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Synthesis and physical gels of pH- and thermo-responsive tertiary amine methacrylate based ABA triblock copolymers and drug release studies

Fadime Fulya Taktak [a], Vural Bütün [b,*]

[a] Department of Chemistry, Faculty of Arts and Science, Afyon Kocatepe University, 03200 Afyonkarahisar, Turkey
[b] Department of Chemistry, Faculty of Arts and Science, Eskişehir Osmangazi University, 26480 Eskişehir, Turkey

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ABSTRACT

A series of novel pH-responsive ABA triblock copolymer gelators have been synthesized by using poly[2-(diisopropylamino)ethyl methacrylate] (PDPA) as the A block and poly[2-(dimethylamino)ethyl methacrylate] (PDMA) as the B block via group transfer polymerization. While the PDPA-*b*-PDMA-*b*-PDPA triblock copolymers are molecularly soluble in acidic aqueous media due to protonation of all tertiary amine groups, they formed either gels by the chain-end hydrophobic interactions with relatively high polymer concentration (10 wt%) or near monodisperse "flower" micelles with low polymer concentration at neutral and basic aqueous solutions. The hydrophobic model drug release was studied in a sustained manner from the gels at pH 7.4 by varying the polymer concentration, the polymer molecular weight and the temperature of the medium. Preliminary studies indicate that both slow, sustained release and fast, triggered release of a model hydrophobic drug, dipyridamole, can be achieved by tuning the solution pH, polymer concentration, polymer molecular weight and temperature of the gel.

1. Introduction

In recent years, hydrogels have been intensively studied as controlled drug-delivery systems to deliver the drugs at desirable times and/or specific sites to achieve the therapeutic objective [1–3]. Hydrogels are three-dimensional polymeric networks, made by chemical or physical cross-linking of hydrophilic polymers [4]. Some hydrogels have great response to the external stimuli such as changes in pH, temperature, ionic strength, solvent type, electric and magnetic fields, light and the presence of chelating species and therefore have received extensive attention in the past several decades [5–9]. Potential applications for hydrogels have been reported in the fields of tissue engineering, synthetic extracellular matrix, implantable devices, biosensors, materials controlling the activity of enzymes, phospholipid bilayer destabilizing agents, materials controlling reversible cell attachment, nanoreactors with precisely placed reactive groups in three-dimensional space, smart microfluidics with responsive hydrogels and energy-conversion systems [10–16]. Stimuli-sensitive block copolymer hydrogels have great potential in biomedical and pharmaceutical applications, especially in drug-delivery systems and tissue engineering [17–20]. Among the stimuli in the biomedical applications, temperature and pH are the most popular physical and chemical stimuli, respectively. Thus, thermo- and/or pH-responsive block copolymer hydrogels have been intensively studied for biomedical applications in recent years [19,21,22].

The pH-sensitive polymers, which contain the ionizable groups and are also called weak polyelectrolytes, show dramatic changes in degree of ionization and water-solubility at a specific pH (pK_a) [5,23]. Typical pH-sensitive polymers for drug delivery are based on the polymers, such as poly(acrylic acid) (PAA) and poly(methacrylic acid) (PMAA) [24–28], poly(imine)s [29,30], poly(L-glutamic acid) (PLG) [31], polymers containing sulfonamide groups [32], poly[2-(dialkylamino)ethyl methacrylate]s [23,33–35], biodegradable poly(β-amino ester) (PAE) [36,37], and poly(2-vinylpyridine) (P2VP) [38].

Armes research group has recently reported novel pH-responsive ABA triblock copolymer gelators by using poly[2-(diisopropylamino)ethyl methacrylate] (PDPA) or poly[2-(diethylamino)ethyl methacrylate] (PDEA) as the A block and poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC) as the B block [33,34]. While both types of ABA triblock copolymers are molecularly soluble in acidic aqueous media, their neutral aqueous solutions with relatively high concentration (10 wt%) form a gel by the chain-end hydrophobic interactions. The hydrophobic model drug release was studied in a sustained manner from the gel at pH 7.4 and 37 °C by varying the polymer concentration. As compared with the PDEA-*b*-PMPC-*b*-PDEA hydrogels, the PDPA-*b*-PMPC-*b*-PDPA hydrogels, which

* Corresponding author. Tel.: +90 222 2393750/2751; fax: +90 222 239357.
E-mail address: vbutun@ogu.edu.tr (V. Bütün).

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contain the more hydrophobic A blocks, showed a lower critical gel concentration and a slower drug release behavior at the same polymer concentration.

Similar gel formation with ABA-type triblock copolymers having hydrophobic end blocks such as poly(methyl methacrylate)-*b*-poly [2-(dimethylamino)ethyl methacrylate]-*b*-poly(methyl methacrylate) (PMMA-*b*-PDMA-*b*-PMMA) and poly(lactic acid)-*b*-poly (ethylene glycol)-*b*-poly(lactic acid) (PLA-*b*-PEG-*b*-PLA) were also reported [39–43]. Such block copolymers form physically cross-linked hydrogels through associates of the hydrophobic blocks. In acidic solution, the PMMA-*b*-PDMA-*b*-PMMA triblock copolymer solution with the concentration above 1 wt% formed a reversible physical network as expected [40]. The viscosity of the gel exhibited a maximum at around pH 4 at which the PDMA chains were highly ionized and formed a stretched conformation. On the other hand, PLA-*b*-PEG-*b*-PLA triblock copolymers form physically associated hard gels typically above 16 wt% polymer concentration [42].

Thermo-responsive ABA triblock copolymers such as poly(lactic acid-*co*-glycolic acid)-*b*-poly(ethylene glycol)-*b*-poly(lactic acid-*co*-glycolic acid) (PLGA-*b*-PEG-*b*-PLGA), poly(ethylene glycol)-*b*-poly (ε-caprolactone)-*b*-poly(ethylene glycol) (PEG-*b*-PCL-*b*-PEG), poly (ε-caprolactone)-*b*-poly(ethylene glycol)-*b*-poly(ε-caprolactone) (PCL-*b*-PEG-*b*-PCL), and Pluronic triblock copolymers were also studied in detail as thermogelling agents in the hydrophobic drug releases [22,44–49].

In recent years, much interest has been devoted to both pH-, and thermo-responsive (dual responsive) amphiphilic polymers [21,22,50]. For example, a dual responsive multiblock poly(ester amino urethane)s (PCL-*b*-PEG-*b*-PCL-*b*-PAU) were reported as novel hydrogelling agent by Dayananda et al. [50]. In the multiblock copolymers, the tertiary amine residues of the poly(amino urethane) (PAU) segments act as pH-responsive moieties, while the PCL-*b*-PEG-*b*-PCL blocks act as biodegradable and temperature-sensitive segments. At a relatively high pH (7.0 or above), the multiblock copolymer aqueous solution showed a sol–gel–sol (aggregation) transition with increasing temperature. In contrast, at a lower pH (below pH 7.0), the polymer solution always existed as a sol state within the experimental temperature range. The gel window covers the physiological conditions. After subcutaneous injection of the 20 wt% multiblock copolymer solutions into mice, polymeric hydrogels were formed in situ in a short time. The in vitro release of an anticancer drug, paclitaxel, persisted over 1 month under physiological conditions [50].

The stimuli-responsive amphiphilic block copolymers have gained great interest in the area of drug-delivery technology over the past few decades [51,52]. The polymers containing hydrophilic PDMA block are of particular scientific interest, since they are sensitive to both temperature and pH [23,35,53–56]. The PDMA block exhibits a low critical solution temperature (LCST) in the range of 34–50 °C at pH 8 depending on molecular weight and pH sensitivity characterized by a critical point between pH 7.0 and 8.0 depending on solution conditions [23,55,56]. Thermo-responsive behaviours of PDMA homopolymers have also been studied in detail by Plamper et al. They have reported that the LCST of PDMA homopolymers in buffer solutions can be tuned by changing solution pH, polymer molecular weight, electrolyte concentration, and polymer concentration [55]. For the first time, they have also succeeded to get an upper critical solution temperature (UCST) for PDMA homopolymer in buffered solution by the addition of trivalent counterions into the solution [56]. This novel thermo-responsive behaviour of the PDMA homopolymers can be switched off by reducing the valency of the counterions from trivalent to divalent via UV illumination (photoinduced dissolution). Although the thermo-responsive PDMA is not really biocompatible due to its partially cationic nature at body pH, it is the most extensively studied polymer and is of particular scientific interest due to its pH- and thermo-responsive nature.

Herein, we describe the synthesis of novel pH- and thermo-responsive gelators based on ABA triblock copolymers, where the central B block comprises DMA and the outer A blocks are composed of DPA, see Fig. 1.

2. Experimental section

2.1. General protocols

Group transfer polymerization (GTP) was used to synthesize tertiary amine methacrylate based ABA-type triblock copolymers with narrow molecular weight distributions and well-controlled molecular weights and comonomer compositions in THF at 20 °C [57]. All chemicals were purchased from Aldrich, unless otherwise stated. All glassware and transfer needles were dried by storing in an oven overnight at 140 °C before use. All reactions were carried out under dry nitrogen. Prior to polymerization, all glassware was dried overnight at 140 °C, assembled hot, flamed under high vacuum to remove residual water and allowed to cool to room temperature. Nitrogen was passed through two P_2O_5 drying columns prior to use.

Tetrahydrofuran (THF; Labscan) was initially dried over sodium wire and refluxed over potassium for 3 days before use. The dried THF was distilled freshly in a Schlenk flask and transferred into the reaction vessel via cannula. Both DPA (SP2) and DMA monomers were each passed in turn through a basic alumina column to remove the hydroquinone methyl ether inhibitor and stirred over calcium hydride, the less volatile 2,2-diphenyl-1-picrylhydrazyl hydrate (DPPH) inhibitor was added and stored at −20 °C. The monomers were each distilled under reduced pressure before transferring into the reaction vessel by cannula under a dry nitrogen atmosphere. 1-Methoxy-1-trimethylsiloxy-2-methyl-1-propene (MTS) was distilled and stored at −20 °C in a graduated Schlenk flask under dry nitrogen prior to use. Tetra-*n*-butylammonium bibenzoate (TBABB) was prepared by the method of Dicker et al. [58].

2.1.1. Synthesis of tertiary amine methacrylate-based triblock copolymers

To synthesize a PDPA-*b*-PDMA-*b*-PDPA triblock copolymer by group transfer polymerization (GTP) via sequential monomer addition, the solid TBABB catalyst (100 mg) was added from a sidearm under a nitrogen purge into a 250 mL three-necked round bottom flask. After transferring both THF (125 mL) and MTS (0.15 mL) into the flask via cannula the solution was stirred for 15 min, and then first monomer (DPA, 3.0 mL) was added. In the meantime, an exotherm was monitored during the addition of monomer with a contact thermocouple attached to the side of the

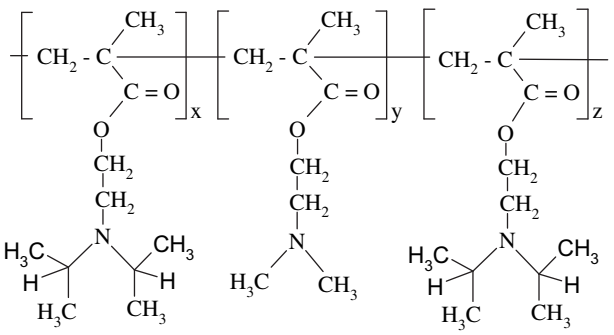

Fig. 1. Chemical structures of the PDPA-*b*-PDMA-*b*-PDPA triblock copolymers synthesized by using group transfer polymerization.

reaction vessel. It was observed that the reaction temperature typically increased by 2 °C. The reaction mixture was stirred until the solution temperature returned to room temperature (approximately 40 min). Then a 5 mL aliquot of the reaction mixture was extracted via syringe for GPC and proton NMR analysis.

To produce an AB diblock copolymer (PDPA-*b*-PDMA), after a 5 mL aliquot was extracted from the polymerizing DPA reaction mixture (as described above), the second monomer (DMA, 10 mL) was added via cannula and a second exotherm was recorded (5.2 °C). The reaction mixture was stirred at room temperature until the exotherm had abated (approximately 40 min). Finally, to produce an ABA triblock copolymer (PDPA-*b*-PDMA-*b*-PDPA), after extraction of a 5 mL aliquot from the polymerizing PDPA-*b*-PDMA reaction mixture (as described above) the third monomer (DPA, 2.5 mL) was added via cannula. The reaction mixture was, again, stirred at room temperature until the exotherm had abated (approximately 50 min). After extraction of a final 0.5 mL aliquot for GPC analysis, the reaction was terminated with methanol (2 mL) prior to recovery using a rotary evaporator. The resulting triblock copolymer was dried on a vacuum line at room temperature for 24 h after removing PDPA and PDPA-*b*-PDMA contaminations as described below. After removal of the contaminants from the diblock copolymer (second step aliquot) and from the triblock copolymer (third step aliquot), the actual DP's of the resulting PDPA-*b*-PDMA-*b*-PDPA (FT14) triblock copolymer were calculated from ^{1}H NMR spectra as being 23, 150 and 20, respectively. All copolymerizations gave very high yields (>98%). A summary of the three synthesized triblock copolymers, including their ^{1}H NMR and GPC data, are listed in Table 1.

As GPC indicated little homopolymer contamination both in diblock copolymer and in the triblock copolymer, the PDPA-*b*-PDMA diblock copolymer (from the second step aliquot) and the PDPA-*b*-PDMA-*b*-PDPA triblock copolymer (from the final product) were precipitated from THF into cold *n*-hexane to remove the related contaminants. For the diblock copolymer purification, after evaporation of the 75% of the THF solvent of the aliquot, the concentrate aliquot was poured into cold *n*-hexane (20 mL). For the triblock copolymer purification, typically, copolymer (15 g) was dissolved in minimum amount of THF (30 mL) and then poured into cold *n*-hexane (500 mL). The precipitated block copolymers were washed with cold *n*-hexane twice before drying under vacuum at room temperature for 24 h. Liquid nitrogen bath was used to cool *n*-hexane. The PDPA homopolymer contaminants were successfully removed from the copolymer as can be seen in Fig. 2. On the other hand, it is very difficult to remove diblock copolymer contaminant from triblock copolymer. But it can be neglected due to very low polydispersity index values of the triblock copolymers ($M_w/M_n < 1.11$).

2.2. Copolymer characterizations

2.2.1. Gel permeation chromatography (THF eluent)

Molecular weights (M_n) and polydispersity index (M_w/M_n) of all polymers were determined by using gel permeation chromatography (GPC). The GPC consisted of an Agilent Iso Pump, a refractive index detector, both Mixed 'D' and Mixed 'E' columns (ex. Polymer Labs), and calibration was carried out using PMMA calibration standards (ex. Polymer Labs), with M_n ranging from 680 g mol^{-1} to 218,600 g mol^{-1}. The GPC eluent was HPLC grade THF stabilized with BHT, at a flow rate of 1.0 mL min^{-1}.

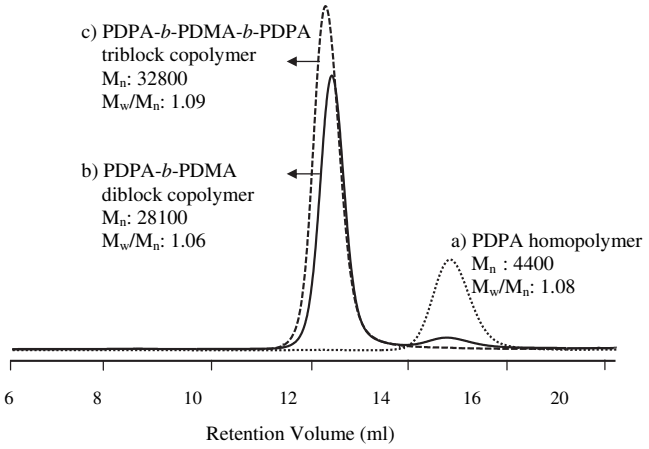

Fig. 2. GPC chromatograms of each step in the synthesis of PDPA-*b*-PDMA-*b*-PDPA triblock copolymer (FT14): a) PDPA homopolymer; b) PDPA-*b*-PDMA diblock copolymer; c) PDPA-*b*-PDMA-*b*-PDPA triblock copolymer (after removal of the contaminants).

2.2.2. Nuclear magnetic resonance spectroscopy (NMR)

A Bruker 400 MHz Avance NMR instrument was used to determine the block copolymer compositions. All proton NMR spectra were recorded in $CDCl_3$ solvent. The methoxy signal at δ 3.6–3.7 due to the MTS initiator fragment was used as end group to estimate the actual DP of the first PDPA block (just before addition of second monomer) [23]. Treating this PDPA block as an end group, the degrees of polymerizations of both second and third blocks were determined by comparing appropriate integrals assigned to the different comonomers. ^{1}H NMR studies were also carried out to characterize micellization and gelation behaviors of the triblock copolymers in D_2O by adjusting the solution pH with DCl and NaOD addition.

2.2.3. Dynamic light scattering

To determine the hydrodynamic radius and the polydispersity index (PDI = μ_2/Γ^2) of the triblock copolymer micelles dynamic light scattering (DLS) studies were conducted using an ALV/CGS-3 compact goniometer system (Malvern, UK) equipped with a 22 mW He–Ne laser operating at λ_o 632.8 nm, an avalanche photodiode detector with high quantum efficiency, and an ALV/LSE-5003 multiple tau digital correlator electronics system. All measurements were performed on the triblock copolymer solutions having concentrations between 0.1 and 1.0% at 20 °C for aqueous solution using a fixed scattering angle of 90°, and the data were fitted using second-order cumulants analysis. The polymer solution (1.0 wt%

Table 1
Copolymer compositions, number-average molecular weights (M_n), polydispersity indexes (M_w/M_n), and actual degrees of polymerizations (DP) for the $PDPA_{0.12}$-*b*-$PDMA_{0.78}$-*b*-$PDPA_{0.10}$ triblock copolymers.

Sample code	M_n (g/mol, theory)	Comonomer composition (mol%, theory)	M_n (g/mol)[a]	M_w/M_n[a]	M_n (g/mol)[b]	Actual DP[b]	Comonomer composition (mol%)[b]
FT14	25,000	15/75/10	32,800	1.09	33,300	23/150/20	12/78/10
FT15	35,000	15/75/10	41,600	1.10	43,600	32/200/25	12/78/10
FT16	45,000	15/75/10	47,600	1.11	50,800	35/230/30	12/78/10

[a] As determined by GPC, calibrated with poly(methyl methacrylate) standards.
[b] As determined by ^{1}H NMR spectroscopy using the GTP initiator fragment as an end group and relevant signals of both blocks.

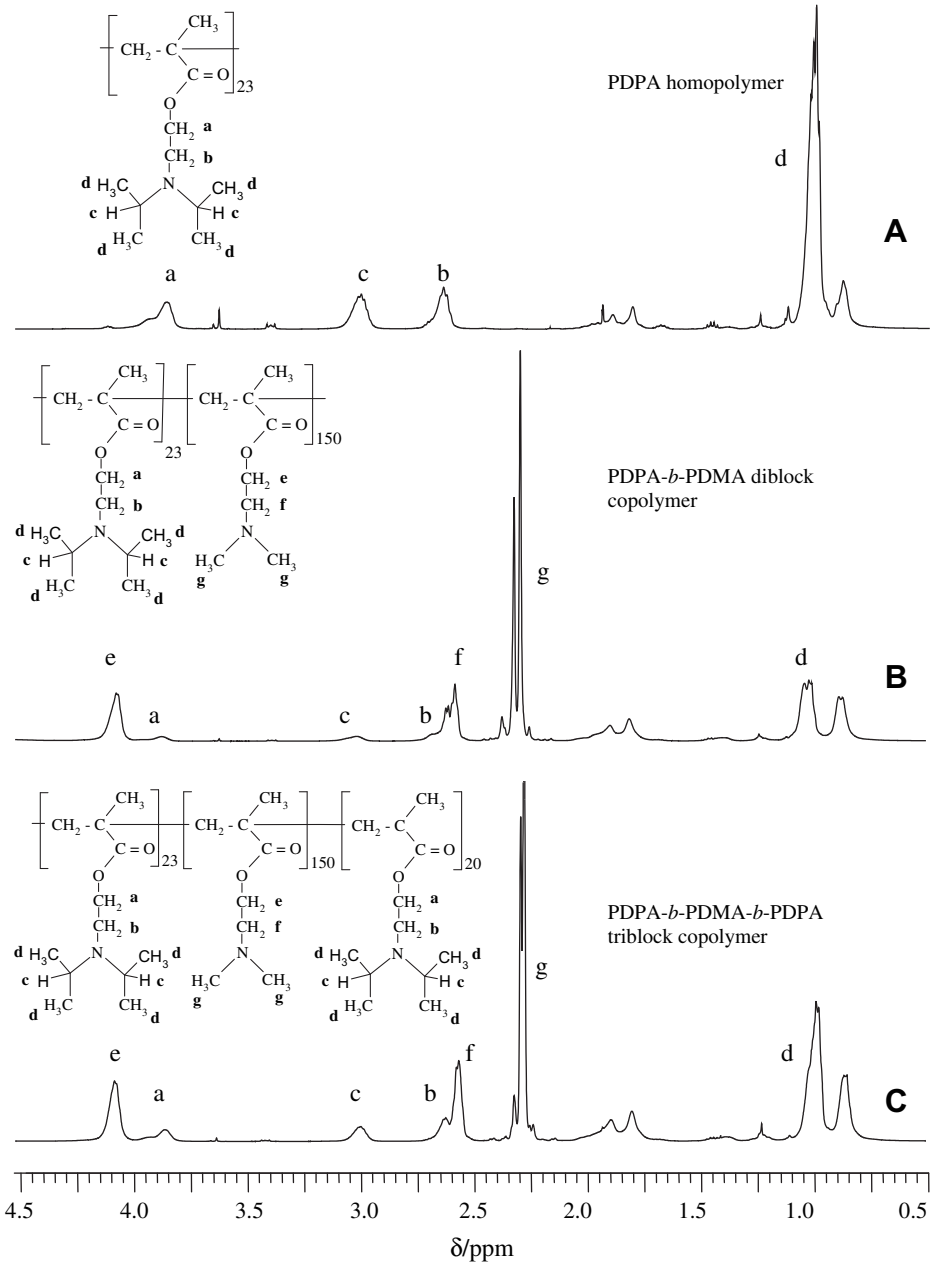

Fig. 3. Proton NMR spectra in $CDCl_3$: A) $PDPA_{23}$ homopolymer (before addition of DMA and DPA monomers); B) $PDPA_{23}$-*b*-$PDMA_{150}$ diblock copolymer and C) $PDPA_{23}$-*b*-$PDMA_{150}$-*b*-$PDPA_{20}$ triblock copolymer.

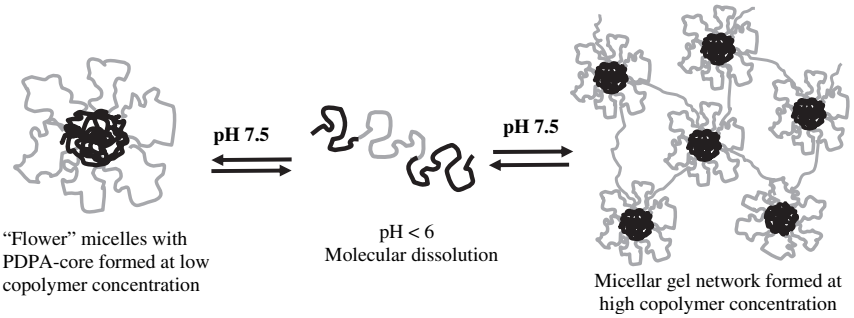

Fig. 4. Schematic representation of the molecular dissolution, "flower" micelle and "macroscopic gel" formations depending on solution pH and PDPA-*b*-PDMA-*b*-PDPA triblock copolymer concentration.

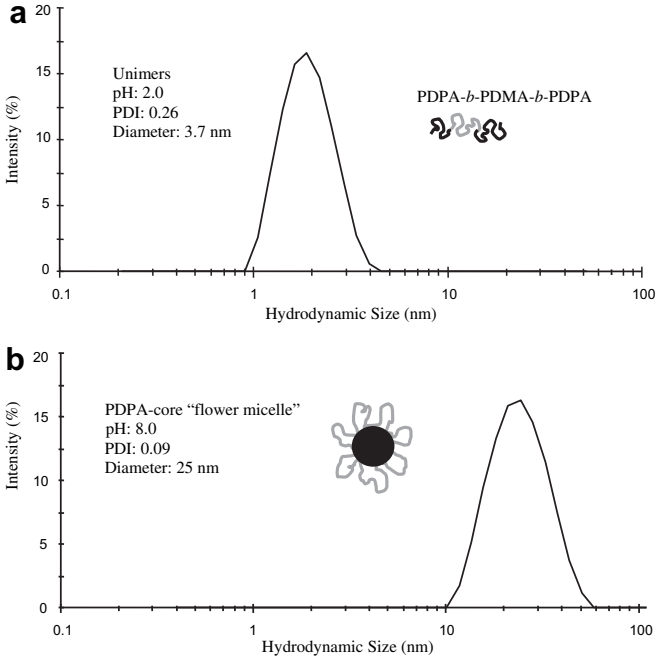

Fig. 5. Hydrodynamic diameter distribution for the $PDPA_{23}$-*b*-$PDMA_{150}$-*b*-DPA_{20} triblock copolymer (FT14, 0.5 wt%) at 20 °C: (a) Unimers at pH 2.0; (b) flower micelles with PDPA-core at pH 8.0.

FT14) was prepared as follows: The polymer FT14 (0.1 g) and deionized water (9.0 ml) were mixed before addition of 1.0 M HCl solution for molecular dissolution of the triblock copolymer at pH 2.0. The solution pH was then increased from pH 2.0 to above pH 7 by adding KOH solution (1.0 M). The concentration of NaCl salt formed by neutralization was calculated to be 0.069 M (0.4 wt%) in the resulting micellar solution. The equilibrium time after adjustment of the pH with KOH addition was minimum 1 h before DLS and UV measurements.

2.2.4. Surface tension measurements

The surface tension measurements were carried out using a Kruss K11 surface tensiometer (platinum ring method) for the PDPA-*b*-PDMA-*b*-PDPA triblock copolymer solutions to determine their both surface activities and critical micelle concentrations in aqueous media.

2.2.5. In vitro drug release studies

Drug release studies from hydrogels were carried out by recording UV/visible absorption spectra of the surrounding solution at 291 nm on a Perkin Elmer UV/vis Lambda 35 spectrophotometer.

3. Results and discussion

3.1. Triblock copolymer syntheses and characterizations

It is possible to make an ABA triblock copolymer by using either monofunctional or bifunctional GTP initiator. In this study, monofunctional MTS initiator has been chosen for the synthesis of PDPA-*b*-PDMA-*b*-PDPA triblock copolymers due to our great experience with it. The PDPA-*b*-PDMA-*b*-PDPA triblock copolymers were successfully synthesized in high yield by using GTP chemistry and characterized by using GPC and ^{1}H NMR spectroscopy. The number-average molecular weights (M_n) and the polydispersity index (M_w/M_n) of the copolymers were determined by GPC and are summarized in Table 1. The GPC traces of each step in the synthesis of a PDPA-*b*-PDMA-*b*-PDPA triblock copolymer (FT14) are given in Fig. 2.

Typical ^{1}H NMR spectra of PDPA homopolymer (just before addition of second and third monomers), PDPA-*b*-PDMA diblock copolymer and the triblock copolymer (PDPA-*b*-PDMA-*b*-PDPA, FT14) are shown in Fig. 3, recorded in $CDCl_3$ with the relevant signals labeled. Absolute DP's and the block compositions were determined by comparing well-defined peak integrals assigned to the different comonomers. It is worth that the proton NMR spectra of both PDPA-*b*-PDMA diblock copolymer and the PDPA-*b*-PDMA-*b*-PDPA triblock copolymer have been taken after removal of the contaminants (PDPA contaminant from diblock copolymer and both PDPA and PDPA-*b*-PDMA diblock contaminants from the triblock copolymer).

The absolute DP of the PDPA homopolymer, first block, was estimated to be 23 by comparing the peak integrals of the three methoxy protons at δ 3.6–3.7 due to the terminal MMA residues derived from the MTS initiator with the oxymethylene protons of the PDPA residues at δ 3.8 (see upper spectrum in Fig. 3) [23,35]. Treating this first PDPA block as an "end group", inspection of the middle and the lower ^{1}H NMR ($CDCl_3$) spectra in Fig. 3 for the PDPA-*b*-PDMA-*b*-PDPA triblock copolymer indicated average degrees of polymerization for the second block PDMA and the third block PDPA of 150 and 20, respectively [by comparing the peak integrals of the six dimethylamino protons in the PDMA residues at δ 2.3 with the CH protons of isopropyl groups in both PDPA blocks at δ 3.0].

In general, good agreement was observed between the theoretical and determined M_n's, DP's and comonomer compositions from the NMR/GPC values (see Table 1). All triblock copolymers had low M_w/M_n's (<1.11), which are typical of polymers synthesized via GTP. The observed small increases in DP compared to theoretical values are almost certainly due to the imperfect MTS initiator efficiency (around 90% in such polymerizations of tertiary amine methacrylate based monomers) and the removal of the PDPA contamination from the triblock copolymers, which results in an increase in the mean DPs of both second block and third block.

3.2. Aqueous solution behavior of the triblock copolymer

The PDMA homopolymer is a weak polybase and it is water-soluble at both neutral and acidic pH at room temperature but less soluble at alkaline solutions and its pK_a value is 7.0 as reported in our previous studies [23]. On the other hand, it was also reported that PDPA homopolymer dissolves as a cationic polyelectrolyte in acidic solution (pH < 6) due to protonation of its tertiary amine residues. Precipitation from aqueous solution occurs when the solution pH exceeds the pK_a of 6.4 for PDPA homopolymer, because the average degree of protonation drops below a critical value and the chains become hydrophobic [23]. Thus, the triblock copolymer was expected to give unimers at acidic pH, and "flower" micelles or gels at around pH 7.4 depending on copolymer concentration (see Fig. 4).

3.2.1. pH-induced formation of "flower micelles" with PDPA-core

Fig. 5 shows DLS distribution functions of the $PDPA_{23}$-*b*-$PDMA_{150}$-*b*-$PDPA_{20}$ triblock copolymer in aqueous solution (0.5 wt %). DLS studies indicated molecular dissolution at low pH (pH < 6), with micellar self-assembly occurring at above pH 7.0. The micelles have polydisperse nature upto pH 8.0. Near-monodisperse micellar self-assembly occurs between pH 8.0 and 9.0. Proton NMR studies indicated dehydration of PDPA end blocks above pH 7.0. Both Figs. 5b and 6b indicated the formation of near-monodisperse PDPA-core micelle at around pH 8.0 and 20 °C.

PDPA-*b*-PDMA-*b*-PDPA triblock copolymers can be molecularly dissolved in acidic solution (pH < 6). The ^{1}H NMR spectra in Fig. 6a

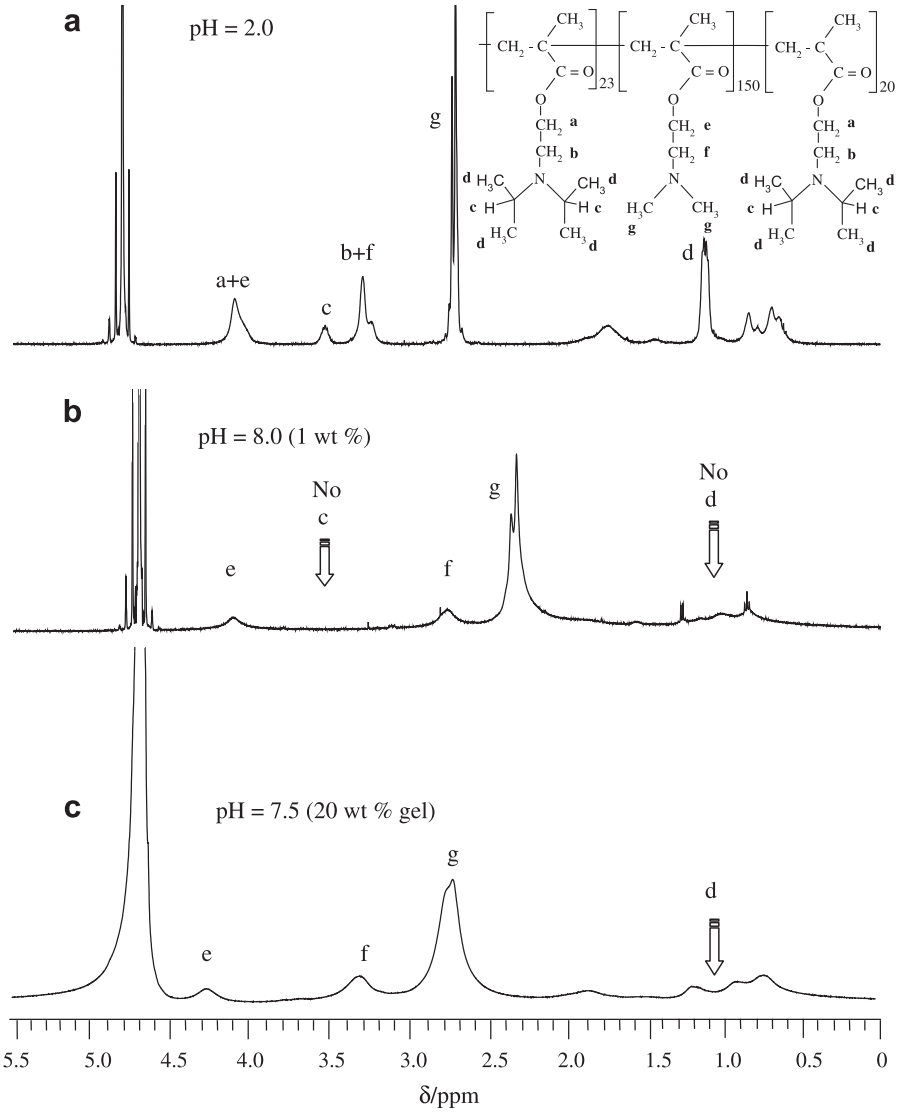

Fig. 6. ^{1}H NMR spectra obtained for the $PDPA_{23}$-*b*-$PDMA_{150}$-*b*-$PDPA_{20}$ triblock copolymer (FT14): (a) as a free-flowing aqueous solution at pH 2 in DCl/D_2O; (b) PDPA-core flower micelles at pH 8.0; (c) as a macroscopic physical gel (20 wt%) at pH 7.5. DCl and NaOD were used to adjust the solution pH. Note that the signals assigned to the protonated PDPA residues in spectrum (a) disappear in spectra (b) and (c) because the deprotonated PDPA blocks become hydrophobic and hence much less solvated in the micelle core.

represent molecular solubility of the $PDPA_{23}$-*b*-$PDMA_{150}$-*b*-$PDPA_{20}$ triblock copolymer (FT14, 1.0 wt%) in D_2O. It is also confirmed that the PDMA blocks remain hydrated even at pH 8.0 (note the prominent signals at δ 2.4 for DMA residues), whereas the signals due to the PDPA block at δ 1.1 and δ 3.4 are suppressed at pH 8.0 (compare parts "a" and "b" of Fig. 6). This is consistent with the PDPA block forming the nonhydrated micelle cores and PDMA block forming the hydrated micelle coronas. DLS and ^{1}H NMR studies confirmed this to be the case. A macroscopic precipitation occurs with the further increase on pH (pH > 9), since deprotonation of the tertiary amine residues of the PDMA causes a decrease on the hydrophilic character of the PDMA block. This pH-induced micellization was completely reversible. Addition of acid led to reprotonation of the both PDPA and PDMA residues, leading to the formation of unimers below pH 6 at 20 °C (see Table 2).

Table 2
A summary of the dynamic light scattering data for unimers at pH 2.0 and PDPA-core micelles at pH between 8.0 and 9.0. All DLS measurements were carried out by using 0.5 wt% triblock copolymer solutions at 20 °C.

Polymer	pH	Diameter (nm)	Polydispersity index (μ_2/Γ^{-2})	Aggregation state
FT14	2.0	3.7	0.26	Unimers
	8.0	25.0	0.09	PDPA-core flower micelles
FT15	2.0	3.8	0.22	Unimers
	9.0	30.0	0.22	PDPA-core flower micelles
FT16	2.0	2.9	0.19	Unimers
	9.0	27.6	0.11	PDPA-core flower micelles

The surface activity of the PDPA-*b*-PDMA-*b*-PDPA triblock copolymer has pH and concentration dependence. As the solution pH is increased, the block copolymer becomes strongly adsorbed at the air–water interface, thus lowering the surface tension of the solution. Above pH 7.0, the limiting surface tension is approximately 40 mN m^{-1} for the $PDPA_{23}$-$PDMA_{150}$-$PDPA_{20}$ triblock copolymer solution. Presumably, the deprotonated hydrophobic PDPA block becomes adsorbed at the air–water interface, thus lowering the surface tension of the solution. This limiting surface tension is similar to that obtained with small molecule surfactants.

It is also possible to identify the so-called critical micelle concentration (CMC) by determining the concentration dependence

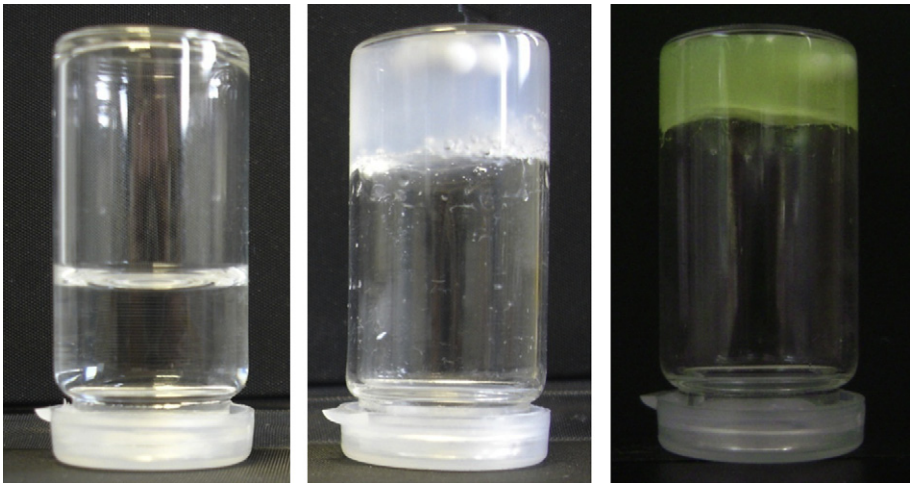

Fig. 7. Digital photographic images of (left) the free-flowing aqueous solution formed at pH 2, (middle) the physical gel (note tube inversion) formed at pH 7.5 and (right) dipyridamole loaded physical gel formed at pH 7.5 by the $PDPA_{23}$-*b*-$PDMA_{150}$-*b*-$PDPA_{20}$ triblock copolymer [FT14 polymer: 20 wt%, drug/polymer ratio: 1/20].

of the surface tension of a block copolymer. The CMC for the $PDPA_{23}$-*b*-$PDMA_{150}$-*b*-$PDPA_{20}$ triblock copolymer is determined to be 0.07 wt% at pH 8.0. This CMC is similar to that obtained with other tertiary amine methacrylate based diblock copolymers [23,53].

3.2.2. Hydrogel preparation

Triblock copolymers PDPA-*b*-PDMA-*b*-PDPA are molecularly dissolved in acidic solution (pH < 6). When the pH is adjusted to between 7.0 and 8.0 with KOH addition, the PDPA blocks become deprotonated, i.e., hydrophobic, which results in attractive interchain interactions (the pK_a of protonated PDPA homopolymer has a pH of around 6.4). In dilute solution, micellization occurs by the PDPA block forming the core due to intra-chain interaction and the PDMA block forming the corona of the micelles, which are termed as "flower micelles". On the other hand, at higher copolymer concentrations (10 wt% and above) it is possible to obtain macroscopic physical gels depending on the triblock copolymer composition. Gelation has been demonstrated by simple tube inversion method. The term "free-standing gel" is referred to describe gels which keep their fixed position following the tube inversion [35]. This is illustrated in Fig. 7 through the digital images of the same triblock copolymer solution at pH 2 and pH 7.5 (with or without drug loading). While the acidic solution is in a fluid state, the neutralized solution forms a "free-standing" gel. In principle, gelation will take place at a given copolymer concentration if the central PDMA block is long enough to bridge between adjacent micelles (micellar bridging) in the aqueous solution.

In the triblock copolymer series, no gelation occurred at a concentration of 5 wt% triblock copolymer, but gelation behavior was observed for 10 wt% and above for all triblock copolymers given in Table 1. All pH-responsive gelations were completely reversible. A decrease on solution pH by addition of HCl causes molecular dissolution of the triblock copolymer. The time shift between the addition of acid–base and the effect taking place was very short (within a second) as reported by Zhu et al. for such block copolymers [59].

The selected PDPA-*b*-PDMA-*b*-PDPA triblock copolymer was examined in terms of its acidic aqueous solution at low pH and the gel produced at above pH 7 using ^{1}H NMR spectroscopy. Fig. 6c presents the ^{1}H NMR spectrum for the 20 wt% concentrated solutions of $PDPA_{23}$-*b*-$PDMA_{150}$-*b*-$PDPA_{20}$ (FT14) in D_2O. At pH 2, all the expected proton NMR peaks due to the protonated central PDMA block and the protonated outer PDPA blocks are visible, which signifie a high degree of solvation and mobility for both types of blocks. In contrast, the peaks stemming from the PDPA blocks are not observed at pH 7.5. This indicates that, becoming deprotonated and hence hydrophobic at this solution pH, the PDPA blocks were significantly reduced in solvation and mobility. As the gels are formed as a result of the bridging between the micelles, it is possible to load hydrophobic drugs into the hydrophobic core parts and use it as a controlled drug release system.

Dipyridamole (DIP) was chosen as the model drug for the controlled release studies from PDPA-*b*-PDMA-*b*-PDPA hydrogel

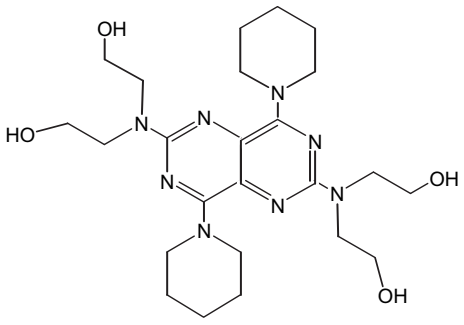

Fig. 8. Chemical structure of dipyridamole.

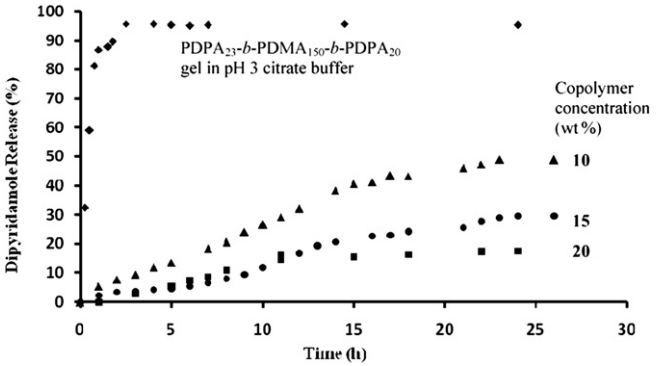

Fig. 9. Effect of both triblock copolymer concentrations and buffer pH on the release of dipyridamole (5 wt% based on triblock copolymer) from a $PDPA_{23}$-*b*-$PDMA_{150}$-*b*-$PDPA_{20}$ triblock copolymer gel at 37 °C. Rapid release of dipyridamole was obtained at pH 3 because of gel dissolution. Slow, sustained release was achieved at pH 7.4 for the 10, 15, and 20 wt% triblock copolymer gels during 24 h.

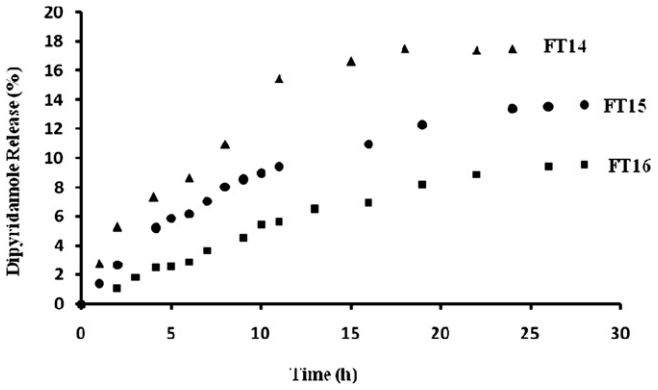

Fig. 10. Effect of the molecular weight of the $PDPA_{0.12}$-*b*-$PDMA_{0.78}$-*b*-$PDPA_{0.10}$ triblock copolymers on the release of dipyridamole (5 wt% based on triblock copolymer) from their gels (20 wt%) at 37 °C and pH 7.4 buffer solution.

system. DIP is a well known coronary vasodilator and a coactivator of antitumor compounds. Dipyridamole contains several tertiary amine groups; thus, like the $PDPA_{23}$-*b*-$PDMA_{150}$-*b*-$PDPA_{20}$ triblock, its solubility in aqueous solution is pH-dependent. DIP is soluble in water below pH 5.9 because of the protonation of its amine groups (see Fig. 8). It becomes water-insoluble above pH 5.9 and precipitates as yellow, needle-shaped crystals in the absence of any copolymer [34]. In the present case, the dipyridamole molecules are solubilized within the PDPA-hydrophobic micellar domains of the gel at around neutral pH.

3.3. Drug-loaded gel production and drug release experiments

DIP (5 wt% of the triblock copolymer mass) was dissolved in 10, 15 and 20 wt% triblock copolymer solutions at pH 2.0, and followed its neutralization with a 2.0 M KOH solution, drug-loaded gels at pH 7.5 were produced (see Fig. 7).

Drug release experiments were performed by loading dipyridamole drug (DIP) into the pH-sensitive $PDPA_{23}$-*b*-$PDMA_{150}$-*b*-$PDPA_{20}$ triblock copolymer hydrogels (10, 15 and 20 wt%) selected as model system. Each of the drug-loaded $PDPA_{23}$-*b*-$PDMA_{150}$-*b*-$PDPA_{20}$ triblock copolymer gel (250 mg, FT14 gel) was replaced into a dialysis membrane (Molecular Weight cutoff of 12–14,000 Da) and inserted into 100 mL phosphate buffer solution of pH 7.4 at 37 °C. The membrane was used to prevent the buffered solution around the gel from any possible micellar dissolution of the gel. The UV absorptions of the buffer solution were measured periodically at 291 nm by using UV–vis spectrophotometer.

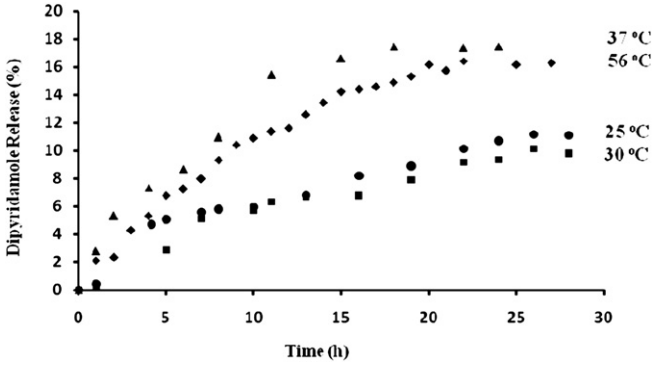

Fig. 11. Effect of the temperature on the release of dipyridamole (5 wt% based on triblock copolymer) from a $PDPA_{23}$-*b*-$PDMA_{150}$-*b*-$PDPA_{20}$ gel (20 wt%) at pH 7.4 buffer solution.

The results are summarized in Fig. 9. For 10, 15 and 20 wt% gels, a slow and long release was obtained during 24 h. DIP was more effectively retained in the higher polymer-concentrated gel. The DIP release from hydrogel was slower if triblock copolymer concentration was higher. On the other hand, on submerging the 20 wt% concentrated gel into a pH 3 citrate buffer solution, quite a rapid release occurred due to molecular dissolution of the gel at this pH. As compared with the $PDPA_{50}$-*b*-$PMPC_{250}$-*b*-$PDPA_{50}$ (M_n: 130,000 g/mol) hydrogels [34], the $PDPA_{23}$-*b*-$PDMA_{150}$-*b*-$PDPA_{20}$ (M_n: 32,800 g/mol) hydrogels (10 wt%) showed three times higher drug release behavior at the same conditions within 4 h.

The effects of both molecular weights of the triblock copolymers having constant comonomer compositions and the temperature on the drug release were also determined by using 20 wt% gel (containing 5 wt% dipyridamole based on triblock copolymer). Fig. 10 shows the molecular weight effect on the release of dipyridamole from their gels (20 wt%) at 37 °C. As the longer polymer chain causes better gel formation [34] and better drug solubilization due to more easy bridge formation among micelles and greater hydrophobic micellar core the DIP release from the hydrogel of high molecular weight polymer was slower than that of the hydrogel of low molecular weight polymer as expected.

On the other hand, as can be seen in Fig. 11, the maximum DIP releases from a $PDPA_{23}$-*b*-$PDMA_{150}$-*b*-$PDPA_{20}$ gel (20 wt%) at 25, 31 37 and 56 °C were determined to be 11, 10, 17.5 and 16.2 wt%, respectively. Fig. 11 indicated similar drug releases at 25 and 31 °C, but higher and faster release at both 37 and 56 °C. To reach the drug release maxima, the period was determined to be 13 h at 37 °C, 18 h at 56 °C and 26 h at both 25 °C and 31 °C. The higher release at both 37 and 56 °C might be related to the LCST of the middle PDMA block. The LCST of the PDMA homopolymer having similar molecular weight was around 35 °C as reported earlier [23]. The thermo-responsive nature of the middle PDMA block has a dramatic effect on the DIP releases from the hydrogel.

4. Conclusions

In summary, a series of well-defined PDPA-*b*-PDMA-*b*-PDPA triblock copolymers have been prepared via GTP. These triblock copolymers could be dissolved molecularly on adjusting the solution pH to below pH 5 with HCl addition. By the addition of KOH into acidic polymer solution, near monodisperse “flower” type micelles were obtained with the diameters to be between 25 and 30 nm in low polymer concentrations and “free-standing gels” were obtained due to formation of bridge between adjacent micelles from concentrated polymer solution (>10 wt%) at around pH 7.4. The gels are believed to be in micellar nature, and the hydrophobic domains can be loaded with hydrophobic drugs. Preliminary studies indicate that both slow, sustained release and fast, triggered release of a model hydrophobic drug can be achieved, by tuning the solution pH, temperature, polymer molecular weight and polymer concentration of the gel. In addition to pH, the thermo-responsive nature of the middle PDMA block has an important effect on the DIP releases from the hydrogel.

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