Research article

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Biodegradable brush copolymer nanomicelles for smart release of doxorubicin

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Abstract

Background and objective: In cancer therapy, smart and biocompatible nanocarriers are the most important features of therapeutic agents. pH-sensitive drug delivery nanocarriers which can be remotely prompted are attractive for patients management and therapeutic purposes. In this paper, a novel nanocarrier was fabricated and investigated for controlled release of Doxorubicin (DOX).

Materials and methods: Self-assembled nanomicelles containing a hydrophilic core and a hydrophobic shell were successfully prepared using poly(2-hydroxyethyl methacrylate-graft-ε-caprolactone)-block-poly (methacrylic acid) [P(HEMA-g-CL)-*b*-(PMAAc)] brush copolymer by combining reversible addition-fragmentation chain transfer polymerization (RAFT) and ring open polymerization (ROP). Morphology, micelles properties, and pH-sensitive behavior were studied by field emission scanning electron microscopy (FESEM), transmission electron microscope (TEM) and distribution laser-scattering (DLS) analysis.

Results and conclusion: Molecular weight of P(HEMA-g-CL) and [P(HEMA-g-CL)-*b*-PMAAc] samples was obtained as 15117 g mol^{-1} and 25887 g mol^{-1}, respectively. The polydispersity index (PDI) of P(HEMA-g-CL) (PDI = 1.14) and [P(HEMA-g-CL)-*b*-PMAAc] (PDI = 1.19) synthesized by RAFT polymerization were relatively low, suggesting good control of the technique over the process. The self-assembled micelles were pH-sensitive and showed low critical concentration in water. TEM showed that the micelles had nanosized spherical shape with average size of 35 nm. The critical micelle concentration (CMC) value of [P(HEMA-g-CL)-*b*-PMAAc] micelle was 0.025 g l^{-1}. Encapsulation efficacy of the nanomicelle was 94.3%. Release behavior of DOX from the nanomicelles revealed that rate of core release could be efficiently controlled by body temperature and pH. In this regard, the release rate at pH of 7.4 and 5.4 was 54.73 and 36.52%, respectively. As a conclusion, structure of the nanocarrier and its controllable characteristics introduced it as appropriate vehicle in drug delivery.

Keywords: Cancer therapy, Doxorubicin, Nanomicelle, pH-sensitive

1. Introduction

In the past decades, progress in nanotechnology has been significant because of its potential in disease prevention, treatment, and diagnosis [1]. Drug delivery systems have been developed

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progressively due to their potential in delivery of therapeutic drugs to target tissues with low toxicity and high efficiency [2-5]. Anticancer drugs pose adverse impacts on healthy tissues in the way of target organs via blood circulation. Targeted drug delivery systems have been developed to administer the cytotoxic drugs specifically in target cancer cells [6-7].

Nano-sized self-assembled polymeric micelles prepared from amphiphilic copolymers are popular because of their applications in gene and drug delivery [8-10]. They have several advantages including water solubility improvement of drugs, decreasing drugs' side effects, prolonging the circulation time of drugs, improving drugs' bioavailability, and passive targeting of drugs in tumor tissues via enhanced permeability and retention (EPR) effect [11-13].

Amphiphilic graft copolymers have been developed from hydrophobic biodegradable polymers grafted to synthetic or natural polymers [14]. Amphiphilic copolymers such as poly (2-hydroxyethylmethacrylate) grafted to aliphatic polyesters such as poly(ε-caprolactone) (PCL), poly(lactide) (PLA), and poly(lactide-co-glycolide) (PLGA) are of the most studied chemicals due to their excellent biocompatibility and *in vivo* biodegradability [15-17].

There are few reports about hydrophobic polymers grafted to hydrophilic polymers because of the difficulties in the synthesis process. Grafted copolymer micelles have several advantages such as enhanced stability, optimized length and density of the graft affecting drug loading, and optimal tumor targeting owing to the high density of targeting ligands prepared by hydrophilic grafting per macromolecule [18-20].

Composition, size of particles and morphology of carriers can be optimized through the synthesis process. Various copolymers are available for controlled/living radical polymerization techniques [21,22]. These include nitroxide-mediated polymerization (NMP) [23], atom transfer radical polymerization (ATRP) [24], reversible addition fragmentation chain transfer (RAFT) [25,26], and ring open polymerization (ROP) [27]. RAFT polymerization is a powerful method for synthesis of amphiphilic block copolymers with predefined composition, well-defined structure, and narrow dispersity [28]. Synthesis by a wide range of various monomers such as 2-hydroxyethylmethacrylate (HEMA) [29], acrylic acid (AA) [30], and N- isopropylacrylamide (NIPAm) [31], which can be inserted into the chain transfer agent (CTA), are feasible by this method.

In the present study, we used a simple way to prepare polymeric nanomicelles for targeted cancer therapy. Synthesis, characterization, and self-assembly behavior of a novel pH-sensitive copolymer of poly(2-hydroxyethyl methacrylate-graft-ε-caprolactone-block-poly (methacrylic acid) [P(HEMA-g-CL)-*b*-(PMAAc)] was investigated. At first, the copolymer of poly(2-hydroxyethyl methacrylate-graft-ε-caprolactone [P(HEMA-g-CL)] were synthesized via ROP and RAFT approaches. Then, MAAc was successfully synthesized using RAFT polymerization. Doxorubicin (DOX) was inserted into the nanomicelles by ionic interaction and hydrogen bonding.

2. Materials and methods

2.1. Materials

RAFT agent (4-cyano-4-[(phenylcarbothioyl) sulfanyl] pentanoic acid) was synthesized in our laboratory [25]. Chemicals of HEMA, methacrylic acid, $Sn(Oct)_2$, and ε-caprolactone (ε-CL) were purchased from Merck (Germany) and azobisisobutyronitrile (AIBN) from Fluka (Switzerland). DOX was prepared from Zhejiang (China). Dimethyl sulfoxide (DMSO) and the other reagents were purchased from Merck (Germany).

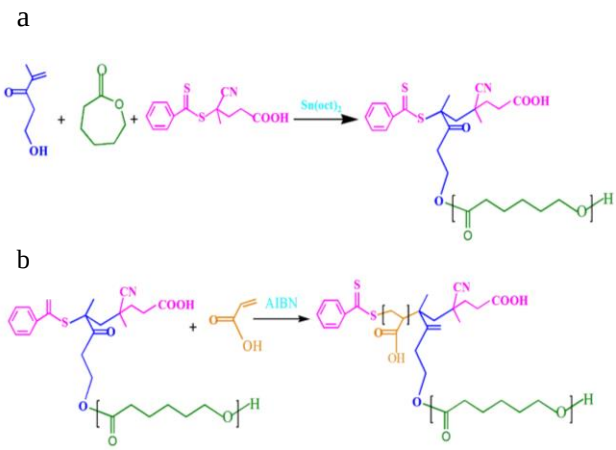
2.2. Synthesis of poly(2-hydroxyethyl methacrylate-graft-ε-caprolactone) P(HEMA-g-CL) copolymers

Reaction was done by glove-box techniques under nitrogen atmosphere. For this purpose, the

RAFT agent (20 mg, 0.07 mmol) and AIBN (3.0 mg, 0.01 mmol) were charged in flask and stirred for 1 h. Then, ε-CL (4.40 g, 38.55 mmol), HEMA (0.5 g, 3.84 mmol), $Sn(Oct)_2$ (0.4 g, 1.97 mmol), and toluene (3 ml) were added to the flask and heated to 110 °C for 3 h. The reaction was stopped by cooling in ice. The P(HEMA-g-CL) copolymer was precipitated in ether. Finally, the product was dried under vacuum for 24 h at room temperature (Scheme 1a).

2.3. Synthesis of poly(2-hydroxyethyl methacrylate-graft-ε-caprolactone)-block-metaacrylic acid copolymer [P(HEMA-g-CL)-*b*-PMAAc]

Block copolymerization was done by using P(HEMA-g-CL) as macro-RAFT agent and MAAc monomer. A flask was charged with P(HEMA-g-CL) (1 g, 0.06 mmol), MAAc monomer (1 g, 11.6 mmol), AIBN (3 mg, 0.01 mmol), and dimethylformamid (10 ml). The mixture was degassed and moved to oil bath at 85 °C for 48 h. Then, the [P(HEMA-g-CL)-*b*-MAAc)] copolymer was precipitated in diethyl ether. Finally, the product was dried under vacuum for 24 h at room temperature (Scheme 1b).



Scheme 1- Synthesis of a) poly(2-hydroxyethyl methacrylate-graft-ε-caprolactone) P(HEMA-g-CL); b) poly(2-hydroxyethylmethacrylate-graft-ε-caprolactone)-block-poly(methacrylic acid) [P(HEMA-g-CL)-*b*-PMAAc]

2.4. Preparation of DOX-[P(HEMA-g-CL)-*b*-PMAAc] nanomicelles

100 mg [P(HEMA-g-CL)-*b*-PMAAc] in 2 ml deionized water and DOX (10 mg) were mixed in a 25-ml vial and stored at 25 ºC for 48 h in darkness. Finally, DOX-[P(HEMA-g-CL)-*b*-PMAAc] was poured into a dialysis bag. The dialysis bag was directly immersed in 500 ml of distilled water. After 48 h, water was refreshed to remove DMSO solvent.

2.5. Characterization of [P(HEMA-g-CL)-*b*-PMAAc] and DOX-[P(HEMA-g-CL)-*b*-PMAAc] nanomicelles (Scheme 2)

Size exclusion analyses was carried out using a Waters 1515 (USA) gel permeation chromatography (GPC) equipped with Breeze 1515 isocratic pump and 7725 manual injector.

Fourier transform infrared (FT-IR) spectra of the samples were obtained by Shimadzu apparatus (Model 8101M, Japan) within the range of 4000 to 400 cm^{-1} wavenumbers.

Proton nuclear magnetic resonance (^{1}HNMR) spectra were obtained at 25 °C using an FT-NMR (400 mHz) Bruker spectrometer (Germany). The samples were prepared in deuterated DMSO.

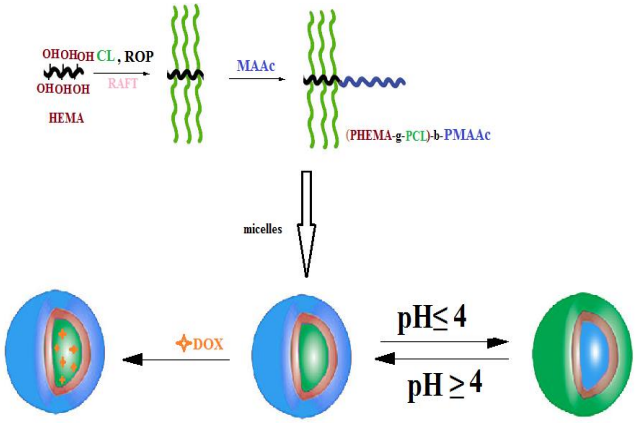
Determination of CMC was done by preparation of pyrene stock solution (6×10^{-7} mol l^{-1}) in acetone that was stored at 5 °C for further use. To measure steady-state fluorescence spectra, the pyrene stock solution was added to deionized water to give pyrene concentration of 12×10^{-7} mol l^{-1}. Then, acetone-free pyrene solution was added followed by solutions of polymeric micelles at concentration of 0.008, 0.01, 0.04, 0.08, and 0.1 g l^{-1} at pH 3-4. Pyrene fluorescence intensity ratios (I337/I333) were plotted against logarithm of the synthesized di-block copolymer concentrations (Log C).

Particle size of the nanocomposite was measured by laser-scattering technique (Zetasizer Nano ZS90, Malvern, UK) at 25 °C. Field emission scanning electron microscope (FESEM) (Model

1430 VP, UK) was applied to determine morphology of the nanomicelles.

Encapsulation efficiency of DOX-[P(HEMA-g-CL)-*b*-PMAAc] nanoparticles was calculated by the following equation.

$$EE(\%) = \frac{\text{Mass of drug in nanomicelles}}{\text{Mass of initial added DTX}} \times 100$$



Scheme 2- Structure of pH-sensitive [P(HEMA-g-CL)-*b*-PMAAc] and DOX-loaded [P(HEMA-g-CL)-*b*-PMAAc] nanomicelles

3. Results and discussion

3.1. Characterization of [P(HEMA-g-CL)-*b*-PMAAc] copolymer

FT-IR spectra of P(HEMA-g-CL) and [P(HEMA-g-CL)-*b*-PMAAc] copolymers are shown in Figure 1. Graph of P(HEMA-g-CL) showed the transmittance bands of stretching C–O–C at 1253 cm^{-1}, stretching C–O at 1303 cm^{-1}, stretching carbonyl at 1722 cm^{-1}, stretching aliphatic C–H at 2935 cm^{-1}, and stretching O–H at 3475 cm^{-1}. Graph of [P(HEMA-g-CL)-*b*-PMAAc] showed the typical bands of both P(HEMA-g-CL) and P(MAAc). The main transmittance bands were included to stretching carbonyl at 1652 cm^{-1}, stretching aliphatic C–H and bending C–H at 2916 and 2850 cm^{-1}, and O–H at 3433 cm^{-1}.

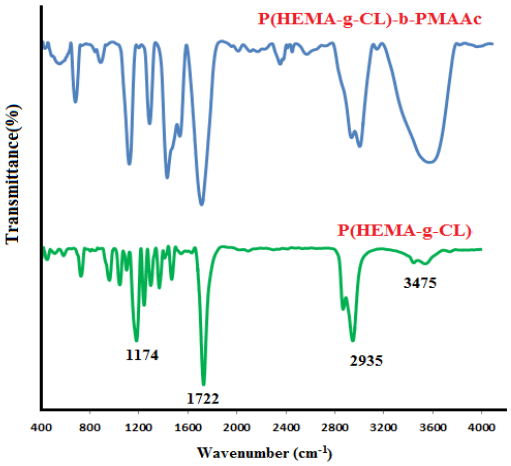
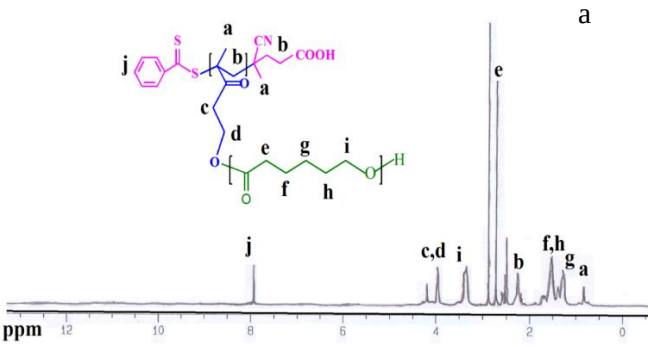


Figure 1- FTIR spectrum of a) P(HEMA-g-CL), and b)[P(HEMA-g-CL)-*b*-PMAAc] copolymers

Successful synthesis of poly(HEMA-g-CL) and [P(HEMA-g-CL)-*b*-PMAAc] copolymers was characterized by ^{1}HNMR spectroscopy. ^{1}HNMR spectrum of P(HEMA-g-CL) demonstrated chemical shifts at 0.76−0.8 ppm (a) and 1.24−1.54 ppm (g,f,h), and 2.26 ppm (b) related to the methylene protons of PCL backbone and RAFT backbone, respectively. Chemical shifts at 2.8 ppm (e) and 3.3 ppm (k) were related to –CO-CH_2 and –CH_2-OH protons, respectively. Chemical shift at 3.9−4.2 ppm (i,c,d) was related to CH_2-O and methylene protons of PCL and PHEMA. In addition, the chemical shift at 7.9 ppm was attributed to aromatic protons of the RAFT agent (Figure 2a). As seen in the ^{1}HNMR spectrum of [P(HEMA-g-CL)-*b*-PMAAc] co-polymers, all the peaks were adopted with the copolymer (Figure 2b).



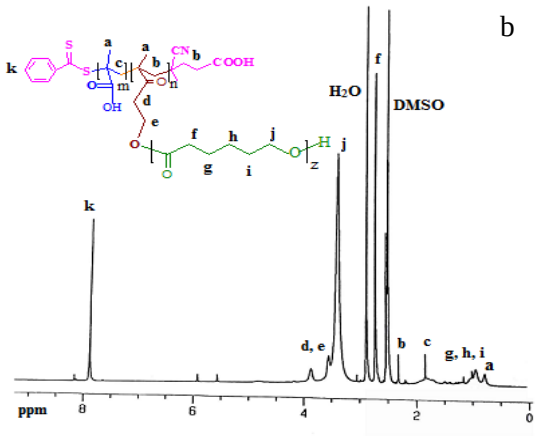


Figure 2- ^{1}HNMR spectrum of a) P(HEMA-g-CL), and b)[P(HEMA-g-CL)-*b*-PMAAc] copolymers

GPC chromatograms of P(HEMA-g-CL) and [P(HEMA-g-CL)-*b*-PMAAc] samples are shown in Figure 3. P(HEMA-g-CL) and [P(HEMA-g-CL)-*b*-PMAAc] synthesized by RAFT polymerization showed relatively low PDI of 1.14 and 1.19, respectively, which suggest an appropriate control of RAFT technique over the process. Molecular weights of the two copolymers obtained from GPC and ^{1}HNMR are compared in Table 1.

Table 1- Comparison of P(HEMA-g-CL) and [P(HEMA-g-CL)-*b*-PMAAc] characteristics achieved by GPC and ^{1}HNMR

Sample	M_n^a (GPC)	M_n^b (^{1}HNMR)	M_w^a	PDI[a]
P(HEMA-g-CL)	15117	14768	17233	1.14
[P(HEMA-g-CL)-*b*-PMAAc]	25887	25345	30705	1.19

M_n: number average molecular weight; M_w: weight average molecular weight; PDI: polydispersity index

3.2. Characterization of [P(HEMA-g-CL)-*b*-PMAAc] nanomicelles

3.2.1. Morphology

Morphology of the micelles was investigated by FESEM and TEM. Images of TEM indicates spherical shape (Figure 3a). The sphericity with approximate diameter of 35 nm is attributed to the primary micelles containing PCL as hydrophobic core and PHEMA and PMAAc blocks as a mixed hydrophilic shell. The morphology was further studied by FESEM (Figure 3b). It also indicated spherical shape and average diameter of 45 ±5 nm for the micelles. It is observed that the self-assembled nanomicelles of the brush copolymers are well dispersed individually in the medium.

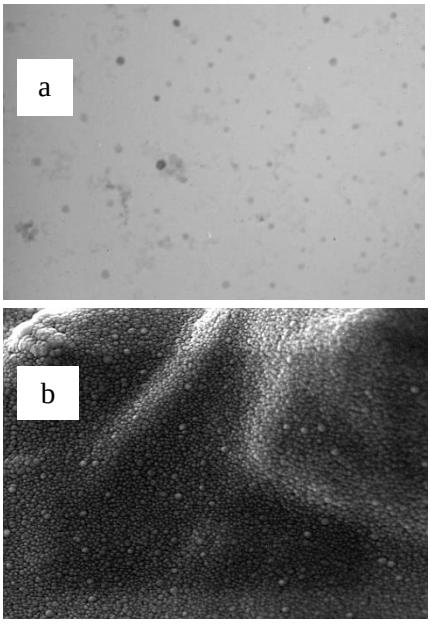


Figure 3- Images of a) TEM and b) FESEM of [P(HEMA-g-CL)-*b*-PMAAc] nanomicelles

3.2.2. Critical micelle concentration of the nanomicelles

Plot of fluorescence intensity for [P(HEMA-g-CL)-*b*-PMAAc] nanoparticles at 25 °C is depicted in Figure 4 and CMC of the nanomicelle was 0.025 g l^{-1} that is the least concentration required for nanomicelles formation.

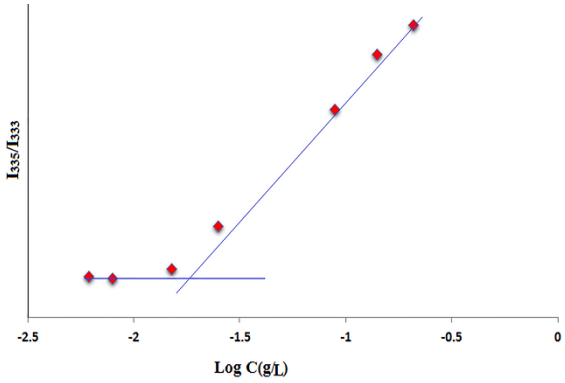
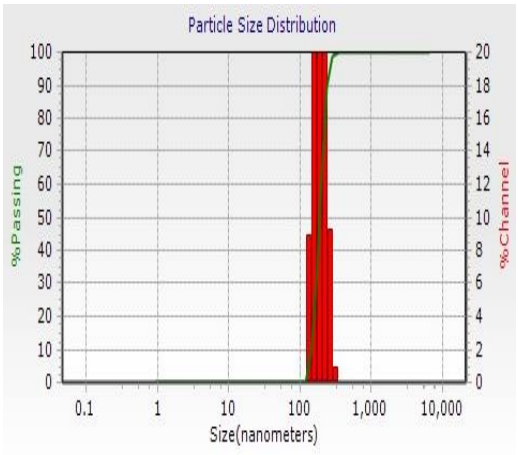


Figure 4- Fluorescence intensity of [P(HEMA-g-CL)-*b*-PMAAc] nanomicelles against logarithm of concentration

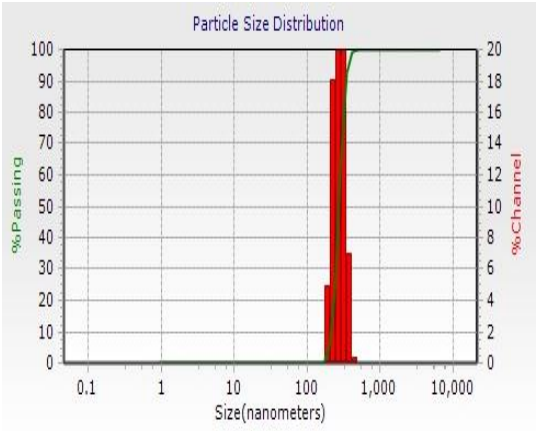
3.2.3. pH-sensitivity of [P(HEMA-g-CL)-b-PMAAc] nanomicelles

pH is an important factor when applying pH-sensitive polymers. The weak acids such as carboxylic acids are able to either release or accept proton under environmental pH changes [33]. DLS results confirmed pH sensitivity of the nanomicelles. Particle size of the brush copolymers was obtained by DLS at different pH (Figure 5). Mean particle size of the nanomicelles was 158, 279, and 124 nm at pH of 7, ≤4, and ≥9, respectively. The least size observed at pH 9, which was probably due to deprotonation of PMAAc at basic pH, leading to reduced diameters. Sizes of the particles obtained by TEM and FESEM were smaller than DLS. TEM imaging is done in the absence of solvent compared to the hydrodynamic diameter determined in a solution by DLS [25].

a



b



c

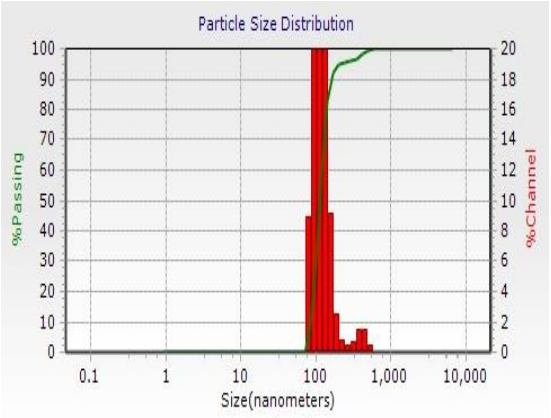


Figure 5- DLS diagrams of [P(HEMA-g-CL)-*b*-PMAAc] nanomicelles at a) pH = 7, b) pH ≤ 4, and c) pH ≥ 9

3.3. Controlled release of DOX from the nanomicelles

Encapsulation was done by a simple dialysis method. For this purpose, DOX and [P(HEMA-g-CL)-*b*-PMAAc] were dissolved in DMSO. Then, dialysis was done against distilled water. During the self-assembling process of the nanomicelles, DOX entered to the hydrophobic core (PCL). As calculated, 94.4% of the drug was loaded into [P(HEMA-g-CL)-*b*-PMAAc] nano-micelles. The drug release profile is shown in Figure 6. *In vitro* release behaviors of DOX at pH of 5.4 and 7.4 at 37 °C were examined. In this regard, the conjugated DOX was mainly released under acidic pH. A burst release was observed at pH = 5.4 after 7 h due to protonation of PMAAc carboxyl groups that weaken the interaction between the copolymer and DOX. Then, drug release continued at slower rate so that about 55% of DOX was released at pH = 5.4 after 102.5 h. In comparison, a lower release rate was observed at pH = 7.4 because of the strong ionic interaction between the copolymer and DOX, thanks to deprotonation of the carboxylic groups in PMAAc copolymer and their interaction with the positively charged drug at internal space of the nanomicelles.

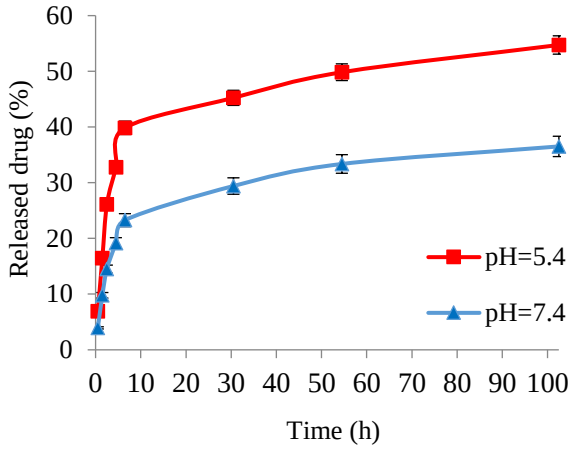


Figure 6- Release profiles of DOX from [P(HEMA-g-CL)-*b*-PMAAc] nanomicelles *In vitro*

4. Conclusions

HEMA and ε-CL were reacted by RAFT and ROP polymerization in the presence of AIBN and $Sn(oct)_2$ to synthesize PHEMA-g-PCL copolymers. Then, P(HEMA-g-CL)-*b*-PMAAc graft copolymer consisting of pH-sensitive PMAAc hydrophilic shell and biodegradable hydrophobic PCL core was synthesized by ROP and RAFT polymerization and macromonomer method. The amphiphilic copolymers showed different behavior at different pH and were able to self-assemble into micelles in water with CMC of 0.025 g l^{-1}. According to TEM and FESEM images, size of P(HEMA-g-CL)-*b*-PMAAc micelles was in the range of 35-45 ±5 nm and they have spherical shape. The biodegradable P(HEMA-g-CL)-*b*-PMAAc nanomicelles released a higher concentration of DOX at higher rate at pH 5.4 compared to 7.4. This feature introduces the complex as a potential carrier to smartly deliver the drugs to the target organs.

5. Conflict of interest

The authors declare that they have no conflict of interest.

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