

Synergistic Effect in Drug Solubility by New Binary Micelles of Poly(ϵ -caprolactone)-poly(ethylene oxide) and F127®

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Binary mixtures of block copolymers E₁₁₄CL₂₀ and E₉₇P₆₉E₉₇ (F127®) were prepared in order to tailor a drug delivery system with synergistic effect, concerning dilution stability and high drug solubility. Both reduction of the critical micelle concentrations (CMC) values and enhance hydrophobic drug solubility in F127/E₁₁₄CL₂₀ mixtures (30-50 wt.%) were observed by addition of the diblock E₁₁₄CL₂₀, also reaching the best enhancement of drug solubility for mangiferin and carbamazepine.

Keywords: poly(ϵ -caprolactone)-poly(ethylene oxide), binary mixtures, synergistic effect, drug solubility

Introduction

Although copolymers-self-assembled micelles have been extensively studied into pharmacological field as drug delivery systems (DDS), hydrophobic drugs solubility and biodisponibility still have been a truth challenge, concerning their biodistribution in the final medicine assays.¹⁻⁴ However, co-micelles with two different copolymers have attracted much attention, once they can act as “smart nanocarriers” through synergistic effect, in which compensate undesirable responses, and tailor desirable physicochemical properties.⁵⁻⁷

In particular, poly(ϵ -caprolactone), a polyether-polyester copolymer class, has been widely researched as anti-cancer DDS, mainly due to amphiphilic properties, also showing good biocompatibility and biodegradability, and low toxicity levels.^{3,8,9} Another class of copolymer, the so-called Pluronic®, also has shown useful properties to pharmacological applications such as sol-gel transition and biocompatibility. Instead, these nonionic surfactants, composed by a generically triblock-type structure E_nP_mE_n (poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene

oxide)), are limited-usage, since the amphiphilic nature of the polymer promotes self-assembly of the molecules into a micelle, composed by a weak hydrophobic core of poly(propylene oxide), forming solutions with high critical micelle concentration (CMC) values.^{3,10-12}

Therefore, the main aim of this work is to provide a “new” drug delivery system, concerning the synergistic property of co-micelles core of poly(ϵ -caprolactone) and poly(propylene oxide) from E₁₁₄CL₂₀ and F127®, respectively, in which may promote a higher drug solubility with low CMC.^{11,13} Three poor water soluble drugs were investigated: carbamazepine,¹⁴ quercetin^{13,15} and mangiferin¹⁶ based on drug/core micelles interactions (see Figure 1). Additionally to their antioxidants properties, quercetin and mangiferin have been gaining much attention due to anti-tumor activity, acting by different mechanisms on cancer cells/tumor.^{17,18}

Experimental

Materials

Copolymer E₁₁₄CL₂₀ (CL₂₀) (E = ethylene oxide and CL = ϵ -caprolactone blocks) was synthesized and

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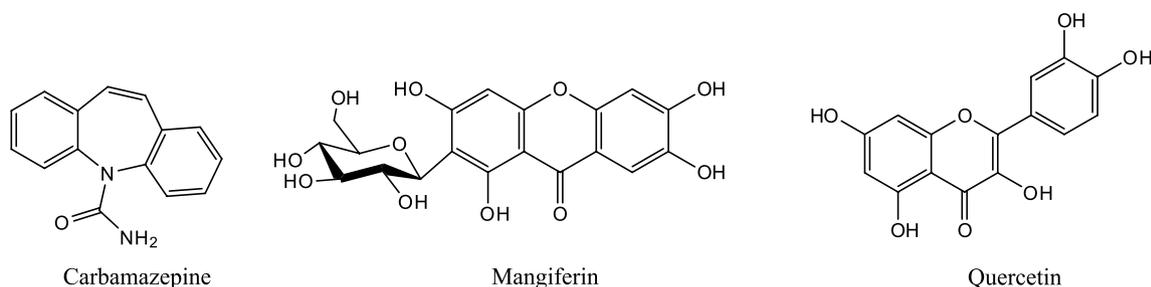


Figure 1. Chemical structure of the drugs.

characterized in the School of Chemistry, Manchester, through anionic polymerization, following well-known synthesis reaction.¹⁹ F127[®] copolymer (E₉₇P₆₉E₉₇, E = ethylene oxide and P = propylene oxide blocks), commercially available as Pluronic[®] or Lutrol[®], was purchased by Uniqema (ICI surfactants, United Kingdom). Molecular characteristics of the copolymers are shown in Table 1. The fluorescent dye DPH (1,6-diphenyl-1,3,5-hexatriene) was supplied by Biochemika (Germany). Carbamazepine was supplied by Sigma-Aldrich (Poole Dorset, UK). Mangiferin and quercetin were donated by Natural Products Laboratory at Federal University of Ceará (Brazil) and Flora Brasil LTDA (Brazil), respectively, and used as received. For aqueous experiments, was used Milli-Q water, and all other reagents were in analytical grade.

Table 1. Molecular characteristics of the copolymers E₁₁₄CL₂₀ and F127[®]

Copolymer	$M_n / (\text{g mol}^{-1})^a$	W_E^b	W_h^c	M_w / M_n^d
E ₁₁₄ CL ₂₀	7296	0.680	0.320	1.36
F127 [®]	12510	0.689	0.311	1.20

^aAverage number of molecular weight by nuclear magnetic resonance (¹³C NMR); ^bmass fraction of hydrophilic portion of copolymer, “E” block; ^cmass fraction of hydrophobic portion of copolymer, “P” and “CL” blocks; ^dpolydispersity index by gel permeation chromatography (GPC).

Binary mixtures preparation

The binary micelle solutions were prepared for each experiment by dissolving the copolymers to desired concentrations: (F127)_{1-x} – (CL₂₀)_x, where “x” is the weight percentage of CL₂₀ in the mixture, 10, 30 and 50%, and were renamed as F/CL₂₀ 10, F/CL₂₀ 30 and F/CL₂₀ 50.

Dynamic light scattering (DLS)

The hydrodynamic diameter (D_h) of the micelles of copolymers F127[®], CL₂₀, and their mixtures F/CL₂₀ 30 and 50, with and without drug, were determined using a Nano Zetasizer, Malvern Zetasizer Nano ZS (ZEN 3500). The

measurements were obtained using the filtered aliquots from the solubilization procedure. The systems were investigated using 30 scans with 30 s acquisition time allowed for each scan. All measurements were made in triplicate.

Critical micelle concentration (CMC)

The following methodology was adapted from Alexandridis *et al.*,²⁰ and already used in other works of our group. Briefly, stock solutions were prepared by dissolving the copolymers in Milli-Q water during 24 h for complete dissolution. Then, the solutions were diluted to the required concentrations within the range 0.0001–1 g dL. DPH was dissolved in methanol and added into copolymer solutions, obtaining 1% (v/v) of copolymers in methanol and 0.004 mM of DPH. An instrument F-4500 Hitachi fluorescence spectrophotometer was used in the experiment. For all solutions, with temperature at 25 and 37 ± 0.2 °C, a fluorescence emission at 428 nm was measured with an excitation wavelength at 350 nm. Plots of fluorescence emission intensity *versus* log of copolymer concentration in mg dm⁻³ were used to determine the CMC.

Solubilization

Solubilization of the drugs was evaluated by the adapted method of dissolution from Richter *et al.*²¹ Previously, the systems were prepared by dissolving CL₂₀, F127[®] and their binary mixtures in acetone (ca. 20 mL), then ca. 10 mg of drug was added into each polymer solution, and stirred for at least 30 min. The solutions were rota-evaporated to remove acetone, forming a film of copolymer and drug. This film was resuspended in 10 mL of Milli-Q water and stirred at room temperature (26 ± 1 °C) for at least 16 h. Then, the resulted suspension was centrifuged at 13,000 rpm during 30 min in a Sorvall[®] centrifuge; model RC5CPlus, under room temperature control, and the supernatant was filtered (Millipore membrane, 0.45 μm) to remove non-soluble drug molecules. The solubilization of the systems (S-S₀), where S and S₀ are attributed to the drug total and water solubility, respectively, were determined measuring the absorbance

in a UV-Vis spectrophotometer (Instrutherm, UV 2000 A) by an absorbance (λ_{maximum}) for each drug (283, 256 and 375 nm for carbamazepine, mangiferin and quercetin, respectively).

Results and Discussion

CMC

The well-established fluorescence-based method, using DPH as the fluorescent probe, was used to determine copolymers onset of micellization in this work. Figure 2a shows the plots of fluorescence emission intensity against $\log C$ for F127 at two temperatures, and Figure 2b shows the results of CMC found in this work compared to theoretical values for all systems, according to equation 1.

In accordance to Chiapetta *et al.*,¹¹ we also used an

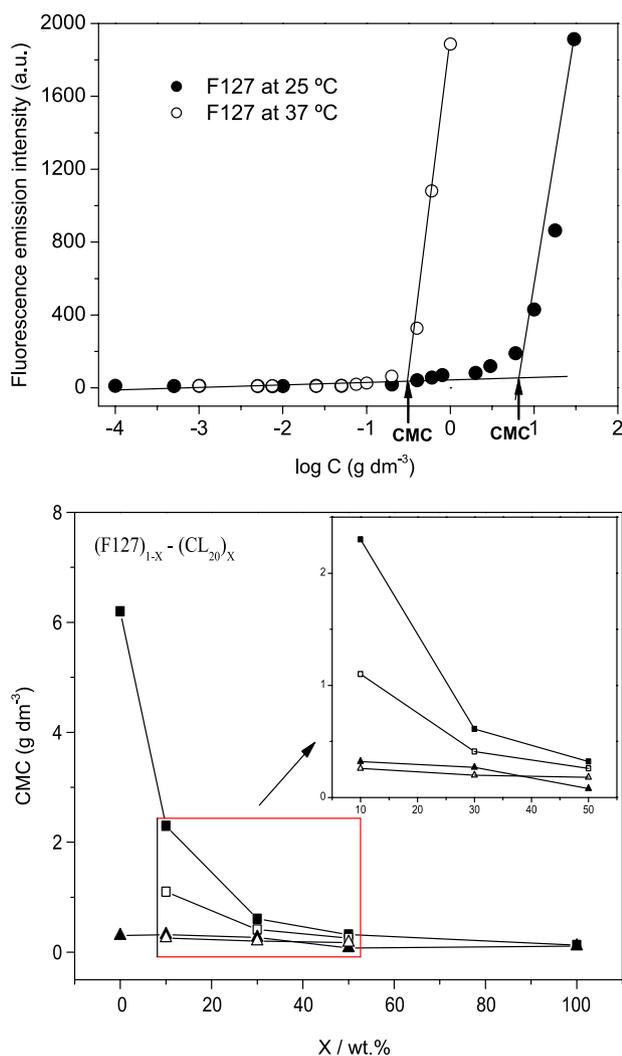


Figure 2. (a) Plots of fluorescence emission intensity of DPH versus $\log C$ of F127 solutions at 25 and 37 °C; (b) CMC values of copolymers and their mixtures: experimental (■) at 25 °C and (▲) 37 °C; theoretical (□) at 25 °C and (△) at 37 °C.

analytical model to preview theoretical values of CMC of binary solutions, in which assumes the formation of an ideal mixture of surfactants in the micelle and a phase separation model:

$$1 / \text{CMC}^* = X_1 / \text{CMC}_1 + X_2 / \text{CMC}_2 \quad (1)$$

where CMC* denotes the theoretical value for a binary mixture when no synergistic effect is present between the surfactants; X is the molar fraction of each surfactant in the total mixed solute, and the numbers 1 and 2 denote the two different surfactants in the mixture.

As expected, the CMC value obtained in this work for F127 (6.2 g dm⁻³ at 25 °C) was in accordance to those obtained by Alexandridis *et al.*,²⁰ 7 g dm⁻³ at 25 °C. For diblock CL₂₀, the CMC value was similar to the value obtained by Liu *et al.*¹⁰ for PEG 10,000-CL 5,000 (around 0.110 g dm⁻³), which also has similar hydrophobic balance. Additionally, Attwood *et al.*²² evaluated the CMC to E-CL diblock copolymers with similar hydrophilic E-block length, varying hydrophobic CL-block units. The CMC value found in this work was slightly higher when compared to E₁₁₄CL₃₆ CMC,²² around 0.110 and 0.003 g dm⁻³, respectively. Thereby, in agreement to the higher hydrophobicity, the lower is CMC.

There was a considerable variation in the copolymers CMC and their mixtures ranging temperature from 25 to 37 °C (Figure 2b), especially for the systems containing more proportion of F127, in accordance to Pluronic[®] thermosensitive properties, which can be explained by previous studies showing a more endothermic micellization process for E_nP_mE_n triblock type, with values of ΔH° micellization around 200 kJ mol⁻¹ or more.^{20,23}

CMC of the systems F/CL₂₀ 10, 30 and 50 decreased with increasing CL₂₀ proportion. This can be observed due to CL₂₀ has a lower CMC than F127, owing to its higher hydrophobicity core compared to poly(propylene oxide) from F127. Additionally, as already observed, CMC of copolymers decreases with increasing hydrophobicity, since micellization process reduces the unfavorable interactions of hydrophobic blocks with water, the so-called "hydrophobic effect".^{20,22,23}

Experimental CMC values of the mixtures obtained in this work are slightly different to the theoretical ones, as shown in Figure 2b. For both determinations, the decrease of CMC is a result of the CL₂₀ incorporation. At 25 °C, the experimental CMCs were lower than theoretical ones, showing a positive deviation from ideal behavior and also evidencing the synergistic effect. Instead, at 37 °C, the experimental CMC values were similar to theoretical ones. In general, they showed slightly greater results.

The formation of co-micelles is favorable when the surfactants have different HLBs (hydrophilic-lipophilic balance) and similar hydrophobic blocks with similar molecular weight.^{7,24} Besides F127 and CL₂₀ do not comply with this rule, once they have the same HLB and different hydrophobic block, in weights and structures (see Table 1), the good agreement of the theoretical with the experimental values may suggest a co-micellization process. As observed by Chiappetta *et al.*,¹¹ which have found co-micelles mixing F127 and poloxamines T304 and T904, also not complying with the stated rule for co-micellization.

Moreover, the obtained CMC values for all systems at 37 °C were slightly lower than at 25 °C, assuring that a copolymer solution saturated with drug at room temperature will not precipitate any drug when applied to the body, since the number of micelles increases at body temperature (ca. 37 °C).

Solubilization

The drug solubility ($S-S_0$, mg dm⁻³) in aqueous copolymers solutions of F127, CL₂₀ and their mixtures, prepared by the previously described “film” method,²¹ are shown in Figure 3. The drug solubilities (S_0) in water found in this work were: (i) 0.015 mg dm⁻³ for quercetin, (ii) 1.91 mg dm⁻³ for carbamazepine and (iii) 1.57 mg dm⁻³ for mangiferin. Additionally, according to Brazilian National Health Surveillance Agency (ANVISA), drug solubility in water around 1000-100 mg dm⁻³ are classified by poorly water soluble and/or non-soluble. Thereby, supporting the solubility results for these hydrophobic drugs, since S_0 values were < 100 mg dm⁻³.

Comparing copolymers $S-S_0$ alone, the solubility value was more efficient to CL₂₀ than F127 for all tested drugs,

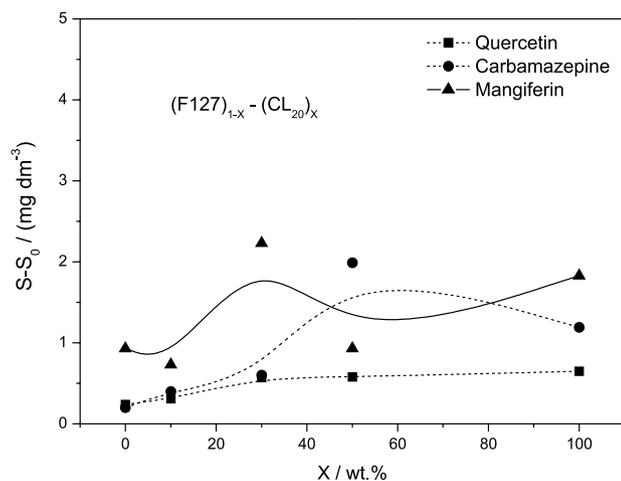


Figure 3. Solubilization ($S-S_0$) of quercetin, carbamazepine and mangiferin in 1 wt.% solutions of binary systems of F127 and CL₂₀ at ca. 25 °C.

due to CL block from E₁₁₄CL₂₀ is much more hydrophobic than P block from F127.

Systems which showed better encapsulation results were: (i) CL₂₀ for quercetin, with a solubility value of 0.65 mg dm⁻³, (ii) F/CL₂₀ 50 for carbamazepine, with 2.0 mg dm⁻³ and (iii) F/CL₂₀ 30 for mangiferin, with 2.2 mg dm⁻³. It is possible to note that each drug had a different optimum system, since different drug chemical structures provide a different micellar core/drug interaction, which may influence their solubility into micelles.⁴ This is a strong evidence of the synergistic effect provided by the higher hydrophobic poly(ϵ -caprolactone) core and longer hydrophobic length of poly(propylene oxide) blocks.

In accordance to our results, a previous work¹³ found higher $S-S_0$ values for binary micelles of copolymers comparing to the copolymers alone, also evidencing a synergistic effect between polymers. According to Ribeiro *et al.*,¹³ F127 (E₉₇P₆₉E₉₇) showed an increase of solubility higher than F87 (E₆₂P₃₉E₆₂) for quercetin. Besides F87 presents a W_h (hydrophobic portion) similar to F127 ($W_h = 0.29$), F127 contains a longer hydrophobic chain, which contributes to its higher $S-S_0$ values.^{19,22}

Additionally, Zhou *et al.*²⁵ studied the solubility for diblocks of E_mB_n (B = unity of oxybutylene) with similar W_h (0.54-0.56) at room temperature (ca. 25 °C) using carbamazepine: (i) E₁₁B₈ (0.53 mg dm⁻³), (ii) E₁₃B₁₀ (1.13 mg dm⁻³) and (iii) E₁₇B₁₂ (1.26 mg dm⁻³). Besides these copolymers show a hydrophobic portion higher than E₁₁₄CL₂₀ (0.31), the relative hydrophobicity of CL to B block is 2:1.²² Thereby, the copolymer CL₂₀ should really present better or similar solubilization results in comparison to E_mB_n diblocks. Thus, as expected, the increase of solubility for carbamazepine in CL₂₀ and F/CL₂₀ 50 solutions was higher than the values found using E_mB_n copolymers.^{22,25}

As far as we know, no works reporting solubilization of mangiferin by DDS have been reported, specifically by binary micelle systems. According to our results, mangiferin showed strongest interactions with copolymer micelles comparing to quercetin and carbamazepine, and even better if compared to F/CL₂₀ mixtures. Therefore, mangiferin-loaded F/CL₂₀ has a great potential for biomedical applications, once the co-micelles can enhance its solubility in water, providing a higher bioavailability.

Micelle size

As a further proof of the synergistic effect of the binary mixtures F/CL₂₀, the micelle size and the size distribution for both unloading and loading systems were investigated, see Figure 4. The mixture F/CL₂₀ 50 was

chosen to be analyzed by DLS, since it is a representative sample, showing the influence of CL₂₀ in half proportion in the mixture. Additionally, drug-loading F/CL₂₀ 50 with mangiferin and quercetin was also evaluated, which can show the influence of both more hydrophilic and hydrophobic drug in the size distribution. Additionally, the mixture was analyzed in different concentrations, 1%, as in the solubilization procedure, and 0.1%; and both concentrations showed the same size distributions patterns.

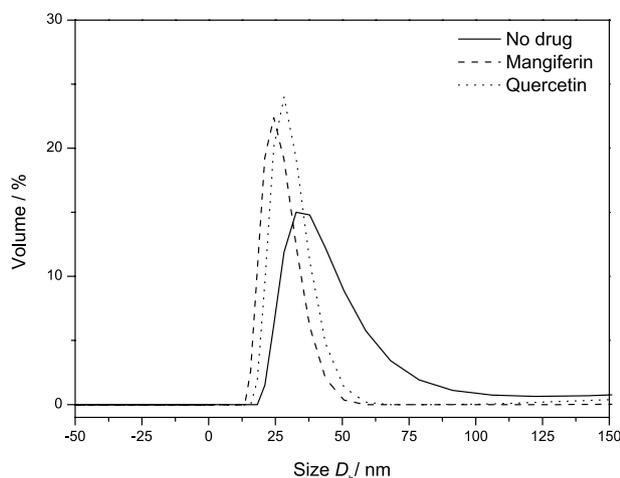


Figure 4. Size distribution of the unloading and loading binary mixture F/CL₂₀ 50 0.1% in solution.

Some works^{26,27} have been investigating mixed micelles of copolymers that have the same hydrophilic blocks but varying block length and different hydrophobic blocks with the same length, in which these parameters can directly influence the self-assembly of the copolymers in solution. In their case, they showed two-stage micellization process: (i) the micelle of one copolymer is first self-assembled, and (ii) the second copolymer is incorporated in the created micelles.²⁶ Interestingly, they also observed that the size distribution profile of the mixed micelles is driven by the asymmetry of the copolymers, in which empirically is tailored by either continuously or discontinuously incorporating the copolymer into the micelles. Herein, when the copolymers have small asymmetries difference, the short copolymer is continuously incorporated into larger copolymer micelles; given an unimodal narrow size distribution indicating co-micelles formation. Instead, when the copolymers have huge asymmetries difference, a finite amount of short copolymers molecules are incorporated into larger copolymer micelles, given a bimodal or unimodal broad size distribution, where the first peak corresponds to co-micelles and the second one to larger copolymer micelles.^{26,27}

In this case, considering the asymmetry difference chemical structure between F127[®] and E₁₁₄CL₂₀, our binary

mixture seems to lead according to the second process of micellization, where both mixed F/CL₂₀ and single E₁₁₄CL₂₀ micelles are formed (see Figure 4). Additionally, as the polymers concentrations in solution are above the CMC, the equilibrium is reached between co-micelles and larger E₁₁₄CL₂₀ micelles.²⁶ Therefore, as seen in Figure 4, the drug-unloading binary mixture F/CL₂₀ showed an unimodal broad micelle size distribution with D_h around 42.03 ± 16.71 (84%), may be an indicative of co-micelles formation but also the presence of some larger single micelles from E₁₁₄CL₂₀.

Controversially, the drug-loading system showed an unimodal narrow size distribution with D_h smaller than F/CL₂₀ no drug. In this case the size distribution can be positively influenced by other parameters such as interactions drug/micelle core and increasing the hydrophobicity of the core, in which provided sharp peaks with stable nano-size co-micelles.

It is possible to notice that the drug-loading co-micelles also showed a tiny smaller hydrodynamic diameter around 22.45 ± 5.5 (95%) and 25.66 ± 5.8 (93%) for mangiferin and quercetin, respectively. Supposedly, these results may be provided by van der Waals forces between drug molecules and P and CL blocks into micelles core, which were not further investigated in this work.

Conclusions

F/CL₂₀ binary micelles have shown to be of great interest to drug delivery applications, owing to the self-assembly of both copolymers into co-micelles. The outstanding results were directly related to the more hydrophobic contribution from poly(ϵ -caprolactone) block into micelles core, where the addition of the E₁₁₄CL₂₀ in the mixtures promoted a positively synergistic effect, since increased the solubility of the hydrophobic drugs, decreased CMC values, even upon high dilutions, and formed stable drug-loading co-micelles.

Acknowledgments

N. M. P. S. R. acknowledges for the support from MCTI/CNPq/Universal 456725/2014-5 (grant number: 304392/2013-8), INCT-NanoBioSimes and Central Analítica UFC/SISNANO and CAPES (I. M. C. and L. M. U. D.).

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Submitted: August 22, 2016

Published online: November 17, 2016