem 1971;41(2):487–91.
[23] Chen G, Hoffman AS. Graft copolymers that exhibit temperature-induced phase transitions over a wide range of pH. Nature 1995;373(6509):49–52.
[24] Sohn YS, Cho YH, Baek H, Jung OS. Synthesis and properties of low molecular weight polyphosphazenes. Macromolecules 1995;28(22):7566–8.
[25] Zhang JX, Qiu LY, Jin Y, Zhu KJ. Controlled nanoparticles formation by self-assembly of novel amphiphilic polyphosphazenes with poly (N-isopropylacrylamide) and ethyl glycinate as side groups. React Funct Polym 2006;66(12):1630–40.
[26] Zhang JX, Qiu LY, Jin Y, Zhu KJ. Multimorphological self-assemblies of amphiphilic graft polyphosphazenes with oligopoly (N-isopropylacrylamide) and ethyl 4-aminobenzoate as side groups. Macromolecules 2006;39(1):451–5.
[27] Wilhelm M, Zhao CL, Wang Y, Xu R, Winnik MA, Mura JL, et al. Poly (styrene-ethylene oxide) block copolymer micelle formation in water: a fluorescence probe study. Macromolecules 1991;24(5):1033–40.
[28] Moriguchi I, Hirono S, Liu Q, Nakagome I, Matsushita Y. Simple method of calculating octanol/water partition coefficient. Chem Pharm Bull 1992;40(1):127–30.
[29] Fedors RF. Method for estimating both the solubility parameters and molar volumes of liquids. Polym Eng Sci 1974;14(2):147–54.
[30] Van Krevelen DW. Properties of polymers-their correlation with chemical structure, their numerical estimation and prediction from additive group contributions. Amsterdam: Elsevier science publisher B.V.; 1990. p. 212.
[31] Zhang JX, Li SH, Li XH, Qiu LY, Li XD, Li XJ, et al. Physicochemical characterization, in vitro, and in vivo evaluation of indomethacin-loaded nanocarriers self-assembled by amphiphilic polyphosphazene. J Biomed Mater Res A 2008;86A(4):914–25.
[32] Greenhalgh DJ, Williams AC, Timmins P, York P. Solubility parameters as predictors of miscibility in solid dispersions. J Pharm Sci 1999;88(11):1182–90.
[33] Hancock BC, York P, Rowe RC. The use of solubility parameters in pharmaceutical dosage form design. Int J Pharm 1997;148(1):1–21.
[34] Liu JB, Xiao YH, Allen C. Polymer-drug compatibility: a guide to the development of delivery systems for the anticancer agent, ellipticine. J Pharm Sci 2004;93(1):132–43.