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Crew cut, flower-like and mixed-shaggy micelles prepared from HLH and LHL triblocks as carriers: a comparative study of encapsulation, stability and release properties

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Abstract Two model triblock copolymers composed of hydrophilic (H) polyethylene glycol (PEG) and lypophilic (L) poly(butylene adipate) (PBA) have been synthesized and characterized with quite same molecular weight of each segments and different segment order: PBA-PEG-PBA and mPEG-PBA-mPEG. While LHL micelles adopt a flower-like arrangement with looped PEG on the shell, HLH micelles form a crew-cut particle with stretched hydrated PEG on the shell. The comparative investigation of the pharmaceutical properties of the obtained crewcut and flower-like micellar nanoparticles displayed advantages and disadvantages over each other. In order to exploit the advantages of both systems, the mixing has been used as a strategy. The mixed micelles with "shaggy arrangement" have been produced from the comicelling of LHL and HLH triblocks. They revealed better drug loading, encapsulation efficiency, more controlled release rates, smaller particle sizes and size distributions.

Keywords Micellar nanoparticles · Mixed micelles · Crew-cut and flower-like nanoparticles · Micelle size stability · Encapsulation capacity

Abbreviations

CMC	Critical micelle concentration
H	Hydrophile
L	Lipophile
PBA	Poly (butylene adipate)
mPEG	Monomethoxy polyethylene glycol
PME	Polymer micellation efficiency
R_h	Radius of Hydration

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Introduction

Polymeric core-shell nanoparticles, typically in the size range of 20–200 nm composed of block copolymers with micellar structure have attracted high attentions as drug carriers for drug delivery systems [1–4]. While hydrophobic inner core of the micelles is capable of solubilizing hydrophobic drug, the hydrophilic outer shell composed of the flexible tethered polymer chains provide stabilization and biocompatibility of particles by prohibiting them from being taken up by RES systems (stealth mechanism) [5–8].

Thermodynamically micellar nanoparticles could be prepared from amphiphile block copolymers in the aqueous medium and above their critical micelle concentration (CMC) [5, 9–13]. Drug-loaded micelles in comparison to the free drug administration have shown promising pharmaceutical performance in the disease likes cancer [11, 14]. The nanometric dimension of the spherical micelles awarded the carriers with more absorbability, bioavailability and targeting of the incorporated drug [15].

The amphiphilic triblock copolymers with lypophile (L)-Hydrophile (H)—L and HLH structures self assemble in the aqueous medium to flower-like and crew cut arrangement respectively (Scheme 1). While LHL micelles adopt a flower-like arrangement with looped swollen PEG on the surface of the particle as a shell, HLH micelles form a crew-cut particle with stretched swollen PEG. Two opposing thermodynamic parameters of the micelle formation in this

regime are first, loss of entropy due to looping and stretching of the H block and second, the free energy gain on the association of hydrophobic segments in the micellar core [13, 16].

LHL triblock can be rapidly swollen in the high concentration regime to form a physically cross-linked, biodegradable hydrogel [17, 18].

Both of the spherical crew cut and flower-like micelles resulted from different order of the lypophile-hyrophile segments have been investigated extensively for encapsulation of the hydrophobic drug [19]. The properties of the gained micelles such as drug loading, stability, size, size distribution and release rate were determined by the physiochemical characteristics of the triblocks. These properties include hydrophile-lypophile balance (HLB) [20], the crystallinity of the core and etc [19].

However, the prepared micelles from single triblocks are often lacking in one or more specialties [21]. One of the proposed strategies to overcome the limits and prepare more desired micelle is mixing. The resulted mixed micelles manifest synergistic properties superior to those of the individual components [22, 23]. The advantages of the mixed micelles over their constituents include lowering CMC, enhancing drug loading, improving size control etc [9, 24–27].

In this study quercetin as anti-carcinogenic, anti-allergic, chemo preventive or/and chemotherapeutic agent for prostate cancer has been used as incorporated hydrophobic drug [28].

Here we report the synthesis and characterization of the two model amphiphilic triblocks with different hydrophile-lypophile segments: poly (butylene adipate)—polyethylene glycol- poly (butylene adipate) (PBA-PEG-PBA) and monomethoxy polyethylene glycol—PBA- monomethoxy polyethylene glycol (mPEG- PBA- mPEG). The lypophile (L) and hydrophile (H) building blocks of the polymers comprise almost same molecular weights. The prepared crew cut and flower-like micelles have been investigated comparatively by their pharmaceutical properties and stabilities. Previously comparative reported studies on these two systems focused more on the length of the blocks [19]. With constant block lengths, the comparison has been alerted on the impact of the micelle structure on the pharmaceutical properties. In order to exploit the advantages of each system, the mixing has been used as a strategy. The binary mixtures of the two copolymers with same chemical structure and same molecular weight of H and L segments have been prepared in the different ratios. The obtained mixed micelles show shaggy-like arrangement by comicelling of HLH and LHL bock copolymers. Here it was demonstrated that flower-like micelles could provide more capacity for the quercetin with enhanced stability, more controlled release rate but increased hydrodynamic radius. In contrast, the crew-cut micelles could offer smaller particles with less cargo space for the encapsulation of the drug in the core, less stability (higher CMC) and faster release. The resulted mixed micelles by morphologies between crew-cut and flower-like (shaggy like micelles) have been characterized with better loading capacity, more controlling drug release, lower sized distribution and more stable micelles over the pure HLH crew-cut micelles. The smaller sizes with improved distribution have been recorded for the mixed micelles over LHL micelles.

Experimental

Materials

1,4-Butanediol, adipoyl chloride, quercetin dihydride (Scheme 2), polyethylene glycol (PEG Mw=2,000 kg/mol) and monomethoxy PEG (MPEG Mw=2,000 kg/mol) were obtained from Fluka. Acetone, dichloromethane (DCM), triethyl amine and pyrene were purchased from Merck Chemical Co. Diethyl ether was purchased from Guandong Guango Chemical Co. (China). All the chemicals were of analytical grade and used without additional purification. All aqueous solutions were prepared by deionized water.

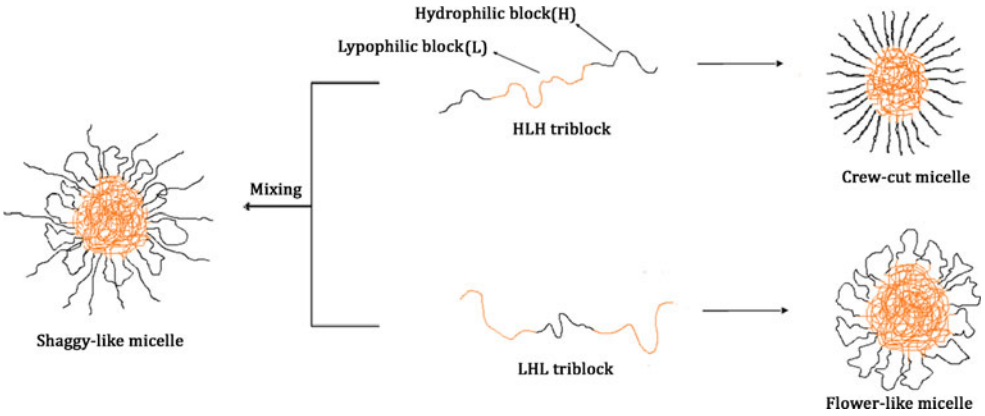
Synthesis of the block copolymers

mPEG-PBA-mPEG

The synthesis of the triblock mPEG-*b*-poly (butylene-co-butyne adipate)-*b*-mPEG has been conducted based on the previously reported procedure [34].

PBA-PEG-PBA

The title compound was synthesized by three steps. First, the hydroxyl-terminated polyester (HO-PBA-OH) was synthesized via solvent free polycondensation reaction of adipoyl chloride and glycols (1: 1.05 mol ratios respectively). The required amount of monomers with the mentioned molar ratios were placed in the cooled 250 mL three-necked round bottomed flask to 4 °C and the reduced pressure applied removing the released HCl. The temperature gradually increased to 70 °C in 30 min and kept for 6 h until the releasing of HCl seased. At the second step, the acid chloride terminated PEG (Mw=2000) was synthesized via reaction of PEG with adipoyl chloride in a 1:2.05 molar ratio. The predetermined amount of HO-PEG-OH (Mw=2000) were placed in a cooled 250 mL three-necked round bottomed flask at 4 °C and mixed with weighted adipoyl chloride. The reduced pressure applied and the temperature gradually increased to 60 °C. The reaction kept for 6–7 h until there were no sign of HCl releasing. At the third step, the reaction conducted to get final triblock from coupling reaction of the



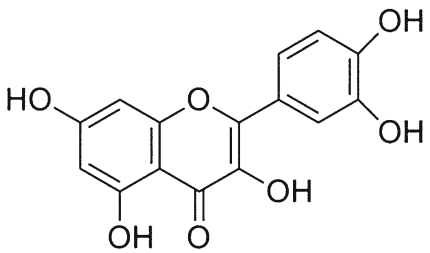
Scheme 1 The schematic representation of the designed pure and mixed micelles

prepared acid chloride terminated PEG and hydroxyl terminated PBA. PBA was dissolved in 40 ml of dichloromethane (DCM). Then a 50 ml dichloromethane solution of each acid chloride PEG and triethyl amine were added dropwisely and consecutively to a stirring solution of PBA under N_2 at room temperature. The applied molar ratios preparing PBA-PEG-PBA were 2.05:1:2 for hydroxyl terminated PBA, acide chloride terminated PEG and triethyl amine; respectively. The reaction mixture was stirred at room temperature under N_2 for 24 h. After completing the reaction, the precipitated triethyl amine hydrochloride salts was removed by filtration. The solvent was concentrated and the product was precipitated in 40 ml cold diethyl ether, filtered, and dried under vacuum for 24 h at 40 °C.

Analysis of the prepared block copolymers

Polymer molecular weights and molecular weight distributions were determined using GPC. An Agilent 1,100 series apparatus equipped with a refractive index (RI) detector using analytical grade of tetra hydrofuran (THF) as mobile phase at a flow rate of 1 $ml\,min^{-1}$ at 23 °C employed for the analysis.

^{1}H-NMR spectrum for the block copolymer was measured using a Bruker drx-500 AVANCE instrument in $CDCl_3$. The IR spectra of copolymers were carried out on the Shimadzu IR-460 from polymer powders. The DSC thermograms of the copolymers were acquired by use of a computer-interfaced calorimeter (Perkin Elmer Pyres DSC) under a nitrogen atmosphere and a heating rate of 10 °C min^{-1} from ambient to 350 °C.



Scheme 2 Quercetin molecule as hydrophobe drug

Preparation of the micelles

Two preparation methods were analyzed primarily in term of the efficiencies and sizes: solvent diffusion [29] and nanoprecipitation method [30]. The only difference between them was the removing step of the miscible organic solvent from dispersion. Upon the size analysis, later one (nanoprecipitation) was chosen as an efficient preparation method of nanometric micelles [30, 31].

Briefly, 10 mg of triblock copolymer and a predetermined amount of quercetin dissolved in an aliquot of acetone (the acetone used as miscible solvent). The optimized drug/ polymer ratio (10 % quercetin/polymer that was extracted from the optimization experiments) was applied. The obtained acetone solution was added dropwisely to 10 ml deionized water under moderate stirring at room temperature. In the solvent diffusion method, acetone will be replaced in the micelles by diffusion mechanisms gradually. In the nanoprecipitation, there is a need to remove miscible organic solvent to have through replacement of the solvent and form solid core. Acetone removed completely under reduced pressure. Finally, to eliminate the aggregated big particles, unincorporated drug crystals and other contaminations, the dispersion has been filtered through 0.45 μm cellulose acetate syringe filter. The final dispersions of the quercetin-loaded micelles were freezed

Table 1 Exponent n and drug release mechanism from polymeric delivery systems of spheres

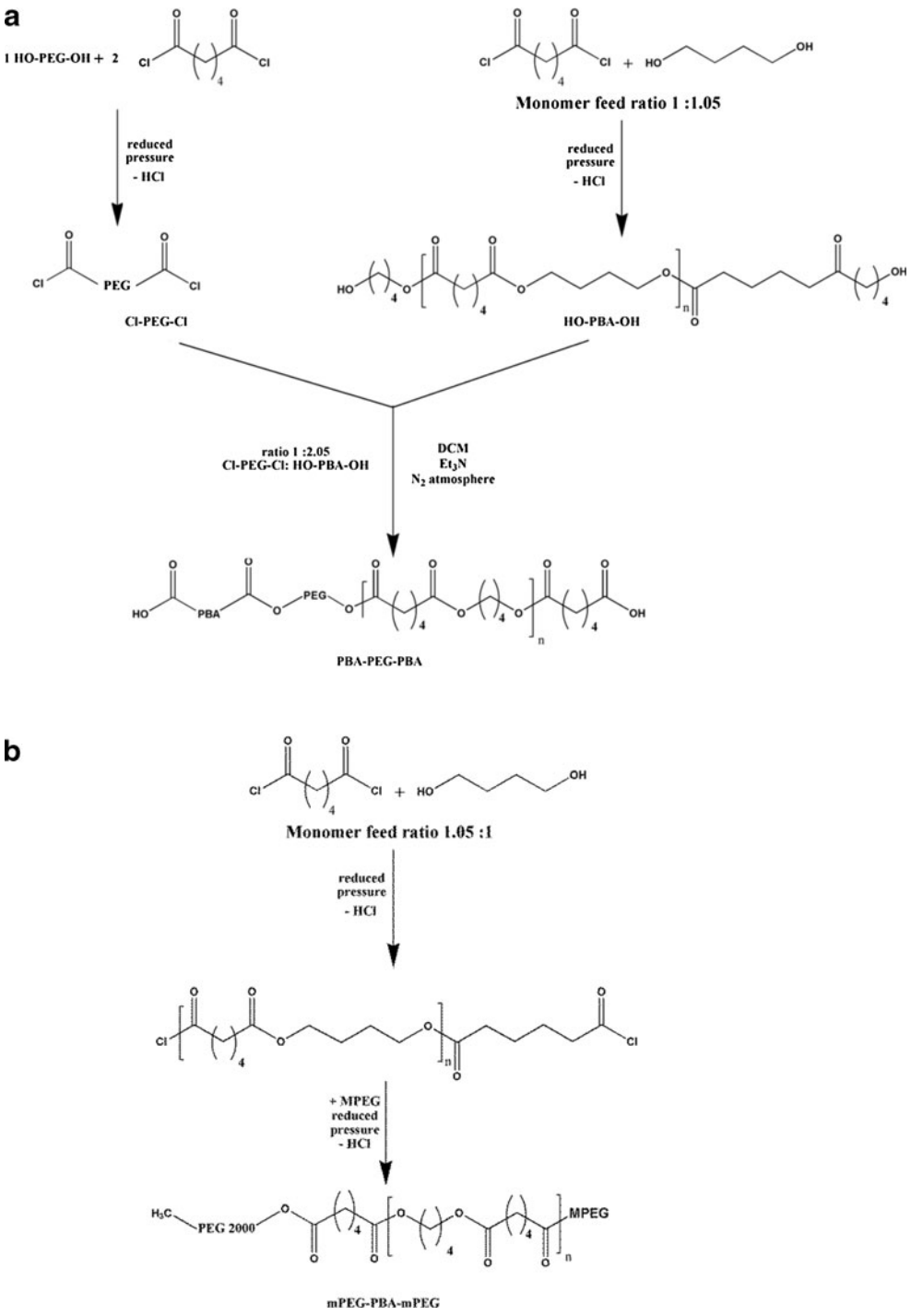
Diffusion exponent (n)	Overall solute diffusion mechanism
$n < 0.43$	Fickian diffusion
$0.43 < n < 1$	Anomalous (non-Fickian) diffusion
$n > 1$	Zero order release

quickly and transferred to the lyophilizer providing fine dried powder.

Characterizations of the micelles

The prepared micelles were characterized by yield, drug loading content and encapsulation efficiency. They were determined gravimetrically by ultraviolet absorption (UV) using Beer-Lambert equation at their maximum wavelength on a Carry 100 Bio spectrophotometer. The equations represented in the Eqs. (1), (2) and (3), respectively:

$$micelles\ yield\ \% = \frac{Weight\ of\ the\ micelles}{Weight\ of\ the\ feeding\ polymer\ and\ drug} \times 100 \quad (1)$$



Scheme 3 The chemical synthesis routes for PBA-PEG-PBA (**a**) and mPEG-PBA-mPEG (**b**) triblock copolymers

Table 2 Major characteristics of the synthesized block copolymers

Copolymers	Mw of PEG	Mn[a]	Mw[a]	Mw/Mn[a]	Tm[b](°C)
HO-PBA-OH	–	7444	10567	1.44	60.34
mPEG-PBA-mPEG	2000	7577	13103	1.73	54.97
PBA-PEG-PBA	2000	11944	22058	1.84	59.83

[a] Obtained from GPC analysis

[b] Obtained from DSC analysis

$$Drug\ loading\ content\,\% = \frac{Weight\ of\ the\ drug\ in\ micelles}{Weight\ of\ the\ micelles} \times 100 \quad (2)$$

$$Encapsulation\ efficiency\,\% = \frac{Weight\ of\ the\ drug\ in\ micelles}{Weight\ of\ the\ feed\ drug} \times 100 \quad (3)$$

The efficiency of the polymer aggregated to prepare nanoparticulate micelles, Polymer micellation efficiency (PME), defined as:

$$Polymer\ micellation\ efficiency\ (\mathrm{PME})\% = \frac{Weight\ of\ the\ polymer\ in\ micelles}{Weight\ of\ the\ feed\ polymer} \times 100 \quad (4)$$

Malvern Zetasizer Nano series instrument was used to perform dynamic light scattering (DLS) experiments. Before the DLS measurements, a 0.45 μm filter was used to remove dust. The morphology of the prepared micelles was studied by atomic force microscope (AFM), Transmission electron microscopy (TEM) and scanning electron microscope (SEM). For AFM Measurements, all samples were cast on mica substrates (cleaned by layer removing) from dilute dispersion of the prepared nanoparticles (10^{-7} mg/ml). The films were dried for 2 days at dust free chamber in the room temperature before AFM observation. Atomic force micrographs were recorded under ambient conditions with silicon cantilever tips (PPP-NCH, 300–330 kHz, 42 N/m from Nanosensors) using an Asylum Research (Santa Barbara, California) MFP-3D-Bio machine in non-contact mode under ambient conditions. SEM analysis was carried out using a Cambridge S-360 SEM scanning electron microscopy. Prior to observation, the samples were gold sputter-coated to render them electrically conductive. For TEM observation, a drop of suspension containing 2 wt% phosphotungstic acid was placed on a copper grid with Formvar® film, dried and negatively stained. TEM images of the samples were obtained using a Philips CM120 machine at an acceleration voltage of 120 keV.

Fluorimetric measurements were recorded on luminescence spectroscopy PERKIN ELMER LS50B to determine CMC values by applying pyrene as a hydrophobic fluorescent probe. The used method was based on the reported one in the literatures [32]. Briefly, the stock pyrene solution of 12×10^{-7} M in

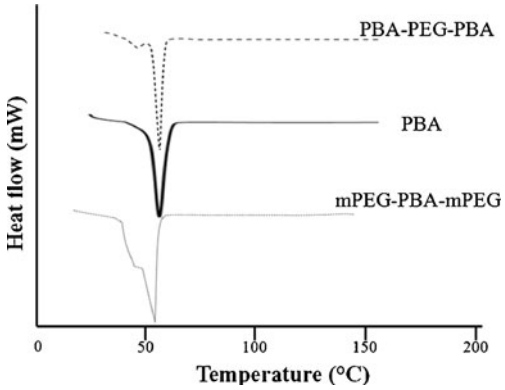
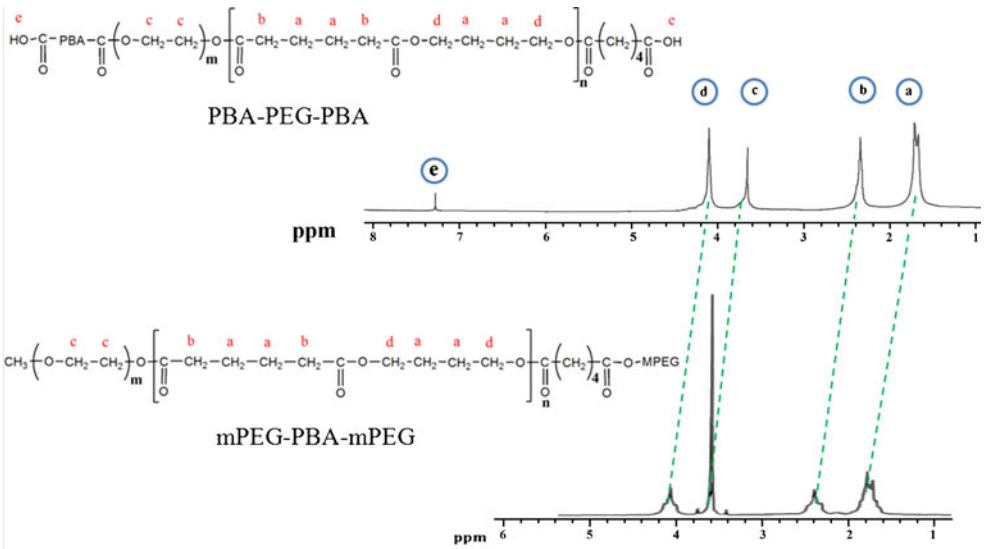


Fig. 2 DSC curves of the prepared PBA and triblocks

Fig. 1 ^{1}H-NMR spectrum of the prepared triblocks



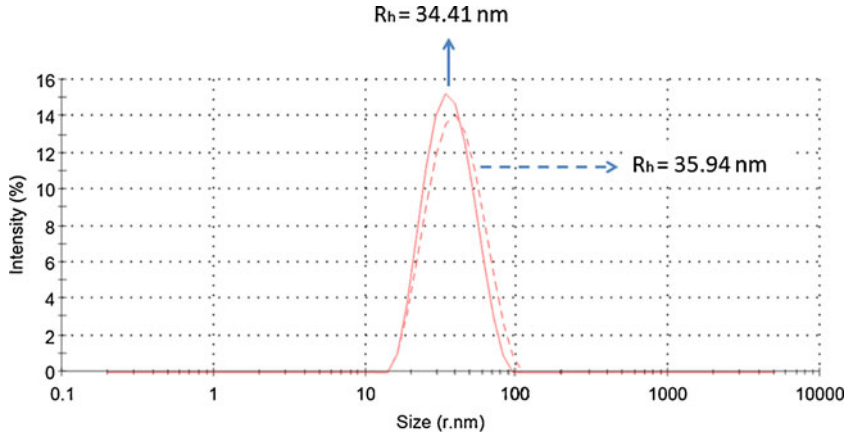


Fig. 3 Drug free micelles prepared by solvent diffusion (- - -) and nanoprecipitation (-) method

deionized water were obtained from the acetone solution of pyrene (6×10^{-2}M). Acetone was then eliminated under reduced pressure at 60°C for 1 h. The pyrene solution was mixed with micellar solutions of concentrations ranging from 5×10^{-7} to 0.5 g/L. These solutions were stirred smoothly for a period of overnight at room temperature to reach the equilibrium state. The fluorescence emission spectra were measured at375 nm while the excitation wavelength (λ_{exc}) was 339 nm. The CMCs were subtracted by depicting of the intensity of the first peak (I_1) of the emission spectrum at 375 nm versus the logarithmic values of the copolymer concentrations. CMCs of the mixed micelles were obtained from the phase separation model. CMC of the mixed micelles were calculated from the individual CMCs, and the respective mole fractions of the two copolymers (α) [33] as follows:

$$\frac{1}{CMC_{mix}} = \frac{\alpha_1}{CMC_1} \times \frac{\alpha_2}{CMC_2} \tag{5}$$

CMC_1 and CMC_2 are the CMC of the pure mPEG-PBA-mPEG and PBA-PEG-PBA copolymers.

The cumulative in vitro release evaluation of quercetin from the micelles was investigated using a dialysis bag 12 kDa by diffusion technique right after the preparation of quercetin-loaded micelles. All the details and methods were previously described in details [34]. The experimental release data were compared based on the two well-known theoretical models to get an insight to the mechanism of the drug transport and release kinetic. The Higuchi and Korsmeyer-Peppas (or Power law) describe the release of drug from the polymeric system as bulk degrading sphere system. While the Higuchi model considers the drug release from the polymeric matrix just through a diffusion process based on Fick's law(Eq. (6)), the power law surveys various mechanisms of transport including the Fickian diffusion, non-Fickian transport as well as zero-order (constant-rate) release behavior (Eq. (7)):

$$\frac{M_t}{M_\infty} = Kt^{1/2} \tag{6}$$

While K constant reflects the formulation characteristics, M_t and M_∞ are the amounts of drug released at time t and as time approaches infinity respectively.

$$\frac{M_t}{M_\infty} = K't^n \tag{7}$$

k' is a constant incorporating geometrical and structural characteristics of the polymeric network and the drug and n is an exponent of release (characterizing the mechanisms). In the first 60 % of the fractional release of degradable spheres, when $n<0.43$ Fickian diffusion is dominated mechanism, when $0.43<n<1$ anomalous non-Fickian

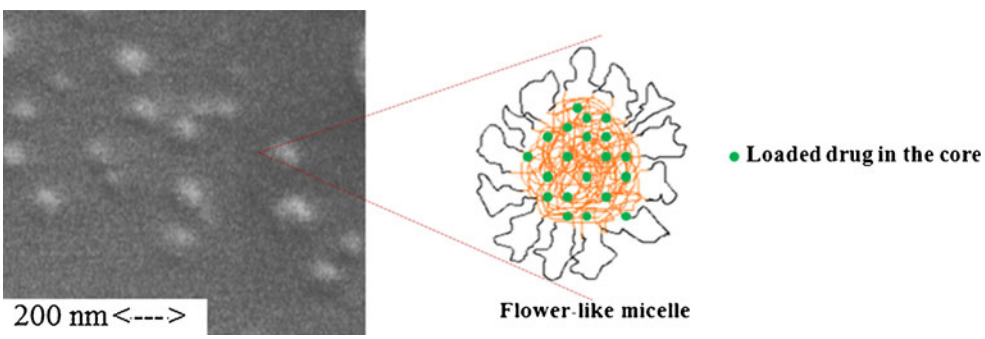


Fig. 4 Typical SEM micrograph of quercetin-loaded LHL flower-like micelles

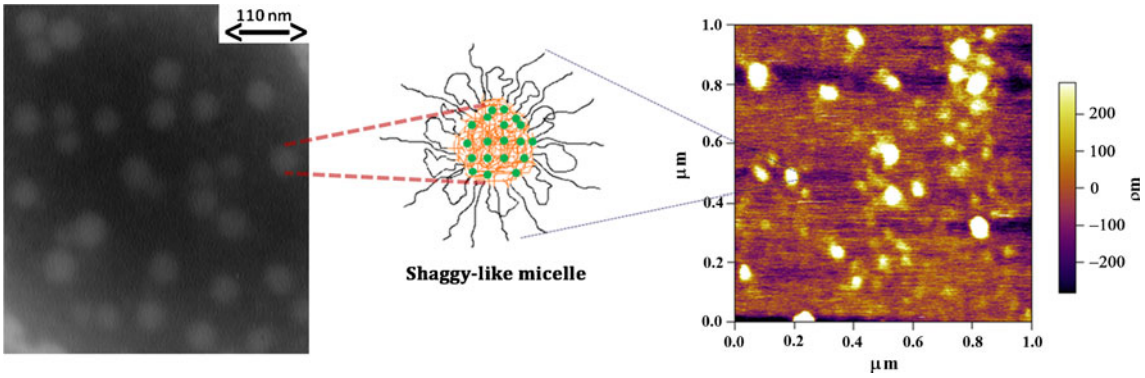


Fig. 5 AFM (*right*) and TEM (*left*) images of quercetin-loaded micelles Mix 25%

transport and finally while n >1 is zero-order release when (Table 1).

Results and discussions

All the synthetic steps for PBA-PEG-PBA (LHL) and mPEG-PBA-mPEG (HLH) are represented in the Scheme 3. Based on the molecular weight of the hydroxyl terminated polyesters in the first step, the determined ratio of the prepared acid chloride terminated PEG was applied to get PBA-PEG-PBA. At the other side, the excess amount of mPEG was added to the acid chloride terminated PBA to get mPEG-PBA-mPEG.

The main object was to prepare hydrophobe segments with almost same molecular weights to minimize the effect of the Mw on the pharmaceutical properties of the micelles. Therefore, the applied ratio of the monomers at the first step (1:1.05), temperature, reaction time and other affective factor kept similar. The molecular weight of the hydroxyl terminated PBA and PBA block in mPEG-PBA-mPEG (known Mw for the mPEGs provide the PBA Mw) confirmed the close molecular weight for the polyesteric segments in the copolymers (10567 in PBA-PEG-PBA and 9103 for mPEG-PBA-mPEG). The GPC curves of the copolymers display unimodal distribution and there are no related peaks for PEG and polyesters. The number and weight average molecular weight (Mn and Mw) and melting points of the prepared polymers are tabulated in the Table 2.

The chemical structures of the prepared triblocks were confirmed by FT-IR and ^{1}H-NMR spectrums (the typical ^{1}H-NMR analyses are displayed in the Fig. 1).

The chemical structures of the triblocks were analyzed by ^{1}H-NMR too. The actual ratios of the PBA/PEG in the copolymers, which is determined from surface integrals of the peaks of ^{1}H-NMR spectra coincide to the designed ratios in the PBA-PEG-PBA and mPEG-PBA-mPEG. Using $CDCl_3$ as solvent, where the polymers dissolved molecularly, presence of resonance peaks corresponded to PBA and PEG segments in both copolymers were detected successfully. The multiple peaks at 1.71 ppm, the peaks at 2.39 ppm and finally the triplet peaks at 4.09 ppm are corresponded to the esteric PBA part of the triblocks. The peak at about 3.64 ppm was assigned to the methylene protons of the PEG block that is present with different ratios of H_c for two triblocks. While the PEG peak in the mPEG-PBA-mPEG has a shoulder at 3.39 ppm as CH3 end group, the occurrence of OH peaks at around 7.15 ppm for PBA-PEG-PBA indicates the successful coupling reaction of the PBA and PEG in the both triblocks.

Table 3 Major characteristics of the prepared quercetin loaded micelles

Polymer	Feed drug/ polymer (%)[a]	Drug loading (%)	Encapsulation Efficiency (%)	PME (%)	Yield (%)	R_h[b] (nm)	R_{core}[c]	PDI[b]	CMC (mol/l)[d]
mPEG-PBA-mPEG (HLH)	10	12.08	87.09±2.3	63.38	65.54	33.26±2.1	23.34	0.111	1.74×10^{-6}
PBA-PEG-PBA(LHL)	10	13.39	93.14±2.1	60.25	63.24	79.13±3.3	41.68	0.095	3.05×10^{-7}
Mix 25%	10	13.11	89.15±2.5	59.09	61.82	71.22±3.6	39.71	0.099	3.84×10^{-7}
Mix 75%	10	12.40	92.93±1.9	65.65	68.13	53.26±2.6	32.47	0.095	7.99×10^{-7}

[a] Drug/copolymer ratio in the feed was optimized in 10 wt%

[b] Obtained from light scattering analysis

[c] Calculated from TEM measurement

[d] Calculated from fluorescence analysis

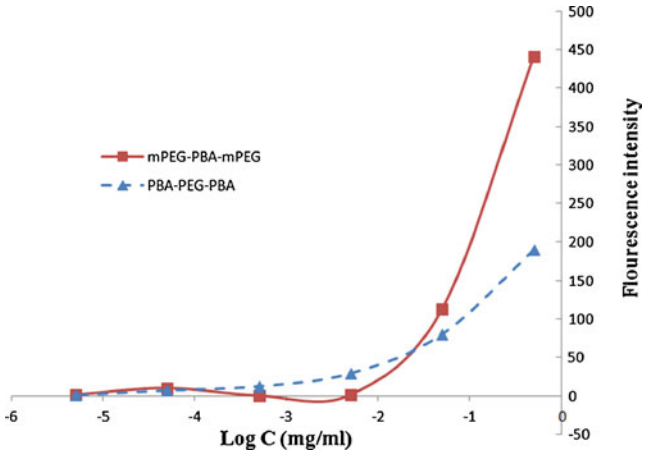


Fig. 6 Determination of the CMC by fluorescence measurement technique

DSC thermograms of the synthesized copolymers exhibited in the Fig. 2. In agreement with the reported results, DSC thermogram of the synthesized PBA polymer (Fig. 2) displays a main transition peak of the melting process with one peak at 60.34 °C [20]. The introduction of PEG segment (Tm=50.8 °C) to PBA has influenced the crystallinity of the polyesteric segment due to the mutual effects in mPEG-PBA-mPEG. The reduced melting peak appears at 54.97 with one peak as a shoulder (corresponds to crystallized PEG). In agreement with those mentioned literature [35] because of the less compatibility of the low ratio PEG with dominant PBA segments at PBA-PEG-PBA, no intermediate melting endotherm was found. This might be due to the lower mutual effect resulted from sufficient phase separation. The synthesized copolymers proved to be semi crystalline.

As soon as the acetone solution (miscible organic solvent), containing copolymers and drug, has diffused into the dispersing medium, the polymer involving entrapped drug would be precipitated. This technique first time was patented by Fessi et al as nanoprecipitation method [30]. Figure 3 indicates the lower size of the drug free micelles prepared from nanoprecpitations in comparison to the solvent-diffusion. It might be due to the shrinkage of solidified core after complete removing of the

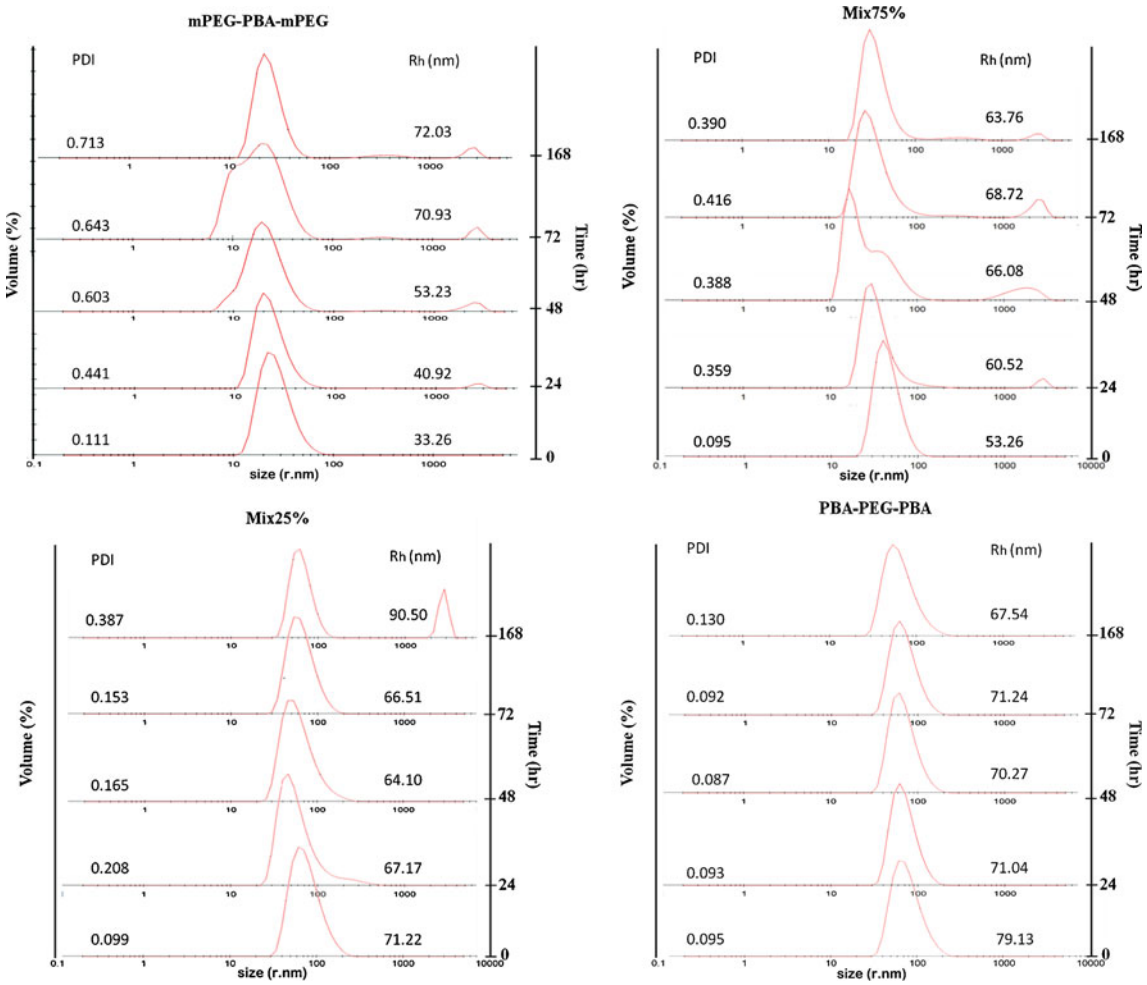


Fig. 7 Time dependent size measurement of the quercetin loaded micelles

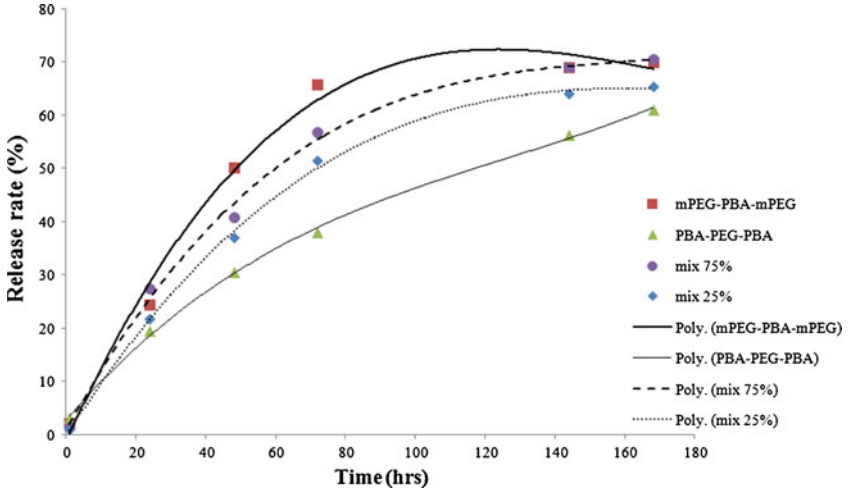


Fig. 8 *In vitro* release profiles quercetin loaded pure and micelles

acetone through the medium and micelles. Thus, all the drug-loaded micelles were prepared via nanoprecipitation method.

In order to discovering the optimized drug/copolymer ratio in the feed, different ratios has been evaluated for HLH. Drug/copolymer weight ratio of 10 % was the best and applied to all other samples. The morphology of the prepared micelles was investigated by AFM, TEM and SEM technique. The typical SEM micrograph of LHL flower-like micelles is illustrated in Fig. 4. Figure 5 displayed the typical AFM and TEM images of the quercetin loaded 25 % mixed micelles. As it is obvious, the semi spherical smooth morphologies for the nanoparticulate micelles can be realized. Due to the negative staining of the dispersions, the hydrophobe core is visible through TEM (R_{core}), while the total core-shell structure of the nanoparticle is visible in the AFM and SEM images ($R_{particle} = R_{core} + R_{shell}$).

The drug loading and encapsulation efficiency calculated from quantitative UV measurements (using Eqs. 2 and 3) of the micelles are listed at Table 3. The other parameters like polymer micellation efficiencies, sizes, size distributions, yields and CMCs are also summarized in the Table 3.

The improved drug loading was observed in the flower-like LHL micelles (13.39 %) in comparison to the crew-cut HLH micelles (12.08 %). While there are no considerable differences in the other parameters such as PME and Yields, it seems the CMCs and particle sizes play key rules for the obtained results on the drug loadings. Commonly, sizes of the micelles are under control of the composition and molecular weight of the polymeric constituents. The higher molecular weight of the LHL blocks, the altered segments order of the lypophile/hydrophile parts and finally bridging ability of the middle PEG between discrete micelles have raised the hydration radius of the LHL micelle into 79.13 nm. Due to the looped swelled PEG on the surface of LHL, the proportion of core to shell increased in comparison to HLH. It provides more space for encapsulation of the drug in every particle. All these together with the decreased CMC of the LHL, causes enhanced loading capacity and higher encapsulation capability of the LHL micelles. The lower CMC of the LHL in comparison to HLH is not surprising, given the lower hydrophile/lypophile balance (HLB) in LHL triblocks (Fig. 6) [24].

Always, more control over micellar shape, size, loading and releasing properties is desirable in the pharmaceutical applications [36]. A promising strategy to tune copolymer structure is mixing, where the mixed micelles with synergistic properties of the components achieved. Both produced LHL and HLH drug loaded micelles offered the advantages and disadvantages. The lower drug loading and stability (CMC) are the weak points of HLH (in comparison to LHL nanoparticles) while the larger R_h is the weak side of the LHL micelles. In order to exploit fully the advantages of the both micelles, the binary mixtures of LHL and HLH

Table 4 The parameters of the Higuichi and Korsmeyer-Peppas models

Entry	Nanoparticles	Higuchi		Korsmeyer-Peppas			Release mechanism
		K	r^2	K'	r^2	n	
1	mPEG-PBA-mPEG	5.910668	0.8985	0.31480	0.9982	0.805730	Anomalous
2	PBA-PEG-PBA	4.911530	0.9880	0.48541	0.9998	0.587521	Anomalous
3	Mix 25%	5.597096	0.9763	0.02022	0.9986	0.924946	Anomalous
4	Mix 75%	5.902186	0.9732	0.17264	0.9961	0.865818	Anomalous

were prepared in the two different molar ratios 0.25/0.75 (is shown as 75 %) and 0.75/0.25 (is shown as sample 25 %). Mixing as a strategy was used to reach a micelle with higher drug loading and stability in proportion to HLH. In addition, the final mixed micelles must offer lower sizes with better stealth characteristics over LHL micelles.

As it is highlighted in the Table 3, the mixed micelles could enhance the loading of the HLH from 12.08 % to 12.40 % and 13.39 % in 75 % and 25 % respectively. As it was reported in the other works [21] the promoted size distributions can be seen as other advantage of the prepared mixed micelles over pure ones. The mono modal narrow size distributions have been observed for the 75 % and 25 % mixed micelles with 0.095 and 0.099 PDIs. Comparing to the 0.111 as PDI of HLH, the better polydispersity achieved. The CMCs of the mixed micelles subtracted from the Eq. 5 emphasized the stabilities between the CMCs of the pure micelles as result of mixing which means more stable particles than HLH. The hydration radiuses decreased in comparison to the LHL micelles to the 71.22 and 53.26 nm for the 25 % and 75 % micelles respectively.

Assessing the micelle stabilities, the time dependant DLS measurement has been performed. The hydrodynamic radiuses of the quercetin-loaded micelles in the 1 mg/ml concentration were measured in the predetermined intervals (Fig. 7).

As it is obvious in the Fig. 7, the LHL micelles have been shown significantly good stability with minimum change on the PDI and Rh from 0.095 to 0.130 and 79.13 to 67.54 respectively after 1 week. At the same time, the change in the PDI and sizes are significant for the HLH micelles. It is due to the lower CMCs and possible higher biodegradability of the triblock copolymers with presence of more hydrophile building blocks in the aqueous medium. The enhanced stabilities with lower PDI and Rh changes have been arrived at mixing less stable HLH to more stable LHL triblocks. When the molar ratio of the LHL increased from mix 75 % (micelles contain 25 % LHL triblock) to mix 25 % (micelles contain 75 % LHL triblock), the stability of the micelles is indicated with minor alterations.

The stability and biodegradability of the micelles comprise direct impact on the release profile too. *In vitro* releases profiles of the pure and mixed micelles are accessible in Fig. 8. Visibly the compositions of the micelles (affecting the stability and degradability of the polymeric matrixes) not only have shaped the drug loading contents, but also have influenced the release trends of the quercetin from nanoparticular micelles.

The Higuchi equation and Korsmeyer-Peppas models have been employed to investigate the mechanism of the drug release from the pure and mixed micelles. The k, k' and n parameters of the models were subtracted by linear regression analysis using Microsoft Excel 2007 software and exhibited in the Table 4. The fitting accuracy of the models was checked from the coefficients of correlation (r^2 in Table 4).

The fitting accuracy of the Korsmeyer-Peppas (power low) model fitted more to the all micelles includes both pure and mixed micelles. The higher r^2 values were attained when the log M_t/M_∞ depicted against "log t" in the power low model in comparison to the r^2 values in the Higuichi model (where the M_t/M_∞ depicted against "$t^{1/2}$"). As a result, the transport mechanism of the quercetin is not pure Fickian diffusion. The value of n in power law model indicates that release trends of the quercetin from all the micelles follow the Anomalous release mechanisms. In this condition, the mechanism of the drug release is association of the "diffusion-controlled" and "swelling-controlled" drug release. As biodegradable polymeric sphere, swelling event was combined to the penetration of water, dissociation and degradation of the micelles and polymers. It is notable that LHL micelles with lowest CMC value, size change (Fig. 7) and highest stabilities exhibited closest n value to the Fickian diffusion release. This result causes more controlled release of the quercetin with reduced rate in comparison to the HLH micelles (Fig. 8). The synergistic properties of the mixed micelles were emphasized by release profiles. The 25 % and 75 % mixed micelles drug release stand between the two pure micelles where more controllable release rate has been achieved. The more ratio of the LHL, the more controlled release rate in the 25 % mixed micelles was seen (Fig. 7). It was demonstrated from the release studies that the mixed micelles as outcome of applying mixing as a strategy, could provide drug loaded micelles with tuned properties of the components.

Conclusion

Two model triblock copolymers PBA-PEG-PBA and mPEG-PBA-mPEG have been synthesized and characterized carefully. These copolymers with a quite same molecular weight of hydrophile and lypophile segments had structure of LHL and HLH. Every resulted crew cut and flower-like micelles displayed advantages and disadvantages. In order to produce a particle with optimized and synergistic properties, the binary mixtures of the triblock copolymers were prepared and compared to the pure ones. The disadvantages of every pure micelle have been covered by the advantages of the other micelles by comicelling of the two systems in the mixed micelles with shaggy morphology.

Better loading capacity, more controlling drug release, lower sized distribution and more stable micelles have been realized for the mixed micelles over the pure HLH micelles. The smaller sizes with better distribution have been produced by blending the LHL with HLH triblocks giving mixed micelles. By minimizing the molecular weight effects, this study not only offers

an indication for the efficiency of mixing strategy to have synergistic properties but also emphasizes the advantages and disadvantages of the either crew cut or flower-like micellar nanoparticles in the comparative experimental approach.

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