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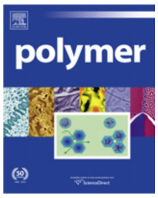

Self-assembly of pH-responsive and fluorescent comb-like amphiphilic copolymers in aqueous media

Min Li [a], Guo Liang Li [a], Zhiguo Zhang [a], Jun Li [b], Koon-Gee Neoh [a], En-Tang Kang [a,*]

[a] *Department of Chemical and Biomolecular Engineering, National University of Singapore, Kent Ridge, Singapore 119260*
[b] *Division of Bioengineering, National University of Singapore, Kent Ridge, Singapore 117574*

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"Comb-like" graft copolymers, consisting of a poly((*N*-vinylcarbazole)-*co*-(4-vinylbenzyl chloride)) (P(NVK-*co*-VBC)) copolymer backbone from free radical polymerization and poly(((2-dimethylamino)ethyl methacrylate)-*co*-(*tert*-butyl acrylate)) (P(DMAEMA-*co*-*t*BA)) side chains from atom transfer radical polymerization (ATRP), were hydrolyzed to produce the acrylic acid (AAc)-containing "comb-like" graft copolymers of P(NVK-*co*-VBC)-*comb*-P(DMAEMA-*co*-AAc). The amphiphilic copolymers possess a fluorescent hydrophobic P(NVK-*co*-VBC) backbone and pH-sensitive hydrophilic P(DMAEMA-*co*-AAc) side chains. Arising from acid–base interaction of the hydrophilic side chains, the copolymers can self-assemble into pH-responsive fluorescent and multi-walled hollow vesicles of well-defined morphology in aqueous media. The size and layered wall thickness of the vesicles are also dependent on the length of the copolymer side chains, while the number of wall layers is dependent on the concentration of the vesicles in the aqueous media. In comparison, a *N*-isopropylacrylamide (NIPAAm)-containing comb-like amphiphilic copolymer (P(NVK-*co*-VBC)-*comb*-P(NIPAAm-*co*-DMAEMA)) of similar structure, albeit with non-interacting hydropholic side chains, self-assembles only into temperature and pH-responsive single-shelled hollow nanoparticles in aqueous media.

1. Introduction

Stimuli-responsive polymers have been extensively investigated in conjunction with the development of "smart materials" [1–3]. Polymers, which response to acid–base interactions or to changes in pH of the environment, are attractive materials for biological applications [4,5]. Poly((2-dimethylamino)ethyl methacrylate) (P(DMAEMA)) and poly(acrylic acid) (P(AAc)) are favorite stimuli-responsive materials because of their good pH-sensitivity in an aqueous medium [6–11]. P(DMAEMA) becomes protonated in a low-pH medium and is rendered hydrophilic. Deprotonation at high pH, however, can render P(DMAEMA) hydrophobic. P(DMAEMA)-based materials can therefore exhibit reversible pH-dependent swelling behavior in an aqueous medium [7,12,13]. P(AAc), on the other hand, is ionized and hydrophilic at high pH, and non-ionized and hydrophobic at low pH. P(AAc) has been widely used in pH-responsive copolymers and microspheres [14–16].

Recently, hollow micro- and nano-spheres have attracted a great deal of attention because of their potential applications in catalysis, controlled drug delivery, paints and coatings, and as electronic materials [17–19]. The vesicular polymerization route to functional hollow spheres has been described [20]. The design and synthesis of hollow microspheres which can respond to environmental stimuli of changing temperature [21,22], electric and magnetic fields [21,23], and pH and ionic strength have been reported [20,24]. Self-assembly of amphiphilic copolymers into vesicles of well-controlled structures has also attracted considerable attention [25–29]. Polymer vesicles result from an equilibrium in the interfacial energy between the core and the external medium, the stretching of the core-forming blocks and the repulsive interaction among corona chains [30]. Many types of amphiphilic copolymers, such as block copolymers [14,31] and graft copolymers [32,33], have been reported to aggregate into vesicles.

In this work, comb-like graft copolymers, consisting of a fluorescent hydrophobic poly((*N*-vinylcarbazole)-*co*-(4-vinylbenzyl chloride)) (P(NVK-*co*-VBC)) copolymer backbone and pH-responsive hydrophilic P(DMAEMA-*co*-AAc) copolymer side chains of controlled length, were synthesized. The amphiphilic copolymers can self-assemble into hollow vesicles with "onion-like" multi-walls in aqueous media of a certain concentration range through acid–base interaction of the side chains. The vesicular morphology can be controlled by pH of the medium, the length of the graft chains (side chains) of the comb-like copolymer and the concentration of copolymer solution. Moreover, the copolymer vesicles exhibit

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* Corresponding author. Tel.: +65 6516 2189; fax: +65 67791936.
E-mail address: cheket@nus.edu.sg (E.-T. Kang).

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a reversible change in fluorescence intensity in aqueous media of pH 3 to 9. In comparison, amphiphilic comb-like copolymers, consisting of the same hydrophobic P(NVK-*co*-VBC) backbone, albeit with non-interacting hydrophilic P(NIPAAm-*co*-DMAEMA) copolymer side chains (NIPAAm = *N*-isopropylacrylamide), can self-assemble only into hollow nanoparticles of single-shell in aqueous media.

2. Experimental section

2.1. Materials

The monomers, 4-vinylbenzyl chloride (VBC, 90%), *N*-vinylcarbazole (NVK, 98%), *N*-isopropylacrylamide (NIPAAm, 97%), (2-dimethylamino)ethyl methacrylate (DMAEMA, 98%), and *tert*-butyl acrylate (*t*BA, 98%) were obtained from Sigma–Aldrich Chemical Co. DMAEMA and *t*BA were used after removal of the inhibitors in a ready-to-use disposable inhibitor-removal column (Sigma–Aldrich Chemical Co.). Azobisisobutyronitrile (AIBN, 98%) was recrystallized from methanol, dried in a vacuum oven and stored under nitrogen. 1,1,4,7,10,10′-Hexamethyltriethyenetetramine (HMTETA, 99%), copper(I) chloride (CuCl, 99%), trifluoroacetic acid (TFA) and all solvents (analytical grade) were also obtained from Sigma–Aldrich Chemical Co. and were used as received. Purified argon was used in all reactions.

2.2. Synthesis of the P(NVK-co-VBC) backbone

Procedures for synthesizing the P(NVK-*co*-VBC) copolymer were similar to those reported in the literature [34]. A mixture of VBC (0.882 g, 5.2 mmol), NVK (1.025 g, 5.2 mmol) and AIBN (6 mg) in 5 mL of tetrahydrofuran (THF) was refluxed at 60 °C under argon for 24 h. The reaction mixture was cooled to room temperature. The crude polymer was purified via several dissolution–reprecipitation cycles, using THF (minimum amount) to dissolve and methanol (100 mL) to precipitate, prior to being dried under reduced pressure. Gel-permeation chromatography (GPC) measurements: number-average molecular weight (M_n) = 6730 and polydispersity index (PDI) = 1.12. ^{1}H Nuclear magnetic resonance (NMR) spectroscopy measurements: copolymer chemical composition (monomer repeat units), [NVK]:[VBC] = 4:39.

2.3. Synthesis of the comb-shaped P(NVK-co-VBC)-comb-P(DMAEMA-co-tBA) copolymers (KVDT copolymers)

Graft copolymerization of DMAEMA and *t*BA was carried out under the typical conditions of atom transfer radical polymerization (ATRP, Scheme 1). Thus, for the synthesis of **KVDT3** copolymer of Table 1, 0.38 mL of *t*BA (2.52 mmol), 3.9 mL of DMAEMA (22.7 mmol), 43.5 mg (0.252 mmol, based on the −Cl moieties) of the P(NVK-*co*-VBC) backbone macroinitiator, 48 μL of HMTETA (0.252 mmol), and 5 mL of THF were introduced into a 25 mL flask equipped with a magnetic stirrer. After the maroinitiator and monomers had dissolved completely, the reaction mixture was degassed by bubbling argon through the solution for 30 min. Then, 25 mg of CuCl (0.252 mmol) was added into the reaction mixture under an argon atmosphere. The reaction mixture was purged with argon for an additional 10 min. The flask was sealed with a rubber stopper under an argon atmosphere. The polymerization was allowed to proceed under continuous stirring at 60 °C for a predetermined period of time. The reaction was terminated by exposing the reaction mixture to air. The copolymer solution was diluted by adding 5 folds the volume of THF. The catalyst complex was removed by passing the blue dilute polymer solution through a short aluminum oxide column. A colorless solution was obtained. After removal of THF in a rotary evaporator, the copolymer was precipitated in excess hexane. The crude polymer was purified by re-precipitation twice in hexane to remove the reactant residues, prior to being dried under reduced pressure. GPC measurements: M_n = 78 370 and PDI = 1.30. ^{1}H NMR spectroscopy measurements: copolymer side chain chemical composition, [DMAEMA]:[*t*BA] = 421:43. Other P(NVK-*co*-VBC)-*comb*-P(DMAEMA-*co*-*t*BA) copolymers (**KVDT1**, **KVDT2** and **KVDT4** of Table 1) were prepared using similar procedures.

2.4. Hydrolysis of the P(NVK-co-VBC)-comb-P(DMAEMA-co-tBA) copolymers

About 0.24 g (0.132 mmol, based on the *t*BA groups) of **KVDT3** of Table 1, 1.01 mL (1.32 mmol) of TFA, and 30 mL of dichloromethane were introduced into a 50 mL round bottom flask. The mixture was stirred at room temperature for 6 h. The product was precipitated by adding the solution to an excess volume of hexane, followed by filtering and drying under reduced pressure to yield the acrylic acid (AAc) containing copolymer, **KVDA3** of Table 1. Other P(NVK-*co*-VBC)-*comb*-P(DMAEMA-*co*-*t*BA) copolymers (**KVDT1**, **KVDT2** and **KVDT4** of Table 1) were hydrolyzed using similar procedures.

2.5. Synthesis of the P(NVK-co-VBC)-comb-P(NIPAAm-co-DMAEMA) copolymer (KVND copolymer)

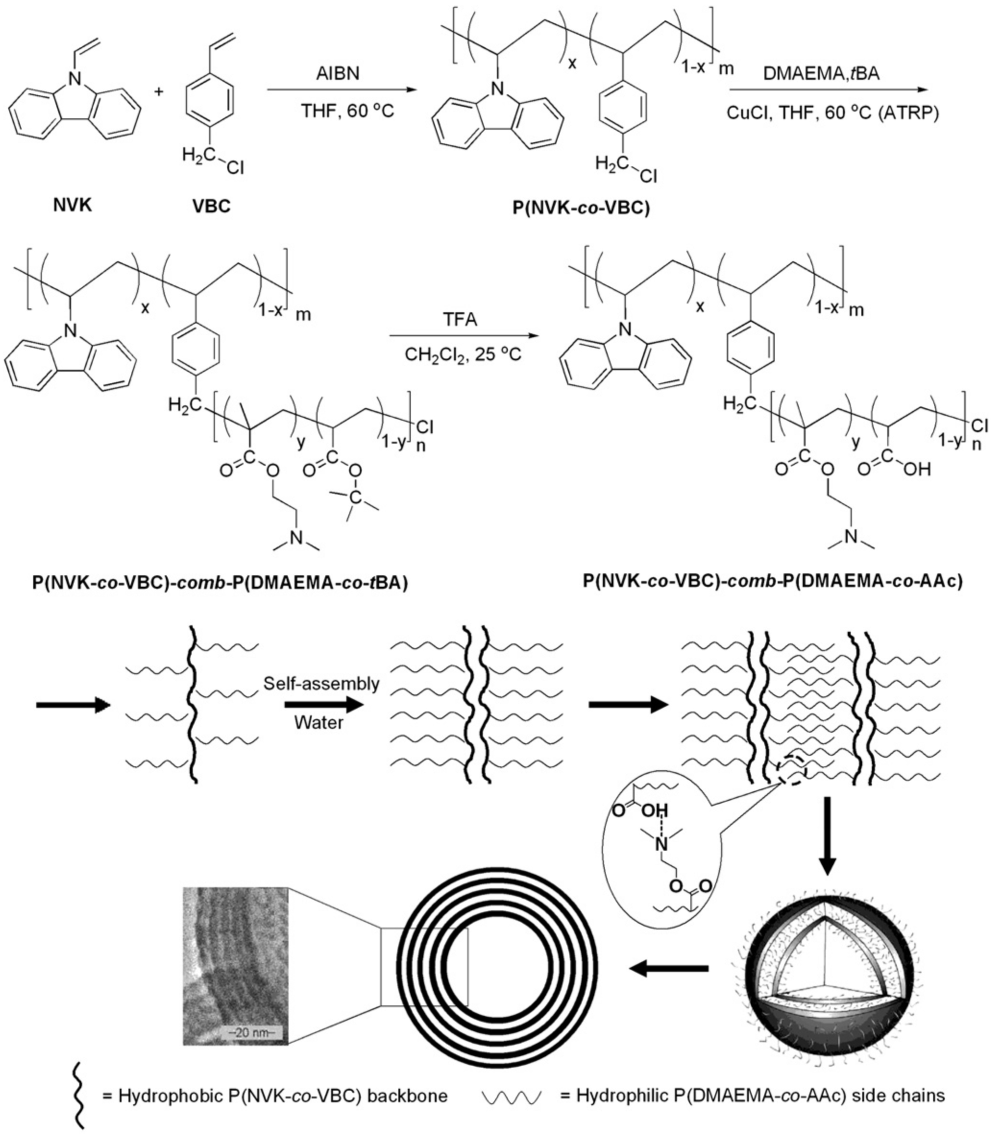
Graft copolymerization of NIPAAm and DMAEMA was carried out under the typical conditions of ATRP (Scheme 2). The synthesis procedures are similar to those employed for the P(NVK-*co*-VBC)-*comb*-P(DMAEMA-*co*-*t*BA) copolymers. Thus, 0.82 g of NIPAAm (7.0 mmol), 0.6 mL of DMAEMA (3.5 mmol), 24.1 mg (0.14 mmol, based on the −Cl moieties) of the P(NVK-*co*-VBC) backbone macroinitiator, 26.6 μL of HMTETA (0.14 mmol), and 3 mL of THF were introduced into a 25 mL flask equipped with a magnetic stirrer. After degassing with argon for 30 min, 13.9 mg of CuCl (0.14 mmol) was added into the reaction mixture under an argon atmosphere. The reaction mixture was purged with argon for an additional 10 min. The flask was sealed and kept in a 60 °C oil bath for a predetermined period of time. The reaction was terminated by exposing the reaction mixture to air. The copolymer solution was diluted with THF and passed through an alumina column to remove the catalyst. After removal of THF in a rotary evaporator, the copolymer was precipitated in hexane, filtered and dried under reduced pressure. GPC measurements: M_n = 31 610 and PDI = 1.37. ^{1}H NMR spectroscopy measurements: copolymer side chain chemical composition, [NIPAAm]:[DMAEMA] = 191:21.

2.6. Preparation of the self-assembled nanoparticles

For the preparation of the self-assembled nanoparticles, 20 mg of the **KVDA1** or **KVND** copolymer was first dissolved in 20 mL of doubly distilled water in an ultrasonic water bath for 5 min. The solution was then stirred vigorously for another 10 min to obtain the 0.1 wt% homogenous copolymer solution. The pH of the solution was adjusted by adding 1 M HCl and 1 M NaOH. NaCl was added to keep the solutions under a constant ionic strength at 0.01 M. The self-assemblies of other comb-like copolymers and in different solution concentrations were carried out using similar procedures.

2.7. Instrumentation

The copolymers prepared in this work were characterized by GPC, Fourier-transform infrared (FT-IR) spectroscopy, and ^{1}H NMR spectroscopy. GPC measurements were performed on a Waters GPC system equipped with Waters Styragel columns, a Waters-2487 dual wavelength UV detector, and a Waters-2414 refractive index detector. THF or *N*,*N*-dimethylformamide (DMF) was used as the



AIBN = azobisisobutyronitrile THF = tetrahydrofuran VBC = 4-vinylbenzyl chloride
NVK = *N*-vinylcarbazole *t*BA = *tert*-butyl acrylate DMAEMA = (2-dimethylamino)ethyl methacrylate
TFA = trifluoroacetic acid ATRP = atom transfer radical polymerization

Scheme 1. Synthesis and self-assembly of the amphiphilic comb-like P(NVK-*co*-VBC)-*comb*-P(DMAEMA-*co*-AAc) copolymer.

eluent at a flow rate of 1.0 mL/min. Monodispersed polystyrene standards were used to generate the calibration curve. It should be noted that some discrepancies in molecular weights can probably arise from the difference in hydrodynamic volumes between the comb-like copolymers prepared in this work and the PS standards used as GPC references. The FT-IR spectra of the samples dispersed in KBr pellets were measured on a Bio-Rad FTS 135 FT-IR spectrophotometer. Each spectrum was obtained by cumulating 64 scans. ^{1}H NMR spectra were measured on a Bruker ARX 300 MHz spectrometer, using $CDCl_3$ or D_2O as the solvent, by accumulating 300 scans at a relaxation time of 2 s. UV–visible absorption spectra were recorded on a Shimadzu UV-1601 spectrophotometer. The fluorescence spectra were measured on a Shimadzu RF-5031 spectrophotometer. The zeta potential of the self-assembled nanoparticles was measured on a Zetasizer Nano ZS (Malvern Instruments, Southborough, MA). The average hydrodynamic diameters of vesicles were determined by dynamic light scattering (DLS). DLS was performed on a Brookhaven 90 plus laser light scattering spectrometer at the scattering angle of 90°. Transmission electron microscopy (TEM) images were obtained on a JEOL JEM-2010 transmission electron microscope and a JEOL JEM-2100F field emission transmission electron microscope. Copolymers dissolved in doubly distilled water were spread onto the surface of a carbon coated copper grid and then dried under reduced pressure. Scanning electron microscopy (SEM) images were obtained on a JEOL JSM-6700 field emission scanning electron microscope. The aqueous copolymer solution was dropped onto a clean copper foil on an electron microscope stub, and dried under reduced pressure. The samples were then sputter coated with a thin Pd layer to enhance the contrast and quality of the images.

3. Results and discussion

3.1. Characterization of the synthesized amphiphilic comb-like copolymers

Details on the synthesis and characterization of the copolymer samples are summarized in Table 1.

Table 1
Characterization of the synthesized amphiphilic comb-like copolymers.

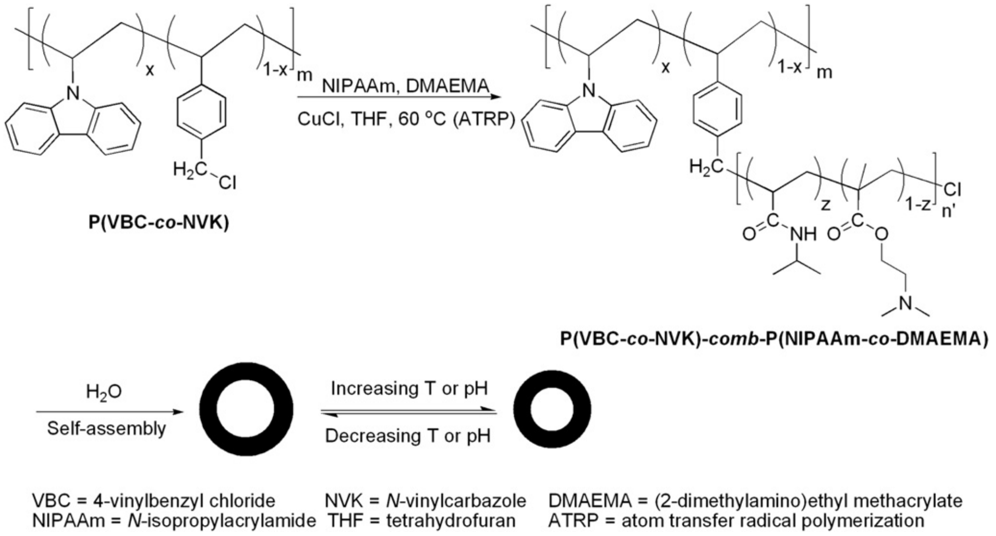
Copolymer	Reaction time (h)	M_n (g/mol)[a]	Polydispersity index (PDI)[a]	Copolymer molar composition[b] [NVK]/[VBC]	Copolymer molar composition[c] [*t*BA]/[DMAEMA]	Copolymer molar composition[d] [AAc]/[DMAEMA]	Copolymer molar composition[e] [NIPAAm]/[DMAEMA]
P(NVK-*co*-VBC)[f]	24	6730	1.12	4/39	–	–	–
KVDT1[g]	2	20 160	1.22	4/39	10/77	–	–
KVDT2[g]	6	57 240	1.28	4/39	27/299	–	–
KVDT3[g]	12	78 370	1.30	4/39	43/421	–	–
KVDT4[h]	12	89 990	1.32	4/39	86/460	–	–
KVDA1[i]	6	19 500	1.23	4/39	–	10/77	–
KVDA2[i]	6	55 650	1.26	4/39	–	27/299	–
KVDA3[i]	6	76 010	1.36	4/39	–	43/421	–
KVDA4[i]	6	85 100	1.40	4/39	–	86/460	–
KVND[j]	3	31 610	1.37	4/39	–	–	191/21

[a] Determined from GPC results. PDI = Weight-average molecular weight/Number-average molecular weight, or M_w/M_n.
[b] Determined from the ^{1}H NMR spectra, M_n's, and the molecular weights of NVK (193.24 g/mol) and VBC (152.62 g/mol).
[c] Determined from the ^{1}H NMR spectra, M_n's, and the molecular weights of DMAEMA (157.22 g/mol) and *t*BA(128.17 g/mol).
[d] Estimated from the corresponding copolymer molar composition of [*t*BA]/[DMAEMA].
[e] Determined from the ^{1}H NMR spectra, M_n's, and the molecular weights of NIPAAm (113.2 g/mol) and DMAEMA (157.22 g/mol).
[f] Synthesized using a molar feed ratio [VBC (0.882 g)]:[NVK]:[AIBN] of 145:145:1 at 60 °C in 5 ml of THF.
[g] P(NVK-*co*-VBC)-*comb*-P(DMAEMA-*co*-*t*BA), synthesized using a molar feed ratio [*t*BA (0.38 mL)]:[DMAEMA]:[initiator]:[CuCl]:[HMTETA] of 10:90:1:1:1 at 60 °C in 5 ml of THF.
[h] P(NVK-*co*-VBC)-*comb*-P(DMAEMA-*co*-*t*BA), synthesized using a molar feed ratio [*t*BA (0.76 mL)]:[DMAEMA]:[initiator]:[CuCl]:[HMTETA] of 20:80:1:1:1 at 60 °C in 5 ml of THF.
[i] From hydrolysis of corresponding P(NVK-*co*-VBC)-*comb*-P(DMAEMA-*co*-*t*BA) copolymer. The molar feed ratio of [TFA]:[*tert*-butyl groups] was about 10:1.
[j] P(VBC-*co*-NVK)-*comb*-P(NIPAAm-*co*-DMAEMA), synthesized using a molar feed ratio [NIPAAm (0.82 g)]:[DMAEMA]:[initiator]:[CuCl]:[HMTETA] of 50:25:1:1:1 at 60 °C in 3 ml of THF.

3.1.1. *Characterization of the P(NVK-co-VBC) copolymer backbone (macroinitiator)*

The copolymer backbone prepared from 1:1 M feed ratio of 4-vinylbenzyl chloride (VBC) and *N*-vinylcarbazole (NVK) is readily soluble in common organic solvents, such as tetrahydrofuran (THF), *N*,*N*-dimethylformamide (DMF) and chloroform. The copolymer has a number-average molecular weight (M_n) and weight-average molecular weight (M_w) of about 6730 and 7540, respectively, giving rise to a polydispersity index (PDI) of 1.12. The composition and chemical structure of the copolymer were analyzed by fourier-transform infrared (FT-IR) and ^{1}H nuclear magnetic resonance (NMR) spectroscopy. For the FT-IR spectrum of P(NVK-*co*-VBC) copolymer in Fig. 1(a), the two strong absorption peaks at 725 and 749 cm^{-1} are associated with the absorption of the carbazole moieties [35,36]. The sharp absorption band at 1265 cm^{-1} is associated with the absorption of the CH_2Cl groups in VBC [37]. The presence of absorption bands associated with both the carbazole and CH_2Cl groups indicates successful copolymerization of VBC and NVK.

For the ^{1}H NMR spectrum of P(NVK-*co*-VBC) copolymer in Fig. 2 (a), the chemical shifts in the region of 6.2–8.1 ppm (signal ***c***) are associated with the protons of the aromatic rings in the carbazole [38,39] and VBC units [34,40]. The chemical shift at $\delta = 4.2$–4.6 ppm (signal ***d***) is attributable to the methylene protons adjacent to the chloride in the VBC groups [40]. The chemical shift at $\delta = 3.5$ (signal ***a***) corresponds to the methyl protons adjacent to the nitrogen atom in the carbazole units [39]. The chemical shifts in the region of 1.2–2.2 ppm (signal ***b***, ***b′*** and ***b″***) are associated with the methylene protons of the NVK and VBC moieties. The composition of the copolymer (NVK:VBC molar ratio) is about 4:39, as



Scheme 2. Synthesis and self-assembly of the amphiphilic comb-like P(NVK-*co*-VBC)-*comb*-P(NIPAAm-*co*-DMAEMA) copolymer.

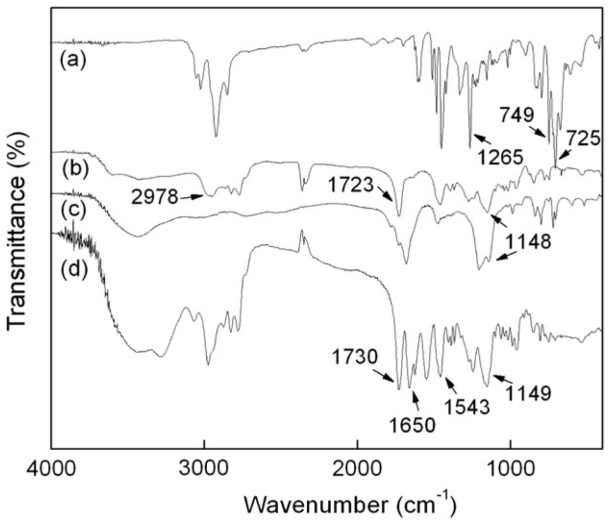


Fig. 1. FT-IR spectra of (a) the P(NVK-*co*-VBC) copolymer, and the (b) **KVDT3** copolymer, (c) **KVDA3** copolymer and (d) **KVND** copolymer of Table 1.

determined from the ratio of chemical shift at $\delta = 3.5$ ppm, associated with the proton adjacent to the nitrogen atom (signal ***a***) of the NVK units, to that at $\delta = 4.2–4.6$ ppm, associated with the two methylene protons adjacent to the chloride atom (signal ***d***) of the VBC units. The difference between the copolymer composition and the initial NVK:VBC molar feed ratio (1:1) indicates a large difference in reactivity between the two monomers under the present experimental conditions, which agrees with the results reported for P(NVK-*co*-VBC) copolymers under free radical polymerization conditions [34].

3.1.2. Characterization of the P(NVK-co-VBC)-comb-P(DMAEMA-co-AAc) copolymers

VBC is a widely used alkyl halide initiator for atom transfer radical polymerization (ATRP) [41–43]. The VBC-containing copolymers can be used as the macroinitiators for ATRP [40]. Molecular brushes or side chains comprising of random copolymers of (2-dimethylamino)ethyl methacrylate (DMAEMA) and *tert*-butyl acrylate (*t*BA) were synthesized by “grafting from” the P(NVK-*co*-VBC) backbone, using the VBC units as the ATRP macroinitiators. The length of the P(DMAEMA-*co*-*t*BA) side chains in the “comb-like” copolymer can be controlled by the ATRP time or the DMAEMA:*t*BA molar feed ratio. Successful graft copolymerization was confirmed by the FT-IR and ^{1}H NMR spectroscopy results. Fig. 1(b) shows the FT-IR spectrum of a P(NVK-*co*-VBC)-*comb*-P(DMAEMA-*co*-*t*BA) copolymer (**KVDT3** of Table 1). The characteristic absorption peak at about 1723 cm^{-1} is associated with the $-$C = O groups of DMAEMA and *t*BA units, that at 1148 cm^{-1} corresponds to the $-$C$-$N group of DMAEMA, while that at 2978 cm^{-1} corresponds to the asymmetric C-H (of *tert*-butyl ester group of *t*BA) stretching vibration [44]. For the ^{1}H NMR spectrum of **KVDT3** copolymer in Fig. 2(b), the characteristic chemical shift at $\delta = 4.1$ ppm (signal **1**) is attributable to the methylene protons adjacent to the oxygen moieties of the ester linkages in the DMAEMA units [45,46]. The chemical shifts at $\delta = 2.3$ (signal **2**) and 2.6 ppm (signal **3**) are associated with the methylene and methyl protons, respectively, of the DMAEMA moieties in the graft copolymer. The characteristic chemical shift at $\delta = 1.4$ ppm (signal **4**) corresponds to the methyl protons of *tert*-butyl groups in the *t*BA units. The compositions of various P(DMAEMA-*co*-*t*BA) graft chains ([DMAEMA]:[*t*BA] molar ratios), as determined from the ^{1}H NMR spectra, are summarized in Table 1.

Trifluoroacetic acid (TFA), in dichloromethane solution, was used for the hydrolysis of the *tert*-butyl groups of *t*BA units in the

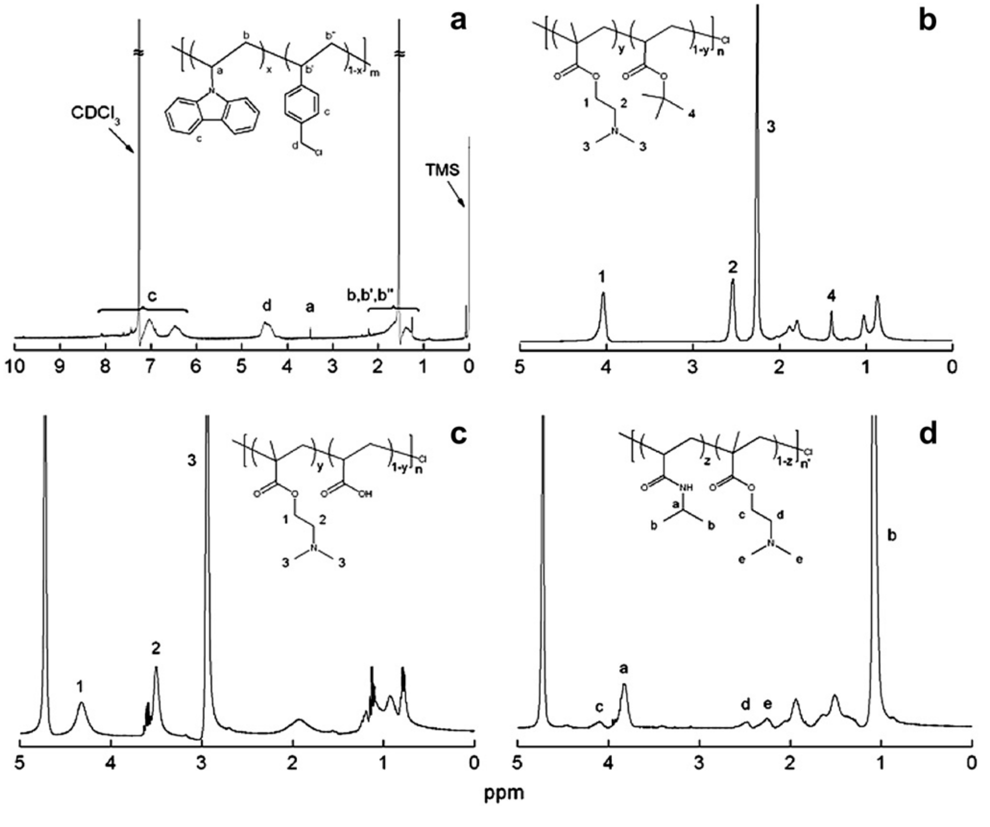


Fig. 2. ^{1}H NMR spectra of (a) the P(NVK-*co*-VBC) copolymer in CDCl$_3$, and the (b) **KVDT3** copolymer in CDCl$_3$, (c) **KVDA3** copolymer and (d) **KVND** copolymer of Table 1 in D$_2$O.

graft copolymer chains. The use of 10:1 M ratio of TFA:*tert*-butyl groups is most effective for the cleavage of *tert*-butyl groups of the copolymers, while retaining a low PDI of the polymers and without affecting the DMAEMA units [46]. The molecular weights and PDIs of the resulting acrylic acid (AAc) containing copolymers are summarized in Table 1. FT-IR and ^{1}H NMR spectroscopy results confirmed the successful hydrolysis of *t*BA to AAc units. The FT-IR spectrum of a P(NVK-*co*-VBC)-*comb*-P(DMAEMA-*co*-AAc) copolymer (**KVDA3** of Table 1) is shown in Fig. 1(c). In comparison with the FT-IR spectrum of the starting **KVDT3** in Fig. 1(b), the presence of the broad absorption (from 3200 to 3600 cm^{-1}), characteristic of a carboxylic acid group, indicate the appearance of carboxylic acid group. For the ^{1}H NMR spectrum of **KVDA3** copolymer in Fig. 2(c), the characteristic chemical shift at 1.4 ppm (signal **4**) associated with methyl protons in the *tert*-butyl group has disappeared after the hydrolysis reaction. The signals associated with the methylene protons (signal **2**) and methyl protons (signal **3**) of DMAEMA units have shifted to 2.9 and 3.4 ppm, respectively, because of the protonation of DMAEMA in D_2O [46–48]. All the other chemical shifts associated with DMAEMA remained intact, suggesting that the DMAEMA units were not affected by TFA hydrolysis [46].

3.1.3. Characterization of the P(NVK-co-VBC)-comb-P(NIPAAm-co-DMAEMA) copolymer

For the FT-IR spectrum of **KVND** copolymer of Table 1 (Fig. 1(d)), the two strong amide absorption bands at about 1650 and 1543 cm^{-1} are associated with the stretching of O=C–NH group and the bending of N–H group of *N*-isopropylacrylamide (NIPAAm) units, respectively. The characteristic absorption peaks at about 1730 and 1149 cm^{-1} are associated with the stretching of O=C–O group and C–N group, respectively, of DMAEMA units. The presence of both the O=C–O and O=C–NH groups indicates successful copolymerization of DMAEMA and NIPAAm. For the ^{1}H NMR spectrum of **KVND** copolymer of Table 1 in Fig. 2(d), the characteristic chemical shift at $\delta = 4.1$ ppm (signal **c**) are attributable to the methylene protons adjacent to the oxygen moieties of the ester linkages in the DMAEMA units. The chemical shifts at $\delta = 2.3$ (signal ***e***) and 2.6 ppm (signal ***d***) are associated with the methylene and methyl protons, respectively, of the DMAEMA units in the copolymer. The characteristic chemical shift at $\delta = 3.9$ ppm (signal ***a***) corresponds to the $-CH(CH_3)_2$ groups in the NIPAAm units. The composition of the P(NIPAAm-*co*-DMAEMA) side chains ([NIPAAm]:[DMAEMA] molar ratios), as determined from the ^{1}H NMR spectrum, is shown in Table 1.

3.2. Self-assembly of the P(NVK-co-VBC)-comb-P(DMAEMA-co-AAc) copolymers in aqueous media

The as-synthesized “comb-like” P(NVK-*co*-VBC)-*comb*-P(DMAEMA-*co*-AAc) copolymers with different length of the side chains (**KVDA1**, **KVDA2**, **KVDA3** and **KVDA4** of Table 1), are soluble in water under vigorous agitation. Their self-assembly behavior in an aqueous medium was first investigated. Fig. 3(a)–(c) show the respective TEM images of the **KVDA1** copolymer self-assembled at room temperature (25 °C) and pH of 3, 7 and 9 in 0.1 wt% solution. Hollow vesicles were observed at all pH values. The dependence of the vesicle size and morphology on the pH and concentration of the medium, as well as on the length of the side chains of the copolymer, will be discussed in the next section. Further examination of the images at higher magnification reveals that the vesicles have “onion-like” multi-walls (Fig. 3(d)). For the multi-walled vesicles self-assembled from the **KVDA1** copolymer at pH 9, the overall wall thickness is about 3 nm, with the thickness of each uniformly spaced layer of about 0.5 nm.

The process of self-assembly of the P(NVK-*co*-VBC)-*comb*-P(DMAEMA-*co*-AAc) copolymers to form the multi-walled vesicles is shown in Scheme 1. In an aqueous medium, the hydrophobic P(NVK-*co*-VBC) backbones are densely packed, arising from their hydrophobic association to minimize the interfacial free energy. Subsequent self-assembly of the multilamellar structure occurs due to supramolecular acid–base interactions, which involve the amino groups of DMAEMA units as the acceptors and the carboxylic acid groups of AAc units as the donors. Complementary ionic interaction and hydrogen bonding for controlled self-assembly of functional supramolecular materials have been widely reported [49,50]. The layered structures then close to form hollow vesicles, similar to that

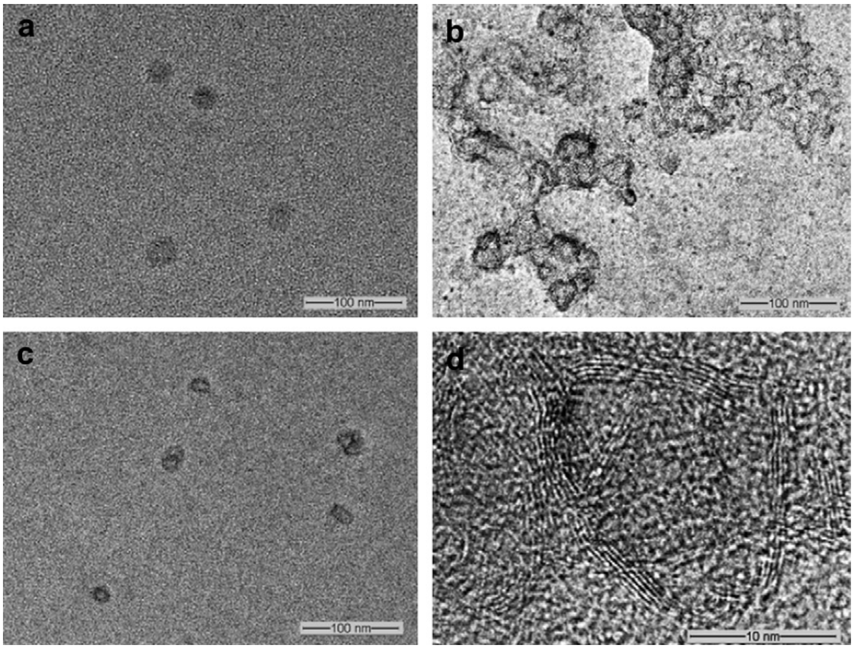


Fig. 3. TEM images of the self-assembled vesicles of the **KVDA1** copolymer of Table 1 in aqueous environment at room temperature (25 °C) and different pH: (a) pH = 3, (b) pH = 7 and (c, d) pH = 9. The concentration of each copolymer solution was 0.1 wt%.

of amphiphilic block copolymers of liposomes dissolved in selected solvents [33,51]. The structure of lamellae is well-defined as the composition of each lamella is composed of one hydrophobic P(NVK-*co*-VBC) layer sandwiched between two hydrophilic P(DMAEMA-*co*-AAc) layers of controlled chain length from ATRP.

To further study the effect of acid–base or hydrogen bonding interaction of side chains on the morphology of the self-assembled micelles, self-assembly behavior of the P(NVK-*co*-VBC)-*comb*-P(NIPAAm-*co*-DMAEMA) copolymer, which was composed of the same P(NVK-*co*-VBC) backbone, albeit with side chains of different chemical nature, was investigated. The TEM images of the hollow nanoparticles self-assembled from **KVND** copolymer of Table 1 at different temperature and pH are shown in Fig. 4. In comparison with the multi-walled hollow vesicles from the P(NVK-*co*-VBC)-*comb*-P(DMAEMA-*co*-AAc) copolymers, the hollow nanoparticles of **KVND** copolymer contain only a single wall or shell, in the absence of acid–base interaction of the hydrophilic side chains.

3.3. *Factors affecting the morphology of the P(NVK-co-VBC)-comb-P(DMAEMA-co-AAc) copolymer vesicles*

The morphology, including the diameter of vesicles and number of lamellar layers in the multi-walls, can be tuned via controlling (i) the pH of the dispersion medium, (ii) the length of the P(DMAEMA-*co*-AAc) side chains, and (iii) the concentration of the copolymer solution.

3.3.1. *Effect of pH of the media*

In an aqueous dispersion, the average size of the P(NVK-*co*-VBC)-*comb*-P(DMAEMA-*co*-AAc) copolymer vesicles is sensitive to the pH of the environment. The TEM images shown in Fig. 3 indicate that the size of the **KVDA1** copolymer vesicles at pH 7 (Fig. 3(b)) is larger than those at pH 3 (Fig. 3(a)) or pH 9 (Fig. 3(c)). The pH-dependent vesicle size is also confirmed by dynamic light scattering (DLS) studies. Fig. 5(a) shows the pH dependence of the average hydrodynamic diameter (D_h) of the copolymer vesicles of **KVDA1**. The copolymer vesicles have the largest D_h at pH 6. D_h of the vesicles decreases when the pH of the medium is reduced to

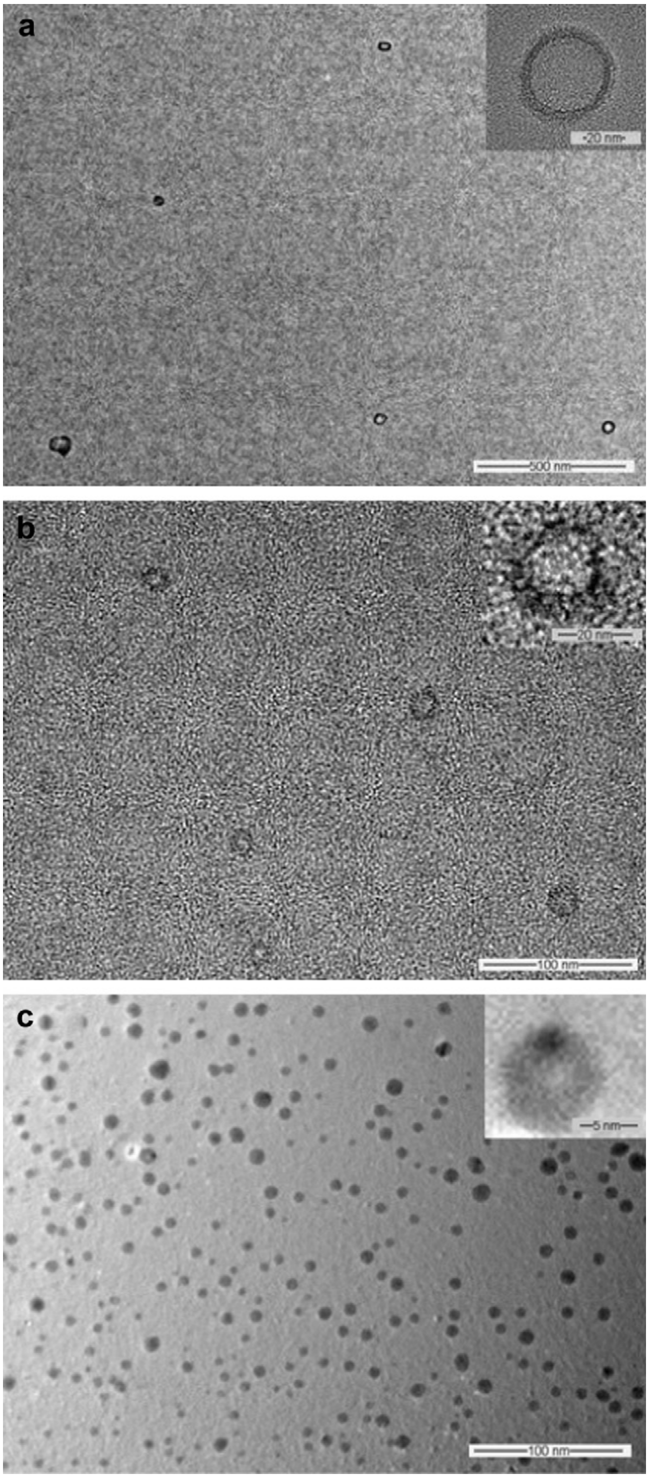


Fig. 4. TEM images of the self-assembled hollow nanoparticles of the **KVND** copolymer of Table 1 in aqueous media: (a) pH 3 and 25 °C, (b) pH 7 and 25 °C and (c) pH 7 and 40 °C. The concentration of the copolymer solution was 0.1 wt%.

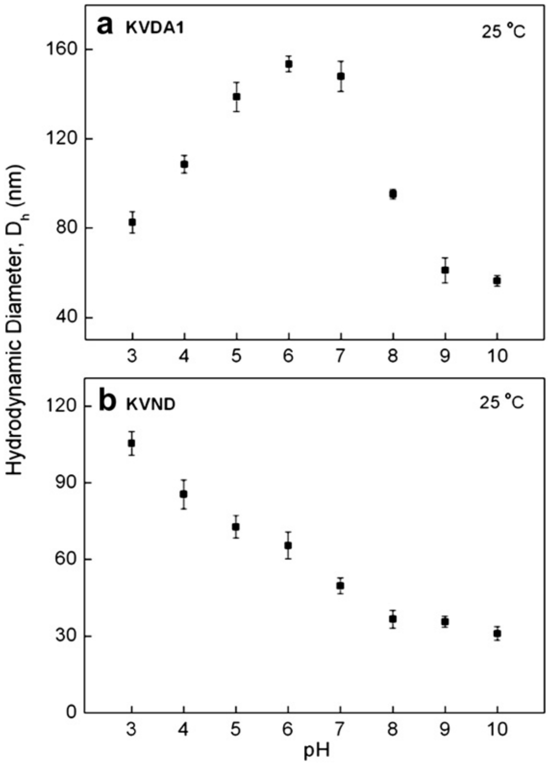


Fig. 5. Effect of pH of aqueous media on the average hydrodynamic diameter (D_h) of the vesicles self-assembled from the (a) **KVDA1** copolymer and the nanoparticles self-assembled from the (b) **KVND** copolymer of Table 1. The concentration of each copolymer solution was 0.1 wt%.

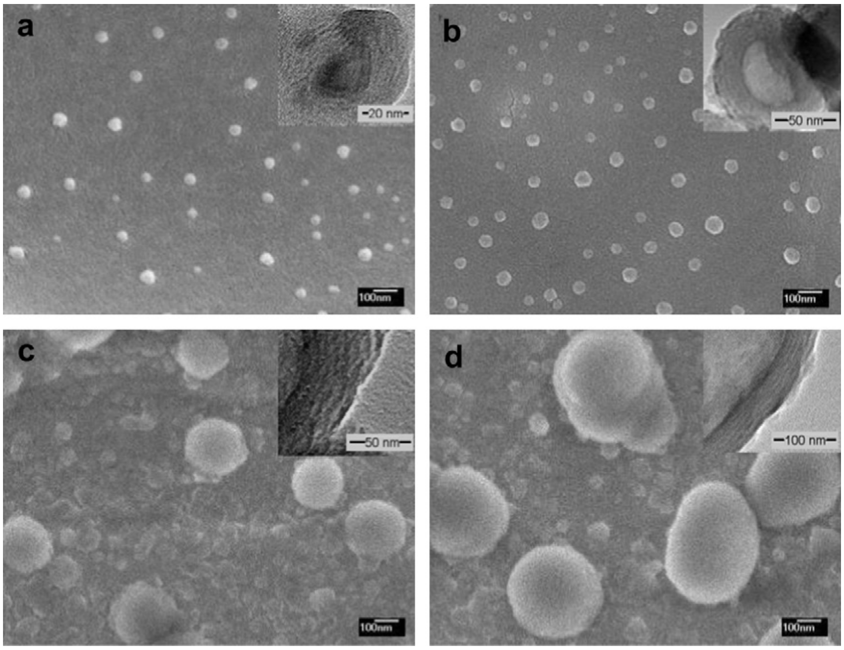


Fig. 6. SEM images of the self-assembled vesicles of the (a) **KVDA1**, (b) **KVDA2**, (c) **KVDA3** and (d) **KVDA4** copolymers of Table 1 in aqueous environment at room temperature (25 °C) and pH 7. The insets show the corresponding TEM images and detailed morphology of the vesicles. The concentration of each of the copolymer solution was 0.1 wt%.

below 5 or raised to above 7. The pH dependence of D_h can be attributed to the simultaneous presence of carboxylic acid groups of the AAc units and tertiary amino groups of the DMAEMA units in the copolymer molecule. AAc and DMAEMA are the well-known pH-sensitive components commonly found in stimuli-responsive polymers. P(AAc) is a polyacid with an apparent dissociation constant (pKa) of about 4.6 [52]. P(DMAEMA), which is water-soluble at neutral pH and in acidic media, is a weak polybase with a pKa of about 7.0–7.3 [47]. The difference in pKa of the two pH-sensitive components allows the copolymer to respond to variation in solution pH. At a low pH (lower than the pKa of P(AAc)), the carboxylic acid groups of AAc units are not ionized while the amino groups of DMAEMA units are protonated. As the solution pH is increased to above 5 (higher than the pKa of P(AAc) and lower than the pKa of P(DMAEMA)), the amio groups are protonated and the carboxylic acid groups become ionized. Zeta potential measurements were further employed to investigate the surface potential of the so obtained copolymer micelles in aqueous media. The zeta potential of vesicles decreased from +34.6 mV at pH 3 to +4.2 mV at pH 6. Under this condition, there are probably inter- and intra-molecular ionic complexation of the ionized AAc units with the protonated DMAEMA units in the side chains [2]. As the pH of medium is further increased to above the pKa of P(DMAEMA), the amino groups of DMAEMA units become deprotonated. The zeta potential of the vesicles at pH 9 decreased further to −25.1 mV. The pH response and the equilibrium size of the hollow vesicles were usually attained within a few minutes after the pH adjustment.

The size of the single-shelled P(NVK-*co*-VBC)-*comb*-P(NIPAAm-*co*-DMAEMA) copolymer hollow nanoparticles is sensitive to both the pH and temperature of the media. TEM images show that the diameter of the **KVND** nanoparticles is maximum at pH = 3 and 25 °C (Fig. 4(a)), decreases when the pH is increased to 7 at the constant temperature of 25 °C (Fig. 4(b)), and decreases even further when the temperature is increased to 40 °C with the pH maintained at 7 (Fig. 4(c)). At all pH, the hollow nanoparticles self-assembled from the **KVND** copolymer have the well-defined single-shell morphology. The pH-dependent particle size is also confirmed by DLS studies. At the constant temperature of 25 °C, the D_h of **KVND** nanoparticles decreases from about 105 nm at pH = 3 to about 31 nm at pH = 10, as shown in Fig. 5(b). The pH response arises from the hydrophobic association of the copolymer in the aqueous medium, as the tertiary amino groups of DMAEMA units become deprotonated and hydrophobic at high pH. P(NIPAAm) is a well-known thermo-responsive polymer and exhibits a lower critical solution temperature (LCST) of about 32 °C in an aqueous medium [7]. The temperature-dependent size of the **KVND** copolymer nanoparticles is consistent with the LCST behavior of P(NIPAAm) in aqueous media [22,53].

3.3.2. *Effect of the side chain length of the copolymer*

Fig. 6 shows the SEM images of the vesicles self-assembled from 0.1 wt% solutions of the respective **KVDA1**, **KVDA2**, **KVDA3** and **KVDA4** copolymers at pH 6. The corresponding TEM images (insets of Fig. 6) show that the vesicles, self-assembled from the copolymers with controlled lengths of the graft chains from the ATRP process, all have the well-defined multi-walled morphology. The size of the vesicles increases with the increase in length of the graft chains of the comb-like copolymers. Fig. 7(a) and (b) show the hydrodynamic diameters and size distribution, obtained from DLS studies, of the vesicles assembled from P(NVK-*co*-VBC)-*comb*-P(DMAEMA-*co*-AAc) copolymers at pH = 7 and pH = 5, respectively. At pH 7, the vesicles of **KVDA1**, which has the shortest side chains, exhibit the smallest average diameter of about 153 nm. On the other hand, the vesicles of **KVDA4**, which has the longest side chains, have the largest average diameter of about 901 nm. DLS measurements at pH 5 (Fig. 7(b)) also reveal that the size of the vesicles increases with increasing length of the side chains. The DLS results are consistent with the observation from the SEM images. The increase in vesicle size can be attributed to the increase in repulsive force, arising from the increased content of ionized AAc components and protonated DMAEMA units in the graft chains or molecular brushes. Moreover, the overall wall thickness of the vesicles increases with the increase in length of the grafted side chains of the comb-like copolymers. Vesicles of **KVDA1** have the thinnest wall of about 18 nm, as measured from the TEM images, while those of **KVDA4** have the thickest wall of about 55 nm. The

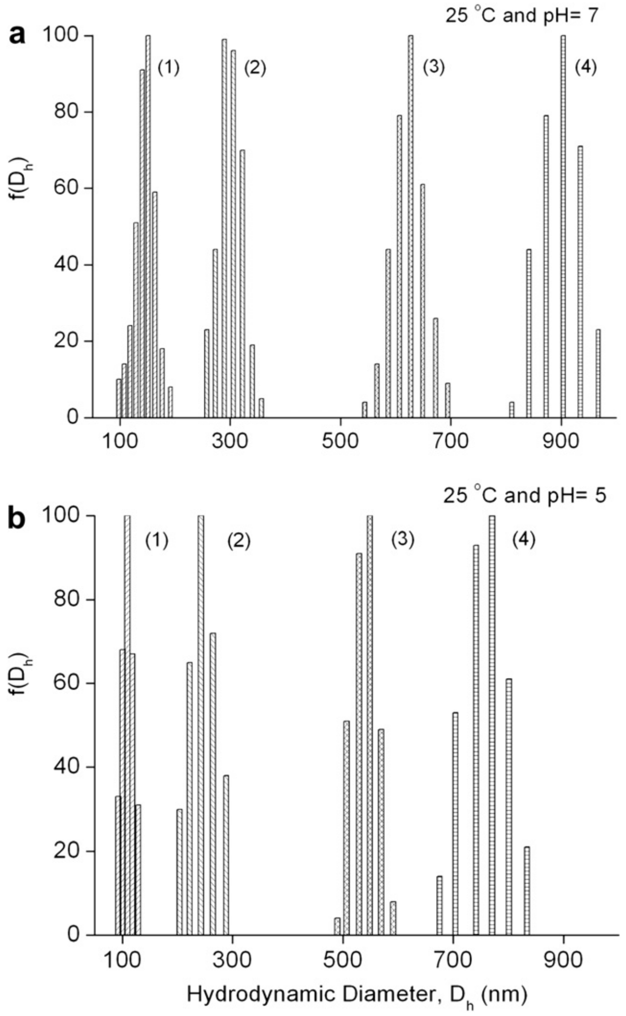


Fig. 7. Hydrodynamic diameter (D_h) and size distribution of the vesicles self-assembled from the comb-like copolymers of (1) **KVDA1**, (2) **KVDA2**, (3) **KVDA3** and (4) **KVDA4** of Table 1 at (a) pH 7 and (b) pH 5. The concentration of each copolymer solution was 0.1 wt%.

average number of wall layers of the vesicles self-assembled from the four copolymers is about 6, which is independent of the length of the side chains. Thus, the morphology, including the size and the wall thickness of the vesicles can be tuned by controlling the length of the grafted P(DMAEMA-*co*-AAc) molecular brushes.

3.3.3. *Effect of copolymer concentration*

Well-defined vesicles of the P(NVK-*co*-VBC)-*comb*-P(DMAEMA-*co*-AAc) copolymers are observed in the aqueous solution concentration range of about 0.04–0.5 wt%. Fig. 8 shows the TEM images of multi-walled vesicles of self-assembled **KVDA3** copolymer from aqueous solutions of different concentrations. The vesicles from 0.04 wt%, 0.05 wt% and 0.1 wt% solutions (Fig. 8(a)–(c), respectively) have a similar hollow architecture, but with different number of layers of the multi-walls. The number of wall layer increases with increasing concentration of the comb-like copolymer in the aqueous medium. Outside the concentration range of 0.04–0.5 wt%, hollow vesicles with the unique morphology can hardly be observed. For copolymer concentrations above 0.5 wt%, "onion-like" solid particles, instead of the hollow vesicles, are observed, as revealed by the TEM image of particles self-assembled in 0.8 wt% solution (Fig. 8(d)).

3.4. *Effect of pH on the optical properties of P(NVK-co-VBC)-comb-P(DMAEMA-co-AAc) copolymers*

The effect of pH on the optical properties of the copolymers was investigated. The fluorescence intensity (I_f) of a 1.0 mg/mL aqueous solution of each copolymer was monitored at the excitation wavelength of 295 nm. Fig. 9 shows the variation in absorption spectra and I_f of **KVDA3** in the aqueous solution pH range of 3–9. The absorption maximum at 295 nm of the copolymer is independent of pH of the medium. On the other hand, the copolymer exhibits the ability of I_f "switching" in response to changes in solution pH. The I_f is maximum when pH of the medium is 3, and is quenched when pH of the medium is increased. The switching of I_f by pH is completely reversible when the pH of medium is reduced. The phenomena can be attributed to the simultaneous presence of the carboxylic acid groups of P(AAc) and the tertiary amino species of P(DMAEMA) in the copolymer molecule. Vinylcarbazole units are well-known luminescent chromophores [54,55] and are covalently

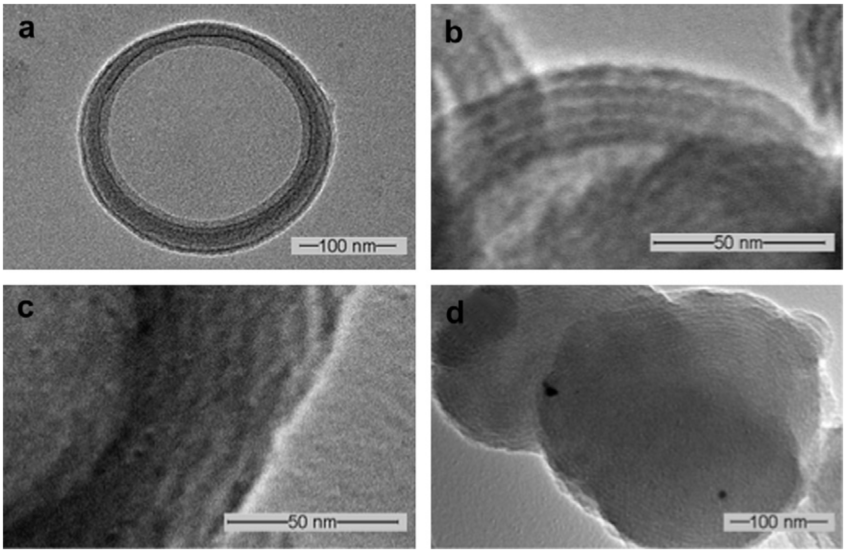


Fig. 8. TEM images of the self-assembled vesicles of the **KVDA3** copolymer of Table 1 at room temperature (25 °C), pH 7 and concentrations of (a) 0.04 wt%, (b) 0.05 wt%, (c) 0.1 wt% and (d) 0.8 wt%.

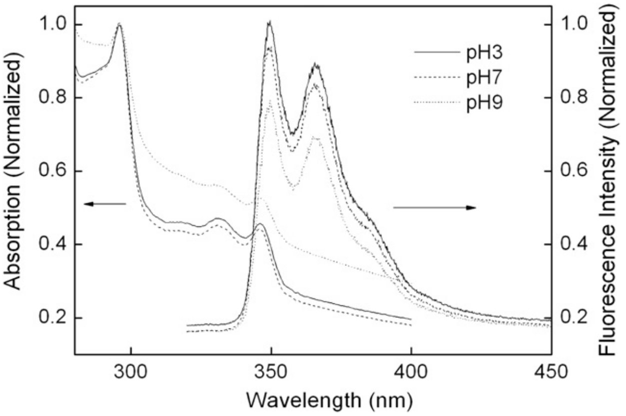


Fig. 9. Effect of pH on the normalized absorption and fluorescence (excitation wavelength at 295 nm) spectra of a 0.1 wt% aqueous solution of the **KVDA3** copolymer of Table 1.

linked to the P(DMAEMA-*co*-AAc) copolymer side chains. For fluorescence of the aromatic hydrocarbon chromophores covalently bound to P(AAc), sharp changes in fluorescent property can occur with changes in pH of the surrounding environment, arising from acid—base equilibrium of -COOH groups in the P(AAc) segments [56]. The tertiary amino species are good electron donors and are known to quench photoelectron transfer (PET) from/to the excited state of polyaromatic molecules [57,58]. The pKa of the carboxylic acid groups of P(AAc) is about 4.6 and the pKa of tertiary amino groups of P(DMAEMA) is about 7.0—7.3 in water. The difference in pKa of the two pH-sensitive groups allows the copolymer to respond to variation in solution pH. At pH lower than the pKa of carboxylic acid groups of P(AAc), most of the carboxylic acid groups are not ionized while the amino groups are protonated, the fluorescence of P(NVK) is in the un-perturbed state. As pH is increased above the pKa of carboxylic acid, the carboxylic acid groups become ionized (while the tertiary amino groups of P(DMAEMA) remain protonated) and the fluorescence is quenched. As pH is increased further to above the pKa of tertiary amino groups of P(DMAEMA), the tertiary amino groups become deprotonated. The fluorescence is further quenched by the tertiary amino groups [57,58]. Therefore, the coexistence of carboxylic acid units and tertiary amino groups result in the reversible fluorescence profile of the copolymer solution in response to changes in pH.

4. Conclusions

"Comb-like" graft copolymers, consisting of a P(NVK-*co*-VBC) copolymer backbone from free radical polymerization and well-defined P(DMAEMA-*co*-AAc) graft (side) chains from controlled radical polymerization and acid hydrolysis, have been prepared. TEM and SEM images reveal that the water-soluble P(NVK-*co*-VBC)-*comb*-P(DMAEMA-*co*-AAc) copolymers can self-assemble in aqueous media into hollow vesicles with multi-walls, arising from the acid—base interaction of the AAc and DMAEMA units in the side chains. In addition to the unique molecular architecture, the copolymer vesicles exhibit reversible pH dependence in size and fluorescence intensity in aqueous media. The vesicular morphology of the copolymer can be tuned by pH of the medium, the length of the hydrophilic P(DMAEMA-*co*-AAc) side chains, and the concentration of the copolymer solution. In comparison, the pH- and temperature-responsive hollow nanoparticles self-assembled from the comb-like graft copolymer P(NVK-*co*-VBC)-*comb*-P(NIPAAm-*co*-DMAEMA) are single-shelled due to the absence of acid—base side chain interaction. Thus, the structure of amphiphilic comb-like copolymers can be tailored for applications as biomaterials and in biomedical systems, such as in stimuli-responsive drug delivery and release systems. The copolymers are probably also useful as optical sensory materials for changes in pH and temperature of the environment.

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