Artigo

BINARY MICELLES ($E_{45}S_8$/F127) FOR QUERCETIN AND GRISEOFULVIN SOLUBILISATION

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Micelles have aroused interest due to their ability to assist in the transport of poorly soluble drugs. In this study the mixture of copolymers F127/$E_{45}S_8$ in different proportions (F/ES 30/70, 50/50 and 70/30) was performed to improve the bioavailability of griseofulvin and quercetin. The results of cytotoxicity (MTT assay) revealed that the copolymers F127 and $E_{45}S_8$ had considerable biocompatibility and did not affect the metabolism of human neutrophils. The binary systems were also evaluated by critical micellar concentration (CMC) and thermoresponsive behavior. The CMC values were intermediate to those of the isolated copolymers. The systems maintained the thermoresponsive properties present in F127 making the systems interesting for subcutaneous administration. The systems presented small size, an average range in size from 17 to 38 nm, and the samples prepared with higher hydrophobic proportion presented more uniform sizes. Results suggest stability and the increasing of the nanosystems circulation time. The F/ES 30/70 system has polydispersity smaller than 0.1 and showed an increase of 129 times for quercetin solubility. Thus, it is possible to consider F127/$E_{45}S_8$ micelles as potential nanosystems for poorly soluble drug delivery.

Keywords: Atomic Force Microscopy; drug solubilisation; cytotoxicity assay; tube inversion.

INTRODUCTION

The pharmaceutical industry constantly faces challenges in obtaining new drugs. These challenges are usually attributed to the low solubility of the drug, that can lead to low bioavailability resulting in suboptimal drug delivery.[1] Among numerous studies, binary micelles of amphiphilic copolymers have been explored, owing to the many advantageous features as drug delivery systems. The success of those binary mixtures is related to the versatility of the chemical composition of block-type copolymers, where the micelle core polarity can be readily tailored according to the choice of hydrophobic and hydrophilic portions and their relative lengths.[2,3]

Generally, triblock copolymers of $E_mP_nE_m$ type, so-called Pluronic® F127 (P = propylene oxide and E = ethylene oxide) are used for solubilisation of hydrophobic drugs due to its amphiphilic profile.[4] Indeed, their production is already well-established in industrial scale, which makes their usage economically feasible. The F127 also presents gelling thermoresponsive property. This characteristic is interesting for subcutaneous administration of drugs because the solution can quickly gel at body temperature.[5,6] However, the polymer in aqueous solution does not present satisfactory or even great values of drug solubilisation efficiency, which is mainly attributed to the low hydrophobicity of poly (propylene oxide) blocks, therefore giving high values of critical micellar concentration (CMC) and critical micellar temperature (CMT), and undesirable features for drug administration.[7,8] Those limitations have been the motivation of several works.[9–11]

Some studies reported block copolymers of E_mS_n, $E_mS_nE_m$, S_nE_m and $S_nE_mS_n$ types (E = ethylene oxide and S = styrene oxide) as smart drug delivery systems, being a potential for pharmaceutical applications.[12–17] This attention is mainly attributed to the high hydrophobicity of S units, where its aromatic rings have great affinity for poorly water-soluble drugs. Despite high solubilisation efficiency, these copolymers also have some limitations concerning dilution and turbidity when in aqueous medium.[18,19]

The combination of copolymers with different hydrophobicity has generated a lot of interest nowadays, as it is a way to overcome the disadvantages of micellar systems formed by copolymers itself for solubilisation of hydrophobic drugs. This combination contributes to the incorporation of various functionalities without the need for synthesis of new copolymers, as these systems have numerous advantages such as improved thermodynamic and kinetic stability, increased bioavailability, encapsulation efficiency and biocompatibility.[8,11,19]

Griseofulvin is used in several works as a model drug to compare solubilisation efficiency in different copolymers.[20–23] Quercetin is a flavonoid which presents anti-inflammatory and antitumor activities.[24] It is a drug in experimental stage,[25] with several ongoing clinical studies, as shown in the U.S. government website.[26] Several works try to solve the problem of its low aqueous solubility (0.5 mg L^{-1} at 25 °C) using solubilising agents,[16,27,28] developing prodrugs[29] or preparing quercetin nanocrystals.[30]

The objective of this work is to study the copolymers $E_{45}S_8$ and their binary mixtures with F127 intending to overcome the limitations of systems formed by copolymers themselves for griseofulvin and quercetin solubilisation for potential pharmacological application.

EXPERIMENTAL

Materials

Copolymer $E_{45}S_8$ (ES) was synthesized by anionic polymerization of styrene oxide followed by ethylene oxide and donated by the

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Table 1. Molecular characteristics of copolymers $E_{45}S_8$ and F127

Copolymer	M_w [a]	W_E [b]	W_h [c]	M_w/M_n [d]	Reference
$E_{45}S_8$ (ES)	2940	0.673	0.327	1.06	[2]
$E_{98}P_{67}E_{98}$ (F127)	12510	0.689	0.311	1.20	[31]

[a]Average number of molecular weight (g mol^{-1}) by Nuclear Magnetic Resonance (^{13}C NMR); [b]W_E is the mass fraction of hydrophilic block in the copolymer chain; [c]W_h is the mass fraction of hydrophobic block; [d]Polydispersity index by Gel Permeation Chromatography (GPC).

School of Chemistry, Manchester University. The copolymer $E_{97}P_{69}E_{97}$ (F127) was purchased from Uniqema LTD. 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. Human leucocyte-rich blood from healthy adults was obtained from blood bank – HEMOCE (Fortaleza, Brazil). The model drug griseofulvin (352.8 g mol^{-1}) was supplied by Sigma-Aldrich (Poole Dorset, UK), and quercetin (302.2 g mol^{-1}) was donated by PADETEC – UFC (Fortaleza, Brazil). Both drugs were used as received. All other reagents were used in analytical grade.

Copolymers characterization

MTT assay

The neutrophils were exposed to $E_{45}S_8$ and F127 (10, 50 and 100 μg mL^{-1}), water (vehicle, control), Hanks' balanced salt solution (HBSS, culture medium, negative control) or Triton X-100 (0.2%, cytotoxic standard) for 15 min at 37 °C and then 200 μL of 3-(-4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) was added. After 3 h the cells were washed with phosphate buffer solution, and the DMSO (100 μL) was added for the solubilisation of the formazan product. The absorbance was measured at 540 nm.[32]

Binary systems

Binary mixtures

The copolymers mixtures F127/$E_{45}S_8$ were prepared by dissolving the desired concentrations. The systems followed the ratios 30/70, 50/50, 70/30 and were denominated F/ES 30/70, F/ES 50/50, F/ES 70/30, respectively.

Critical micelle concentration (CMC)

Firstly, stock solutions were prepared by dissolving the systems in Milli-Q water, allowing 24 h for complete dissolution before diluting to required concentrations within the range 0.0001 – 1 g L^{-1}. The 1,6-diphenyl-1,3,5-hexatriene (DPH) was used as a probe to determine micelles formation. The DPH fluorescent dye was dissolved in methanol (0.4 mmol l^{-1}) in the dark and then added (30 μL DPH in 3 mL solution) to the solutions of the copolymers. The solutions containing DPH were kept in the dark and the absorbance measurements of the systems were taken 12 h after the addition of DPH. This well-established methodology was first reported by Alexandridis *et al.*,[33] where in this assay, an F-4500 Hitachi Fluorescence Spectrophotometer was used to determine the onset of micellisation for each copolymer solution. During the whole experiment, the temperature was kept at 25 °C and 37 ± 0.2 °C, and the fluorescence emission at 428 nm, being measured with an excitation wavelength at 350 nm.

Thermoresponsive behavior

Aqueous solutions (0.5 g with concentration range from 15 – 35 wt% of copolymers/system) were prepared, enclosed into small tubes (10 mm in diameter) and slowly heated (0.1 °C min^{-1}) in a water bath through the temperature range 10 – 90 °C. Gelation was recognized by immobility of the solution when the tube was inverted at intervals of ±1 °C.[22]

Drug solubilisation

In this assay, copolymers and their mixtures were dissolved in Milli-Q water (1% w/w, 10 mL), where a further mass of drug (w ≈ 10 mg) was added. Then, the prepared system was slowly stirred at 25 ± 0.1 °C) in a thermostatic bath for 4 days. The supernatant was filtered (0.45 μm Millipore) to remove any non-solubilised drug and diluted with methanol. The drug concentration was monitored by UV/Visible spectroscopy (Thermo Scientific Genesys 6) at 292 and 375 nm for griseofulvin and quercetin, respectively, using a calibration curve, as described in Crothers *et al.*.[2] Blank experiments, without copolymer, were done to determine the solubility of the drug in water. All measurements were carried out in triplicate and the results are shown averaged with standard deviation.

Particle size

Dynamic light scattering (DLS) technique was used to determine the hydrodynamic diameter (D_h) of the copolymers micelles in diluted solution, with and without drug. Aliquots were filtered in Millipore 0.45 μm and analyzed using a Malvern Zetasizer Nano ZS90 equipment. The systems were investigated using 30 scans with 30 s acquisition time allowed for each scan. All measurements were made in triplicate.

RESULTS AND DISCUSSION

Cytotoxicity tests

Figure 1 shows the effect of copolymers on human neutrophil viability evaluated through MTT test. The results demonstrated that both $E_{45}S_8$ and F127 at concentrations from 10 to 100 μg mL^{-1} did not reduce the viability of cells when compared to the control group (vehicle, DMSO 1%). The MTT test measured cell viability evaluating its ability to reduce tetrazolium compound to formazan crystals by mitochondrial succinate dehydrogenase.[34] The results suggest that $E_{45}S_8$ or F127 did not affect the metabolism of human neutrophils at the evaluated concentrations. These results were corroborated by previous studies where $E_{45}S_8$ and its mixtures with Pluronic® P123 were not toxic for plasma membrane of human neutrophils.[19] The absence of toxic effect of $E_{45}S_8$ and F127 is very important considering that human neutrophils are the most abundant white blood cells having a central role on innate immune response.[35]

Critical micelle concentration (CMC)

The fluorescence emission spectra of DPH in diluted polymer systems was used to plot graphs of DPH emission intensity at a wavelength of 428 nm *versus* the logarithm of the concentration (g L^{-1}) of polymer systems. The CMC of each system was estimated as the point where it starts a sharp increase in emission intensity of DPH, due to its solubilisation in the hydrophobic cores of the micelles.[33]

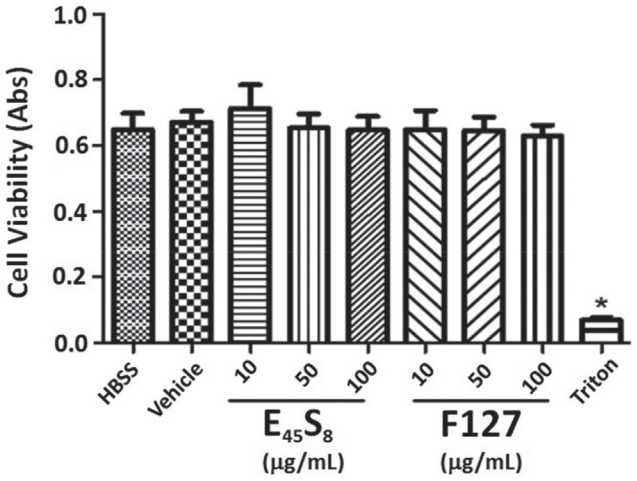

Figure 1. *Evaluation of toxicity of polymers, $E_{45}S_8$ and F127, measured by MTT assay in human neutrophils *vs. HBSS (untreated cells). Results represent means ± SEM. ($p < 0.05$; ANOVA and Tukey's post hoc test)*

Values of CMC of ES expressed in mmol L^{-1} are much lower than those for F127 (Table 2). The length of E_m does not affect the CMC, but the hydrophobic character of hydrophobic block does.[17,36,37] So, the higher is the hydrophobic effect, the lower is the surfactant's CMC. This explains why the CMC of $E_{45}S_8$ is lower. The relative hydrophobicity between P and S units is 1:12.[7]

The systems presented a decrease in CMC values with the temperature increase, especially for F127, representing a higher micelle formation capacity at higher temperatures, due to dehydration of the ethylene oxide chains leading to increased segregation between the PEO and PPO blocks.[4,38] This behavior also can be explained by previous studies that show a more endothermic micellisation process for $E_nP_mE_n$ type triblocks, with micellisation $\Delta H°$ values around 200 kJ mol^{-1} or more. This results in instability of micelles in aqueous solution, increasing the number of micelles with increasing temperature.[33,37] The copolymers E_nS_m type presented a reduced micellisation enthalpy ($\Delta H_m \approx 0$), which can be related to the interaction of the S block with water hydrophobic effect. This results in stability of micelles over a wide temperature range.[36,39] These standard micellisation enthalpy values are calculated from the log ratio (CMC) *versus* 1 / T (Equation 1):

$$\Delta H°_m = RT^2 \, (\text{dln CMC/dT}) \text{ or } \Delta H°_m = R \, [\text{dln CMC/d(1/T)}] \qquad (1)$$

However, the contribution of entropy generally dominates the micellisation process in aqueous surfactant solutions with the enthalpy playing a minor role. The unfavorable enthalpy of triblocks copolymers $E_nP_mE_n$ type is outweighed by a stronger entropy effect. The presence of hydrocarbon molecules in water causes a reduction in water entropy, inducing an increase in the degree of structuring of the water molecules due to cavity formation. However, this decrease in entropy is restored when hydrocarbon molecules aggregate to form micelles due to hydrogen bond formation.[33]

The mixtures presented intermediate CMC values to the isolated copolymers and the increased proportion of the less hydrophobic core copolymer in the mixture results in an increase in the CMC value of the systems. Similar results were found in the literature.[22,40–42]

Yet the mixtures retain low values of CMC, which make them promising for pharmacological applications due to the potential stability of their micelles after dilution in the blood, causing a greater circulation time of the drug in the blood,[43,44] in addition of minimizing the side effects caused by the drug in its free form.

Table 2. Critical micelle concentration (CMC) of copolymers ES, F127 and its mixtures at 25 and 37 ºC

Systems	CMC (g L^{-1})	
	25 ºC	37 ºC
F127	0.2300	0.0790
F/ES 70/30	0.0074	0.0074
F/ES 50/50	0.0220	0.0190
F/ES 30/70	0.0047	0.0031
$E_{45}S_8$	0.0030	0.0020

Taken together the results, it was observed that the CMC of $E_{45}S_8$ and F127 at 37 °C were in the concentration range considered as non-toxic for human neutrophils. However, we are discussing about distinct methods (chemical and biological) being important additional studies to determine the bioavailability of these materials.

Thermoresponsive behavior

Figure 2 shows the phase diagrams of F127, diblock ES and their mixtures. The copolymer ES did not present the interesting thermoresponsive properties of F127, which was already expected for copolymers with poly(styrene oxide).[41,45] For the mixtures of F127 and ES, it was not possible to produce a stable curve of mobile-hard transition when the ratio of F127 was 30% (F/ES 30/70), therefore the results are not shown in Figure 2.

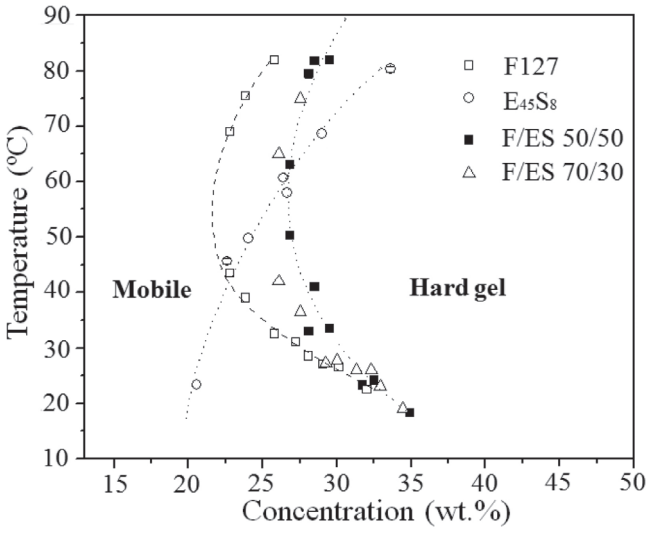

Figure 2. *Phase diagram of the systems (□) F127, (○) ES, (■) F/ES 50/50 and (△) F/ES 70/30*

The F127's thermoresponsive property is a capacity of reversibly transforming from moving fluids to immobile gels and to return to moving fluids in the range temperature and can be related to the triblocks $E_nP_mE_n$ type with high $\Delta H°_m$ value. Due to the endothermic nature of the micellisation process, the number of micelles increases with increasing temperature and a compacted micellar gel forms at a critical gelling temperature.[37]

However, E_nS_m copolymers do not have thermoresponsive properties due to their low $\Delta H°_m$ value, presenting micellar stability over a wide temperature range, in addition to the kinetic stabilization of micelles by vitrification of the S block core when the temperature tends to 0 °C.[39]

The systems F/ES 50/50 and F/ES 70/30 had curves of transition

which almost overlap, retaining the thermoresponsive properties of F127, gelling upon heating. The gel formation of these systems with a transition temperature in the range between room temperature and body temperature (25 – 37 °C) makes them interesting for application in subcutaneous drug delivery.

Drug solubilisation

The aqueous solubilities (S_0) for both drugs were obtained according to the well-established solubilisation procedure "Shake-Flask".[2,4] For quercetin S_0 was 0.05 mg dL^{-1} at 25 ºC similar to that obtained by Saija *et al.*,[46] 0.0514 mg dL^{-1} at room temperature. For griseofulvin S_0 was 3.6 mg dL^{-1} at 25 ºC, also compatible with the literature.[4]

After obtaining the drug solubilities data, two parameters were investigated for the copolymers and their mixtures: S_{cp}, the solubilisation capacity expressed in mg of drug per g of polymer (Equation 2), and S_h, the solubilisation capacity expressed in mg of drug per g of hydrophobic block (W_h) (Equation 3), where S is the solubility of drug in micellar solution, S_0 is the aqueous solubility of drug and m_{cop} is the mass of copolymer used in the solution composition. The results are shown in Figure 3 and Table 3.

$$S_{cp} = S\text{-}S_0/m_{cop} \quad (2)$$

$$S_h = S_{cp}/W_h \quad (3)$$

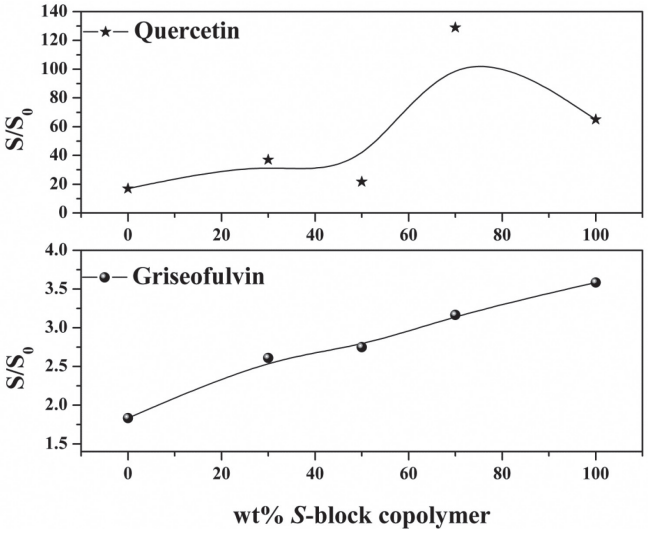

Figure 3. *Increased solubility of 1wt.% solutions of F127, $E_{45}S_8$ (ES) and their mixtures at 25 °C*

The S_{cp} values presented by the isolated and mixed systems show that the use of poly(styrene oxide) core copolymer favors drug solubilisation. The hydrophobic character of the poly(styrene oxide) core is superior to the poly(propylene oxide) present in F127, leading to a greater affinity of drugs for the micelle core.[37]

The drugs presented different optimal system. For griseofulvin the best system was $E_{45}S_8$, while the mixtures presented solubility values intermediate to those of the isolated systems, a result similar to Pinho *et al.* and Oliveira *et al.*[19,41] Quercetin presented better results for the F/ES 30/70 system. That result may be due to different chemical structures of the drugs (Figure 4) and their different interactions with the micelle core, evidencing a synergistic effect in the quercetin system.[42] The synergistic effect is provided in this work by mixing between the diblock $E_{45}S_8$ and the F127 triblock, which resulted in an increase of quercetin solubility by more than 100 times. We can

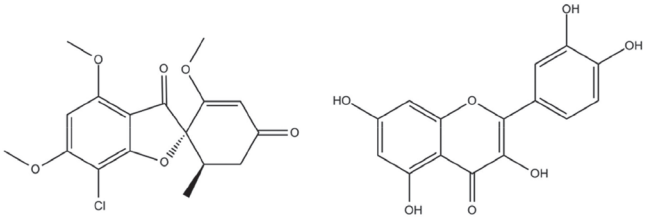

Figure 4. *Chemical structures of griseofulvin (a) and quercetin (b)*

suggest that the presence of hydroxyls in the quercetin structure makes it more compatible with the system.

The value of S_h provides a direct measure of the efficiency of solubilisation in micellar core and it is not so dependent of copolymer characteristics, such as hydrophilic block length and copolymer architecture.[16] As expected, S_h values obtained for both drugs at 25 °C were much higher for ES than for F127, mainly due to the higher hydrophobicity of S-block chains when compared to P-block chains, due the aromatic groups from styrene oxide monomer.

The values found for the mixtures, F/ES, approached an average of the copolymers alone. For instance, for griseofulvin, the F/ES 70/30 system showed S_h values of 18.2, which is in accordance to comicellisation process of diblock with F127. Besides, the same profile was also observed for the drug quercetin. All S_h values were much lower when compared to griseofulvin values, being attributed to the lower water solubility of quercetin, and also showing the dependence between the S_h of copolymer and the drug, as observed by Crothers *et al.*[15]

Solubilisation can be considered as a drug partition between two phases: aqueous and micellar. The partition coefficient (P) is the ratio of drug concentration in micelle phase to the drug concentration in water for a specific surfactant concentration,[47] according to Equation 4.

$$P = S_{cp}/S_0 \quad (4)$$

The solubilisation results show an increase of *P* with increasing hydrophobicity in the systems, a result similar to the literature.[4,40] This suggests a direct relationship between the values of S_h and P.

The variation of Gibbs energy (ΔG°) of solubilisation can be calculated as a function of temperature and partition coefficient (Equation 5), where R is gas constant, T is temperature in Kelvin and *P* is partition coefficient.

$$\Delta G° = -RT\ln P \quad (5)$$

The results indicate spontaneous solubilisation for the systems, at standard conditions, manifested by the negative values of ΔG°, except for the F127 system with griseofulvin. The obtained data indicate that the increase of the hydrophobic character of the micelles decreases the ΔG°, favoring the spontaneity of the solubilisation. This is in accordance with the literature.[40]

The hydrophobicity effect is usually even more pronounced for process of micellisation for non-ionic surfactants.[48] This effect is greater for diblock due the relative hydrophobicity between P and S units is 1:12.[7] The presence of hydrophobes causes a reduction in the entropy of water that is restored when the surfactant molecules aggregate to form micelles. The objective of this aggregation is to restore hydrogen bonds and the degree of freedom of the hydrophobe, as well as to increase the entropy of water making the phenomenon of micellisation entropically favorable.[33,37,49] Thus, systems with molecules of greater hydrophobic character form systems more spontaneously.

Table 3. Solubility parameters of 1 wt.% solutions of F127, $E_{45}S_8$ (ES) and their mixtures at 25 °C for griseofulvin (S_0 = 3.6 mg dL^{-1}) and quercetin (S_0 = 0.05 mg dL^{-1})

Systems	Griseofulvin					Quercetin				
	S_{cp}[a](mg dL^{-1})	S_h[b](mg dL^{-1})	S/S_0[c]	P[d]	ΔG° (kJ mol^{-1})	S_{cp}[a](mg dL^{-1})	S_h[b](mg dL^{-1})	S/S_0[c]	P[d]	ΔG° (kJ mol^{-1})
F127	3.0	9.3	1.83	0.83	+0.462	0.8	2.6	17	16	-6.87
F/ES 70/30	5.8	18.2	2.61	1.61	-1.18	1.8	5.8	37	36	-8.88
F/ES 50/50	6.3	19.7	2.75	1.75	-1.4	1.04	3.26	28	20.8	-7.52
F/ES 30/70	7.8	24.1	3.2	2.17	-1.92	6.4	19.86	129	128	-12.0
$E_{45}S_8$	9.3	28.5	3.58	2.58	-2.35	3.2	9.9	65	64	-10.3

[a] Solubilisation capacities expressed in mg of drug per g of polymer; [b] Solubilisation capacities expressed in mg of drug per g of hydrophobic block; [c] Increased solubilities; [d] Partition coefficient.

Table 4. Hydrodynamic diameter (D_h) and polydispersity of copolymers systems at 25 °C: without and with drugs

Samples	Unloaded		Griseofulvin		Quercetin	
	D_h/nm	PdI	D_h/nm	PdI	D_h/nm	PdI
F127	17.43 ± 0.7	0.475	38.02 ± 4.5	0.76	28.79 ± 0.8	0.50
F/ES 70/30	23.52 ± 0.2	0.177	29.9 ± 0.6	0.46	25.86 ± 0.6	0.31
F/ES 50/50	23.13 ± 0.4	0.244	25.8 ± 1.94	0.33	29.43 ± 0.6	0.47
F/ES 30/70	19.15 ± 0.2	0.068	24.13 ± 0.0	0.22	22.33 ± 0.2	0.28
$E_{45}S_8$	18.30 ± 0.4	0.073	21.63 ± 0.52	0.32	18.52 ± 0.4	0.18

Particle size

Particle size distribution by DLS measurements were performed to evaluate the comicellisation of ES with F127 at 1 wt.% aqueous solution. The appearance of a single peak for binary mixture samples confirms the formation of comicelle self-assembly. Table 4 shows the average hydrodynamic diameter (D_h) values for all analyzed systems.

The average hydrodynamic diameter (D_h) of the systems unloaded has an average size range from 17.43 to 23.53 nm and the systems loaded was between 18.52 to 38.02. The incorporation of drugs results in an increase in the systems' diameter, which can be associated with drug interaction with the hydrophobic micelle core.[8]

The size of systems providing stability and long running time, since nanoparticles ranging in size from 10 to 100 nm can prevent rapid metabolisation by the liver or filtered out by the kidneys.[9] The small size of the systems also favors their accumulation in tumors, since nanoparticles with a diameter between 10 - 200 nm favor the accumulation in tumors via the EPR effect.[50]

The F/ES 70/30, F/ES 50/50 and F127 unloaded systems showed moderate polydispersity with PdI between 0.1 – 0.4, while the $E_{45}S_8$ and F/ES 30/70 systems presented PdI less than 0.1 indicating a monodisperse particle size distribution.[22,51] After drug addition, an increase in polydispersity was observed for all systems. The nanosystems formed with higher hydrophobic proportion presented more uniform sizes and stability evidenced by the lower polydispersity index and reduced particle sizes.

Several studies show micelles systems with PdI between 0 and 0.4 as promising for intravenous and subcutaneous application.[44,52–55] With the exception of the sample with F127 the systems obtained can be used in intravenous and subcutaneous application.

The systems, after the incorporation of the drugs, presented bimodal distribution profile. Based on this fact, it is possible to suggest that the structure of the drugs can affects the packing parameter.[56] Observed aggregates have variable sizes, but the largest proportion is formed by micellar nanoparticles (Tables 1S, 2S and 3S in Supplementary Material). These aggregates can be spherical micelles, vesicles or cylindrical (threadlike or wormlike) micelles.[56] Vesicles have variable sizes, usually >100 nm in diameter while threadlike micelles have very slow-diffusing and sizes lying near the DLS upper detection limit. These micelles feature typical values size ranging from 100 to 10,000 nm.[57]

CONCLUSIONS

In this study, micellar nanosystems formed by copolymers $E_{45}S_8$, F127 and their binary mixtures were investigated. Research has shown that the mixtures resulted in systems that have a combination of properties of their constituents, such as thermoresponsive property and low CMC values, which make them potential candidates for pharmacological use such as for application in subcutaneous drug delivery. The sample demonstrated better solubilisation capacity for systems with greater hydrophobicity. However, the drugs presented different optimal system. For griseofulvin, they presented $E_{45}S_8$ and for quercetin they presented F/ES 30/70. Those results may be related to the structural differences of drugs that interact differently with the micelle nucleus. In the particle size study, the systems presented small size and the samples formed with higher hydrophobic proportion presented more uniform sizes, which can provide stability and increase the nanosystems circulation time. Therefore, the micellar nanosystems formed by binary mixture F127 and $E_{45}S_8$ copolymers are interesting hydrophobic drug nanocarriers.

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