



Synthesis, characterization and surfactant properties of cholic acid containing linear and star polymers

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Abstract

Surface-active linear and star-shaped miktoarm polymers containing cholic acid as a bile acid derivative are prepared by esterification of poly(ethylene glycol methyl ether) and subsequent ring-opening polymerization of epsilon caprolactone, respectively. The chemical structures, molecular characteristics and thermal properties of obtained polymers are clearly confirmed by FT-IR, ¹H-NMR, GPC, DSC and TGA analysis. The presence of characteristic bands of cholic acid, ether and ester groups in FT-IR and ¹H-NMR spectroscopies is proof of the successful synthesis of linear and star-shaped polymers. The melting point of star-shaped polymer is slightly lower than the linear polymer. Additionally, their surface activity properties have been investigated by AFM, SEM and contact angle studies and it is found that they can be self-assembled into micelles. Furthermore, the size of the micelles and contact angle properties has been changed with the addition of both hydrophilic (poly(ethylene glycol)) and hydrophobic (poly(epsilon caprolactone)) blocks on the cholic acid head. It is also clearly seen the effect of hydrophobic poly(epsilon caprolactone) functions increased contact angle and surface tension values after the formation of star-shaped polymer.

Keywords Bile acids · Polymer micelles · Polymeric surfactants · Star polymer · Surface tension

Introduction

Surface active polymers are used as wetting agents, dispersants and emulsifying agents in industrial and medical applications such as cosmetic, paint, detergent, agricultural and food industries [1]. Surface active agents (surfactants) might be synthesized and produced from both synthetic and natural feedstocks (coconut, palm kernel and animal oils, and micro-organisms). Due to cost, availability and process flexibility, bigger parts of the production capacity are utilized by petrochemical origin surfactants [2, 3]. In recent years, biosurfactants produced by microorganisms gained high

interest as they are amphiphilic molecules that have ability to reduce surface and interfacial tension of the medium [4]. Glycolipids, phospholipids and fatty acids are the main biosurfactant classes with a particular surface activity [5, 6]. Despite petrochemical-based surfactants are the majority in various applications, biosurfactants are gaining an interest over their synthetic counterparts due to their better biodegradability, low toxicity and being environmentally friendly [7, 8]. However, biosurfactants are still not competitive in the surfactant market in terms of cost point of view. They might be used in formulations if the cost is not considered as a priority [9, 10].

Cholic acid (CA) is the most known and studied primary bile acid. It comprises a steroid unit with three hydroxyl groups and a carboxyl group [11]. CA is a biosurfactant formed by a hydrophilic head group, which is a carboxylic function that optimize their enterohepatic circulation [12] and an amphiphilic steroid group, which improves the transfection efficiency [13]. Especially, the spectacular features like convenient bioavailability, inimitable facial amphiphilicity, excellent biocompatibility, and trivial cytotoxicity make CA unique as multifunctional molecule to design numerous cholic acid-based polymers (CAPs) in the several

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research such as pharmacological, biomedical, and others [14]. CA is not soluble in a water and this features effect its bioavailability and provides disadvantage in biomedical applications. CAPs supply a greater target specificity, ample fidelity on the appliance, superior control over amphiphilicity to overcome these drawbacks of CA. CA is an important building block to prepare well-defined macromolecules that has shown promising aggregation features and notable biocompatibility properties [15–20]. Due to rigid polycyclic structure and a well-spaced array of multifunctional groups CA is a excellent starting compound for functionalized molecular architectures having well-defined geometrical features [17, 21–23].

The most widely studied block linear amphiphilic copolymers such as diblock and triblock copolymer for their micelles in the drug delivery system. The conventional micelles made from these polymers suffer from fast initial release rate and low encapsulation efficiency, which limit their applications in drug delivery systems [11, 24, 25]. Because the molecular architecture of amphiphilic copolymers is the most important factor that determines the micelle property and structure, it has been recently focused on the design of the molecular structure of amphiphilic copolymers with nonlinear structure and it has been shown that they exhibit more advanced properties as drug carriers [11, 26]. Star-shaped polymers with variety of functional arms up to hundreds of units employed in drug carriers have generally advantageous due to their smaller hydrodynamic radius, lower solution viscosity, higher density of functional groups and encapsulation efficiency, compact structure and loading content relative to linear polymers [18, 27–29]. The most important point for star-shaped polymer-based micelles is its marked enhancement of micelle stability compared with linear polymers of the same molecular weight.

So, star-shaped block copolymers based on poly(ethylene glycol) (PEG), poly(lactide) (PLA), and poly(ϵ -caprolactone) (PCL) as biomaterials with CA could potentially be utilized to prepare excellent biodegradable drug-carriers system [29, 30].

Due to the asymmetrically positioned functional groups of CA, star-shaped polymers with CA core have a unique structural asymmetry. The facial amphiphilicity of the CA core imparts a spatial asymmetric distribution of the polymer chains [15, 31]. PCL is an important family member of the aliphatic polyester which exhibits good biodegradability, biocompatibility, non-cytotoxicity, non-immunogenicity, and miscible properties with other polymers. Due to these features, is extensively studied as biomaterials for biomedical applications such as prosthetics, sutures, controlled drug delivery and tissue engineering [11, 29, 32–35].

Among them, PEG-based star-shaped polymers providing biocompatible and biodegradable features have been widely used in biomedical studies, drug delivery systems

and health applications [36–38]. Because of the PEG has hydrophilicity and nontoxicity features, star-shaped polymers consisting of PEG chain can easily obtain micelles structure in aqueous solution and this feature helps in biomedical applications [32]. The use of CA as a core center with PEG as arms led to corresponding star polymers, which showed critical aggregation concentrations in surface tension experiments and forming spherical shape micelle aggregates in water determined by transmission emission microscopy [15, 39]. In our case, CA was utilized as a building block for the functionalization of poly(ethylene glycol) methyl ether and the synthesis of a star-shaped miktoarm polymer having two distinct polymer chains (PEG and PCL) [11, 15, 16, 29, 40, 41].

Nevertheless, compared to many literature studies published on micelles obtained from block linear copolymers, studies of micelles obtained from star-shaped copolymers, especially environmental sensitive copolymers, are relatively less and more studies are needed to understand important features such as controlled release behavior and self-assembly [16]. Our objective here is to obtain CA containing linear and star polymers to study their surface properties by changing hydrophobic and hydrophilic units (Scheme 1). CA was selected in our molecular design due to its biological origin, which was exhibit to have better biocompatibility and performances in biomedical applications than those without a CA moiety [17, 21, 22, 29, 42]. So, we designed a novel CA-cored amphiphilic star-shaped polymer with and a hydrophilic PEG chain and three hydrophobic PCL branches, was chosen as the joint to combine PCL and PEG segments in terms of their excellent biocompatibility and biodegradability. For this purpose, cholic acid was esterified with poly(ethylene glycol) methyl ether (mPEG2000) to obtain linear polymer containing cholic acid head. As part of our continuing interest in the development of surface-active polymers, we reported a series of octa-functionalized star-shaped PEG polymers having different chain lengths from 750 and 2000 g/mol. Among them, the POSS-based star-shaped polymer having 2000 g/mol PEG chains exhibited the better surface activity compared to others [43]. Furthermore, the obtained linear polymer was used as a precursor of miktoarm star polymer synthesis by ring opening polymerization (ROP) of ϵ -caprolactone. The chemical characteristics of the obtained linear and miktoarm star polymers were analyzed by FT-IR and ^1H -NMR spectroscopies. In addition, their thermal properties were investigated by thermogravimetric analysis and differential scanning calorimeter. Molecular weights and molecular weight distributions of obtained polymers were also investigated by gel permeation chromatography. Additionally, the surface and micelle properties of them were determined by contact angle measurements, atomic-force microscopy and scanning electron microscopy measurements.

The reaction scheme illustrates the synthesis of (PCL)₅-Cholic-mPEG. It begins with Cholic acid, a steroid with three hydroxyl groups and a carboxylic acid group, reacting with mPEG (methoxy poly(ethylene glycol)) in the presence of EDCI and DMAP. This step forms Cholic-mPEG, where the carboxylic acid group of cholic acid is esterified with the hydroxyl group of mPEG. The second step involves the reaction of Cholic-mPEG with PCL (poly(ε-caprolactone)) in the presence of ε-CL and Sn(Oct)₂ at 120 °C. This step results in the formation of (PCL)₅-Cholic-mPEG, where the three hydroxyl groups of the cholic acid core are esterified with five PCL chains, and the mPEG chain remains attached to the esterified carboxylic acid group.

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introduced into a solution of cholic acid (1.062 g, 2.60 mmol) and mPEG2000 (4.0 g, 2.0 mmol) in dry THF (50 mL) under inert argon atmosphere at ice-cooled temperature. The solution was stirred for 48 h and warmed up to 28 °C. After being cooled to room temperature, the reaction mixture charged into an extraction funnel and diluted with ethyl acetate (100 mL) and transferred to an extraction funnel. Next, the reaction mixture washed with brine (2 × 100 mL) and deionized water (3 × 100 mL), respectively.

The organic residue was then collected and dried over anhydrous sodium sulfate. After the volatiles were removed by rotary evaporator under completely closed vacuum system, the organic residue was purified by column chromatography to give pure alkyne compounds.

Yield: 4.09 g (85%, gravimetrically) $M_{n,NMR}$: 2320 g/mol; $M_{n,GPC}$: 1980 g/mol; M_w/M_n : 1.48. FT-IR (cm^{-1}): 3445 (O-H); 2887 (C-H); 1734 (C=O). ^1H NMR (500 MHz, CDCl_3 , δ , ppm): 3.74 (t, $-\text{COOCH}_2\text{CH}_2\text{O}$ in mPEG); 3.65 (t, $-(\text{OCH}_2\text{CH}_2)\text{O}-$ and $-\text{COOCH}_2\text{CH}_2\text{O}-$ in mPEG); 3.38 (s, CH_3OCH_2- in mPEG); 0.69, 0.89, 0.96 (d, $-\text{CH}_3$ moiety in cholic acid).

Synthesis of miktoarm star-shaped polymer (mPEG-CA-(PCL)₃)

Miktoarm star-shaped polymer was synthesized by ROP of ϵ -CL using mPEG-CA as core center. The mPEG-CA (2.0 g, 0.830 mmol), 2-ethylhexanoic acid tin(II) salt ($\text{Sn}(\text{Oct})_2$) (0.067 g, 0.166 mmol), and ϵ -CL (5.687 g, 49.822 mmol) were added to a flask which filled and discharged three times with inert argon atmosphere. Under the inert argon atmosphere, solution was degassed via flushing for 5 min. After the reaction flask was safely and properly closed, it was stirred at 125 °C for 24 h. The solution was dropped to the room temperature and the organic residue was dissolved in DCM (5 mL) and precipitated in cold methanol. The raw product was collected by vacuum filtration with a G4 glass filter and then the obtained powder form polymer was dried at room temperature in reduced pressure until having a constant weight.

Yield: 7.22 g (94%, gravimetrically) $M_{n,NMR}$: 9210 g/mol; $M_{n,GPC}$: 7980 g/mol; M_w/M_n : 1.36. FT-IR (cm^{-1}): 2947 (C-H); 2875 (C-H); 1722 (C=O); ^1H NMR (500 MHz, CDCl_3 , δ , ppm): 4.06 (m, $\text{CH}_2\text{O}(\text{C}=\text{O})$ in PCL); 3.77 (t, $-\text{COOCH}_2\text{CH}_2\text{O}$ in mPEG); 3.65 (t, $-(\text{OCH}_2\text{CH}_2)\text{O}-$ and $-\text{COOCH}_2\text{CH}_2\text{O}-$ in mPEG); 3.38 (s, CH_3OCH_2- in mPEG); 2.31 (m, $\text{O}(\text{C}=\text{O})\text{CH}_2$ in PCL); 1.64 (m, $\text{O}(\text{C}=\text{O})\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{O}(\text{C}=\text{O})$ in PCL); 1.38 (m, $\text{O}(\text{C}=\text{O})\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{O}(\text{C}=\text{O})$ in PCL); 0.68, 0.91, 0.97 ppm (d, $-\text{CH}_3$ moiety in cholic acid).

Preparation of thin films and wettability performance

The thin films of mPEG-CA-(PCL)₃ and mPEG-CA polymers were dissolved in water at room temperature with a concentration of 50 mg/mL. Glass slides (76 × 26 mm) were cleaned with warm chromic acid solution, washed with deionized water, and dried. The obtained polymer solutions (1.5 mL) were coated onto the glass slides by spin coating method at 1500 rpm for 20 s using Laurell EDC-650-15B spin coater. The coated glasses were dried at 150 °C for 12 h in a vacuum drier [44]. The equilibrium Contact Angle (θ_{eq}) and tilt angle (θ_{Tilt}) values were collected by using by an attention theta optical tensiometer with tilt angle apparatus. The θ_{eq} values were determined after the needle was removed from 5- μL droplets by a dispenser-controlled hypodermic syringe-needle. The θ_{Tilt} measurements were determined according to the tilting method, the solid sample has to be tilted from its horizontal position at 1.5° / second surface rotation speed. In image analysis, the slope of the surface when the liquid first moves on the surface is reported as the tilt contact angle [44]. Five different θ_{eq} and θ_{Tilt} measurements were done on each polymer film surface, and the average values were reported with standard deviations of $\pm 1^\circ$.

Surface tension measurement of the polymer films

Surface tension measurements were carried out via pendant drop method by using Attension Theta Optical Tensiometer [44]. Polymer solutions were homogenized in ultrasonic bath at room temperature for 30 min and mixed with magnetic stirrer for next 8 h at room temperature. The surface tension experiments were established to measure the polymer solution from high concentration (400 mg/mL) to low concentration (5 mg/mL) by diluting with water. The concentration was plotted against the surface tension values obtained. Each measurement was repeated 5 times.

Results and discussion

The synthesis of AB₃ type miktoarm star-shaped polymers was accomplished by the combination of esterification reaction and ring opening polymerization (ROP) method with two-step synthetic procedure. In the first step, esterification reaction of CA with mPEG enabled to prepare linear polymer (mPEG-CA), which was utilized as a precursor of ROP of ϵ -caprolactone to obtain star-shaped miktoarm polymer (mPEG-CA-(PCL)₃) (Scheme 1). The esterification reaction of CA with mPEG was conveniently monitored by FT-IR and ^1H -NMR spectroscopy techniques. After the esterification reaction, the characteristic peaks of both

CA and PEG components including (C=O) at 1734 cm^{-1} , (C-H) at 2887 cm^{-1} , (O-H) at 3445 cm^{-1} , and C-O-C at 1120 cm^{-1} were observed in FT-IR spectrum of mPEG-CA as shown in Fig. 1. Upon ROP of ϵ -caprolactone in the last step, the carbonyl (C=O) peak at 1734 cm^{-1} showed a stronger intensity confirmed the presence of PCL arms in the mPEG-CA-(PCL)₃.

The chemical structures of linear and star polymers containing CA unit were also confirmed by ^1H -NMR spectroscopy measured in CDCl_3 . The characteristic methyl ($-\text{CH}_3$), methylene ($-\text{CH}_2-$) and methine ($-\text{CH}-$) protons of CA were seen in Fig. 2a. The methyl protons ($-\text{CH}_3$) of CA and methoxyl protons ($(-\text{O}-\text{CH}_3)$, $(-\text{OCH}_2\text{CH}_2)\text{O}-$ and $-\text{COOCH}_2\text{CH}_2\text{O}-$) coming from mPEG chains were determined at 0.55–0.93 ppm (He, Hg and Hf), 3.38 ppm (Ha), 3.65 ppm (Hb and Hc) and 3.74 ppm (Hd), respectively (Fig. 2b). The successful synthesis of (PCL)₃-CA-mPEG star polymer was confirmed by the appearance of new peaks belonging to PCL repeating units at 2.31 ppm (Hh) (repeating unit $\text{O}(\text{C}=\text{O})\text{CH}_2$), 1.64 ppm (Hi) (repeating unit $\text{O}(\text{C}=\text{O})\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{O}(\text{C}=\text{O})$), 1.38 ppm (Hj) ($\text{O}(\text{C}=\text{O})\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{O}(\text{C}=\text{O})$) in the spectrum (Fig. 2c).

In order to support these data, the molecular weights characteristics (average molecular weight and molecular weight distribution) of linear and star-shaped polymers were examined by using GPC and ^1H -NMR. Due to the difficulty on seeing methylene groups next to OH groups of PCL and methylene protons (He) of cholic acid, calculation of molecular weights ($M_{n,\text{NMR}}$) was done by using the peak area ratios of much clear methyl group protons (Ha) of cholic acid core to one of the repeating unit's proton (Hj) of PCL block. This calculation method gave $M_{n,\text{NMR}} = 2320$ and 9210 g mol^{-1} for mPEG-CA and mPEG-CA-(PCL)₃, respectively. There was a good correlation between molecular weight ($M_{n,\text{GPC}}$) measured by GPC and molecular weight ($M_{n,\text{NMR}}$) calculated

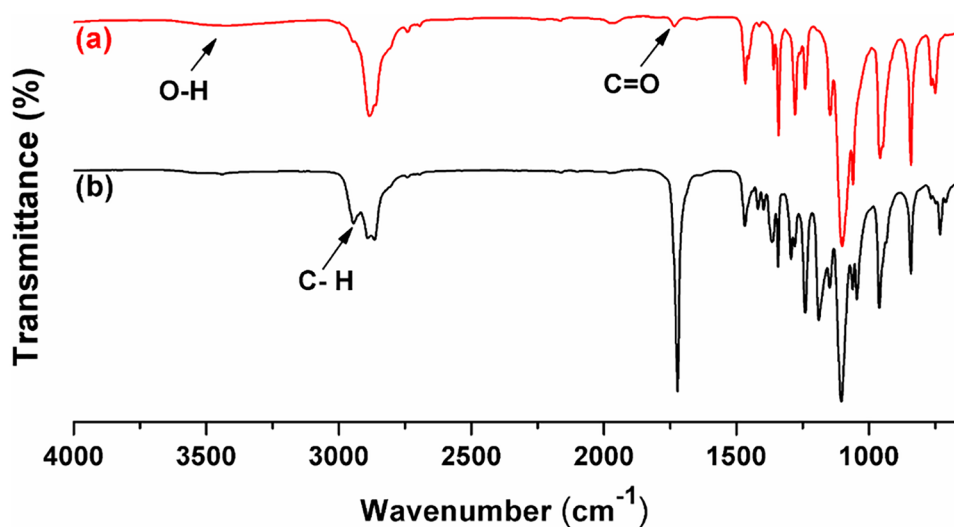
by ^1H -NMR analysis (Fig. S1). Furthermore, both linear and star-shaped polymers displayed low polydispersity indexes ($\bar{D}$) below 1.5. The reaction yields were determined by gravimetric analysis and they were found as 85 and 94% for linear and star polymers. The monomodal molecular weight characteristic and narrow polydispersity indexes were proof of the successful syntheses of them.

Thermal transitions of linear and star-shaped polymers were also investigated by DSC analysis under nitrogen atmosphere (Fig. S2) and the data are shown in Table 1. The melting temperature (T_m) of mPEG-CA-(PCL)₃ showed slightly lower temperature value than that of the precursor mPEG-CA. This could be the effect of PCL arms that acted as plasticizer for mPEG-CA. Since the PCL melted first, the previously melted blocks depressed the T_m of the mPEG-CA-(PCL)₃ [45–47].

Thermal stabilities of synthesized mPEG-CA and mPEG-CA-(PCL)₃ were also investigated by TGA analyses (Fig. S3) and related data were shown as a summary in Table 1. Having three more PCL arms in the structure of miktoarm star polymers didn't make any significant change on maximum degradation temperature (T_{max}) and char yield as compared with the linear polymer. Both polymers gave single decomposition stage between 278.6 and $393.6\text{ }^\circ\text{C}$, however the incorporation of PCL reduced the onset temperature from 354.1 to $278.6\text{ }^\circ\text{C}$. Additionally, the final weight residue at $650\text{ }^\circ\text{C}$, for mPEG-CA and mPEG-CA-(PCL)₃ were 2.8 and 4.3 wt%, respectively.

Surface properties including θ_{eq} , θ_{Tilt} , root mean square (RMS) roughness, and surface tension (ST) data of the mPEG-CA and mPEG-CA-(PCL)₃ polymers were collected in Table 2. According to these results, both mPEG-CA and mPEG-CA-(PCL)₃ films displayed hydrophilic character. However, the θ_{eq} and θ_{Tilt} values were increased when the PCL unit was added the polymer structure due to not only

Fig. 1 FT-IR spectra of (a) mPEG-CA, and (b) mPEG-CA-(PCL)₃



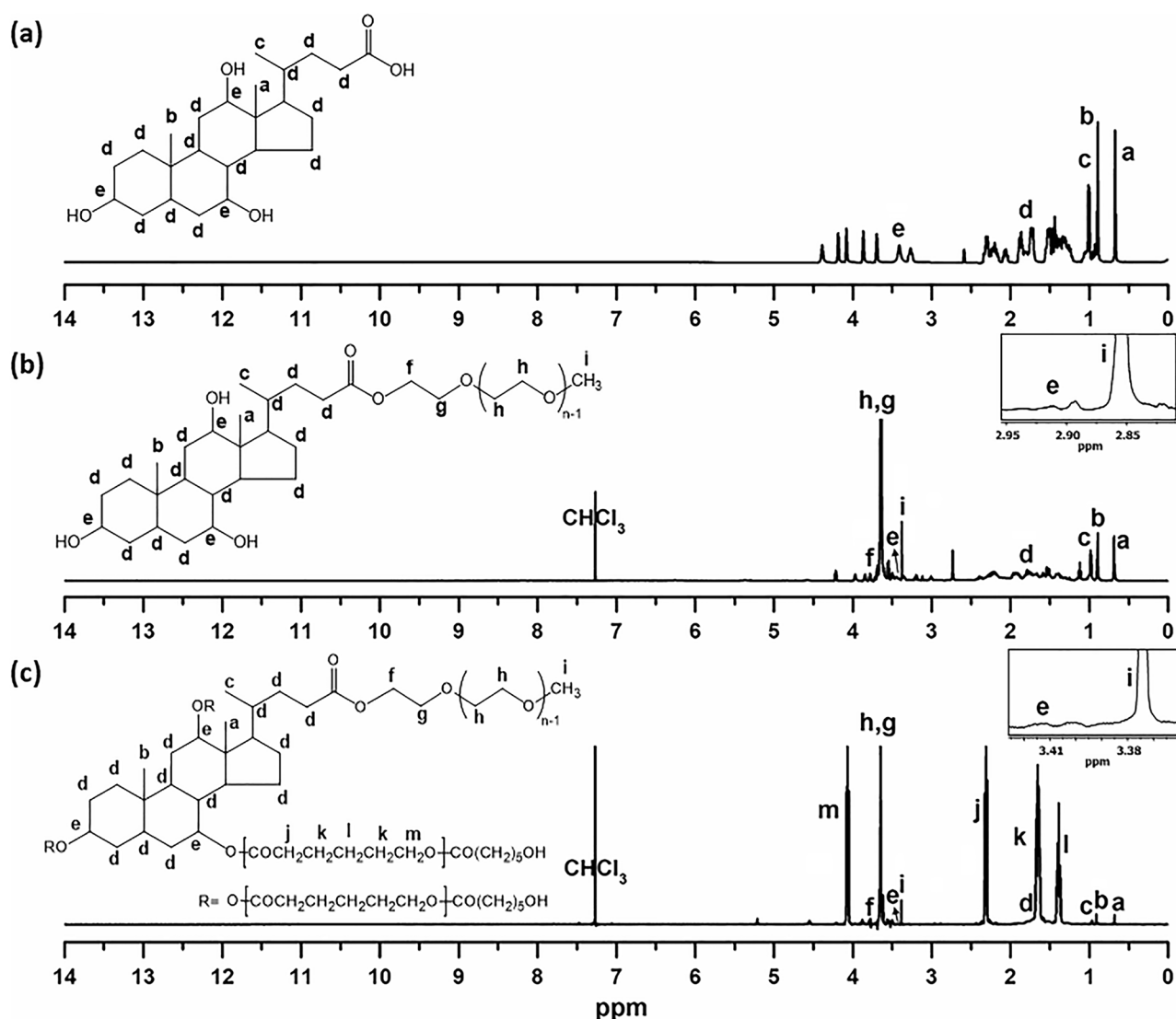


Fig. 2 ^1H NMR spectra of (a) CA, (b) mPEG-CA and (c) mPEG-CA-(PCL) $_3$ in CDCl_3

the PCL more hydrophobic properties but also increasing the surface roughness. In addition to this, water drop is also pinned the PCL pendant group, so the θ_{Tilt} value is increased (Table 2).

Surface tension values of the linear mPEG-CA and star mPEG-CA-(PCL) $_3$ solutions were found as 45.5 mN/m and 52.3 mN/m, respectively (Table 2). These values were calculated the critical micelle concentration (CMC) graph

Table 1 Molecular characteristics and thermal properties of the linear and miktoarm star polymers

Entry	$M_{n,NMR}$ (g mol^{-1}) ^a	$M_{n,GPC}$ (g mol^{-1}) ^b	$\bar{D}$ ^b	T_m ($^{\circ}\text{C}$) ^c	T_{onset} ($^{\circ}\text{C}$) ^d	T_{max} ($^{\circ}\text{C}$) ^d	Char Yield (%) ^e
mPEG-CA	2320	1980	1.5	55.5	354.1	391.2	2.8
mPEG-CA-(PCL) $_3$	9210	7980	1.4	51.4	278.6	393.6	4.3

^a $M_{n,NMR}$ was calculated according to ^1H NMR analysis

^b $M_{n,GPC}$ and $\bar{D}$ were obtained via GPC analysis using linear polystyrenes with narrow molecular weight distributions as calibration standards. THF was used as the mobile phase

^c T_m denotes the melting point of the polymers in the second heating run of the DSC experiments

^d T_{onset} is the onset decomposition temperature of the polymers in TGA experiments

^e T_{max} is the temperature corresponding to the maximum rate of weight loss in TGA experiments

^fThe percent of char yield at 600 $^{\circ}\text{C}$ in TGA experiments

Table 2 Surface and self-assembly properties of mPEG-CA and mPEG-CA-(PCL)₃

Sample	θ_{eq} (°)	θ_{Tilt} (°)	RMS (nm)	ST (mN/m)
mPEG-CA	13	8	13	45.5
mPEG-CA-(PCL) ₃	35	25	26	52.3

(Fig. 3). An increase in the polymer concentration resulted in a decrease in the surface tension values that was compatible with the typical surfactant graphics. The red line of the graphs was showed the constant values of the surface tension of polymer solution that implied polymer saturated on the liquid surface. This polymer saturated constant region was called the CMC values [48]. The surface tension differences between mPEG-CA and mPEG-CA-(PCL)₃ (their structures were illustrated in Scheme 1) were due to the adding pendant PCL structure. The mPEG-CA-(PCL)₃ solution reached the CMC values earlier due to the not only attaching the functional pendant group on the cholic acid head but also reaching liquid saturation with fewer polymer molecules. The reason for the mPEG-CA-(PCL)₃ having higher surface tension values was that it took up more space on the liquid surface of the additional arm length coming from PCL.

As clearly seen in SEM images, spherical micelles were formed in both mPEG-CA and mPEG-CA-(PCL)₃ thin films (Fig. 4). However, there was a significant difference between them, where several crack structures occurred in mPEG-CA-(PCL)₃ film. This crack should be caused by the long PCL unit stress delocalization due to the phase segregation on the thin films during evaporation compatible with literature [49–52]. Katsumata et. al. fabricated the PCL containing blend materials. They indicated that increase in the PCL content in the blend resulted in increase in the void (or crack) size due to the inhomogeneous stress delocalization [49]. Han et al. also reported the stress

delocalization as an unbalanced stress cause of the lamellar twisting. They emphasized that the PCL containing polymers has a spherulite morphology due to the lamellar twisting [51, 52]. Ye et al. fabricated six-arm star-shaped PCL based on cyclophosphazene core by using a hydroxy-terminated cyclophosphazene derivative. They indicated that the morphology of the star shape polymer having 1 wt % PCL was very smooth and few cracks is observed. However, they also reported the tremendous cracks morphology with increasing the PCL content [50]. In addition to this, the contact angle value of mPEG-CA-(PCL)₃ film was much higher than the mPEG-CA's value (Table 1). The pinning on the crack structure of mPEG-CA-(PCL)₃ films increased the contact angle value of the sample. The AFM analysis demonstrated that RMS values were measured as 13 nm to 26 nm for mPEG-CA and mPEG-CA-(PCL)₃ accordingly. Although there was an increase on the values by having the long PCL units of the structure (Fig. 4), this effect was not expected impact of PCL due to entanglement (agglomeration) of the chains. When the SEM images and the RMS differences were examined, it was speculated that the long PCL unit agglomerated at the thin film formations. This might be explained as an agglomeration caused the RMS differences and crack structure of the mPEG-CA-(PCL)₃ films. In our case, PCL units interacted at the liquid surface and saturated on the liquid surfaces at the less polymer concentration (Fig. 3). Thus, the surface tension of the mPEG-CA-(PCL)₃ was slightly increased compared to the mPEG-CA.

The obtained polymer micelles were characterized by SEM and particle size and distribution analysis program. With the help of these programs, micelle lengths and surface areas were determined on the SEM image. At least 100 micelles on average were determined, their lengths were measured and their surface areas were determined with the help of the program. According to these data, the average

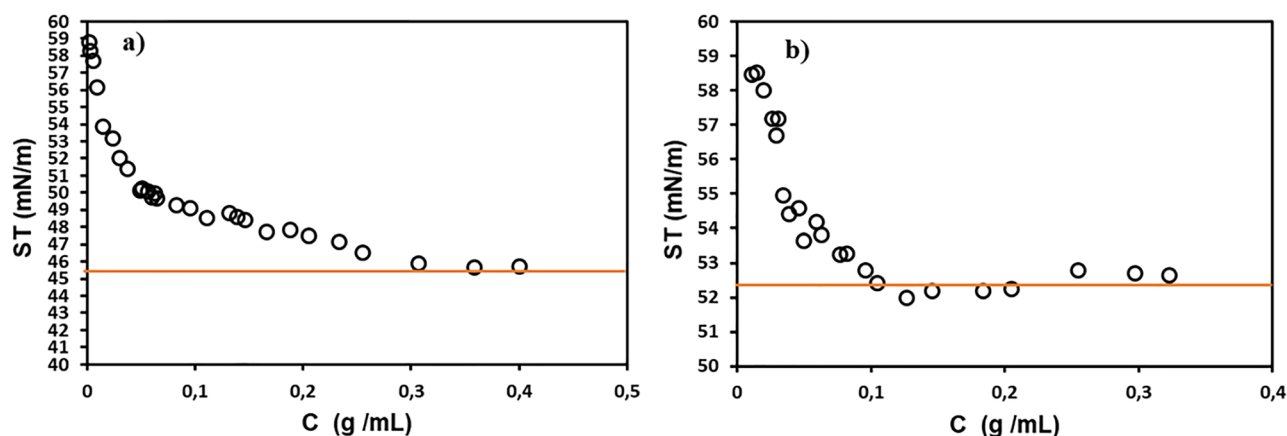
**Fig. 3** Surface tension measurements of **a)** mPEG-CA and **b)** mPEG-CA-(PCL)₃ samples

Fig. 4 SEM and AFM images of **a)** mPEG-CA and **b)** mPEG-CA-(PCL)₃

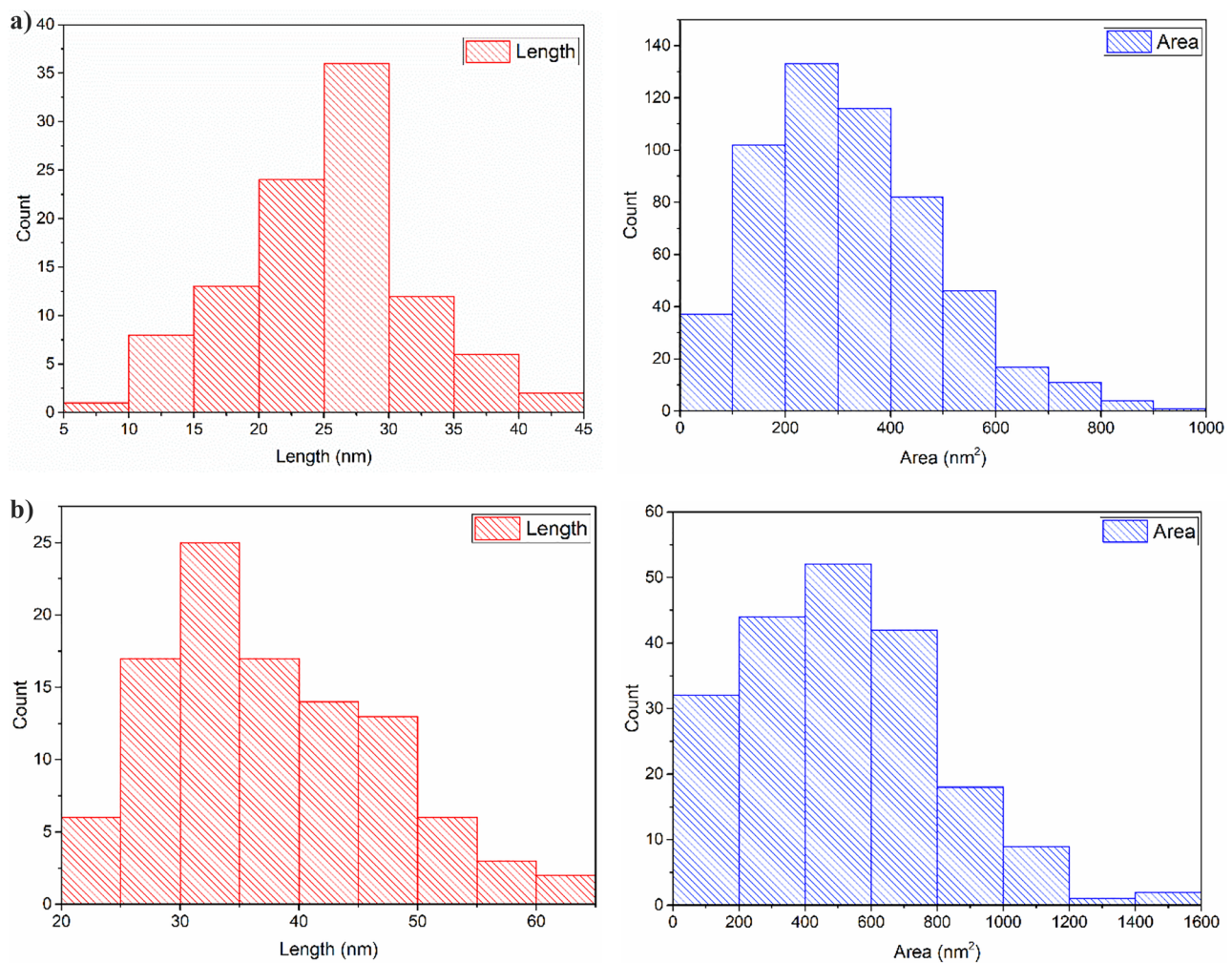
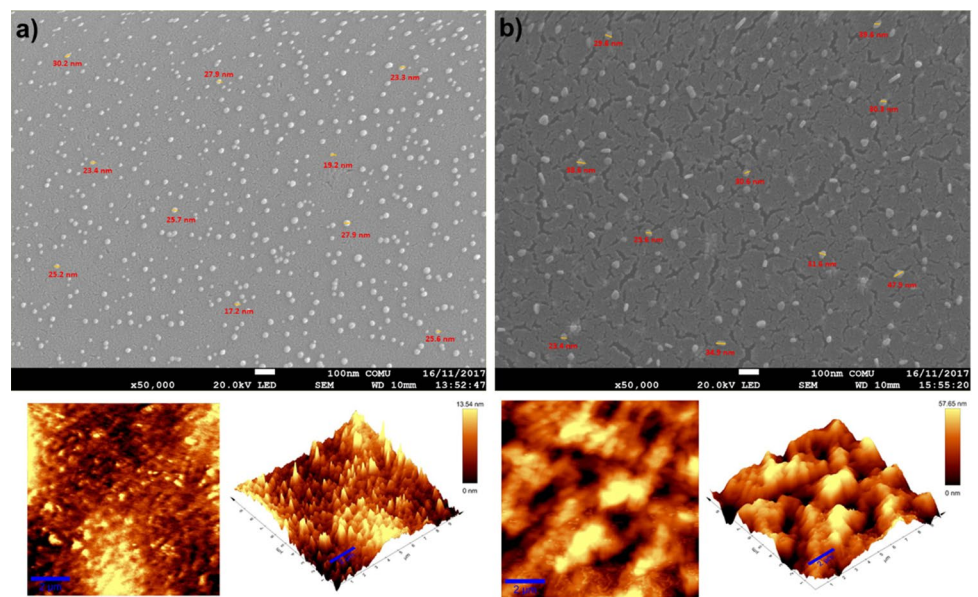


Fig. 5 Histogram of the particle size distribution of **a)** mPEG-CA and **b)** mPEG-CA-(PCL)₃

lengths of the mPEG-CA and mPEG-CA-(PCL)₃ polymers were found to be 25.2 nm and 37.4 nm respectively, while their surface areas were found to be 323.9 nm² and 469.0 nm², respectively (Fig. 5). According to these results, it has been observed that the micelle lengths and surface areas of the mPEG-CA-(PCL)₃ polymer are higher than the mPEG-CA polymer. The increase in the molecular weight of the polymer with the increase in the number of polymer arms causes the mPEG-CA-(PCL)₃ polymer to be a larger structure than the mPEG-CA polymer [53]. Therefore, the micelle dimensions of the mPEG-CA-(PCL)₃ polymer were found to be higher in the micelles obtained. In addition, the star shape of the mPEG-CA-(PCL)₃ polymer makes a structural difference compared to the linear mPEG-CA polymer. The CMC increased with the increase of crystallinity of mPEG-CA-(PCL)₃ due to having PCL arms compared to linear polymers [54].

Conclusions

Cholic acid containing linear mPEG-CA and star-shaped miktoarm mPEG-CA-(PCL)₃ polymers were successfully synthesized with esterification of mPEG and following ring-opening polymerization of ϵ -caprolactone. The characteristic ether and ester bands of mPEG and PCL arms in both FTIR and ¹HNMR spectroscopy as well as a clear shift of GPC chromatogram clearly proved the chemical structure of miktoarm star-shaped polymer. On the other hand, the melting temperature of mPEG-CA-(PCL)₃ was slightly lower than that of the precursor mPEG-CA. By monitoring of the obtained mPEG-CA and mPEG-CA-(PCL)₃ polymers with AFM and SEM analyses, spherical micelles were formed in water. It was also found that the size of the micelles and contact angle properties was changed with the addition of PCL and PEG functional arms on the cholic acid head. Furthermore, the surface tension of the water decreases with addition of two polymers due to the increasing polymer concentration and remained constant at certain critical micelle concentration values. The obtained polymers could self-assemble into micelles with low critical micellar concentrations. The micelles were in spherical shapes and nano-scale particle sizes (less than 100 nm) with narrow size distributions. With adding hydrophobic PCL block chains, micelle sizes increased. As a result of the experiments, cholic acid containing linear mPEG-CA and star-shaped miktoarm mPEG-CA-(PCL)₃ exhibit surfactant behavior and micelle formation. Therefore, synthesized amphiphilic linear and star-shaped polymers based on a cholic acid core can be used in several applications to obtain wetting effects with low foam properties compare to anionic surfactants due to their nonionic nature. Moreover, These polymers as a biosurfactant can

be used in biomedical applications such as drug delivery systems, biomedical devices due to their biocompatible and biodegradable properties.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s10965-021-02564-4>.

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