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SYNTHESIS AND CHARACTERIZATION OF POLY(ε-CAPROLACTONE)–POLY(ETHYLENE GLYCOL)–POLY(ε-CAPROLACTONE) COPOLYMERS: INVESTIGATION OF THE EFFECT OF BLOCKS ON MICELLIZATION

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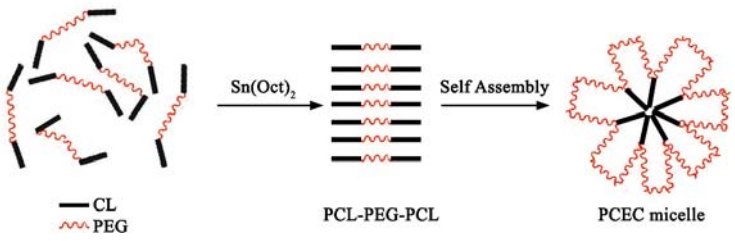
Poly(ε-caprolactone)–poly(ethylene glycol)–poly(ε-caprolactone) copolymers are important synthetic biomedical materials with amphiphilicity, controlled biodegradability and great biocompatibility. This work reports synthesis and characterization of Poly(ε-caprolactone)–poly(ethylene glycol)–poly(ε-caprolactone) triblock copolymers for drug delivery. Triblock copolymers were synthesized by ring-opening polymerization. Molecular weight of used poly(ethylene glycol) is 1450, 3350 and 12000 g/mol and weight ratios of epsilon-caprolactone / poly(ethylene glycol) are 0.5, 1, 2, 24. Synthesis of triblock copolymers was confirmed by ^{1}H-NMR and triblock copolymer micelle formation was studied by fluorescent technique. According to the ^{1}H-NMR spectra peaks at 1.42, 1.62, 2.34, and 4.09 ppm are assigned to methylene protons of – $(CH_2)_3$–, –$OCCH_2$–, and –CH_2OOC– in poly(ε-caprolactone) units, respectively. The sharp single peak at 3.66 ppm is attributed to the methylene protons of homosequences of the poly(ethylene glycol) oxyethylene units. The critical micelle concentrations of the polymers were in the range of 0.000293–0.019202 mg/mL indicating an excellent dynamic stability.



INTRODUCTION

Most studies to date involve ABA triblock copolymers.[1] Among the investigated amphiphilic block copolymers, the biodegradable copolymers are of special interest. The biodegradable polymers, such as poly (ε-caprolactone) (PCL) have been used as important biomaterials for a wide variety of drug delivery carriers because of their biocompatibility and biodegradability.[2] However, the potential applications of PCL are considerably restrained by the high hydrophobicity, rather high crystallinity and the inadequate interaction between PCL and cells, leading to in vivo foreign body reactions, such as inflammation, infections, local tissue necrosis, and implant encapsulation as well as thrombosis. These drawbacks may obstruct its application in drug-controlled release systems. So as to improve these problems, hydrophilic polyether blocks, poly(ethylene glycol) (PEG), or poly(ethylene oxide) (PEO), have been incorporated into degradable polyester backbones for its hydrophilicity, non-toxicity, biocompatibility, nonimmunogenicity and filterability through kidney when the molar mass is below 40,000.[3]

Like their low-molecular-weight counterparts, amphiphilic polymers associate in water to form "polymeric micelles", consisting of a hydrophobic

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core stabilized by a corona of hydrophilic polymeric chains exposed to the aqueous environment.[4] Micelles composed of amphiphilic block copolymers have been shown to possess significant potential as delivery systems providing several advantages when used as a drug-delivery system for hydrophobic drugs. The aqueous solubilities of a number of drugs have been significantly increased by incorporation into copolymer micelles.[5, 6]

There are two components to the stability of a micellar system, thermodynamic stability and kinetic stability.[7] At very low concentrations, the molecules are freely dispersed in aqueous media. As the concentration is increased, the free energy of the system rises because of unfavourable interactions between the hydrophobic domains and surrounding water molecules. At a specific concentration, termed the critical micelle concentrations (CMC), amphiphilic molecules with the appropriate geometry, orient themselves in such a way that the hydrophobic segments are isolated from the aqueous environment, achieving a state of minimum energy that leads to the formation of colloidal assemblies termed micelles. At concentrations above the CMC, the micelles are in dynamic equilibrium with free molecules but are thermodynamically stable and tend to resist disassembly. Below the CMC, however, the micelles will dissociate at a rate that depends mainly on the nature of the amphiphile and the degree of interaction between the molecules. Compared to low molecular weight surfactants, block copolymer micelles generally exhibit lower CMC values and greater resistance to dissociation upon dilution.[8,9] Thus, polymeric micelles are more stable toward dilution in biological fluids. They can increase drug bioavailability and retention, since the drug is well protected from possible inactivation under the effect of their biological surroundings.[4] It has been known for many years that block copolymers form micelles upon dissolution into a solvent selective for one of the blocks.[10] The theories of polymer micellization predict that in the presence of micelles, the concentration of free, unassociated block copolymers is close in magnitude to that of the CMC.[11]

Various techniques are routinely used to determine the CMC in aqueous solution. The most commonly applied methods are conductivity, voltammetry, calorimetry, scattering techniques, surface tension, UV/vis, and fluorescence spectroscopy, which all are based on an abrupt change in the related physical properties upon micelle formation. Since fluorescence spectroscopy is quite more sensitive than optical absorption, the design of new fluorescent probe molecules is a subject of intense research.[12]

Since the assembled micelles prepared from large molecular weight PEG has not been characterized before, in this study the CMC of the mentioned assembled structures were investigated. In these experiments the effect of PCL/PEG ratio and caprolactone quantity on the fluorescence of pyrene present in water at near saturation, 6 x 10^{-7} M was examined.

RESULTS AND DISCUSSION

PCEC triblock copolymers were prepared by ring-opening polymerization of ε-CL monomer in the presence of PEG without any other catalysts. An active hydrogen atom at one end of PEG chains acts as an initiator and induces a selective acyl-oxygen cleavage of ε-CL (Figure 1).

Characterization of PCEC triblock copolymers

The PCEC triblock copolymers with different molecular weight were prepared by changing the molar ratio of ε-CL monomer / PEG homopolymer and PEG molecular weights and composition were determined by ^{1}H NMR spectroscopy. The molecular weights of the triblock products were determined by ^{1}H NMR end group analysis following Eq. (1):[13]

$$M_n^{NMR} = \frac{I^h / 2x114}{(I^a + I^b + I^c)/4x44} x M_n^{DHPEG} + M_n^{DHPEG} = \left[\frac{I^h / 2x114}{\left(I^{a,b,i}\right)/4x44} + 1 \right] x M_n^{DHPEG} \quad (1)$$

where M_n^{NMR} (g/mol) and M_n^{DHPEG} (g/mol) is molecular weight of PCEC and PEG, respectively and I^h, $I^{a,\ b,\ i}$ were integral intensities of peaks at about 4.09 and 3.66 ppm, respectively in ^{1}H-NMR spectrum of PCEC copolymers (Figure 2).

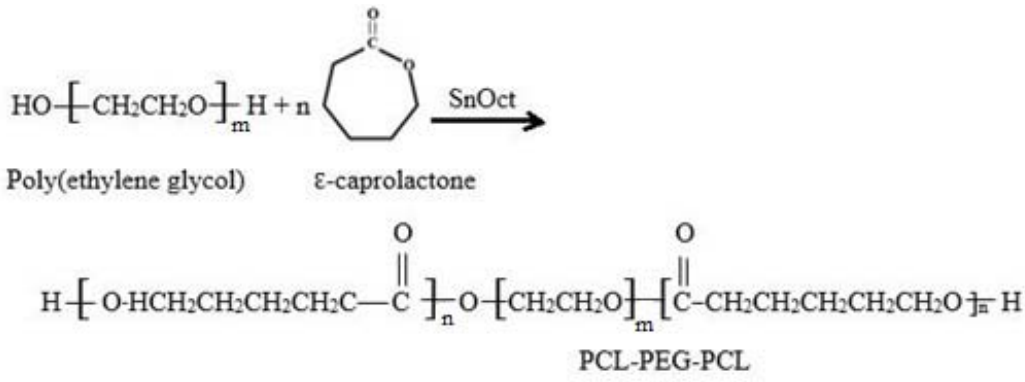

Fig. 1 – Synthesis scheme of PCEC triblock copolymers.

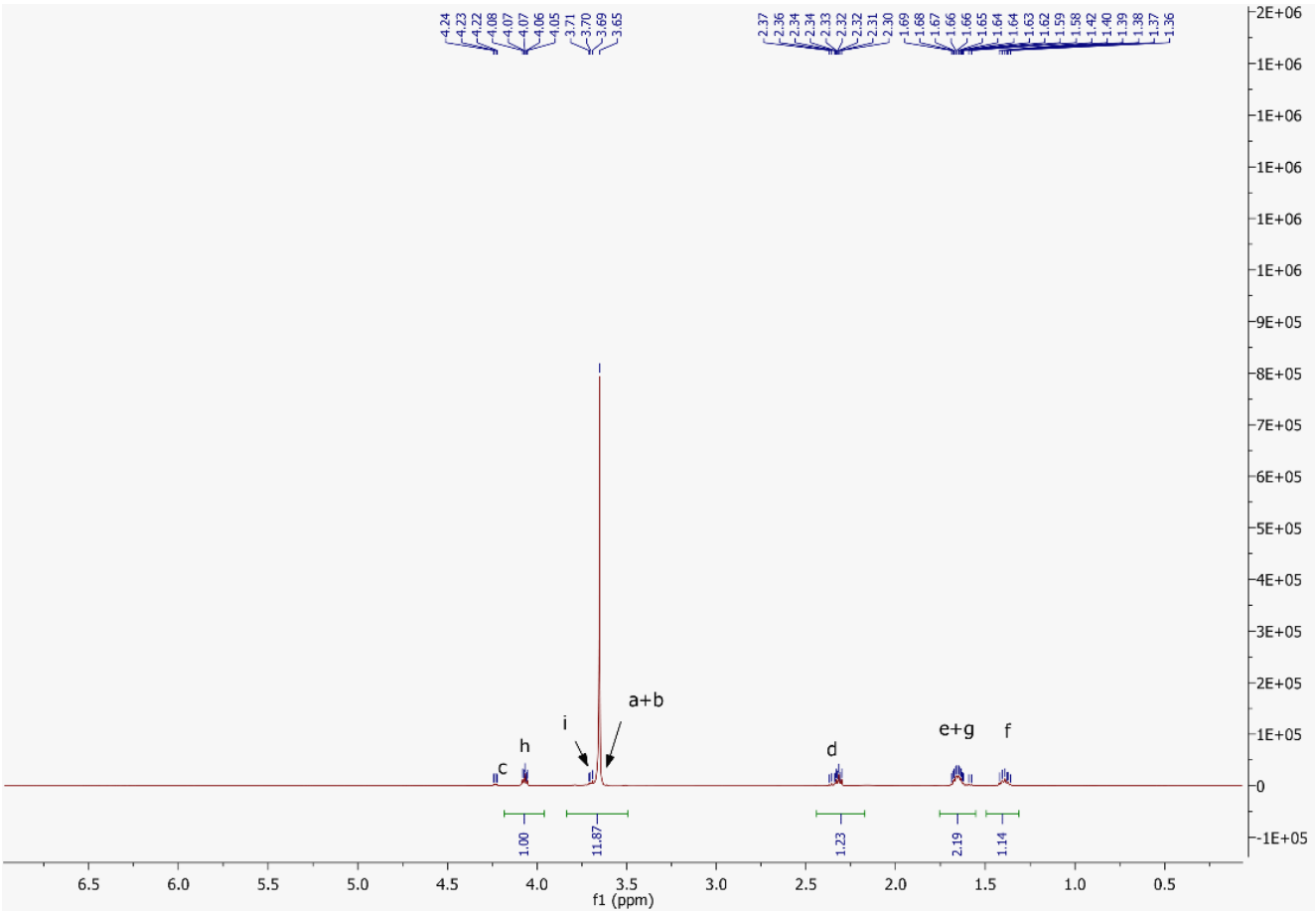

Fig. 2 – ^{1}H NMR spectrum of numbered PCEC8 in $CDCl_3$.

Table 1

Characterization of PCEC triblock copolymers

Sample	Mn of PCL[a] (g/mol)	Mn of PCL[b] (g/mol)	Total Mn[c] (g/mol)	CMC[d] (mg/mL)	Hydrophobic/Hydrophilic Ratio (g/mol)
PCEC 1	35779	34800	37229	*	24.68
PCEC 2	2645	2900	4095	0.002081	1.82
PCEC 3	1218	1450	2671	0.010000	0.84
PCEC 4	486	725	1936	0.019202	0.34
PCEC 5	52595	80400	55953	*	15.70
PCEC 6	5583	6700	9234	0.000841	1.76
PCEC 7	2231	3350	5581	0.002818	0.67

Table 1 (continued)

PCEC 8	1461	1675	4812	0.004467	0.44
PCEC 9	259080	288000	271090	*	21.59
PCEC 10	20724	24000	32727	0.000293	1.73
PCEC 11	10428	12000	22433	0.000457	0.87
PCEC 12	5412	6000	17411	0.001000	0.45

[a] Number average molecular weight of PCL was estimated from the results of ^{1}H NMR.
[b] Number average molecular weight of PCL was estimated from the results of theoretical calculations.
[c] Calculated from the ^{1}H NMR results of PCL and PEG. MW of PEG was 1450, 3350 and 12000.
[d] Evaluated from fluorescence spectroscopy measurement using pyrene as a hydrophobic probe.
* No formation.

^{1}H NMR spectrum of numbered PCEC8 in $CDCl_3$ is given in Figure 2. The unit ratio of PEG and ε -caprolactone was obtained from peak intensities of the methylene proton of the PEG chain and methylene proton in ε-caprolactone units, respectively. According to the H NMR spectra peaks at 1.42, 1.62, 2.34, and 4.09 ppm are assigned to methylene protons of $-(CH_2)_3-$, $-OCCH_2-$, and $-CH_2OOC-$ in PCL units, respectively. The sharp single peak at 3.66 ppm is attributed to the methylene protons of homosequences of the PEG oxyethylene units.[14] NMR spectra showed any evidence of additional compounds such as unreacted monomer or side-reaction products. The degree of polymerization could be controlled by varying the ratio of ε-caprolactone to PEG in the feed stock. The percentage compositions determined from ^{1}HNMR spectroscopy were close to those expected theoretically on the basis of the comonomer feed ratios, confirming full monomer-to-polymer conversion during synthesis. The molecular weights and composition of the block copolymers were obtained from ^{1}HNMR summarized in Table 1. The PCL block molecular weights were 35779, 2645, 1218, 486, 52595, 5583, 2231, 1461, 259080, 20724, 10428, 5412 g/mol as determined by ^{1}H NMR corresponding to the prepared block copolymers of PCEC1, PCEC2, PCEC3, PCEC4, PCEC5, PCEC6, PCEC7, PCEC8, PCEC9, PCEC10, PCEC11, PCEC12, respectively (Table 1). The PCEC molecular weights were 37229, 4095, 2671, 1936, 55953, 9234, 5581, 4812, 271090, 32727, 22433, and 17411 g/mol as determined by ^{1}H NMR corresponding to the prepared block copolymers of PCEC1, PCEC2, PCEC3, PCEC4, PCEC5, PCEC6, PCEC7, PCEC8, PCEC9, PCEC10, PCEC11, PCEC12, respectively (Table 1).

Measurement of critical micelle concentration

Below the CMC amphiphilic molecules have a strong tendency to be adsorbed at the air–water interface. With the increase of amphiphile concentration in the system, a point is reached when both the interface and the bulk of the solvent (water) become saturated with monomeric amphiphiles. At this point (CMC point), any further increase in amphiphile concentration leads to the formation of micelles within the bulk phase and subsequent decrease in the free energy of the system. Below the CMC, the increase in amphiphile concentration leads to the decrease of the surface tension, while above CMC the surface tension remains constant at increasing concentrations of an amphiphile, evidencing the saturation of the interface with an amphiphile and micelle formation in the bulk phase. At CMC and slightly above it, the micelles are still loose and contain some water in the core. With further increase in amphiphile concentration in the medium, the unimer:micelle equilibrium shifts towards micelle formation, micelles become more tight and stable, lose the residual solvent from the core and decrease their size.[15]

The CMC is an effective parameter of micellar stability and a low critical value is desired.[3] The CMC is used to estimate the lowest formation concentration of the micelles in water. The CMC is a particularly important parameter for micelle-based drug delivery systems to avoid burst release upon injection into the bloodstream.[16] The CMC of the copolymer indicates at which point the micelles will disassemble upon dilution.[17]

The CMC of a copolymer can be determined with methods using the fluorescence probe pyrene.[5] Pyrene has been used for more than 50 years as fluorescent probe par excellence for microheterogeneous systems such as micelles, polymers, proteins, peptides and biological membranes. The sensitivity of the pyrene fluorescence intensity to the solvent polarity is widely used for the determination of the CMC of micellar systems.[18]

Two methods exist for determining the critical association concentration (CAC) of polymeric micelles with pyrene fluorescence. The original method, proposed by Kalyanasundaram *et al.*, takes advantage of the changes in the vibronic fine

structure of the pyrene emission and monitors the changes in the ratio of the intensities I_1 and I_3 of the [0,0] and [0,2] bands, respectively. More recently, it has been suggested that a more accurate determination of the CAC can be obtained by monitoring the changes in the ratio of the pyrene excitation spectra intensities at λ = 333 nm for pyrene in water and λ = 336 nm for pyrene in a hydrophobic medium. When micelles are formed in an aqueous medium, the pyrene tends to locate itself inside the hydrophobic core, increasing I_3 intensity. By plotting the I_{336}/I_{333} intensity ratios vs. the logarithm of the concentration of the aqueous solutions of copolymer, sigmoidal curves are obtained, where, at the CAC, a sharp increase is observed in the fluorescence intensity ratio (I_{336}/I_{333}) as the polymer concentration increases.[4,19,20] Luo and co-workers also reported shift in the (0,0) band of pyrene to 337 nm in the micelles of $PMAA_{25}$-b-$PNIPAAm_{48}$.[21] The latter method was used in this study to estimate the CMC of the PCEC triblock copolymers.

The excitation spectra of pyrene in copolymer solutions with the different concentration of PCEC copolymers were acquired and the intensities of absorptions at 337 (I_3) and 334 (I_1) nm were plotted as I_3/I_1 ratio versus polymer concentration in solution (Figure 3). The I_{337}/I_{334} versus log C plots presents a sigmoid curve, which reflects the whole process of micellization. A negligible change of intensity ratio of I_{337}/I_{334} was observed at low concentration range for each PCEC copolymer. With an increase in the copolymer concentration, the intensity ratio exhibits a substantial increase at a certain concentration; reflecting the incorporation of pyrene into the hydrophobic core region of the micelles. The intersection of the lower horizontal tangent and the slope tangent corresponds to the cmc for each micelle in Figures 3, 4, and 5.

Inset in Figure 3 shows the fluorescence excitation spectra of pyrene as a function of the polymer concentration for Sample PCEC1 in water solution. The intensity increased with increasing polymer concentration, and the characteristic (0,0) band of pyrene shifted from 334 to 337 nm when the polymer concentration increased from 3.16 x 10^{-6} to 1 mg/mL which was the threshold concentration of self-aggregation formation of PCEC micelles by intra- and/or intermolecular association in an aqueous solution. The same shift was observed earlier for PCEC.[22] The pyrene probe transferred from water phase into the micelle during this process due to its hydrophobicity and the microenvironment of pyrene changed from high-polar water to less-polar micelle. Obviously, the fluorescence intensity increases tremendously and the maximal peak position shifts with increasing polymer concentration depended mainly on the relative hydrophobic nature of the micelle cores, *i.e.*, more hydrophobicity of the micelle core should induce a larger maximal peak shift.[23] As the concentration of block copolymer molecules increases, an equilibrium of state shifts to a micellar form. Insoluble blocks in micellar cores rearrange to find their low-energy conformation, and the solvent molecules are gradually driven out of the micellar cores. At higher polymer concentrations, large collapsed micellar cores consisting of many insoluble blocks are present, surrounded by diffuse outer shells (coronas) formed from the soluble blocks.[24]

As shown in Table 1, the critical micelle concentrations of the polymers were in the range of 0.000293–0.019202 mg/mL. For products PCEC1, PCEC5 and PCEC9 no shift for pyrene peaks were observed, means micelle formation was not seen due to their high hydrophobic/hydrophilic ratio, so other measurements did not performed using these products.

As shown in Table 1 CMC values of PCEC block copolymers were decreased with the increase of PCL block chain length, *i.e.* the shorter the PCL chain length, the higher the CMC values. These results displayed a similar tendency to the previous reports.[3, 22, 25-27] According to Table 1 CMC values are in the following order: PCEC10 < PCEC11 < PCEC6 < PCEC12 < PCEC2 < PCEC7 < PCEC8 < PCEC3 < PCEC4. In our results, we observed that CMC values were influenced by hydrophobic PCL chain length, not by molecular weight (Table 1).

The CAC of amphiphilic copolymers is determined by many factors, such as the nature and length of the core-forming segments, and the length of the hydrophilic chain. Amphiphilic copolymers that contain highly hydrophobic residues have lower CAC values in water than those that include the less hydrophobic residues. For a series of copolymers, if the corona-forming chain is kept constant, an increase in the molecular weight of the core-forming segment will decrease the CAC. This is because when the chain length of PCL is increased, the hydrophobic segments of PCL can pack more efficiently, and the hydrophobic interactions within the core of micelles are increased. Hence, the PCEC copolymers can more easily self-assemble in aqueous solution to form micelles, resulting in increased micelle stability in aqueous solutions at relatively lower concentrations. To a lesser extent, if the length of the core-forming segment is maintained at a constant length, an increase in the

length of the hydrophilic chain will cause an increase in the value of the CAC.[4,16] The CMC is decreased for higher PCL/PEG block ratios. Low CMC can be attained by modulating the copolymer compositional ratios, which is important for improving the micellar stability, preventing the disassociation and non-targeted drug release of the micelles in the bloodstream, and reducing the toxicity of drug.[21]

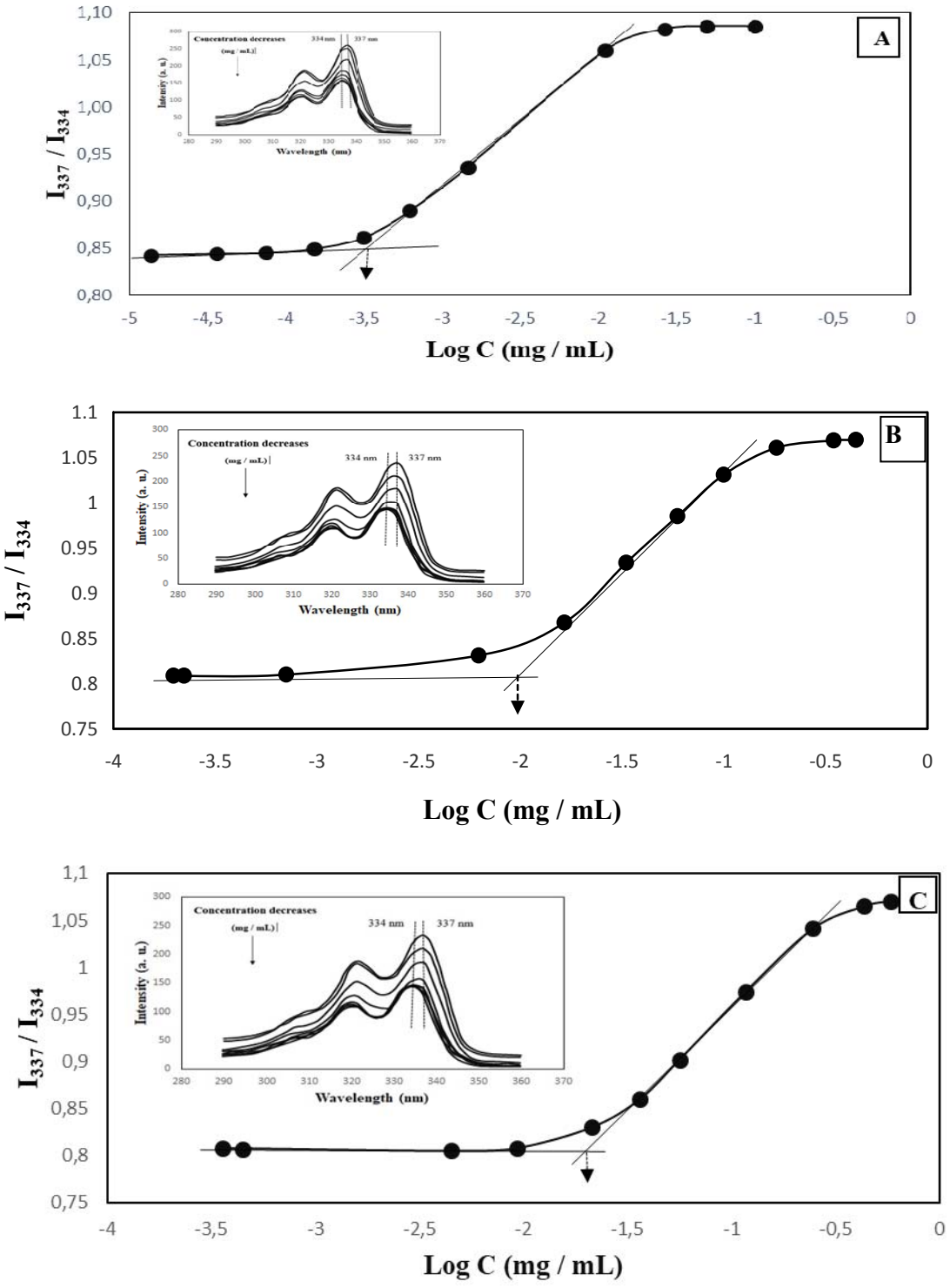

Fig. 3 – Plot of the intensity ratio I_{337}/I_{334} (from pyrene excitation spectra) versus log C (mg/mL) of: PCEC2 (**A**); PCEC3 (**B**); PCEC4 (**C**) in the distilled water. Inset: Fluorescence excitation spectra of pyrene (6.0×10^{-7} M) against PCEC concentration in distilled water (emission wavelength: 390 nm).

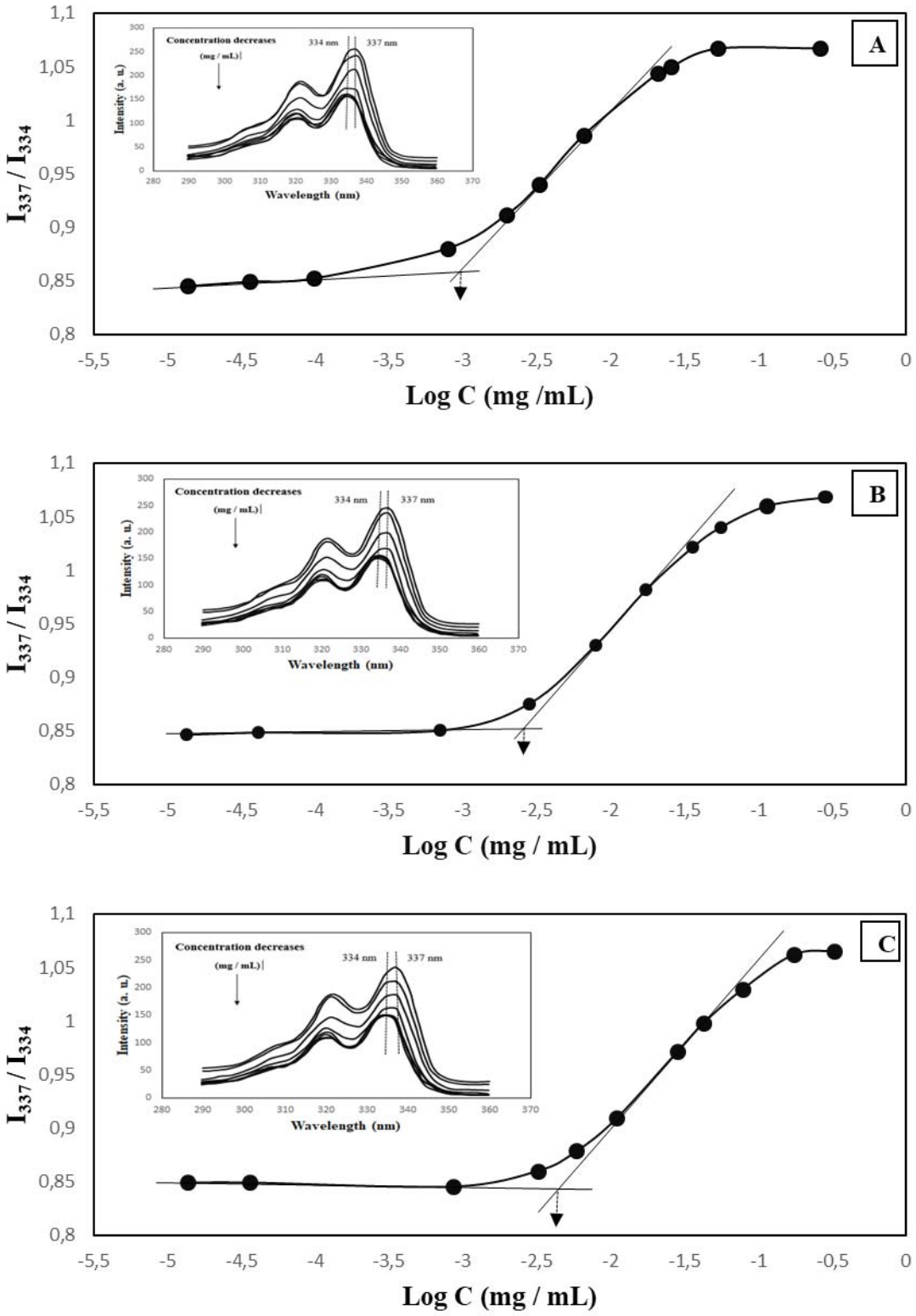

Fig. 4 – Plot of the intensity ratio I_{337}/I_{334} (from pyrene excitation spectra) versus log C (mg/mL) of: PCEC6 (**A**); PCEC7 (**B**); PCEC8 (**C**) in the distilled water. Inset: Fluorescence excitation spectra of pyrene (6.0×10^{-7} M) against PCEC concentration in distilled water (emission wavelength: 390 nm).

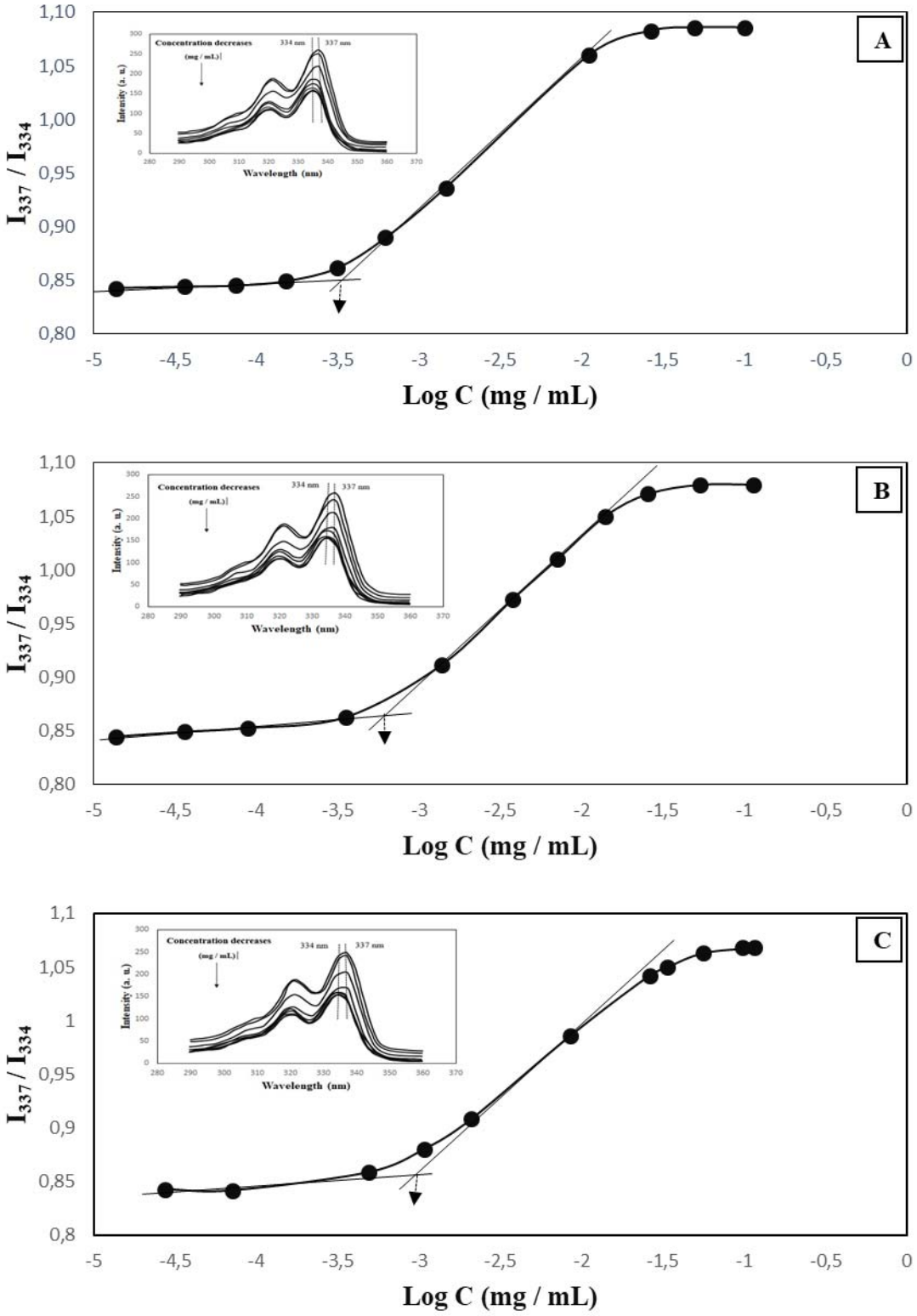

Fig. 5 – Plot of the intensity ratio I_{337}/I_{334} (from pyrene excitation spectra) versus log C (mg/mL) of: PCEC10 (**A**); PCEC11 (**B**); PCEC12 (**C**) in the distilled water. Inset: Fluorescence excitation spectra of pyrene (6.0×10^{-7} M) against PCEC concentration in distilled water (emission wavelength: 390 nm).

An increase in the length of the core-forming block also cause an increase in the core size per micelle which, in turn, results in an increased loading capacity per micelle. For example, for any one block copolymer system, as one increases the length of the core-forming block the aggregation

number increases, resulting in a larger core size and thus a larger cargo space per micelle. However, due to the increase in the aggregation number per micelle, the total number of micelles in solution will decrease per unit mass of polymer.[28]

The CMC for PCEC triblock copolymers decreased from 0,019202 to 0,000293 mg/mL as the proportion of incorporated hydrophobic PCL increased (Table 1). The modest increases in the PCL block length still produced a decrease in CMC confirming previous reports that the hydrophobic block length is the primary factor determining the CMC.[17] The CMC values for these triblock copolymers show that these triblock copolymers all have lower CMC values, providing evidence for an apparent stability of micelles and allowing their use in very dilute aqueous milieu such as body fluid. These CMC values are similar those reported for PCEC (2–3.89 μg/mL);[26] PCL_{1000}–PEG_{6000}–PCL_{1000}, PCL_{1250}–PEG_{6000}–PCL_{1250}, PCL_{1350}–PEG_{6000}–PCL_{1350} were 6.35 x 10^{-3} mg mL^{-1}, 2.54 x 10^{-3} mg mL^{-1} and 3.53 x 10^{-3} mg mL^{-1}, respectively; [29] PCEC-1, PCEC-2, PCEC-3 and PCEC-4 were 11.3, 7.71, 6.23 and 1.44 mg/L, respectively;[3] core cross-linked PCEC micelles showed CMC in the range of 1 x 10^{-3} to 2 x 10^{-3} mg/mL [30]; PCL-PEG-PCL copolymer was determined to be 0.0002 g/L;[22] PC_{20}-E_{40}-C_{20}, PC_{50}-E_{40}-C_{50} and PC_{100}-E_{40}-C_{100} were 4.94 x 10^{-3}, 4.33 x 10^{-3} and 2.63 x 10^{-3} wt %, respectively;[27] PC_{20}-E_{40}-C_{20}, PC_{50}-E_{40}-C_{50} and PC_{100}-E_{40}-C_{100} were 5.43 x 10^{-3}, 4.17 x 10^{-3} and 2.16 x 10^{-3} wt %, respectively[16] which were much lower than those of low molecular weight surfactants, *e.g.*, 2.3 g/L for sodium dodecyl sulfate (SDS) in water.[31] Also the CMC of PCECs was generally lower than that of low-molecular-weight surfactant micelles, such as deoxycholic acid with a CMC of 1.0 x 10^{3} mg L^{-1} and sodium dodecyl sulphate with a CMC of 2.3 x 10^{3} mg L^{-1}.[32]

The use of a copolymer system with a low CMC value may increase the in vivo stability of the micelles. However, in many papers, the disassembly of micelles into single chains is mentioned to be advantageous since this will facilitate elimination of the copolymer material from the body via the kidneys. Therefore, the ideal micelle system will be stable to sink conditions encountered upon injection and will facilitate elimination by eventual disassembly into single chains.[28]

EXPERIMENTAL

Materials

Poly (ethylene glycol) (Mn = 1450, 3350, and 12000 g/mol) purchased from Sigma and was dried by azeotropic distillation using anhydrous toluene. ε-Caprolactone (CL) was purchased from Sigma and purified with CaH_2 by vacuum distillation. Stannous octoate $Sn(Oct)_2$ was purchased from Aldrich and used as received. All other commercially available solvents were purchased from Merck Chemical Co. and were used reagent grade without further purification.

Poly(ε-caprolactone)–poly(ethylene glycol)–poly(ε-caprolactone) (PCEC) triblock copolymer synthesis

The polymerization of the PCEC copolymer, based on ε-CL ring-opening, is illustrated schematically in Figure 1. The PCEC triblock copolymer was synthesized using a ring-opening polymerization of ε-caprolactone in the presence of PEG as a macroinitiator and $Sn(Oct)_2$ as a catalyst.[33] The advantage of this method was avoiding toxic substances that resided in the resulting copolymers. Briefly, PCEC copolymer was prepared by introducing a known amount of ε-caprolactone and PEG (weight ratios of ε-CL/PEG: 0.5, 1, 2, 24, respectively) under nitrogen atmosphere into a two necked vessel equipped with a stirrer, a thermometer and a gas inlet tube, and several drops of $Sn(Oct)_2$ were added. The vessel was kept at 130^0C. During polymerization, the system was stirred slowly, and the viscosity increased with time. After 6h, the reaction system was rapidly heated to 180^0C under vacuum for another 20 min. After cooled to room temperature under nitrogen atmosphere, the PCEC copolymer was first dissolved in methylene chloride and reprecipitated from the filtrate using AR grade excess cold petroleum ether. Then the mixture was filtered and vacuum dried to constant weight. The obtained purified PCEC copolymers were kept in air-tight bags in desiccators before use.[34] PCEC1; PCEC2; PCEC3; PCEC4; PCEC5; PCEC6; PCEC7; PCEC8; PCEC9; PCEC10; PCEC11 and PCEC12 represent PEG1450, ε-CL/PEG 24; PEG1450, ε-CL/PEG 2; PEG1450, ε-CL/PEG 1; PEG1450, ε-CL/PEG 0.5; PEG3350, ε-CL/PEG 24; PEG3350, ε-CL/PEG 2; PEG3350, ε-CL/PEG 1; PEG3350, ε-CL/PEG 0.5; PEG12000, ε-CL/PEG 24; PEG12000, ε-CL/PEG 2; PEG12000, ε-CL/PEG 1, and PEG12000, ε-CL/PEG 0.5, respectively.

Characterization of copolymers

^{1}H nuclear magnetic resonance spectrometer (NMR) measurement

^{1}H NMR spectra (in $CDCl_3$) were recorded on Bruker Avance III 500 MHz Spectrometer (Bruker, Germany) at 500 MHz using tetramethylsilane 0.03% (v/v) as an internal reference standard. The d_1 waiting time was set to 2 s, and the number of scans was 128. Spectra were recorded from -4 to 16 ppm. Peak positions and areas were analyzed using MestReNova Pro software to determine the compositions of the block copolymers. As the number-average molecular weight (M_n) of PEG is known, one can estimate the number-average molecular weights of the PCL block and the copolymer composition as calculated from the peak intensities in the spectrum assigned to both polymers. The number average molecular weight and the PEG/PCL ratio were estimated by integrating the signals pertaining to each monomer, such as the peaks from CH_2 of ethylene glycol and CH_2 of PCL.

Measurement of fluorescence spectroscopy

Pyrene molecule had a strong hydrophobic character and a very low solubility in water. Because pyrene preferentially solubilised itself into the hydrophobic region of micelles, the fluorescence intensity was greatly affected by the environmental change around pyrene.[32] To estimate the critical micelle concentrations of block copolymers,

pyren[24,31,35,36] was used as a hydrophobic probe. CMC of the PCEC triblock copolymers were estimated to prove the potential of core-shell type micelle formation by the measurement of fluorescence spectroscopy (Fluorescence excitation and emission spectra were recorded on a Varian Eclipse spectrofluorometer using 1 cm pathlength cuvettes at room temperature. To obtain sample solutions, a known amount of pyrene in acetone was added to each of a series of 20 ml amber vial and the acetone was also removed under vacuo. The final concentration of pyrene was 6.0×10^{-7} M. A volume of 10 mL of various concentrations (3.16×10^{-6} to 1 mg/mL) of block copolymer solutions was added to each vial and then heated for 3 h at 65°C to equilibrate the pyrene and the block copolymer solution, and left to cool overnight at room temperature. Emission wavelength was 390 nm for excitation spectra. Excitation and emission bandwidths were 1.5 and 1.5 nm, respectively. The ratio of fluorescence intensity at 334 and 337 nm (I_{337}/I_{334}) was calculated and plotted against the logarithm of the copolymer concentrations.

CONCLUSIONS

Varying the ratio of ε-caprolactone to PEG in the initial reaction mixture could control the composition of the copolymer.

The low CMC values for PCEC triblock copolymers indicate a high tendency of the triblock copolymers toward formation of micelles in aqueous solutions and the low CMCs thus signify that the micelle aggregates are comparatively stable and can be applicable to highly dilute aqueous media such as body fluids. The encapsulated drug would not prematurely release from the micelles during the circulation until it reaches a specific target.[21]

As can be seen in Table 1 and as previously discussed, as the hydrophobic block length increased, the CMC decreased. It has been shown that a decrease in the CMC results in an increase in the total number of copolymer molecules participating in the formation of micelles thus increasing the number of micelles in solution available for the solubilization of the solute.

Stability of micelles both in vitro and in vivo, as well as their clearance from the body, depends on their CMC values. The low CMC values of polymers showed that they form stable micellar forms and keep their intact structure upon dilutions with body liquids thus they would protect the drug until the target site.

The following general regularities can be applied to characterize the role of different blocks in micelle stability: (a) the increase in the length of a hydrophobic block at a given length of a hydrophilic block causes a noticeable decrease in CMC value and increase in micelle stability; (b) the increase in the length of a hydrophilic block at a given length of a hydrophobic block results in only a small rise of the CMC value; (c) the increase in the molecular weight of the unimer at a given hydrophilic/hydrophobic ratio causes some decrease in the CMC value.[15]

These CMC values are good enough for a drug carrying micelle. We suggest that these copolymers may be suitable micellar carriers of hydrophobic P-glycoprotein (P-gp) drug substrates, given the formation of stable micellar solutions, shown in this work. Work is going on to explore the loading characteristics of these micelles with hydrophobic drugs.

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SYMBOLS and ABBREVIATIONS

PCEC	Poly(ε-caprolactone)–poly(ethylene glycol)–poly(ε-caprolactone)
PEG	Poly(ethylene glycol)
PCL	Poly(ε-caprolactone)
PEO	Poly(ethylene oxide)
CMC	Critical micelle concentration
M	Molarity (mol / L)
Mn	Molecular weight (g / mol)
$Sn(Oct)_2$	Stannous octoate
h	Time (hour)
s	Time (second)
I^h, $I^{a,\,b,\,i}$	Integral intensities of peaks
CAC	Critical association concentration
λ	Wavelength

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