Supporting Informations

Biocompatible Thermoresponsive *N*-isopropyl-*N*-(3-(isopropylamino)-3-oxopropyl)acrylamide - Based Random Copolymer : Synthesis and Studies of Its Composition Dependent Properties and Anticancer Drug Delivery Efficiency

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Experimental

Materials, Equipment and Analytical Methods

Hexane (S.D. fine, India), ethyl acetate (S.D. fine, India), dichloromethane (DCM) (S.D. fine, India), potassium hydroxide (Qualigens, India), isopropyl amine (Aldrich, USA), acryloyl chloride (Alfa Aesar, India), and anhydrous magnesium sulfate (LobaChemie, India) were

used as received. Triethyl amine (LobaChemie, India) was dried over potassium hydroxide and then distilled. *N*-isopropylacrylamide (TCI, Japan) was purified by recrystallization from *n* hexane. 2, 2/- Azobis(isobutyronitrile) (AIBN) (Spectrochem, India) was re-crystallized from methanol. Ethanol (Saraya Distilliary, India) was left over CaO for overnight and distilled over fresh CaO. *N,N*-dimethyl formamide (DMF) (Spectrochem, India) was dried firstly by azeotropic distillation after mixing with benzene and further dried over magnesium sulfate and distilled under vacuum. Tetrahydrofuran (LobaChemie, India) was dried over sodium and benzophenone and then distilled freshly. Deionized water was prepared by redistillation of the double distilled water in an all-glass distillation apparatus. Poly(ethylene glycol) methyl ether acrylate (PEGA: CH_2=$CHCO_2(CH_2CH_2O)_n$Me, M_n = 480, n = 9 on average, (Aldrich, USA), and 2-(dodecylthiocarbonothioylthio)-2-methylpropionic acid (DDMAT)(TCI, Japan) were used as received. 1-Phenylethyl phenyl dithioacetate (PEPD) were synthesized using reported literature.[1]

^{1}H and ^{13}C NMR. JEOL JNM-ECZ500R FTNMR (500 MHz) was used to record ^{1}H NMR and ^{13}C NMR spectra at room temperature (RT) in $CDCl_3$, DMSO-d_6, D_2O and CD_3COCD_3 as solvents. For polymers prepared using DDMAT as RAFT agent,the conversions of the polymerization have been calculated from the ^{1}H NMR study of the final polymerization mixtures in $CDCl_3$. For polyM3i, it has been determined by comparing the integrated peak area of the residual vinylic signals at 5.61 ppm (1H) of the monomer M3i with the peak area of the methane proton of the isopropyl group in the formed polymer as well as in the monomer at 1.2 ppm. For polyPEGA, it has been determined by comparing the integrated peak area of the residual vinylic signals at 5.8 ppm (1H) of the monomer with the peak area of the methoxy proton of PEGA in the formed polymer as well as in the monomer at 3.32 ppm. The composition of copolymers has been calculated from their ^{1}H NMR in $CD_3COCD_3$by comparing the peak area of the 3 methyl protons of the pendent part of the

polyethylene glycol (–OCH_3)at 3.32 ppm with the peak area of the 12 methane protons of the isopropyl group in the formed polymer at 1.2 ppm. The number-average molar masses based on NMR [M_n (NMR)] of the homopolymers and random copolymers poly(M3i-*ra*-PEGA)were determined also from the ^{1}H NMR spectra taken in CD_3COCD_3 by end-group analysis. For polyM3i as homopolymer or, as segment in its random copolymer, the average peak area of methine proton of −CH(CH_3)$_2$ at 4.2 ppm and of –CONH- at 7.4 ppm with the one third of the peak area of the chain end methyl group (–CH_3)derived fromDDMAT at 0.8 ppm has been used for calculation. For polyPEGA as homopolymer or, as segment in its random copolymer, the constitutional characteristic repeating unit signal is methyl ether peak of pendent part polyethylene glycol (–OCH_3) at 3.32 ppm which is compared with the one third of the peak area of the chain end methyl group(–CH_3) derived from DDMAT at 0.8 ppm. For random copolymer, above two methods have been combined.

For polymers prepared using PEPD as RAFT agent, the conversions of the polymerization have been calculated from the ^{1}H NMR study of the final polymerization mixtures in $CDCl_3$ following the procedure for the polymers prepared using DDMAT. The compositions of the copolymers are calculated from the ^{1}H NMR study of the polymers in CD_3COCD_3 by comparing the peak area of the 3 methyl protons of the pendent part of polyethylene glycol (–OCH_3) at ~3.27 ppm with the peak area of the 12 methane protons of the isopropyl group in the formed polymer at ~1.0 ppm. M_n(NMR) of the polymers are calculated from the ^{1}H NMR in DMSO-d_6.For polyM3i as its homopolymer or, in its random copolymer, the average proton peak area of the aromatic protons of the PEPD derived chain-ends has been calculated by considering the peaks at ~7.4 ppm for 10 aromatic protons at the chain-end and one –CONH proton of the repeating unit at 7.0 ppm and one methine proton of −CH(CH_3)$_2$ of the repeating unit at ~3.7 ppm and then used for the calculation of M_n (NMR) by comparing with the one methine proton of −CH(CH_3)$_2$ of the repeating unit at

~3.7 ppm. For polyPEGA as its homopolymer or, in its random copolymer, the constitutional characteristic repeating unit peak of methyl ether ($–OCH_3$) of the pendent polyethylene glycol at 3.27 ppm has been compared with the average aromatic proton peak of the chain-end. For random copolymer poly(M3i-*ra*-PEGA), above two methods have been combined.

Gel Permeation Chromatography (GPC). GPC5140 Viscotek TDA 305040, Malvern Panalytical Ltd, UK equipped with one CLM3009 general mixed org. and one CLM3008 org. post guard column connected in series, RI detector and GPC0042 Omnisec software was used to determine the number average molar mass (M_n) and molar massdispersity (PDI) in DMF at 50°C with flow rate 1.0 mL/min. Calibrations were made against seven polystyrene (PSt) standard samples (PolymerLab, PSt Calibration Kit, S-M2-10).

Thermogravimetric Analysis (TGA). TGA was conducted under N_2 atmosphere using a PerkinElmer STA 6000 instrument, in the temperature range of 25−900 °C with a heating rate of 10 $^oC\ min^{-1}$.

Differential Scanning Calorimetry (DSC). DSC was carried out under N_2 atmosphere using Mettler STAR SW 10.00 instrument. The instrument was calibrated with indium before use and DSC curves were presented from the second heating run at the rate of $10^oC\ min^{-1}$.[2]

Fourier Transform Infrared (FTIR) Spectroscopy. FTIR spectra were recorded using a PerkinElmer Spectrum version 10.03.05 Spectrometer in the range of 400-4000 cm^{-1} using KBr pallets of the samples.

High Resolution Mass Spectrometry (HRMS). HRMS was performed using a X500R QTOF spectrometer.

Turbidimetry. The phase transition temperatures of homopolymers and copolymers were measured using Carry Bio 100, Agilent UV/vis spectrophotometer connected with a Peltier system, over a temperature range between 5^{o}C and 65^{o}C at theheating/cooling rate of 1 ^{o}C/min with the holding time of 6 min. The percentage transmission (% T) was determined

by normalizing the transmitted light intensity at 550 nm to the maximum value in the actual sample measurement. The cloud point temperature (LCST) was identified as the temperature at 50% transmittance during heating run.

Fluorescence spectra were recorded on a Cary-Eclipse fluorescence spectrophotometer (Agilent Technologies) using solutions of requisite concentrations in deionized water at ambient temperature using pyrene as fluorescence probe.[3]

Dynamic Light Scattering (DLS) studyhas been carried out usingZetasizer Ultra (ZSU5700) [Malvern Panalytical (UK)] at 25^{o}C to determine the hydrodynamic volume (D_h) of the polymers in water.

Transmission Electron Microscopy has been made using HRTEM (Technai G220 TWIN, FEI Company of USA) operating at an accelerating voltage of 200 kV. Samples were prepared by drop casting of polymer solution of 0.1 mg/mL on the carbon coated copper grid. Before measurement, solvent was slowly evaporated at room temperature by keeping it overnight.

Polymer Modelling Spatial orientation of the two pendant groups of each repeating structural units in the formed polymer has also been explored using density functional theory study of the polymer assuming degree of polymerization = 6 and chain ends are ended with methyl group at B3LYP/6-31G** level of theory using Gaussian 09 suits of program.[4]

Synthesis of *N*-isopropyl-3-(isopropylamino) propanamide(SAM3i)

NIPAM (5g, 44.18mmol) was degassed under high vacuum pump followed by purging with under nitrogen three times and then dissolved in 35.4mL of anhydrous ethanol. To it, 2-propyl-amine (3.13 g, 53.016mmol) was then added under stirring and the reaction mixture was kept under reflux condition for 48h *via* dipping in an oil bath maintained at 80^{o}C. Ethanol was evaporated under reduced pressure *via* rotary evaporator and the residue was

purified by column chromatography on silica gel (230-400 mesh size) eluted with ethanol/ethyl acetate (v:v = 5:95) along with 0.5% triethylamine (R_f value=0.40) to give 5.1g (67.5%) (gravimetric yield) of the corresponding secondary amine as light yellow oil.

^{1}H NMR (500 MHz, $CDCl_3$) [Figure S1(a)]: δ(ppm) 0.95 (6H, CH(CH_3)$_2$)1.12 (6H, CH(CH_3)$_2$), 1.50 (1H, CH_2NH), 1.35 (2H, CH_2CH_3), 2.20 (2H, $COCH_2$), 2.71 (1H, NHC*H*), 2.71 (1H, CH_2NH), 3.90 (1H, C*H*(CH_3)$_2$), 7.65 (1H, CON*H*).

^{13}C NMR (125 MHz, $CDCl_3$[Figure S1(b)]: δ (ppm) 13.80, 20.30, 22.59, 29.32, 31.99, 35.64, 40.66, 45.64, 48.95, 171.71.

FT-IR [v(cm^{-1})][Figure S1(c)]: 3268 (N-H Str), 2969, 2930, 2873 (C-H strech), 1637 (C=O Str), 1550 (N-H Bend), 1461(C-H, Bend), 1336 (C-H, Bend), 1127 (C-N strech).

Synthesis of *N*-isopropyl-*N*-(3-(isopropylamino)-3-oxopropyl)acrylamide monomer (M3i)

N-isopropyl-3-(propylamino)propanamide(5.0g, 29.02mmol) was degassed three times with high vacuum pump under nitrogen atmosphere and then dissolved in 81mL anhydrous dichloromethane and triethylamine (NEt_3) (9.673mL, 69.35mmol) was added to it. The resultant solution was cooled to 0^{o}C under nitrogen atmosphere using an ice bath. To it, acryloylchloride (3.1 mL, 34.70mmol) was added drop wise over 10min under stirring. The reaction mixture was slowly warmed up to the room temperate (RT)and kept under stirring at RT for 12h. The formed precipitate was removed by filtration. The organic layer was evaporated using rotary evaporator, and the residue was purified by column chromatography on silica gel (size=230-400mesh size), eluting with by 95% ethyl acetate/hexane along with

0.5% triethylamine (R_f value=0.73) to give pure 4.1g (gravimetric yield=67.21%) desired product.

^{1}H NMR (500 MHz, $CDCl_3$) [Figure S2(a)]: δ(ppm) 1.01 (6H, $CH(CH_3)_2$), 1.21 (6H, $CH(CH_3)_2$), 2.44 (2H,$COCH_2$), 3.43-3.54 (1H,NCH), 3.84-3.94 (2H,CH_2N), 4.25,4.55 (1H,$CH(CH_3)_2$), 5.72, 6.29, 6.86 ($CH=CH_2$), 7.90 (1H, CONH).

^{13}C NMR (125 MHz, $CDCl_3$)[Figure S2(b)]: 19.49, 22.13, 35.68, 38.30, 34.01, 45.95, 48.22, 127.75,129.50, 165.73, 169.60.

FT-IR [ν(cm^{-1})][Figure S2(c)]: 3261 (N-H Str), 3079 (=C-H Str), 2938, 2938, 2871 (C-H Str), 1629 (C=O Str), 1606 (C=C Str), 1560 (N-H Bend), 1458 (C-H, Bend),1383 (C-H Bend), 1127(C-N)

HRMS (TOF-MS) $[M+H]^+$[Figure S2(d)]calculated C12H22N2O2 for 227.1759; found 227.1736 $[M+H]^+$

Synthesis of Polymers

Typical RAFT homo polymerization of *N*-isopropyl-N-(3-(isopropylamino)-3-oxopropyl)acrylamide monomer (M3i) (Run 1, Tables S1 and 1)

Homopolymer of M3i was synthesized *via* RAFT polymerizationin DMF solvent using 2-(dodecylthiocarbonothioylthio)-2-methylpropionic acid (DDMAT)as RAFT reagent and AIBN as initiator. For a typical polymerization using [M3i] : [DDMAT] : [AIBN] = 100 : 1 : 0.2, M3i (208.08mg, 0.9166mmol), DDMAT (3.31 mg, 9.1 x 10^{-6}mol), and AIBN (0.2988 mg, 1.82 x 10^{-6} mol) were taken in a Schlenk tube with a teflon-coated magnetic needle and dissolved in 1.5 mL dry DMF. The Schlenk tube content was then degassed with N_2 gas at RT

for 30 min and then polymerization was performed by dipping the tube into a preheated oil bath maintained at 70^{o}C for 24 h under stirring. After completion, polymerization mixture was quenched by rapid cooling of Schlenk tube *via* immersion in liquid N_2. To get the monomer conversion a small amount of polymerization mixture was taken out for ^{1}H-NMR analysis in $CDCl_3$. Solvent DMF was removed from the remaining polymerization mixture using a high vacuum pump. The obtained residue was then dissolved in THF and precipitated from diethyl ether at RT and separated by centrifugation. This procedure of dissolution followed precipitation and separation by centrifugation was repeated two moretimes. Finally, the purified separated polymer was dried under vacuum at 50^{o}C for 24 h and obtained as white powder.

For polymerization using PEPD as RAFT agent, similar procedure has been followed using 2.07 mg (9.1 x 10^{-6}mol) PEPD in place DDMAT keeping other conditions remained unchanged. **(Run 1$^{/}$, Tables S2 and 2)**

^{1}H NMR (500 MHz, CD_3COCD_3) (Figure 1): δ(ppm) 0.86(3H,C*H*$_3$), 0.9-1.5[12H,CH(C*H*$_3$)$_2$) + 20H, $(CH_2)_{10}$), 2.2 (2H,COC*H*$_2$),3.64 (2H,C*H*$_2$N), 4.25, (1H,C*H*(CH_3)2),4.02 (1H, C*H*(CH_3)$_2$), 7.80 (1H, CON*H*), 1.23 (20H,$CH_3C_{10}H_{20}CH_2$)

FT-IR [ν(cm^{-1})](Figure S3):3301 (N-H Str), 2972, 2934, 2877 (C-H Str), 1636 (C=O Str), 1550 (N-H Bend), 1451 (C-H, Bend), 1367 (C-H Bend), 1127 (C-N Str).

Typical RAFT homo polymerization of poly(ethylene glycol) methyl ether acrylate (PEGA) (Run 2, Tables S1 and 1)

Homopolymer of poly(ethylene glycol) methyl ether acrylate (PEGA)was synthesized *via* RAFT polymerizationof PEGA in DMF solvent using DDMAT as RAFT reagent and AIBN

as initiator. For a typical polymerization using [Monomer]: [RAFT] : [AIBN] = 100 : 1 : 0.2, PEGA (0.427mL, 0.91 mmol), DDMAT (3.31 mg, 9.1 x 10^{-6} mol), and AIBN (0.3 mg, 1.82 x 10^{-6} mol) were taken in a Schlenk tube with a Teflon-coated magnetic needle and 1.07 mL dry DMF was added. The Schlenk tube content was then degassed with N_2 gas at RT for 30 min and then polymerization was performed by dipping the tube into a preheated oil bath maintained at 70^{o}C for 24 h under stirring. After completion, polymerization mixture was quenched by rapid cooling of Schlenk tube *via* immersion in liquid N_2. To get the monomer conversion, a small amount of polymerization mixture was taken out for ^{1}HNMR analysis in $CDCl_3$. Solvent DMF was removed from the remaining polymerization mixture using a high vacuum pump. The obtained residue was then dissolved in THF and precipitated from hexane. This procedure of dissolution followed precipitation was repeated two more times. Finally, the purified separated polymer was dried under vacuum at 50^{o}C for 24 h.

For polymerization using PEPD as RAFT agent, similar procedure has been followed using 2.07 mg (9.1 x 10^{-6}mol) PEPD in place DDMAT keeping other conditions remained unchanged. **(Run 2/, Tables S2 and 2)**

^{1}H NMR (500 MHz, CD_3COCD_3)[Figure 2]:δ(ppm) 0.88 (3H,CH_3), 3.44 (3H, OCH_3), 3.54 (30H,$COCH_2CH_2OC$), 4.17 (2H, CH_2OCO).

FT-IR [ν (cm^{-1})] [Figure S4]:2969, 2930, 2873 (C-H strech), 1637 (C=O Str), 1336 (C-H, Bend), 1461(C-H, Bend), 1060-1150 (asystr C-O-C)

Typical RAFT random copolymerization of *N*-isopropyl-*N*-(3-(isopropylamino)-3-oxopropyl)acrylamide monomer (M3i) andpoly(ethylene glycol) methyl ether acrylate(PEGA) (Run 3, Tables S1 and 1)

Random copolymers were synthesized *via* RAFT polymerization in DMF solvent using DDMAT as RAFT reagent and AIBN as initiator. For a typical polymerization, Run 2, (Table 1) with [Monomer] : [RAFT] : [AIBN] = 100 : 1 : 0.2, M3i (125 mg, 0.55 mmol), DDMAT (3.31 mg, 9.1 x 10^{-6} mol), and AIBN (0.2988mg, 0.88 x 10^{-6} mol), PEGA (0.76 mmol, 0.174 mL) were added sequentially into a Schlenk tube containing a Teflon-coated magnetic bar at 25 °C under nitrogen (total volume: 1.5 mL) in dry DMF. The reaction mixture was then degassed with N_2 gas at RT for 30 min and then polymerization was performed by dipping the tube into a preheated oil bath maintained at 70^{o}C for 24 h under stirring. After completion, polymerization mixture was quenched by rapid cooling of Schlenk tube *via* immersion in liquid N_2. To get the monomer conversion a small amount of polymerization mixture was taken out for ^{1}HNMR analysis in $CDCl_3$. Solvent DMF was removed from the remaining polymerization mixture using a high vacuum pump. The obtained residue was then dissolved in THF and precipitated from hexane at RT. This procedure of dissolution followed precipitation and separation two moretimes. Finally, the purified copolymer obtained as a yellow paste was dried under vacuum at 50^{o}C for 24.

For polymerization using PEPD as RAFT agent, similar procedure has been followed using 2.07 mg (9.1 x 10^{-6}mol) PEPD in place DDMAT keeping other conditions remained unchanged. **(Run 3$^{/}$, Tables S2 and 2)**

^{1}H NMR (500 MHz, CD_3COCD_3) [Figure 3]: δ(ppm) 0.86(3H,CH_3), 1.01-1.24 (12H,CH(CH_3)$_2$), 2.44 (2H,COCH_2), 3.64 (2H,CH_2N), 4.25, (1H,CH(CH_3)2),4.02 (1H, CH(CH_3)$_2$), 7.80 (1H, CONH), 1.23 (20H,$CH_3C_{10}H_{20}CH_2$), 3.34 (3H, OCH_3), 3.54-3.84 (30H,$COCH_2CH_2OC$), 4.27 (2H, CH_2OCO)

FT-IR [ν (cm^{-1})] [Figure S5]:3280 (N-H Str), 2977, 2948, 2848 (C-H strech), 1649 (C=O Str), 1550 (N-H Bend), 1456 (C-H, Bend), 1368 (C-H, Bend), 1127 (C-N strech).

Drug loading

The copolymers (Runs 6 and 6' , Tables 2, and 3, respectively) (35 mg) were dissolved in deionized water (35 mL deionized water) for 4 h. Solution of Dox.HCl (3.75 mL) in 2 mL DMF was treated with TEA (10 mol eq. to Dox.HCl) for 4 h. The neutralized Dox solution was then filtered and added into the polymer solution. The mixture was stirred at room temperature for 24 h.[5] Then, the final mixture was dialyzed against distilled water using a dialysis membrane [molecular weight cut off (MWCO) = 3500 g mol^{-1}] for 8 h. During the first 3 h, the water was exchanged three times (every hour) and then twice during the following 5 h. The dialyzed solution was finally lyophilized to yield the solid micelle sample. Lyophilized drug-loaded micelle was then dissolved in water and analyzed by UV absorbance at 485 nm, using a standard calibration curve experimentally obtained with the DOX solutions in water having known concentrations. Drug loading content (DLC) and drug loading efficiency (DLE) were calculated according to the following formula:

Drug loading content (DLC) (wt. %) = (weight of loaded drug /weight of polymer) ×100%

Drug loading efficiency (DLE) (wt. %) = (weight of loaded drug /weight of drug in feed) ×100%

Drug Release study

5 mg of lyophilized drug-loaded polymeric micelle dissolved in 1 mL phosphate buffer saline (PBS) of 6.4/7.4 pH was taken in a dialysis bag with a MWCO of 3500 g mol^{-1}, which was placed into 20 mL PBS solution.[6] At different intervals, 2.0 mL was removed from the outer aqueous solution and replaced by fresh release medium (PBS solution). The released drug was quantified spectrophotometrically. The test was performed at 37 ^{o}C.

Cell lines and cell culture

Dalton's lymphoma (DL), a murine lymphoma was maintained in the peritoneum of BALB/c mice and was also maintained in vitro in RPMI-1640 (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT), 100 U/mL penicillin and 100 μg/mL streptomycin (Invitrogen, Carlsbad, CA), henceforth considered as complete medium.

Cell viability assay

Effect of free doxorubicin, copolymers 6′ and 6 (P1 and P2, respectively) as well as their corresponding DOX-loaded micelles (P1-DOX and P2-DOX, respectively) on the viability of tumor cells was evaluated by a colorimetric XTT (sodium 3-[1-(phenylaminocarbonyl)-3,4-tetrazolium]-bis(4-methoxy-6-nitro) assay (Roche, Indianapolis, IN). DL tumor cells were plated (5×10^3 cells/well) in 96-well culture dish and incubated with different concentrations of the above-mentioned compounds and incubated at 37°C, 5% CO_2 for 18 hours. OD was taken at 450 nm in a plate reader (Synergy HT, BioTek, USA). The percent viable cell was calculated employing the formula below.[7]

$$\% \text{ Cell Viability} = \frac{\text{Experimental OD}_{450}}{\text{Control OD}_{450}} \times 100$$

Cell growth inhibition assay

Growth inhibitory potential by the above compounds against DL tumor cells was studied by MTT assay.[7]Tumor cells (5×10^3 cells /well) in a 96 well culture dish were treated with serial concentrations of the compounds. Following incubation at 37°C, 5% CO_2, for 48 hours, the proliferation of the tumor cells was assessed by MTT assay using CellTiter 96 kit (Promega, USA). The measurement of absorbance (OD value) was made at 570 nm in a plate reader (BioTek, USA). Percent inhibition of the tumor cells was calculated using under mentioned formula

$$\% \text{ Growth Inhibition} = [1 - \frac{\text{Experimental } OD_{570}}{\text{Target } OD_{570}}] \times 100$$

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where Experimental OD value indicates the values of tumor cells in the presence of the indicated formulations and Target OD indicates the corresponding values of tumor cell alone, cultured in complete medium only.

Cytotoxicity assay

The lytic activity of free doxorubicin or P1-DOX or P2-DOX against DL cells was measured by cytotoxicity assay using the CytoTox 96 Cytotoxicity assay kit from Promega, USA. Target cells (5×10^3) were co-cultured with varying concentrations of the indicated formulations in a 96 well culture dish. The culture dish was incubated for 18 hours at 37^{o}C, 5% CO_2. Percent-specific lysis was determined from the under mentioned formula.[7]

$$\% \text{ Cytotoxicity} = \frac{(\text{Experimental - Effector Spontaneous - Target Spontaneous})}{(\text{Target Maximum - Target Spontaneous})} \times 100$$

Apoptosis &cellular uptake assay

Evaluation of temporal uptake of DOX and apoptotic cell death in DL cells was made following treatment with free DOX or P1-DOX/P2-DOX (30 nM each) for 12 hours. Only polymer was used as positive control along with the untreated cells. The cells were washed in PBS and were stained with FITC-conjugated Annexin V for 30 minutes. These cells were washed in Annexin buffer. FITC-conjugated Annexin V positive cells was visualized under a fluorescence microscope (EVOS FL Cell Imaging System equipped with Plan Fluor, 40X, NA 0.75 objective, Life Technologies, USA) as describe earlier.[8]

Statistical analysis

Unpaired student's t-test or one-way ANOVA followed by Tukey's post hoc test was conducted while comparing between the treated groups. Each experiment was performed in

triplicate and the data are presented as mean ± SD (standard deviation). Differences were considered significant for 'p' value < 0.05. *< 0.05, **< 0.01, ***< 0.001, and ****< 0.0001.

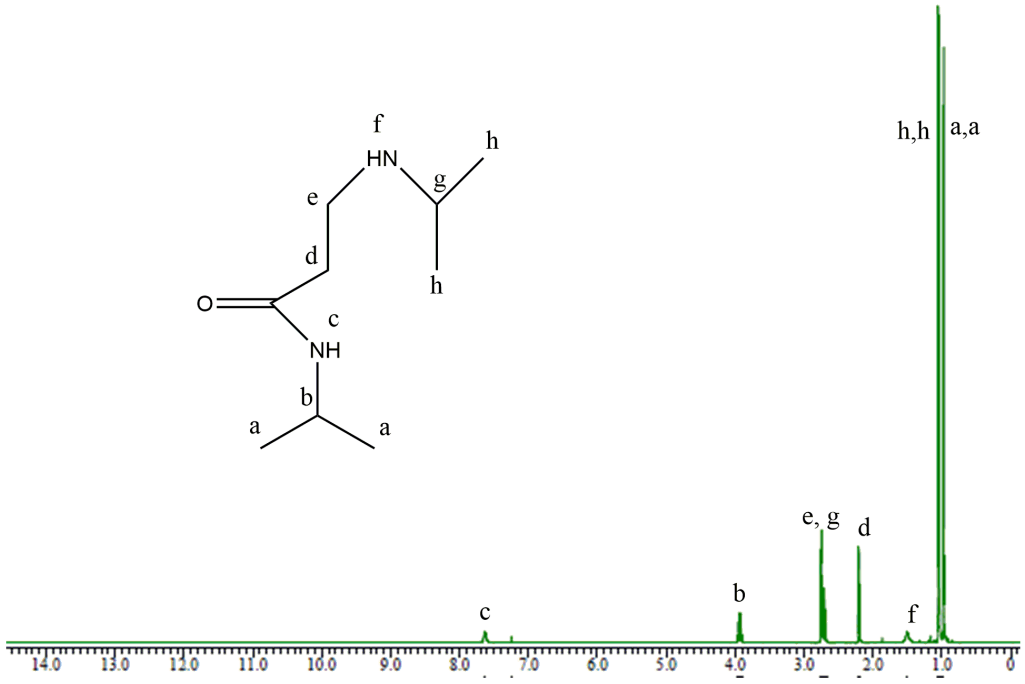

Figure S1(a). ^{1}HNMR (500 MHz, $CDCl_3$) spectrum of *N*- isopropyl-3-(isopropylamino) propanamide (SAM3i)

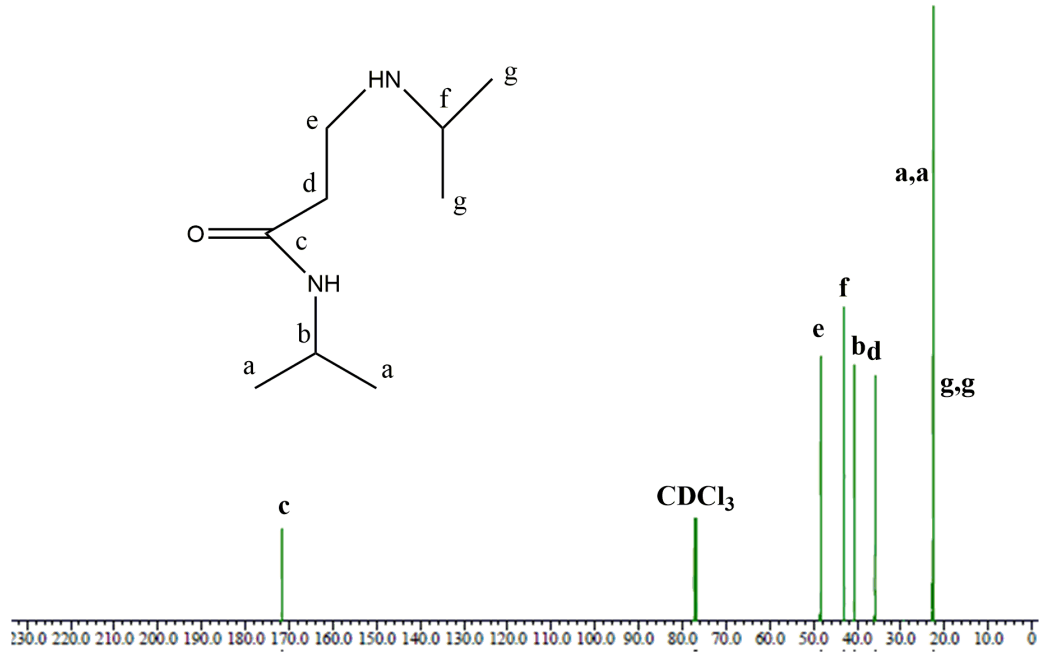

Figure S1(b). ^{13}C-NMR (500 MHz, $CDCl_3$) spectrum of *N*- isopropyl-3- (isopropylamino) propanamide (SAM3i)

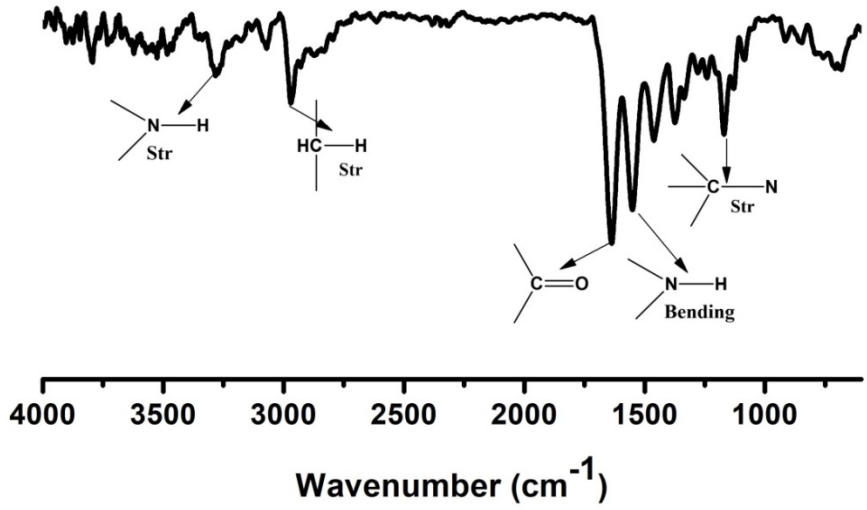

Figure S1(c). FT-IR spectrum of *N*-isopropyl-3-(isopropylamino) propanamide (SAM3i) as KBr pellet

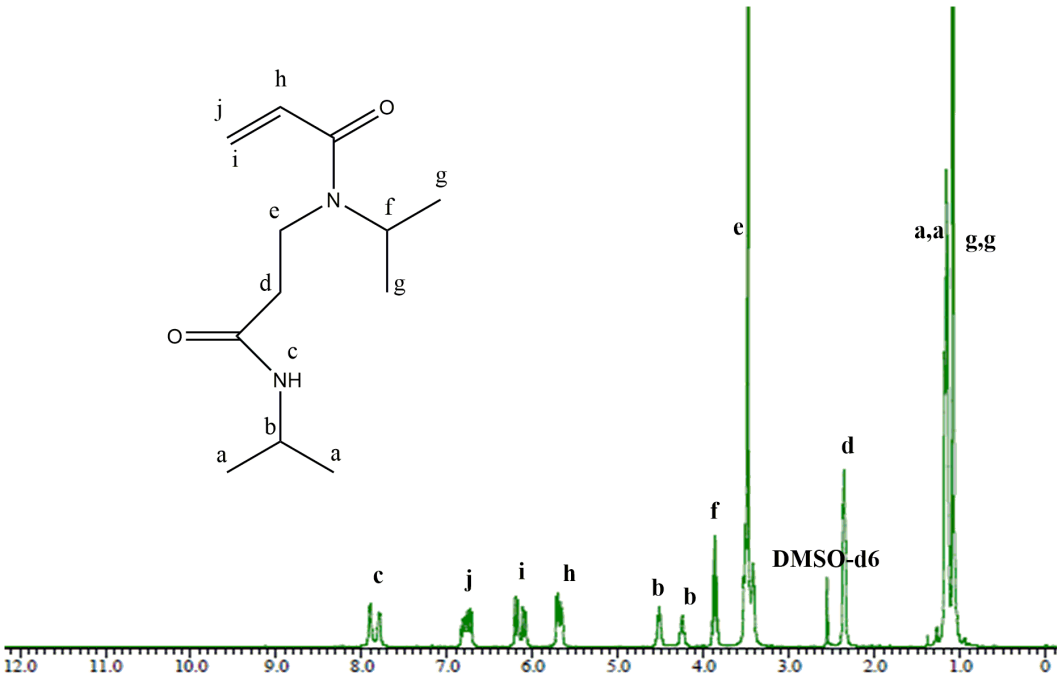

Figure S2(a). ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of *N*-isopropyl-*N*-(3-(isopropylamino)-3-oxopropyl)acrylamide (M3i)

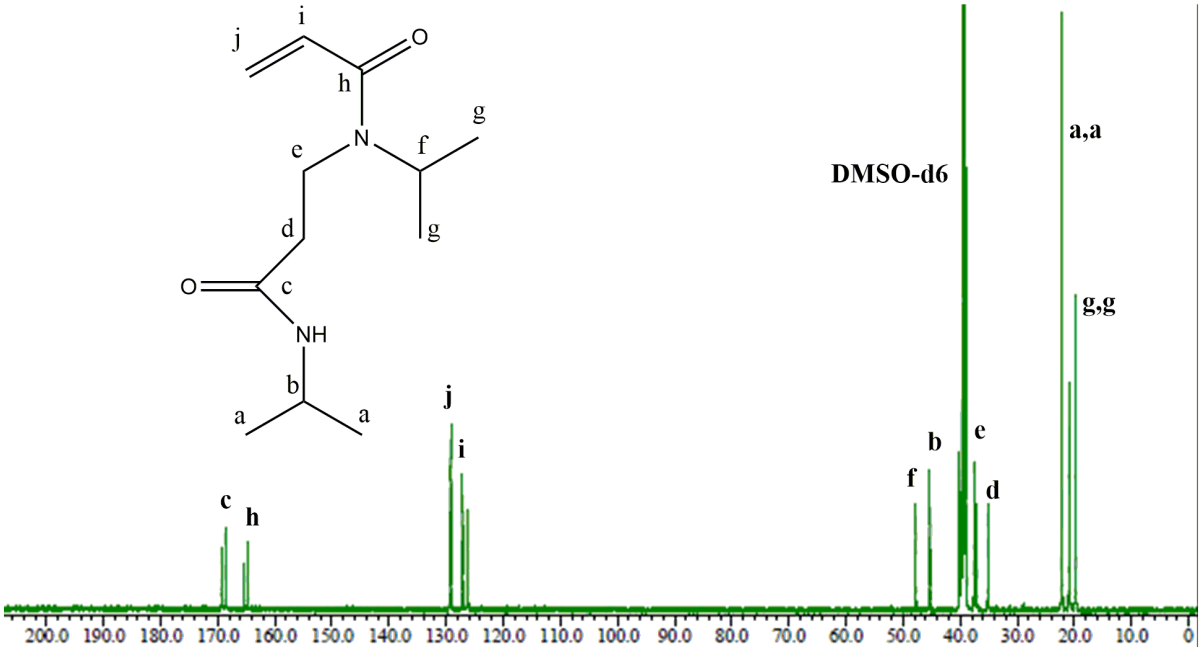

Figure S2(b). ^{13}C NMR (125 MHz, DMSO-d_6) spectrum of *N*-isopropyl-*N*-(3-(isopropylamino)-3-oxopropyl)acrylamide (M3i)

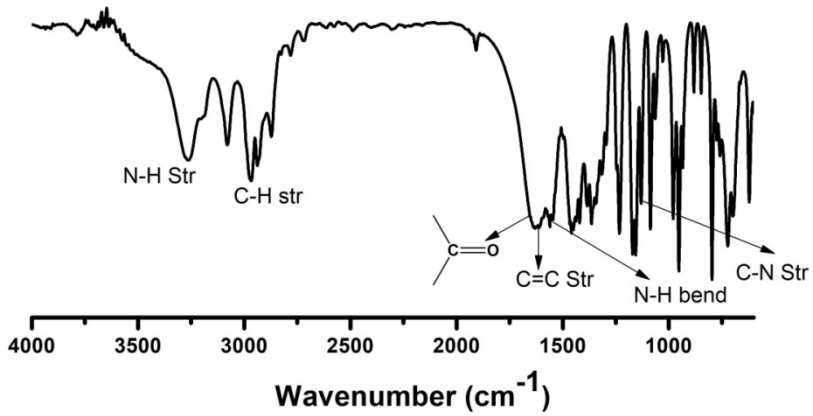

Figure S2(c). FT-IR spectrum of the KBr pellet of *N*-isopropyl-*N*-(3-(isopropylamino)-3-oxopropyl)acrylamide (M3i)

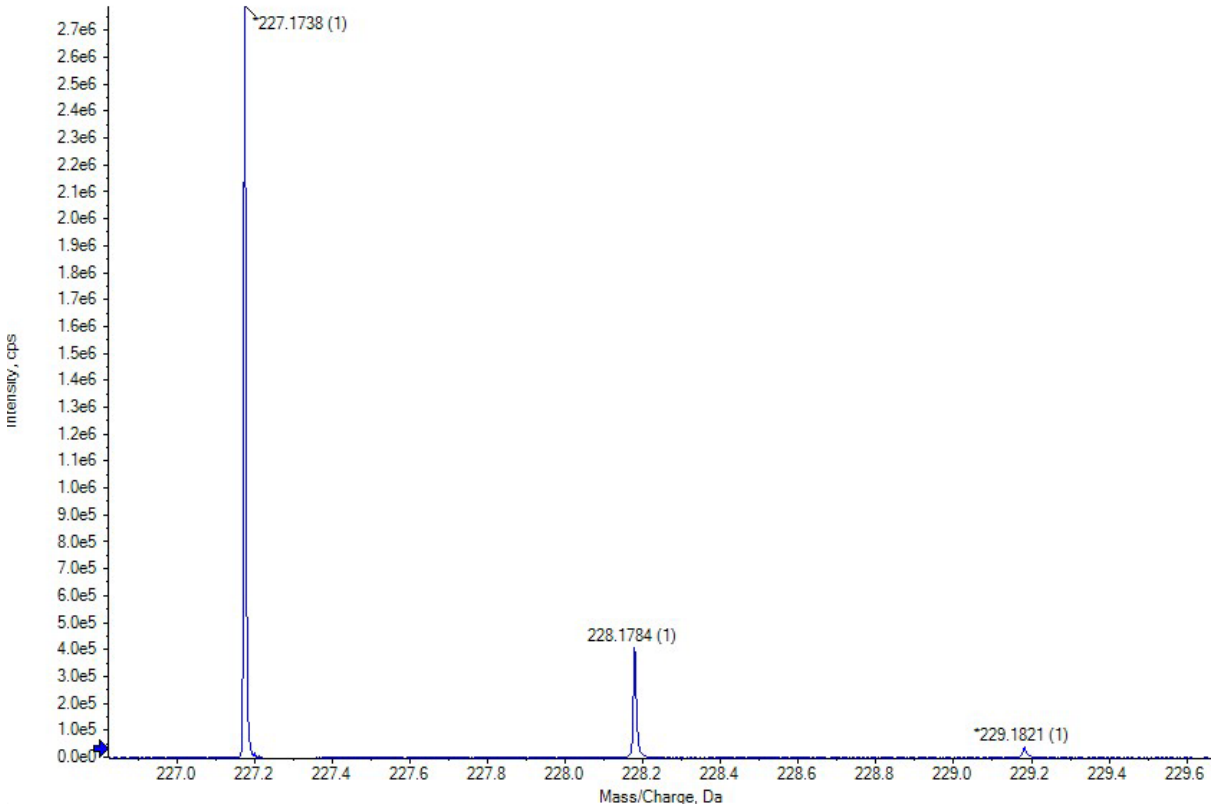

Figure S2(d) HRMS (TOF-MS)of monomer *N*-isopropyl-*N*-(3-(isopropylamino)-3-oxopropyl)acrylamide (M3i)

Table S1. Recipe for the synthesis of polymers by RAFT polymerization process

	Run ID							
Intake amount from Stock	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Monomer Composition Ratio (PEGA:M3i) (molar)	0:100	100:0	40:60	35:65	30:70	25:75	20:80	10:90
M3i (mg)	208.08	-	125	135	145	156	166	187
PEGA(mL)[a]	-	Pure 0.420	0.5	0.428	0.367	0.304	0.243	0.122
DDMAT (mL)[b]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
AIBN(mL)[c]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
DMF(mL)	0.5	0.073	-	0.072	0.133	0.196	0.257	0.378

[a]PEGA = 1.137g in 3.24 mL DMF;

[b]DDMAT = 35.4 mg in 5.33 mL DMF; [c]AIBN = 3.4mg in 5.7mL

Table S2. Recipe for the synthesis of polymers by PEPD RAFT polymerization process

Run ID								
Intake amount from Stock	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Monomer Composition Ratio (PEGA:M3i) (molar)	0:100	0:100	40:60	35:65	30:70	25:75	20:80	10:90
M3i (mg)	208.08	-	125	135	145	156	166	187
PEGA(mL)[a]	-	Raw 0.420	0.5	0.428	0.367	0.304	0.243	0.122
PEPD (mL)[b]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
AIBN(mL)[c]	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
DMF(mL)	0.5	0.073	-	0.072	0.133	0.196	0.257	0.378

[a]PEGA = 1.13g in 3.24 mL DMF;

[b]PEPD = 23.2mg in 5.60mL DMF; [c]AIBN = 3.4mg in 5.7mL

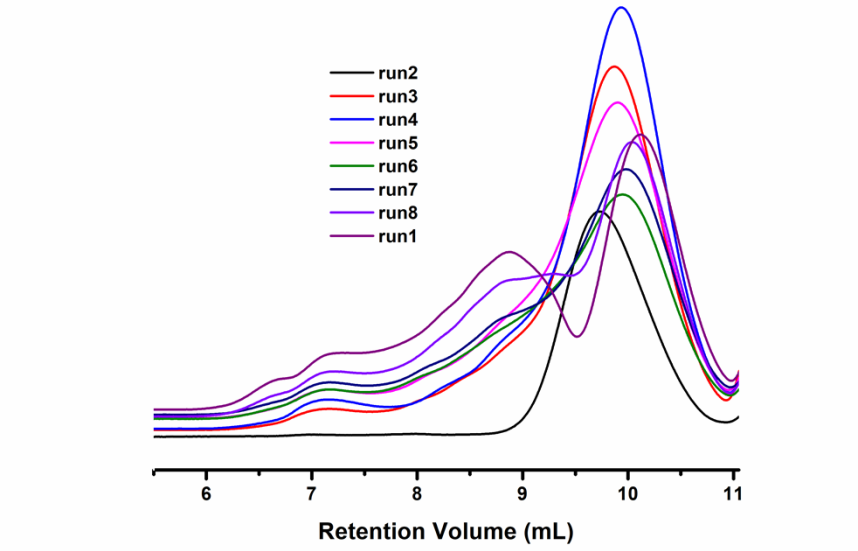

Figure S3. Typical GPC chromatograms of the polymers prepared using DDMAT as RAFT (**Table 1**)

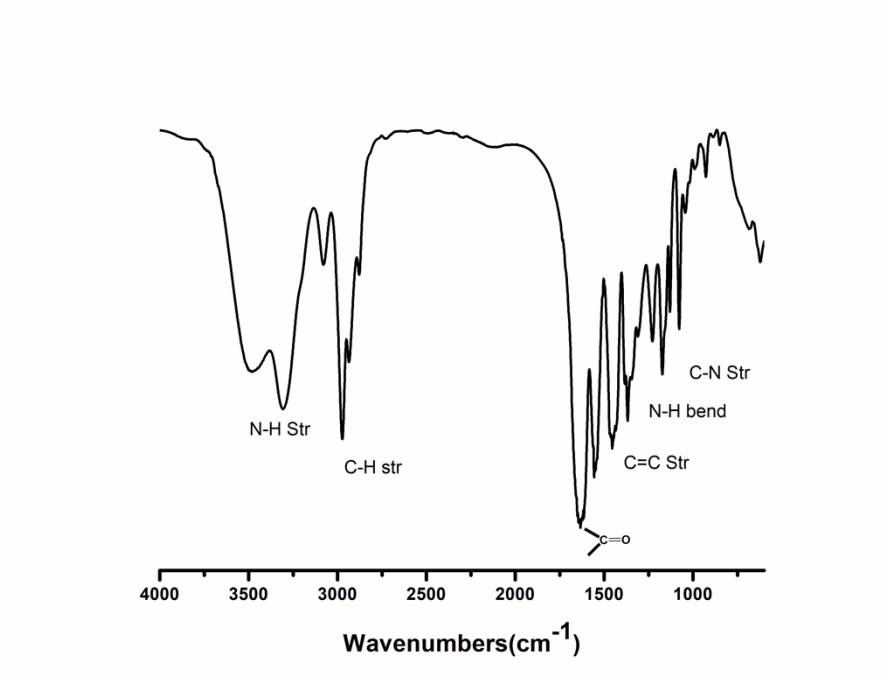

Figure S4(a). FT-IR spectrum of the KBr pellet of poly[N-isopropyl-N-(3-(isopropylamino)-3-oxopropyl)acrylamide] (polyM3i)(Run 1, Tables S1 and 1)

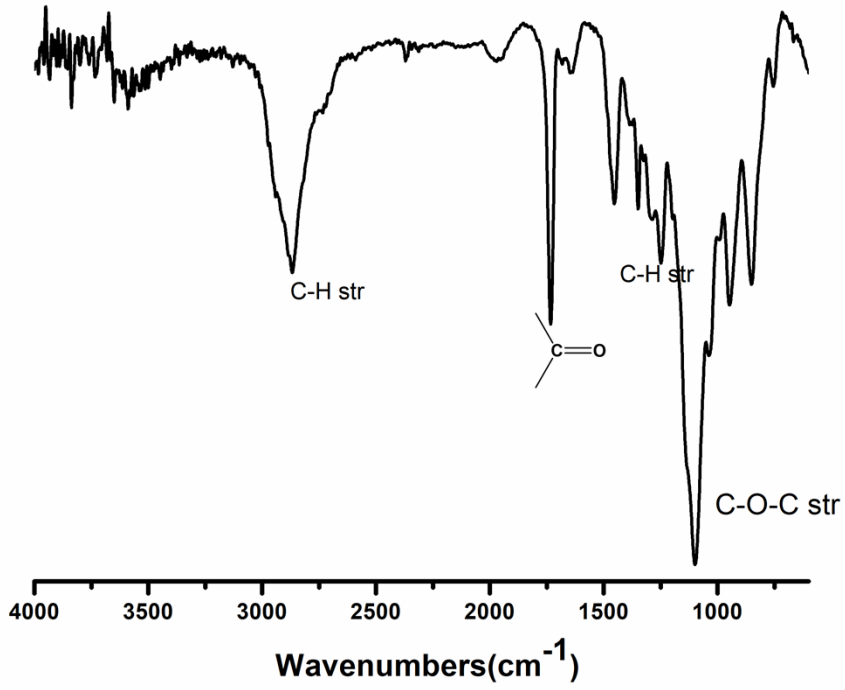

Figure S4(b). FT-IR spectrum of the KBr pellet of polyPEGA (Run 2, Tables S1 and 1)

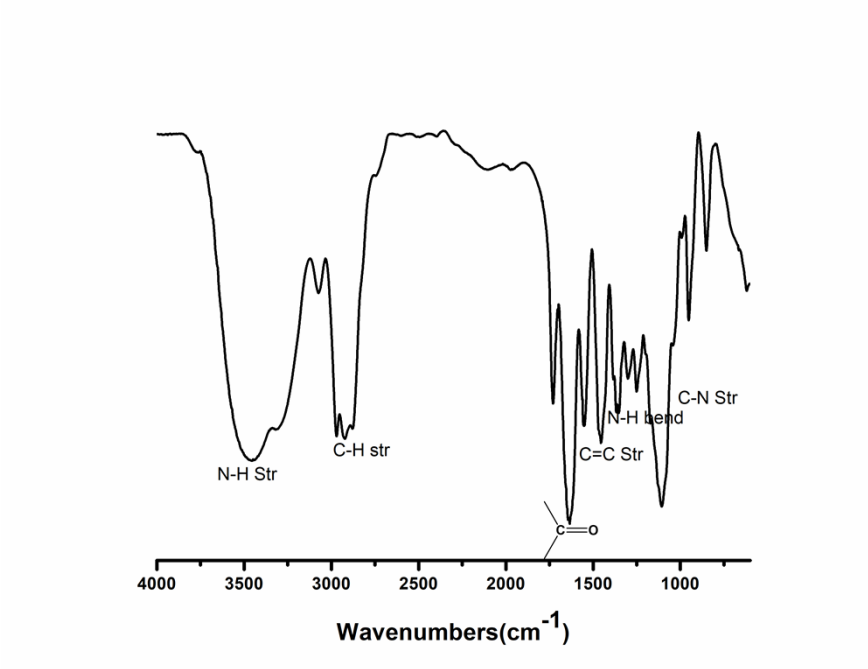

Figure S4(c). FT-IR spectrum of the KBr pellet of copolymer (Run 3, Table 1)

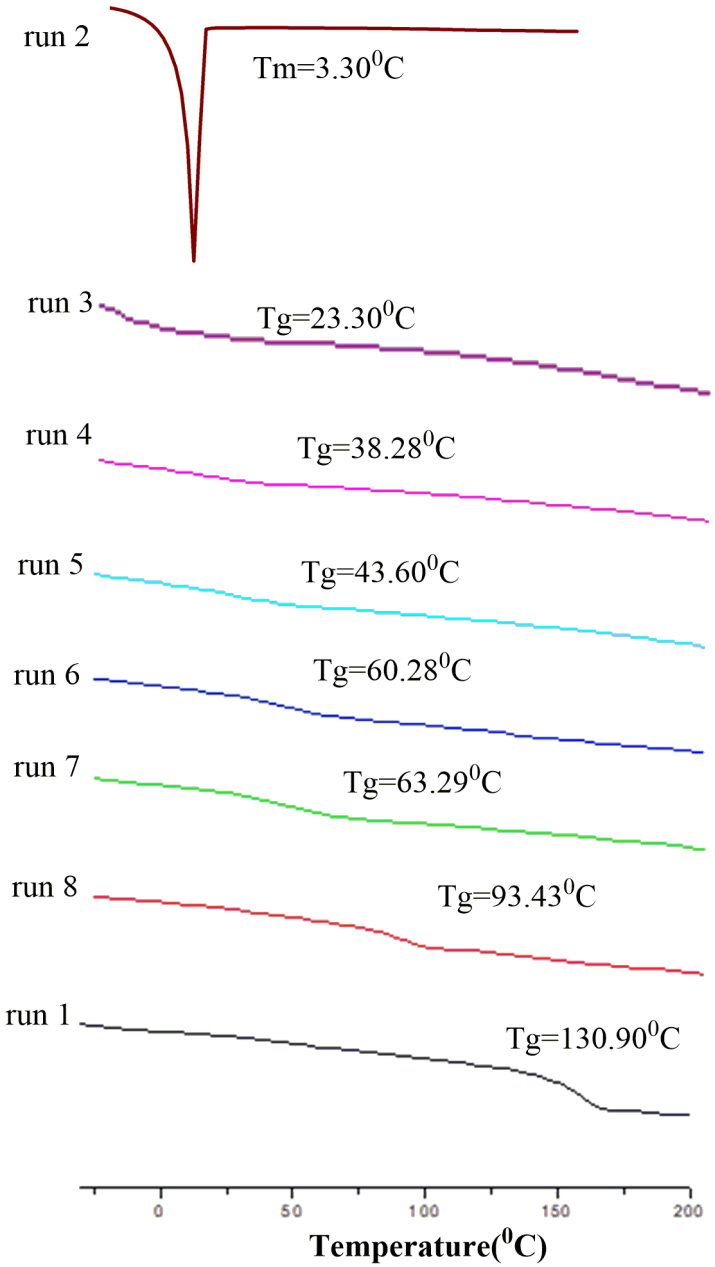

Figure S5. DSC thermograms showing the T_g of polyM3i homopolymer (run 1) and the copolymers having different compositions (runs 3 - 8) and the T_m of polyPEGA homopolymer (run 2) (Table 1**)**

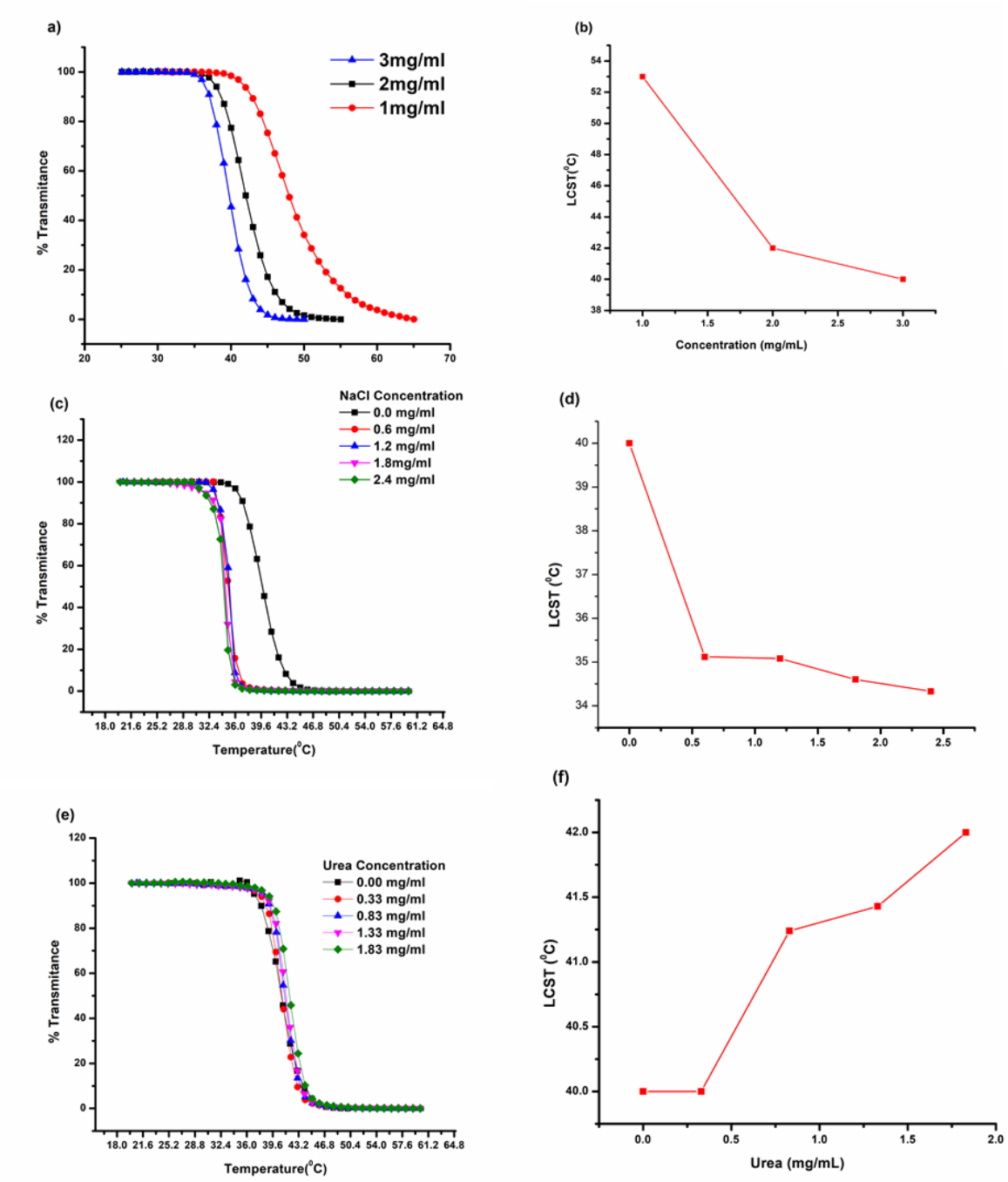

Figure S6. Temperature-dependent transmittance study of the aqueous solution of the copolymer **6** (run 6) **(a)**at different concentrations (1, 2 and 3 mg/mL), and the corresponding plot of the LCST *vs.* concentration of the copolymer **(b)**; at different concentrations of NaCl using 3mg/mL copolymer solution**(c)**and the corresponding plot of the LCST *vs.* concentration of NaCl**(d);**and at different concentrations of urea using 3mg/mL copolymer solution **(e)**and the corresponding plot of the LCST *vs.* concentration of urea **(f)**.

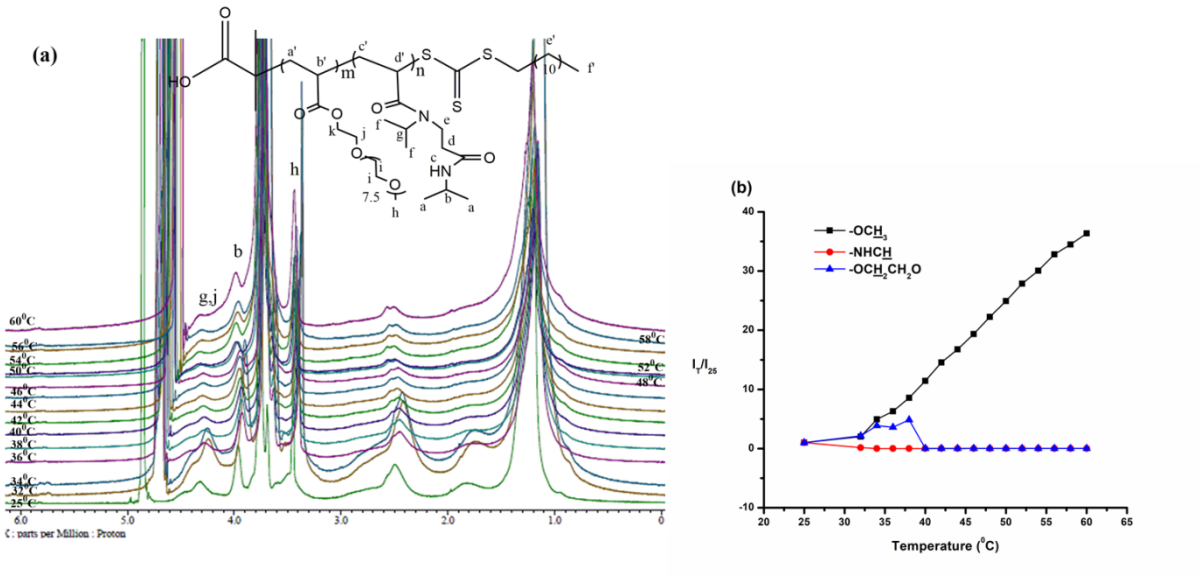

Figure S7. (a) Temperature-dependent 1H NMR spectra of copolymer **6** in D_2O (4.0 mg/mL recorded at 25−60 °C), and **(b)** the corresponding plot of the intensity ratio (I_t/I_{25}) (where I_t = peak intensity at t °C temperature while I_{25} is the same at 25°C) of peaks for the methoxy proton (h, ~3.32 ppm), amidic methine proton (b, ~3.91 ppm), and ethylene glycolic methylene proton (j, ~ 4.25 ppm) against temperature

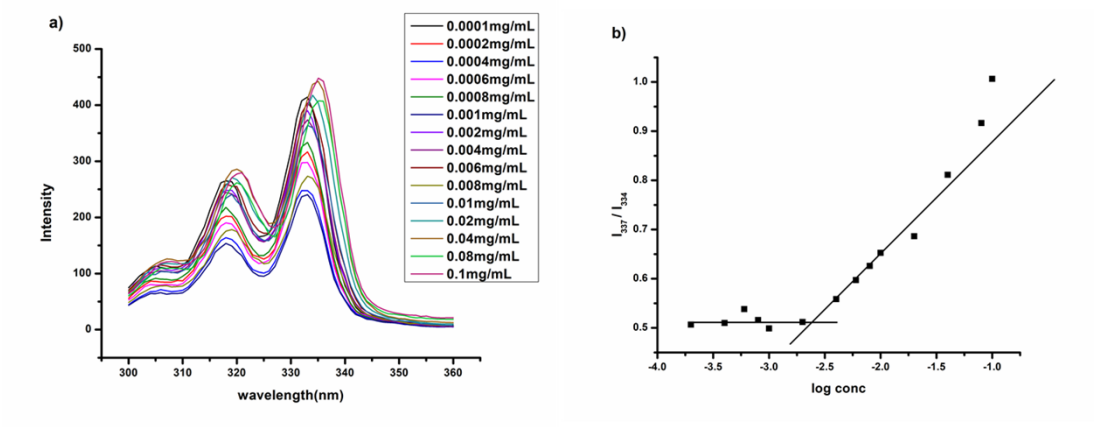

Figure S8. CMC measurement of polyM3i homo polymer (run 1): (a) excitation spectra (emission wavelength 394 nm) of polymer at different concentration with pyrene. (b) the corresponding I_{337}/I_{334} *vs* concentration plot.

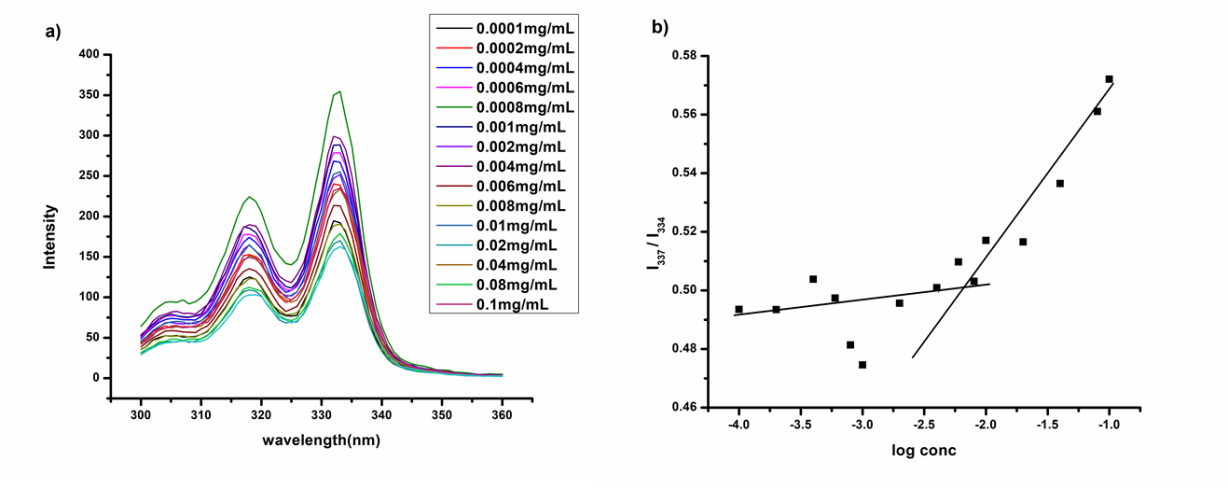

Figure S9. CMC measurement of random copolymer 6 (run 6): (a) excitation spectra (emission wavelength 394 nm) of polymer at different concentration with pyrene, and (b) the corresponding I_{337}/I_{334} *vs* concentration plot.

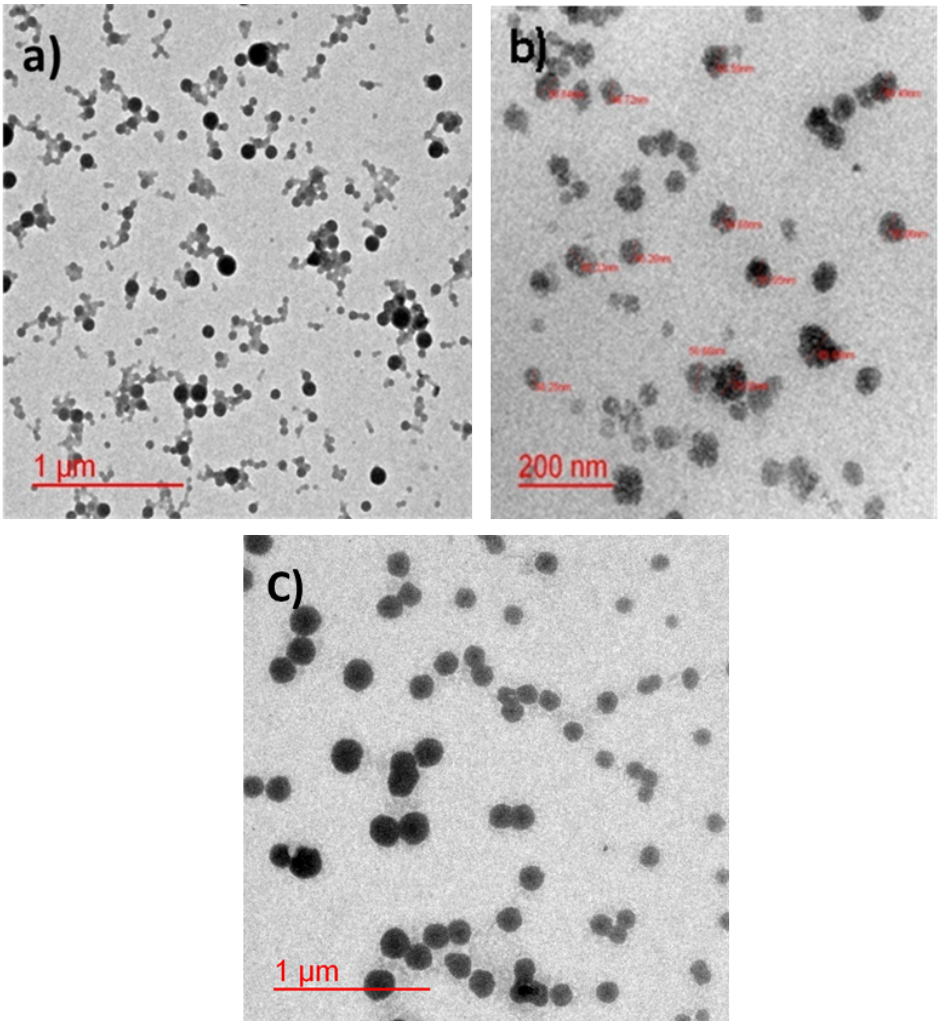

Figure S10. Transmission electron micrographs of the micelles of the **(a)** polyM3i (run 1, Table 1), **(b)** copolymer 6 (P2)(run 6, Table 1) and **(c)** copolymer 6′ (P1) (run 6′, Table 2).

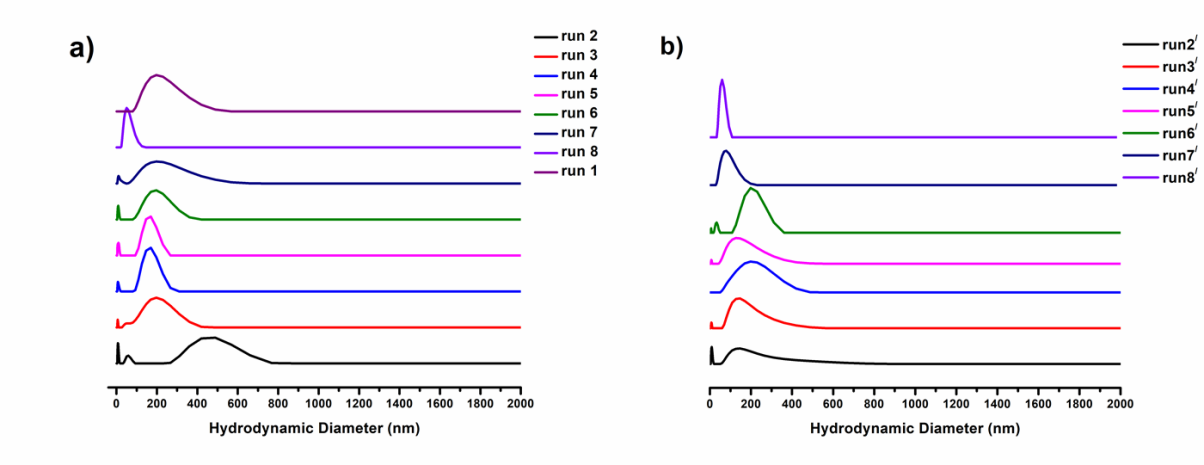

Figure S11. DLS plots:**(a)** polyPEGA (run 2) and its copolymers (runs 3-8) using 3.0 mg/mL solution and polyM3i (run 1) using 0.1 mg/mL at 25°C (Table 1); **(b)** polyPEGA (run 2/) and its copolymers (runs 3/ - 8/) using 0.1 mg/mL aqueous solution at 25°C (Table 2).

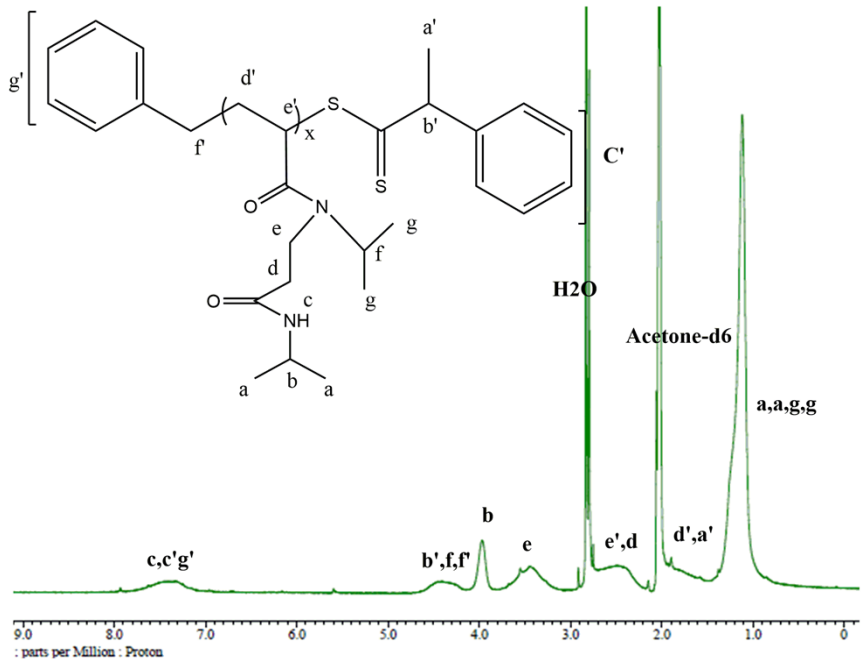

Figure S12. ^{1}H NMR (500 MHz, CD_3COCD_3) spectrum of poly[*N*-isopropyl-N-(3-(isopropylamino)-3-oxopropyl)acrylamide] (polyM3i)(Run 1',Tables S2 and 2)

^{1}H NMR (500 MHz, CD_3COCD_3) [Figure S12]: δ(ppm) 1.01-1.24 (12H,CH(CH_3)$_2$), 2.44 (2H,COCH_2), 3.64 (2H,CH_2N), 4.25, (1H,CH(CH_3)2),4.02 (1H, CH(CH_3)$_2$), 7.10-7.90 (1H, CONH)+(10H, C_6H_5) , 3.34 (3H, OCH_3), 3.54-3.84 (30H,$COCH_2CH_2OC$), 4.27 (2H, CH_2OCO), 4.35 (2H,PhCH'_2)+(PhCH')

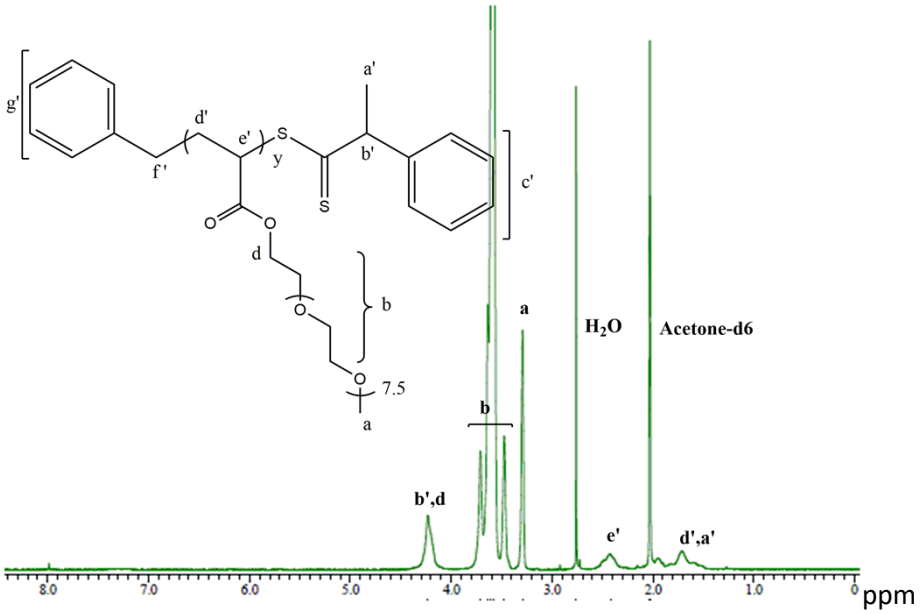

Figure S13. ^{1}H NMR (500 MHz, CD_3COCD_3) spectrum of the homopolymer of poly(ethylene glycol) methyl ether acrylate (polyPEGA) (Run 2', Tables S2 and 2)

^{1}H NMR (500 MHz, CD_3COCD_3) [Figure S13]: δ(ppm) 0.88 (3H,CH_3), 3.44 (3H, OCH_3), 3.54 (30H,$COCH_2CH_2OC$), 4.17 (2H, CH_2OCO).

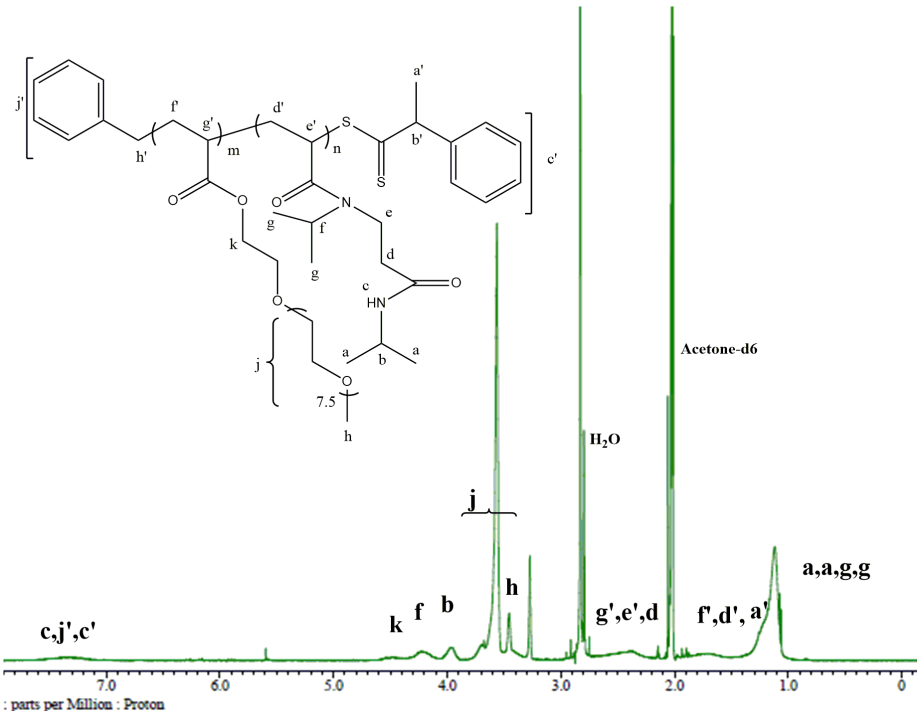

Figure S14. ^{1}H NMR (500 MHz, CD_3COCD_3) spectrum of copolymer (Run 3',Tables S2 and 2)

^{1}H NMR (500 MHz, CD_3COCD_3) [Figure S14]: δ(ppm) 1.01-1.24 (12H,CH(CH_3)$_2$), 2.44 (2H,COCH_2), 3.64 (2H,CH_2N), 4.25, (1H,CH(CH$_3$)2),4.02 (1H, CH(CH$_3$)$_2$), 7.10-7.90 (1H, CONH)+(10H, C_6H_5) , 3.34 (3H, OCH_3), 3.54-3.84 (30H,$COCH_2CH_2OC$), 4.27 (2H, CH_2OCO), 4.35 (2H,PhCH'$_2$)+(PhCH')

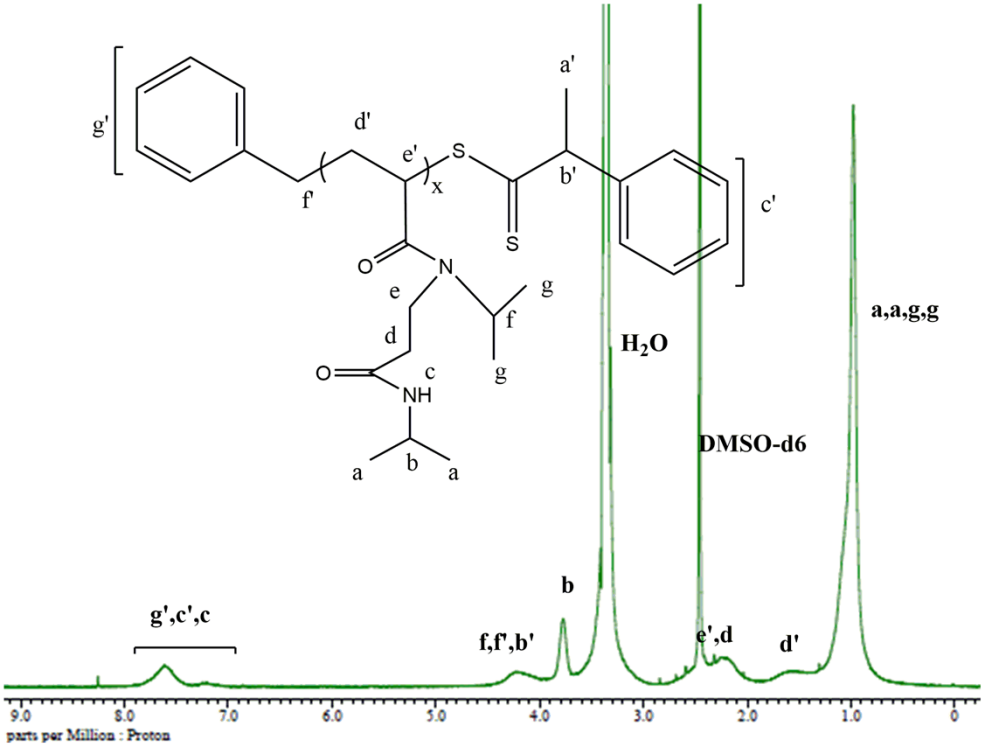

Figure S15. ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of poly[*N*-isopropyl-N-(3-(isopropylamino)-3-oxopropyl)acrylamide] (polyM3i)(Run 1', Tables S2 and 2)

^{1}H NMR (500 MHz, DMSO-d_6) (Figure S15): δ(ppm) 0.6-1.33 [12H,CH(CH_3)$_2$), 2.2 (2H,COCH_2), 3.6, (1H,CH(CH$_3$)2),3.9 (1H, CH(CH$_3$)$_2$), 7.10-7.90 (1H, CONH)+(10H, C_6H_5)

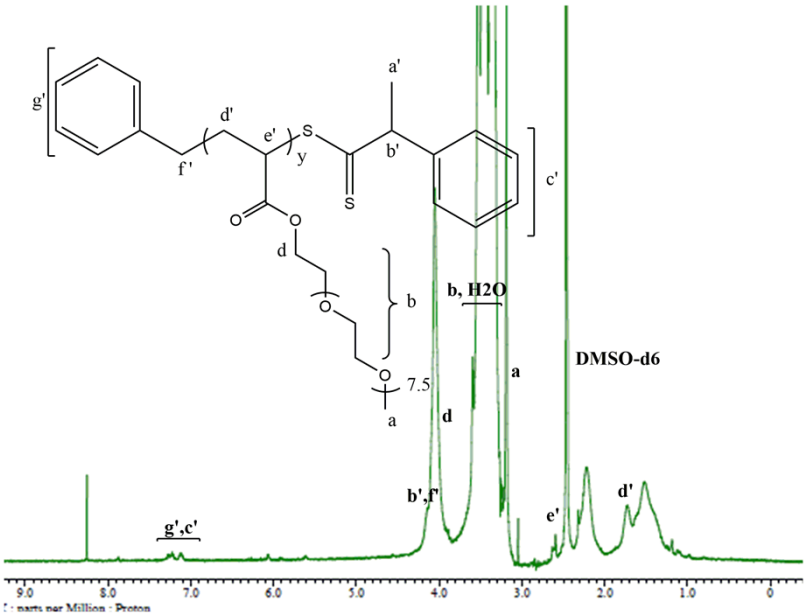

Figure S16. ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of the homopolymer of poly(ethylene glycol) methyl ether acrylate (polyPEGA) (Run 2', Tables S2 and 2)

^{1}H NMR (500 MHz, DMSO-d6) [Figure S16]: δ(ppm) 3.44 (3H, OCH_3), 3.54 (30H,$COCH_2CH_2OC$), 4.17 (2H, CH_2OCO). 4.35 (2H,$PhCH'_2$)+(PhCH')

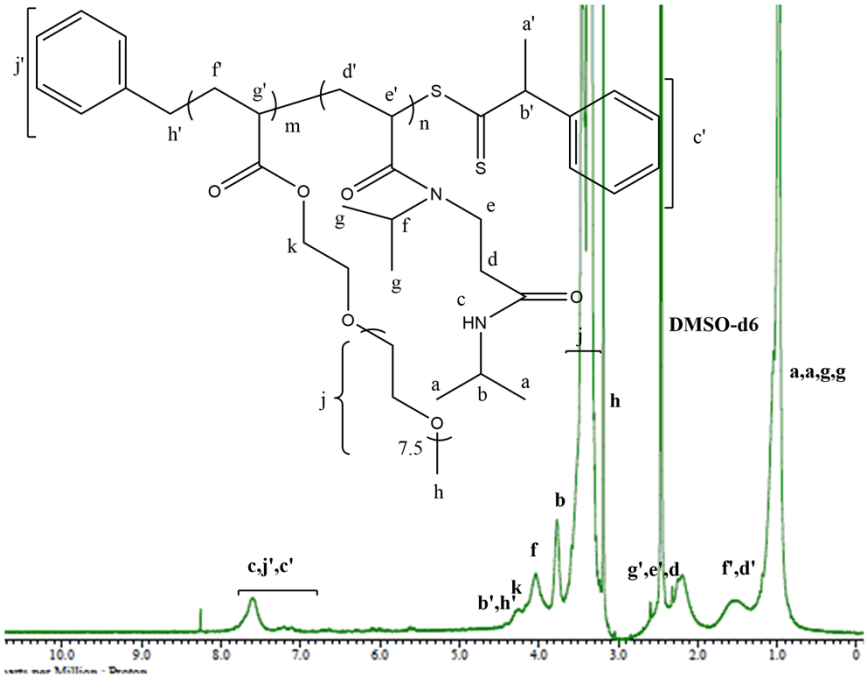

Figure S17. ^{1}H NMR (500 MHz, DMSO-d_6) spectrum of copolymer (Run 3', Tables S2 and 2)

^{1}H NMR (500 MHz, DMSO-d6) [Figure S17]: δ(ppm) 0.7-1.24 (12H,CH(CH_3)$_2$), 2.44 (2H,COCH_2), 3.6, (1H,CH(CH_3)2),3.9 (1H, CH(CH_3)$_2$), 7.10-7.90 (1H, CONH)+(10H, C_6H_5) , 3.34 (3H, OCH_3), 3.54-3.84 (30H,$COCH_2CH_2OC$), 4.27 (2H, CH_2OCO),4.35 (2H,PhCH'$_2$)+(PhCH')

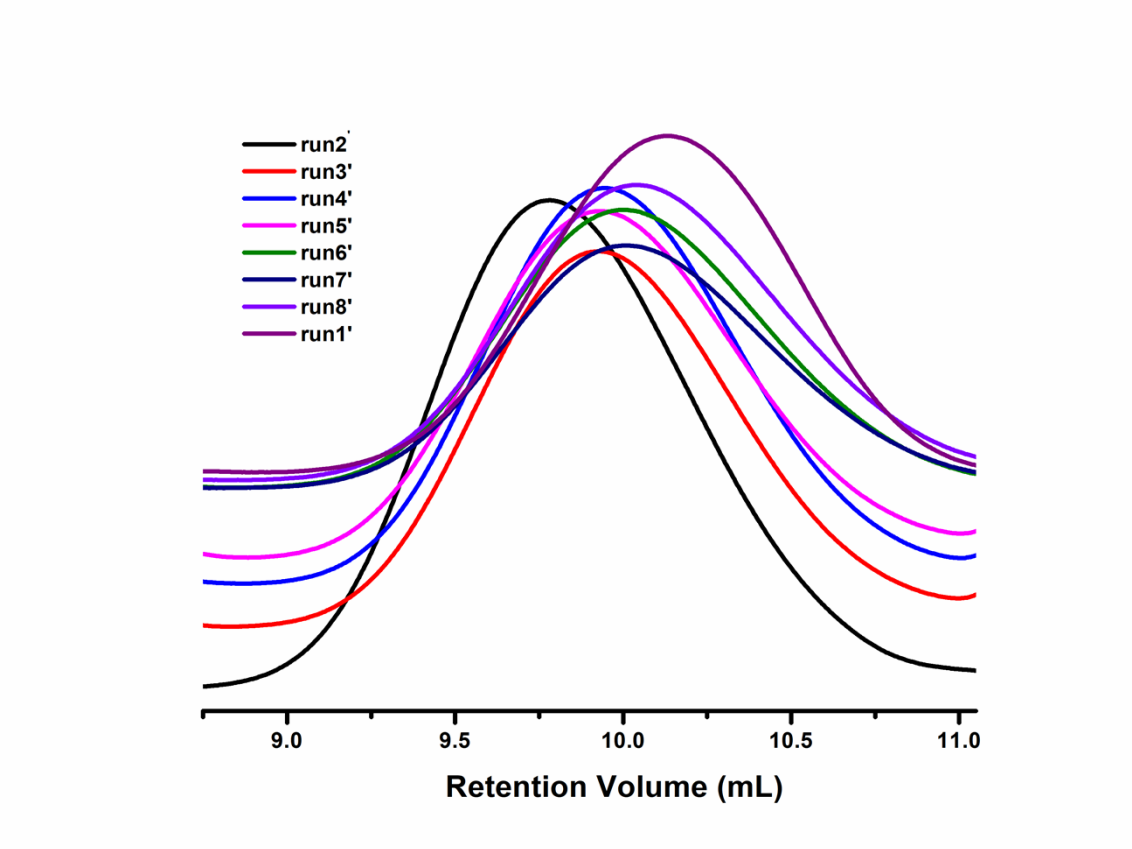

Figure S18. Typical GPC chromatograms of the polymers prepared using PEPD as RAFT agent (**Table 2**).

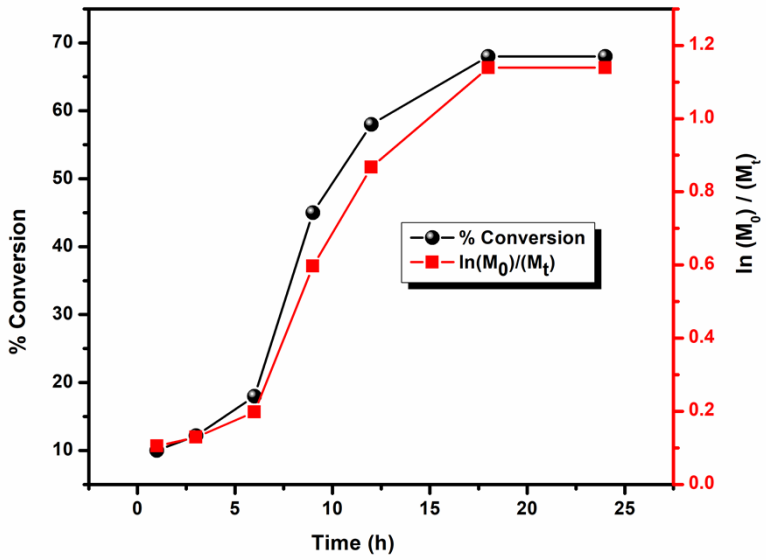

Figure S19(a). % conversion and ln([M]o / [M]t) vs. time plot

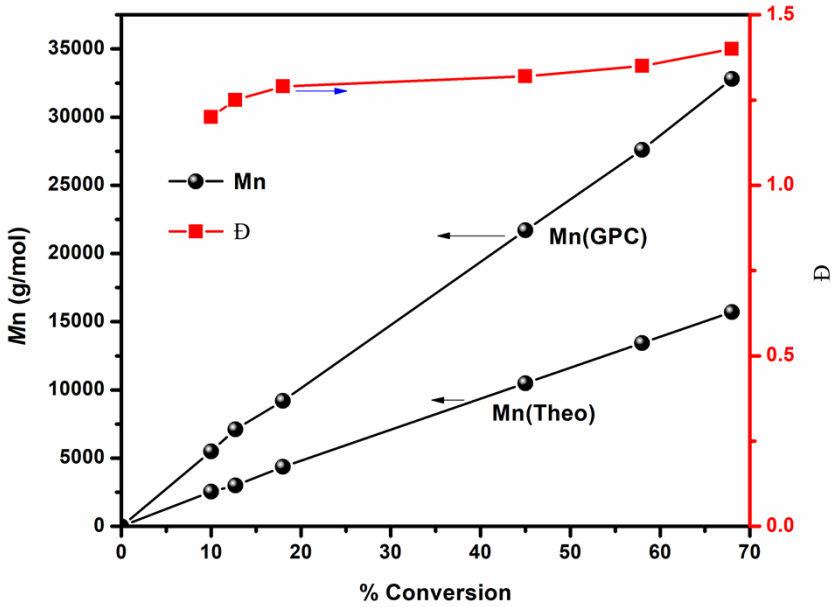

Figure S19(b). The corresponding plot of Mn(GPC), Mn(theo) and dispersity (*Đ*) vs. Monomer conversion (%).

Table S3. Homo- and Hetero-chain extension experiments using PEPED RAFT agent

Reaction ID	Monomer/	Conv. (NMR) (%) (Monomer)[c]	Copolymer Composition Ratio ([PEGA]: [M3i])[d]	Gravi-metric Yield (%)	M_n [e] (Theo) (g/mol)	M_n (NMR) (g/mol)	M_n (GPC) (g/mol)	*Đ*
Run A[a]	M3i	90	0:100	-	8444	3677	17500	1.2
Run B[b]	M3i	-	0:100	49	9238	-	28600	1.5
Run C[b]	PEGA	49	76:24	-	15437	37000	41500	1.99

[a] Using [Monomer]:[[M3i macroinitiator]: [AIBN] = 50:1:0.2 equivalent in 1 mL DMF at 70 ºC for 24 h

[b] Using [Monomer]:[[M3i macroinitiator]: [AIBN] = 50:1:0.2 equivalent in 1 mL DMF at 70 ºC for 24 h

[c] Determined by 1H NMR $CDCl_3$ for **polyM3i macro RAFT agent** (**Run A**) by comparing the integrated peak area of the residual vinylic signals at 5.61 ppm (1H) of the monomer with the peak area of the 12 methane protons of the isopropyl group at 1.2 ppm, For **polyM3i – *b* – polyPEGA (Run C)** by comparing the integrated peak area of the residual vinylic signals at 5.81 ppm (1H) of the monomer with methyl ether peak of pendent part polyethylene glycol (–OCH_3) at 3.32 ppm.

[d] Compositions of the copolymers have been calculated from the ^{1}H NMR in DMSO-d6. For **polyM3i – *b* –polyPEGA (Run C)** by comparing the peak area of the 3 methyl protons of the pendent part of polyethylene glycol (–O*C*H_3) at ~3.27 ppm with the peak area of the 12 methane protons of the isopropyl groups in the formed polymer at ~1.0 ppm.

[e]

$$\overline{M}_{\mathrm{n}}\,(\mathrm{theor}) = \left[\frac{[\mathrm{PEGA}]_{\mathrm{o}}}{[\mathrm{RAFT}]_{\mathrm{o}}} \cdot x_{\mathrm{PEGA}} \cdot M_{\mathrm{PEGA}}\right] + \left[\frac{[\mathrm{M3i}]_{\mathrm{o}}}{[\mathrm{RAFT}]_{\mathrm{o}}} \cdot x_{M3i} \cdot M_{M3i}\right] + M_{\mathrm{RAFT}}$$

where M_{PEGA} is the molar mass of the PEGA, M_{M3i} is the molar mass of the M3i, x_{M3i} is percentage conversion of M3i monomer from 1H NMR / gravimetrically, x_{PEGA} is the percentage conversion of PEGA monomer obtained from 1H NMR, $[M]_{RAFT}$ is the molar mass of the Raft reagent.

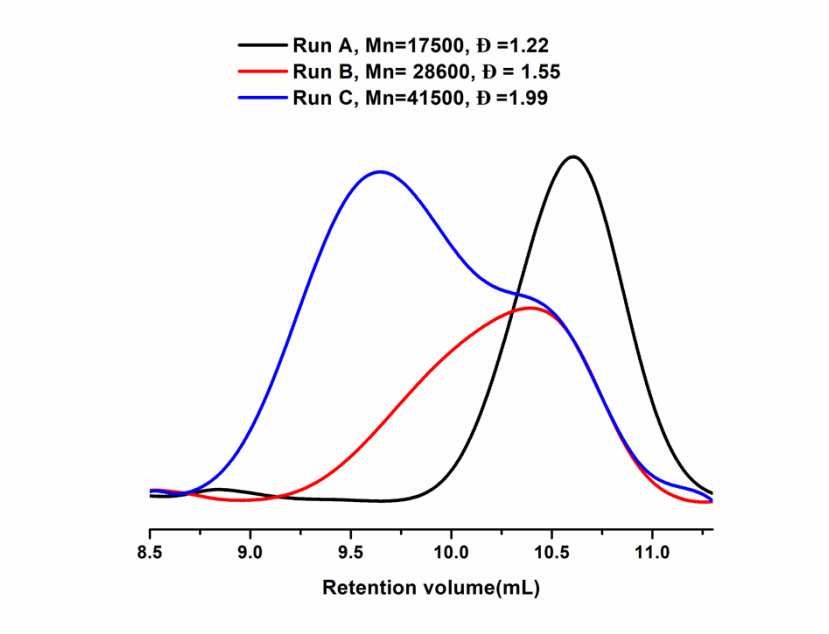

Figure S20. GPC Chromatograms of the PolyM3i macroinitiator and the obtained polymers in the homo and hetero chain extension experiments (**Table S3**)

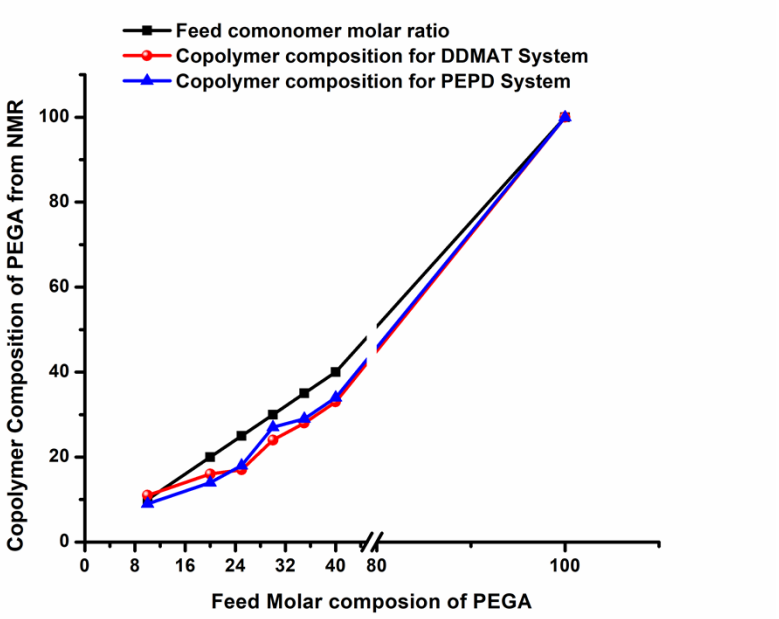

Figure S21. Plots of the feed composition of comonomers and the observed copolymer composition as determined by 1H NMR (**Tables 1** and **2**)

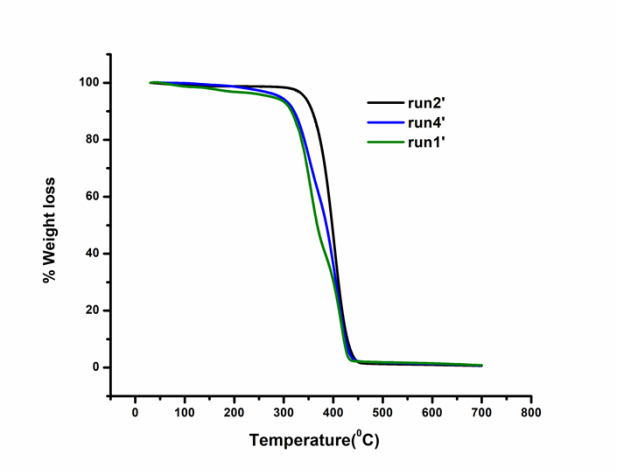

Figure S22(a). Typical TGA thermograms of polyM3i, polyPEGA and random copolypolymer poly(M3i-*ra*-PEGA) prepared using PEPD (runs 1/, 2/ and 4/, respectively, Table 2)

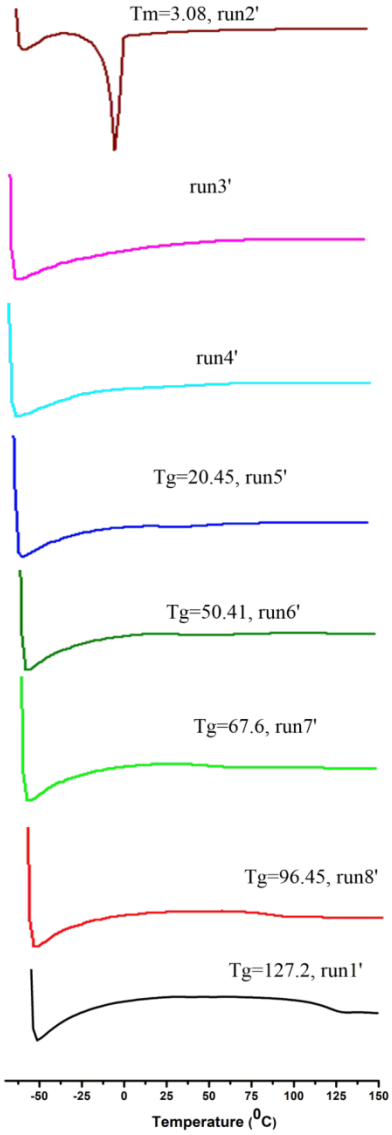

Figure S22(b). DSC thermograms of polymers prepared using PEPD (runs 1/-8/, Table 2)

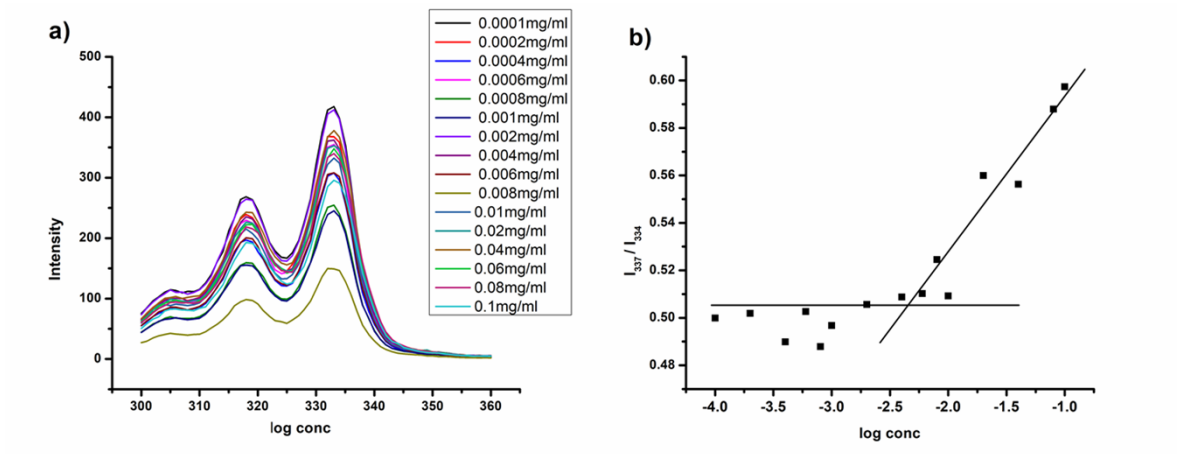

Figure S23.CMC measurement of random copolymer **6′** (run 6′, Table 2): **(a)** excitation spectra (emission wavelength 394 nm) of polymer at different concentration with pyrene, and **(b)** the corresponding I_{337}/I_{334} *vs* concentration plot.

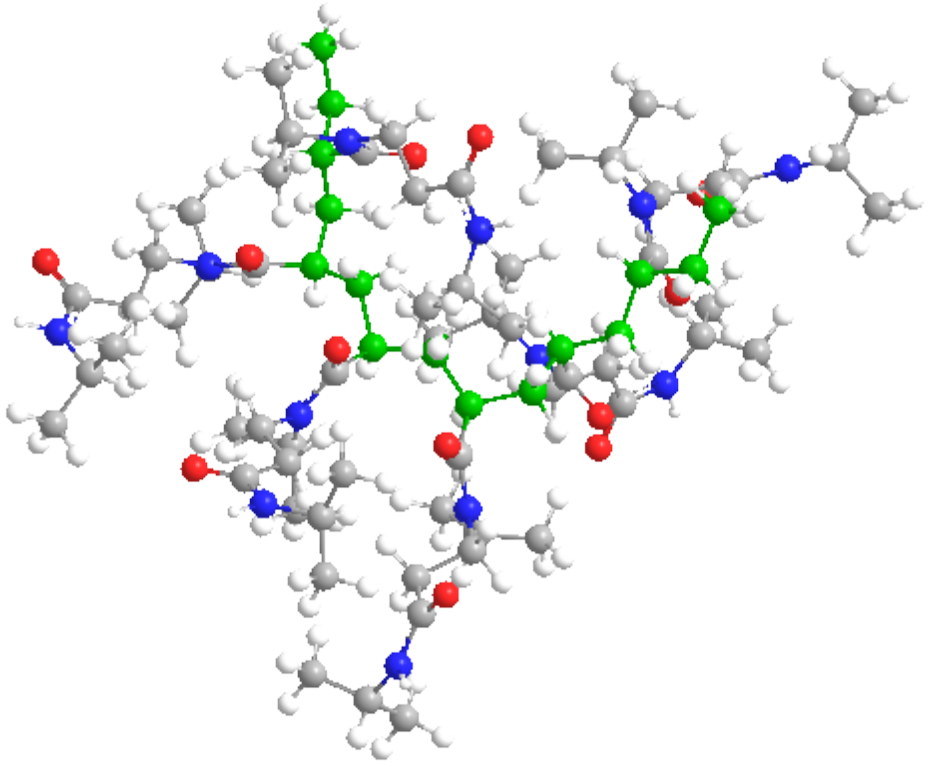

Figure S24. Optimized structure of polyM3i (considering degree of polymerization, n = 6 and with methyl end group) computed at B3LYP/6-31G** level of theory using Gaussian 09 suits of program.

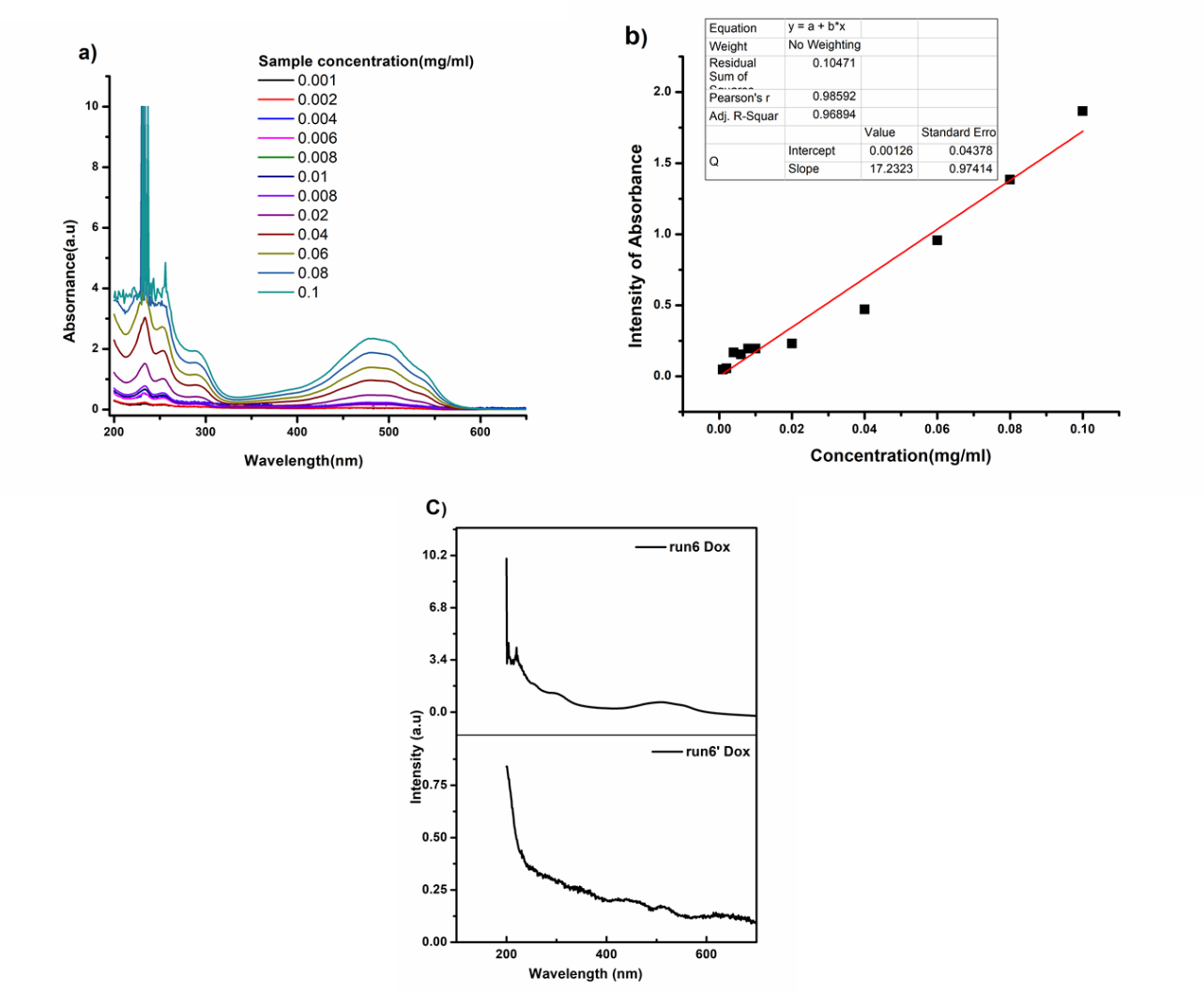

Figure S25. (a) UV-Vis absorbance spectra of different concentrations (mg/mL) of DOX in water; **(b)** Calibration curve for DOX obtained by plotting the absorbance at 485 nm against the concentration of known aqueous DOX solutions; and (c) UV-Vis absorbance spectra of DOX-loaded copolymers 6 (run 6, Table 1) and 6/ (run 6/, Table 2) in water.

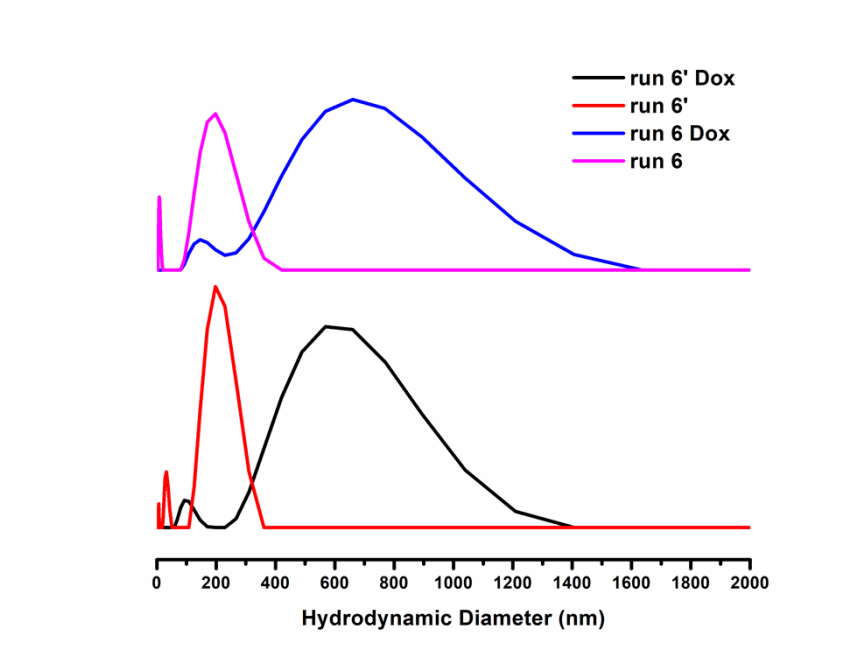

Figure S26. Increase in the hydrodynamic diameter of the micelles of copolymer 6 (run 6) and copolymer 6/ (run 6/) upon DOX drug loading.

Linear fitting of the drug release profiles with different kinetic models

The mathematical forms of the different kinetic models studied to fit the *in vitro* drug release profiles are:

(1) The zero-order model explains the process of constant drug release from a drug delivery device and is expressed generally as

$$M_0 - M_t = k_0 t$$

where M_t is the amount of drug dissolved in time t, M_0 is the initial amount of drug in the solution (most times, M_0 = 0) and k_0 is the zero order release constant expressed in units of concentration/time. Here, the plot of M_t *vs.* t will be a linear one.

(2) The first-order model describes that the release kinetics depend on the amount of the drug present initially and generally expressed as

$$\log\ (M_t/M_0) = \ -k_1 t$$

where, M_t is the drug released at time t, M_o is the amount of drug loaded in the matrix, and k_1 is the first-order rate constant. Here, the plot of (M_t/M_o) *vs.* t will be a linear one.

(3) Higuchi model can be simply expressed as

$$f_t = \ Q \ = \ k_H \sqrt{t}$$

where Q amount of drug is released in time t, and k_H is the Higuchi release constant. Here, the plot of Q *vs.* √t will be a linear one.

(4) Korsmeyer-Peppas model considers the fitting of first 60% drug release data and can be represented as

$$M_t/M_\infty = \ k_{KP} t^n$$

where M_t is the drug released at time t and M_∞ is the amount drug released at the equilibrium, k_{KP} is the release rate constant (related to the structural modifications and geometrical characteristics of the system as well as the release velocity) and n is the release exponent (related to the drug release mechanism). Here, the plot of log(M_t / M_∞) vs. log t will be a linear one.

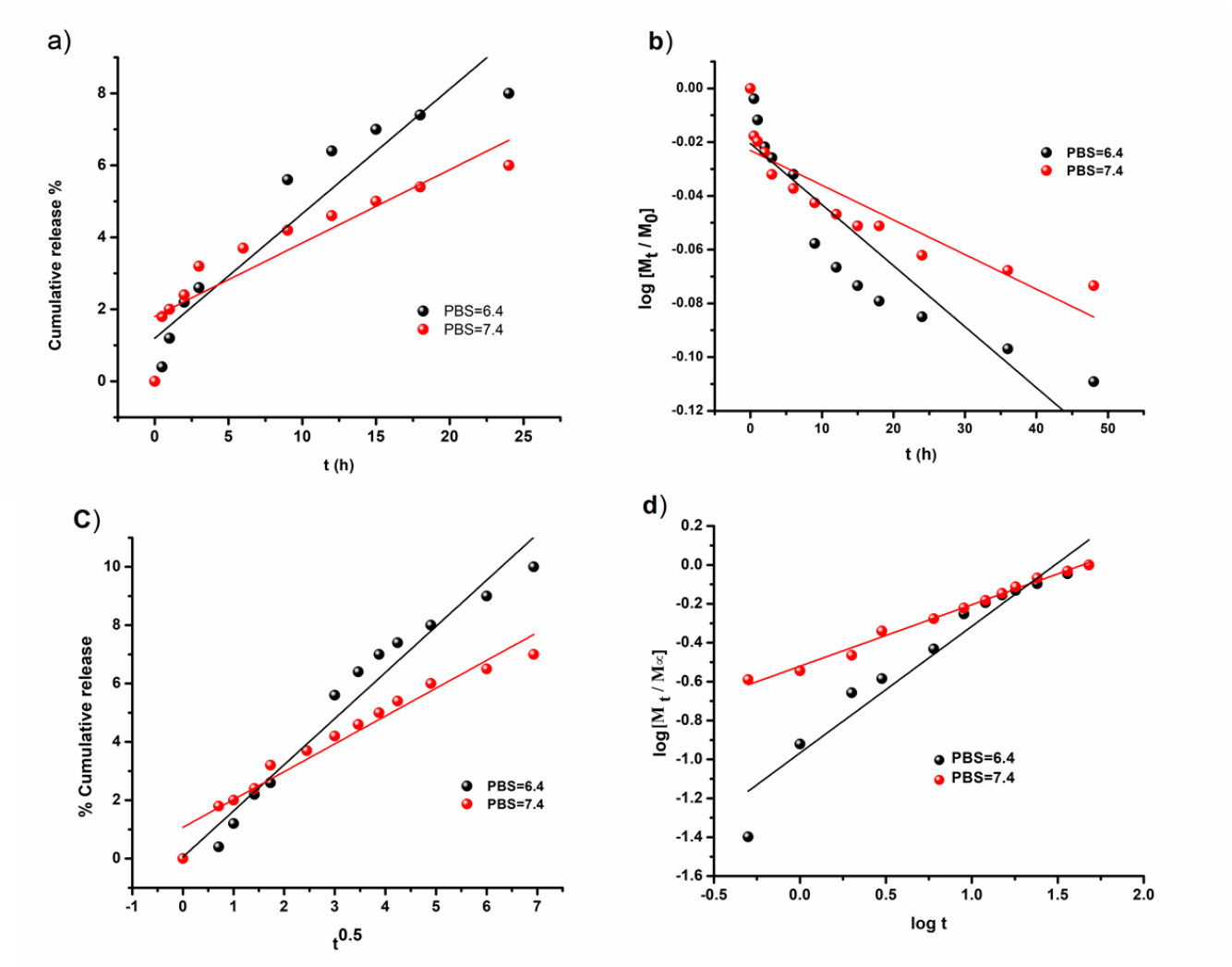

Figure 27. Linear fitting plots for the DOX release profiles of P2-DOX in the PBS of 6.4 and 7.4 pHs using the drug diffusion kinetic models: (a) zero order, (b) first order, (c) Higuchi, and (d) Korsmeyer-Peppas.

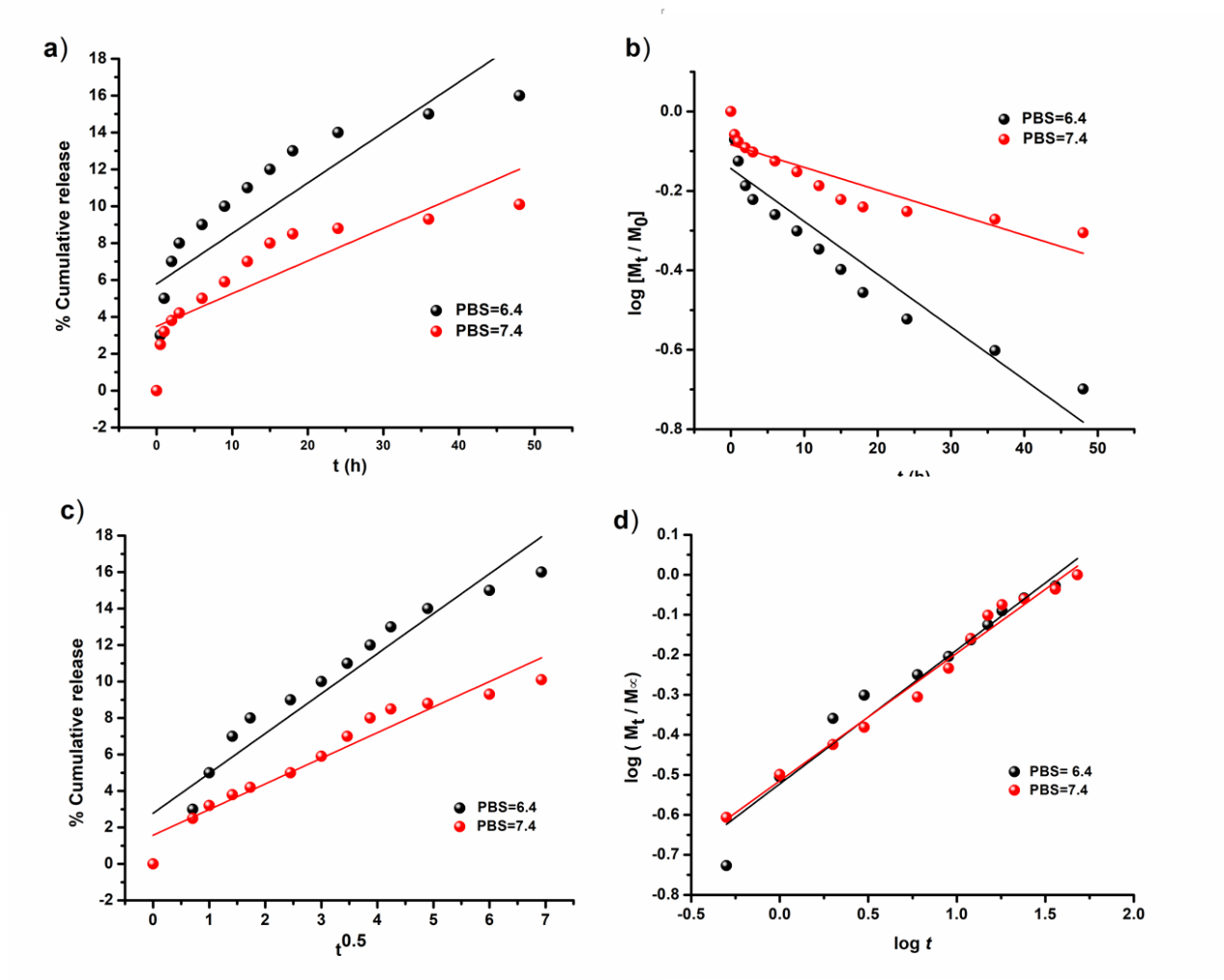

Figure 28. Linear fitting plots for the DOX release profiles of P1-DOX in the PBS of 6.4 and 7.4 pHs using the drug diffusion kinetic models: (a) zero order, (b) first order, (c) Higuchi, and (d) Korsmeyer-Peppas.

Table S4. Rate constants, linear correlation coefficients (r^2) and diffusional exponent (n) obtained by fitting different models for the drug release profiles of DOX-loaded micelles - P1-DOX and P2-DOX.

Sample	Zero-order		First-order		Higuchi		Korsmeyer-Peppas	
	k_0	r^2	k_1	r^2	k_H	r^2	r^2	n
P1-DOX (PBS=6.4)	0.27±0.05	0.69	0.14±0.02	0.88	2.7±0.68	0.91	0.95	0.34±0.02
P1-DOX (PBS=7.4)	0.17±0.03	0.72	-.08±0.01	0.80	1.5±0.39	0.93	0.98	0.32±0.01
P2-DOX (PBS=6.4)	0.34±0.03	0.89	0.02±0.005	0.81	0.05±0.32	0.96	0.93	0.65±0.05
P2-DOX (PBS=7.4)	0.20±0.01	0.81	0.02±0.003	0.78	1.07±0.23	0.94	0.98	0.31±0.009

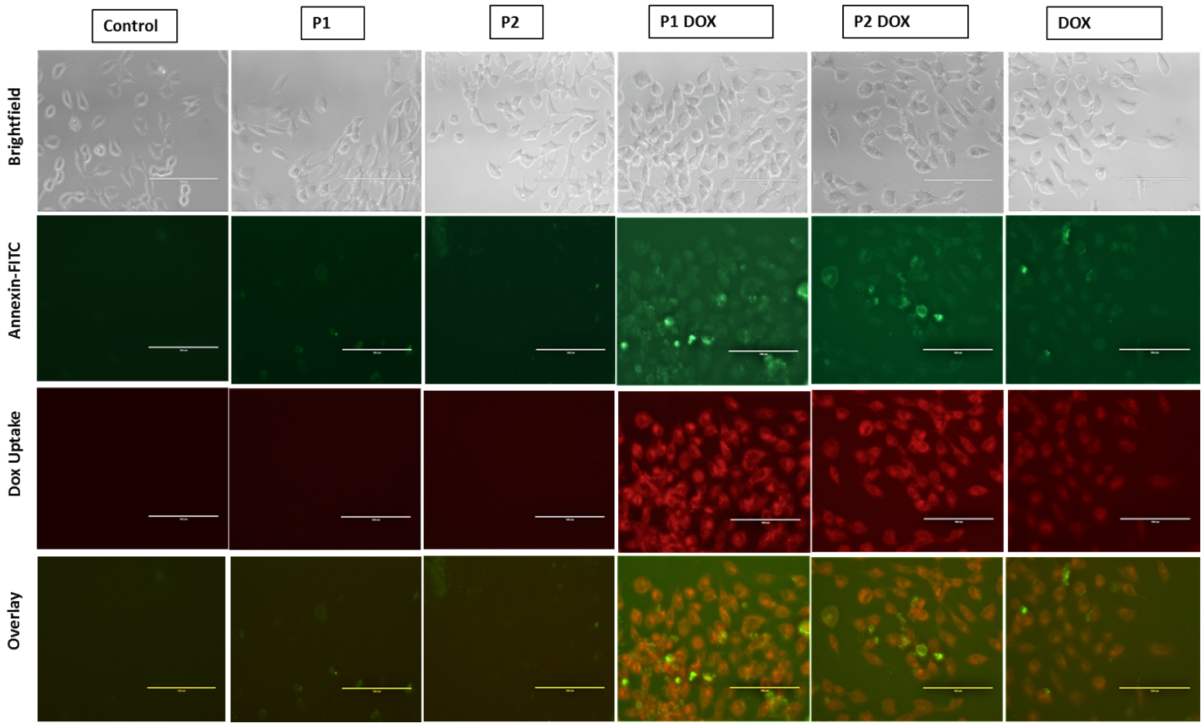

Figure S29. Apoptosis and temporal uptake of DOX in DL cells following treatment with free DOX or DOX loaded copolymeric micelles.

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