

# Physicochemical assessment of Dextran-g-Poly ( $\epsilon$ -caprolactone) micellar nanoaggregates as drug nanocarriers



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## ABSTRACT

Self-assembling polymers in aqueous solution have attracted significant attention with recent research efforts focused on the development of new strategies to design devices useful in the field of controlled drug delivery. In this context, amphiphilic copolymers having specific structural features and self-assembling behaviors in aqueous media that would enable controlled drug release over longer time periods. In this work, we report on the synthesis and characterization of a Poly ( $\epsilon$ -caprolactone)-grafted Dextran copolymer and its use in the preparation of micellar nanoaggregates. The characterization and study of the morphology, topography, size distribution and stability of micellar nanoaggregates by Transmission Electron Microscopy (TEM), Atomic Force Microscopy (AFM), Dynamic Light Scattering (DLS) and Zeta Potential ( $\zeta$ ), respectively, were carried out. Spherical-shaped morphologies and an average size of approximately 83 nm, for drug-free nanoaggregates, were observed. In addition, Zeta Potential studies showed that drug-free nanoaggregates are more stable than drug-loaded structures measured in a phosphate buffer (pH 7.2) medium. UV–vis spectrophotometry of both the drug entrapment efficiency (EE%) and *in vitro* drug release behavior were assessed. The EE% was determined to be 78% (w/w), and a combination of diffusion and eroding polymer matrix mechanisms for drug release were established. Finally, these results indicate that Dx-g-PCL micellar nanoaggregates are suitable for use as a potential nanocarrier having both biodegradable and biocompatible properties.

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## 1. Introduction

Water-soluble polymers offer numerous advantages in applications that are mainly focused on the food (Stephen, Phillips, & Williams, 2006), pharmacology (Craig, 2002), medicine (Kainthan et al., 2006) and tissue engineering (Nair & Laurencin, 2006) industries. This is predominantly due to their biocompatible, biodegradable, bioresorbable and non-toxic properties (Baines, Billingham, & Armes, 1996; Nair & Laurencin, 2007; Noettelet, Vert, & Coudane, 2008; Schmaljohann, 2006; Tao, Zhang, & Cheung, 2006). In addition, many efforts have been made to gain insight into the preparation and understanding of the behavior of polymer nanoaggregates in aqueous solutions for use as nanocarriers for several drug types (Bovere et al., 2014; Howard, Jay, Dziubla, & Lu, 2008; Malam, Loizidou, & Seifalian, 2009). In this context, a class of polymer that has received recent attention is the polysaccharides.

The presence of several chemical functional groups along the chain backbone confers the ability to be modified with other functional groups and/or to generate sites that could be covalently bonded to small molecules, oligomers, or other polymers (Oskuee, Dehshahri, Shiler, & Ramezani, 2009; Torchilin, 2012). A very interesting polysaccharide system that has undergone chemically modification is Dextran, which is obtained mainly from enzymatic processes performed by specific bacterial species (Shamala & Prasad, 1995; Su & Robyt, 1994). This polysaccharide is composed of chains of D-glucose monomeric units connected by  $\alpha$  (1–6) linkages; consequently, a large quantity of hydroxyl groups are available per monomer unit. This fact suggests the possibility of chemical modification with either hydrophilic or hydrophobic moieties. Accordingly, different authors have reported hydrophobic modification and further nanostructure formation of modified Dextran over the last decade (Covis, Ladaviere, Desbrieres, Marie, & Durand, 2013; Fulop, Nielsen, Larsen, & Loftsson, 2013; Kaewprapan et al., 2011; Rouzes, Gref, Leonard, De Sousa, & Dellacherie, 2000).

It is well known that polymer nanostructuration in aqueous media occurs *via* selective solvation of the hydrophilic blocks by

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water molecules, whereas the hydrophobic blocks are excluded from the aqueous medium. Hence, these unfavorable interactions can be minimized by polymer self-assembly, generating, for example, micellar aggregates in aqueous solutions. These nanostructures are constituted as a hydrophobic nucleus surrounded by a hydrophilic crown (Mortensen, 1996; Wang, Tong, & Zhao, 2004). In this respect, a detailed study of Dextran ( $\bar{M}_w = 30,000$  g/mol) covalently modified with bile acid molecules was synthesized over a decade ago by Nichifor, Lopes, Carpov, and Melo (1999). They studied and characterized the aggregation phenomena of this amphiphilic copolymer by dynamic and static light scattering. From these results, the particle size distribution and radius of gyration of the aggregates were determined. In addition, fluorescence measurements using N-phenyl-1-naphthylamine as a hydrophobic fluorescence probe were carried out to obtain the critical aggregation concentration (CAC). The authors found that the nature and degree of Dextran substitution with hydrophobic moieties play a key role, as they influence the behavior of the copolymer in solution as well as the aggregation types, size distribution and colloidal stability.

Recently, Long and coworkers reported on the preparation of nanocapsules using Dextran previously modified with cholesterol (Dex-Chol) and Poly (Lactic Acid) (PLA). The method used for this preparation was dialysis of a Dimethyl sulfoxide (DMSO) solution containing the polymers against water. The results showed that the morphology, size distribution and polydispersity depend on the molecular weight of PLA and the weight ratio of Dex-Chol (Nagahama, Ouchi, & Ohya, 2008).

Shi and coworkers prepared films based on Poly ( $\epsilon$ -caprolactone)-grafted Dextran loaded with Paclitaxel using a solution-casting method. The morphologies of the films were studied by optical microscopy techniques. These analyses showed that loadings of Paclitaxel above 10% (w/w) resulted in homogeneous films. This fact was attributed to the presence of a single-phase morphology. In addition, the release behavior was also studied. From these analyses, the authors observed a strong influence on the release rates *in vitro* due to drug hydration (Shi & Burt, 2004). Liu and co-workers prepared spherical micellar aggregates with a diameter range of 20–50 nm from amino-functionalized Dextran modified with Poly ( $\epsilon$ -caprolactone) blocks in an aqueous medium (Liu & Zhang, 2007). More recent works on micellar nanoaggregate preparation from Dextran-Poly ( $\epsilon$ -caprolactone) copolymers have also been reported (Li, Wang, Wang, Wang, & Jiang, 2013; Sun et al., 2010). Considering the outstanding biocompatibility and biodegradability properties of Dextran modified with Poly ( $\epsilon$ -caprolactone), this class of macromolecules presents an interesting system to be studied and assessed with different types of drugs.

Keeping in mind the previous work, the pursuit of biodegradable and biocompatible macromolecular systems exploited for controlled release with a broad range of drugs is still a challenging task. Consequently, we believe that it is necessary to provide more information from the physical-chemistry point of view on nanoaggregates and their role in drug delivery. Hence, this work proposes to take advantage of the large number of available functional sites along the Dextran chain to synthesize Poly ( $\epsilon$ -caprolactone)-grafted Dextran amphiphilic copolymers. The main goal is to achieve a better understanding of the entrapment process and release behavior for a specific drug loaded into Dx-g-PCL nanoaggregates.

## 2. Experimental

### 2.1. Materials

Dextran ( $\bar{M}_w = 10,000$  g/mol), tin(II) 2-ethylhexanoate,  $\epsilon$ -caprolactone monomer, Poly ( $\epsilon$ -caprolactone) ( $\bar{M}_n = 2000$ ), Poly

(ethylene glycol) ( $\bar{M}_n = 10,000$ ), Pyrene and Amoxicillin from Sigma–Aldrich and Tetrahydrofuran (THF), Dimethyl sulfoxide (DMSO) and chloroform solvents from Merck were used. The following procedures were performed prior to use. Dextran was dried in vacuum until a constant weight was achieved, and  $\epsilon$ -caprolactone monomer was distilled under reduced pressure. All other reagents were used without previous treatment.

### 2.2. Synthesis and characterization of Poly ( $\epsilon$ -caprolactone)-grafted Dextran (Dx-g-PCL)

The synthesis of graft copolymers containing Dextran as the main chain and Poly ( $\epsilon$ -caprolactone) (PCL) grafted blocks was carried out according to a previously reported method (Ydens et al., 2000) with minor modifications in the synthetic route. The monomer of  $\epsilon$ -caprolactone was polymerized by ring-opening onto the Dextran homopolymer backbone to obtain the respective Dextran-g-Poly ( $\epsilon$ -caprolactone) (Dx-g-PCL) graft copolymer.

The synthetic route was as follows: 3 g of Dextran was dissolved in 15 mL of DMSO and placed into a glass flask carefully dried and purged with nitrogen gas prior to use. Later,  $\epsilon$ -caprolactone monomer and tin(II) 2-ethylhexanoate as catalysts were added. The feed ratio of Dextran: $\epsilon$ -caprolactone:tin(II) 2-ethylhexanoate was 1:3:0.05 (w/w/w). The reaction was vigorously stirred at 373 K for 24 h. Then, the copolymer sample was precipitated by a fractionation technique using cold chloroform as the non-solvent. The yield for the grafting reaction was determined by weighing the previously purified and dried product. A value of 73% was obtained.

In order to determine the molecular weight of the copolymers static light scattering (SLS) unit Dawn EOS system inline with Optilab DSP interferometric refractometer, both from Wyatt Technology connected online with three size exclusion chromatographic (SEC) columns (absolute method for determining of average molecular weights of polymers) were used. The solvent used as eluent was THF.

The characterization of the copolymer was performed by a Vector Bruker infrared spectrophotometer (FT-IR) using a KBr pellet technique. Additionally, a nuclear magnetic resonance ( $^1\text{H}$  NMR) spectrum was recorded in a Bruker ACP-200 system using a solvent mixture of DMSO- $d_6$ :D $_2$ O (50:50, v/v). The crystalline morphology of PCL, Dextran and Dx-g-PCL polymers was observed using an Olympus BX60 and CCD digital camera QImaging MP5. Each polymeric film was deposited onto a microscope slide by a solution-casting method. To ensure complete solvent evaporation, the microscope slides were dried under vacuum for 24 h. The optical images were recorded and analyzed via the QImaging QCapture Pro software.

### 2.3. Preparation of drug-free and Amoxicillin-loaded Dx-g-PCL micellar nanoaggregates

Dx-g-PCL micellar nanoaggregates were prepared via simple emulsion (W/O) and solvent-evaporation methods. To 5 mL of an aqueous solution of 1.0 mg/mL Dx-g-PCL was added 5 mL of a chloroform solution containing 0.5 mg/mL Poly (ethylene glycol) (PEG) under sonication for 6 h at 50 °C to allow for complete solvent evaporation. Once the solvent was evaporated, Dx-g-PCL micellar nanoaggregates were dried under vacuum for 24 h and then resuspended in Milli-Q water. Later, the suspension was put into a dialysis system with a molecular mass cut-off of 6–8 kDa under stirring for 12 h against a Tetrahydrofuran–water (80:20, v/v) mixture. After the dialysis, micellar nanoaggregates were concentrated by centrifugation, collected and dried under vacuum for 24 h. Finally, Dx-g-PCL micellar nanoaggregates were resuspended in Milli-Q water and stored at 15 °C.

#### 2.4. Characterization of Dx-g-PCL micellar nanoaggregates

The micellar nanoaggregates were characterized by Low-Voltage Electron Microscopy (LVEM), Atomic Force Microscopy (AFM), UV-vis spectrophotometry (UV-vis), Dynamic Light Scattering (DLS) and Zeta Potential (ZP). The equipment used for the characterization of this work were a Low-Voltage Electron Microscopy LVEM5 electron microscope at a nominal operating voltage of 5 kV, an AFM Dimension 3110-Nanoscope IV, a Shimadzu UV-2450 UV-vis Spectrophotometer, a Fluorolog Spex 1681 spectrofluorimeter, a Nicomp 370 DLS Particle Size Analyzer and a Z-Meter 3.0.

### 2.5. *In vitro* release assay

To determine the entrapment efficiency, the drug-loaded nanoaggregates were dissolved in DMF. The concentration of Amoxicillin released in the solution was then determined in triplicate by UV-vis spectrophotometry, following the absorbance at 274 nm using a previously performed calibration curve. The theoretical and experimental drug loadings, as well as the entrapment efficiency, were calculated using the following formulas:

$$\text{Theoretical drug loading} = \frac{\text{weight of total drug}}{\text{weight of total drug} + \text{polymer}}$$

$$\text{Actual drug loading} = \frac{\text{weight of entrapment drug}}{\text{weight of total drug + polymer}}$$

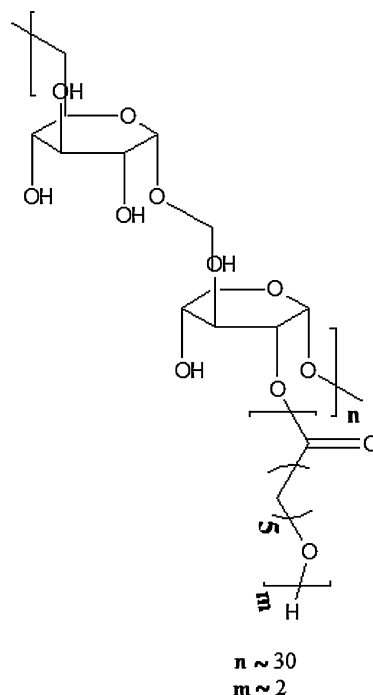
$$\text{Entrapment efficiency (EE \%)} = \frac{\text{actual drug loading}}{\text{theoretical drug loading}} \times 100$$

The entire procedure, from preparation of the micellar aggregates to the determination of entrapment efficiency was performed in triplicate to ensure the reproducibility.

### 3. Results and discussions

### 3.1. Copolymer characterization

<sup>1</sup>H NMR spectroscopy techniques were used to characterize the Dx-g-PCL graft copolymer. Fig. 1 shows the spectrum that confirms that PCL was grafted onto the Dextran backbone chain (Bajgai,

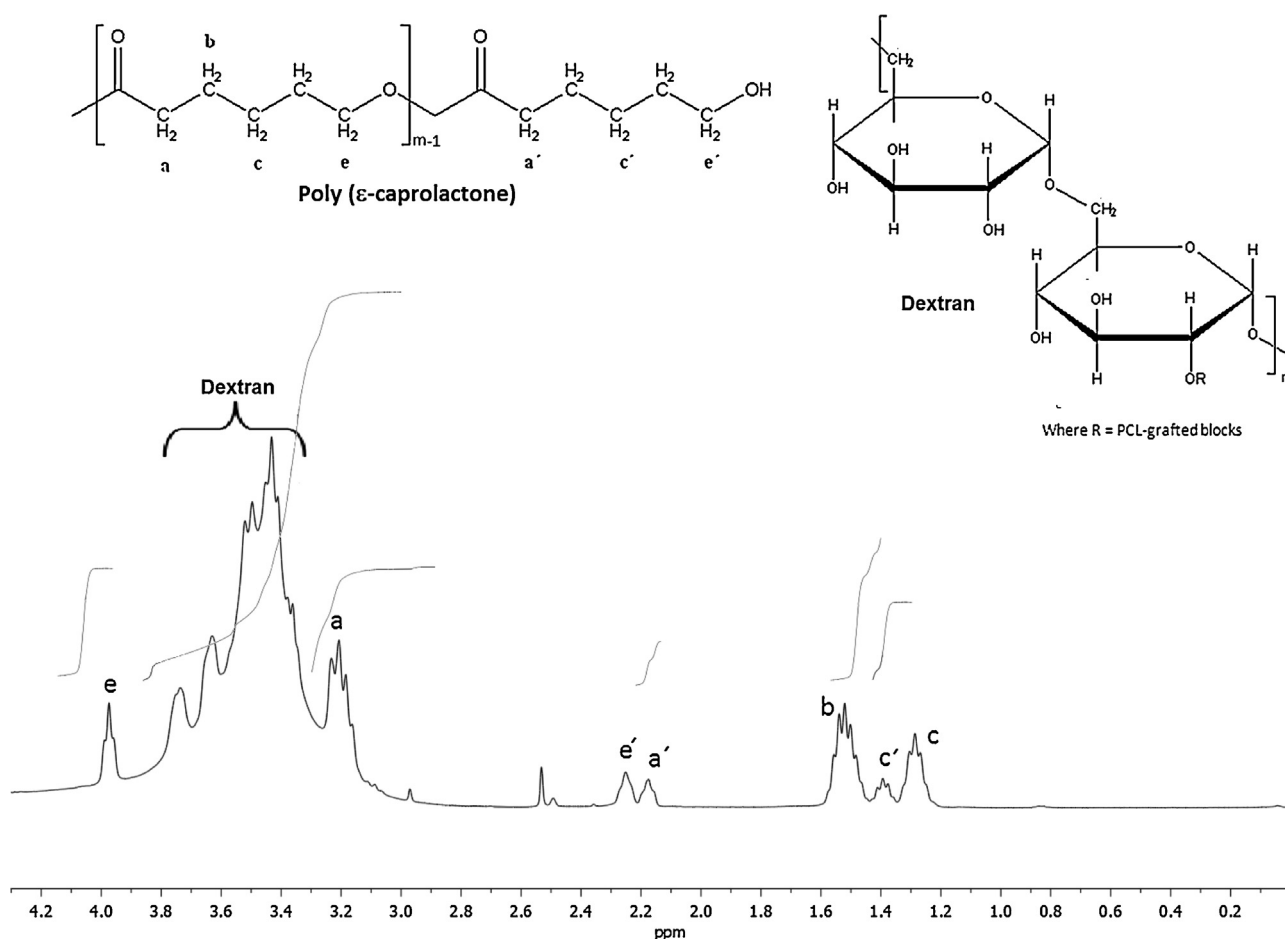


**Scheme 1.** Schematic structure of Poly ( $\epsilon$ -caprolactone)-grafted Dextran copolymer.

Aryal, Lee, Park, & Kim, 2008; Shi and Burt, 2004). The main signals corresponding to Dextran and PCL were the glucosidic proton and methylene groups, respectively, as can be observed from the spectrum. The schematic structure of the copolymer is shown in Scheme 1.

The weight-average molecular weights ( $\bar{M}_w$ ) and the respective polydispersity index for the copolymer fraction was determined by static light scattering (SLS) using Tetrahydrofuran as the eluent. Because  $\epsilon$ -caprolactone monomers were polymerized onto the backbone chain of Dextran,  $10,000 \text{ g mol}^{-1}$ , the increment in the molecular weight of the copolymer, would correspond to the incorporation of the Poly ( $\epsilon$ -caprolactone) blocks. Considering this, the content of lactone was estimated by the difference between the molecular weight of the grafted copolymer and the Dextran precursor. The weight-average molecular weight ( $\bar{M}_w$ ), the content of  $\epsilon$ -caprolactone and the polydispersity were approximately 15,000, 35% and 1.3, respectively. By  $^1\text{H}$  NMR spectrum the weight-average molecular weight was determined to be approximately 16,500. The difference of results by both techniques should be attributed to the solubility issues of copolymer because a solvent mixture was used to record NMR spectrum.

The images of polymer morphologies obtained from Polarized Light Microscopy (PLM) are shown in Fig. 2. It is noted that the semi-crystalline behavior and spherulitic growth of PCL at different molecular weights have been widely studied, mainly by calorimetric techniques and optical microscopy (An, Kim, Chung, Lee, & Kim, 2001; Muller et al., 2004). In contrast, Dextran did not show a remarkable crystalline behavior, in comparison with PCL, as observed by the microscope image. This observation is in good agreement with reported studies on Dextran with high molecular weight ( $\bar{M}_w > 2000$ ). Previous studies propose that depending on the molecular weight, Dextran could engage in rod-like molecular organization, which is associated with liquid-crystalline behavior (Edgar and Gray, 2002; Gekko, 1981, pp. 415–438; Icoz, 2008). Therefore, it is expected that the morphology image of the copolymer would show deviations in both the shape and size of the spherulites isolated from the PCL homopolymer. This could be



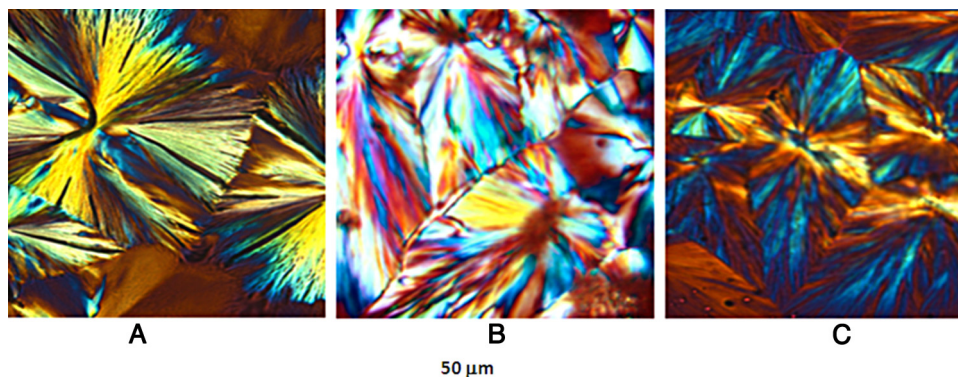
**Fig. 1.**  $^1\text{H}$  NMR spectrum of Poly ( $\epsilon$ -caprolactone)-grafted Dextran. The measurement was carried out at room temperature using a solvent mixture DMSO- $d_6$ : $\text{D}_2\text{O}$  (50:50, v/v).

due to the presence of Dextran domains restricting the crystalline growth of the PCL crystalline fraction.

### 3.2. Determination of critical aggregation concentration (CAC) by fluorescence spectroscopy

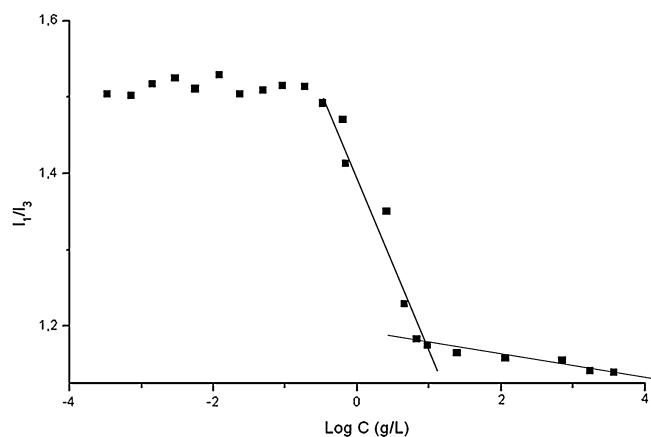
To determine the CAC of Dx-g-PCL micellar nanoaggregates, studies in aqueous medium using Pyrene  $5 \times 10^{-5}$  M as a fluorescence probe molecule were carried out. This probe molecule has been widely used because it exhibits a high sensitivity to

the micropolarity of the surrounding environment (Bains, Patel, & Narayanaswami, 2011; Matsui, Mitsuishi, & Miyashita, 1999). The preferential solubility of this molecule into reservoirs with hydrophobic characteristics is well known, which results in the variation of the absorbance intensity of the specific bands, as Pyrene is an aqueous medium. Hence, the intensity of the bands designated as  $I_1$  and  $I_3$  at 372 nm and 384 nm, respectively, varies. These bands provide information about the micropolarity of the environment where the Pyrene molecules are located. Consequently, a diminishing of the ratio  $I_1/I_3$  indicates an increase in the hydrophobicity of



**Fig. 2.** Polarized optical microscopy pictures of (A) Poly ( $\epsilon$ -caprolactone) ( $\bar{M}_n = 2000$ ), (B) Dextran ( $\bar{M}_n = 10,000$ ) and (C) Poly ( $\epsilon$ -caprolactone)-grafted Dextran ( $\bar{M}_w = 15,000$ ). The samples were prepared onto a microscope slide by solution-casting method.





**Fig. 3.** Plot of  $I_1/I_3$  ratio versus the logarithm of concentration of Dx-g-PCL copolymer. The concentration of Pyrene used was  $5 \times 10^{-5}$  M.

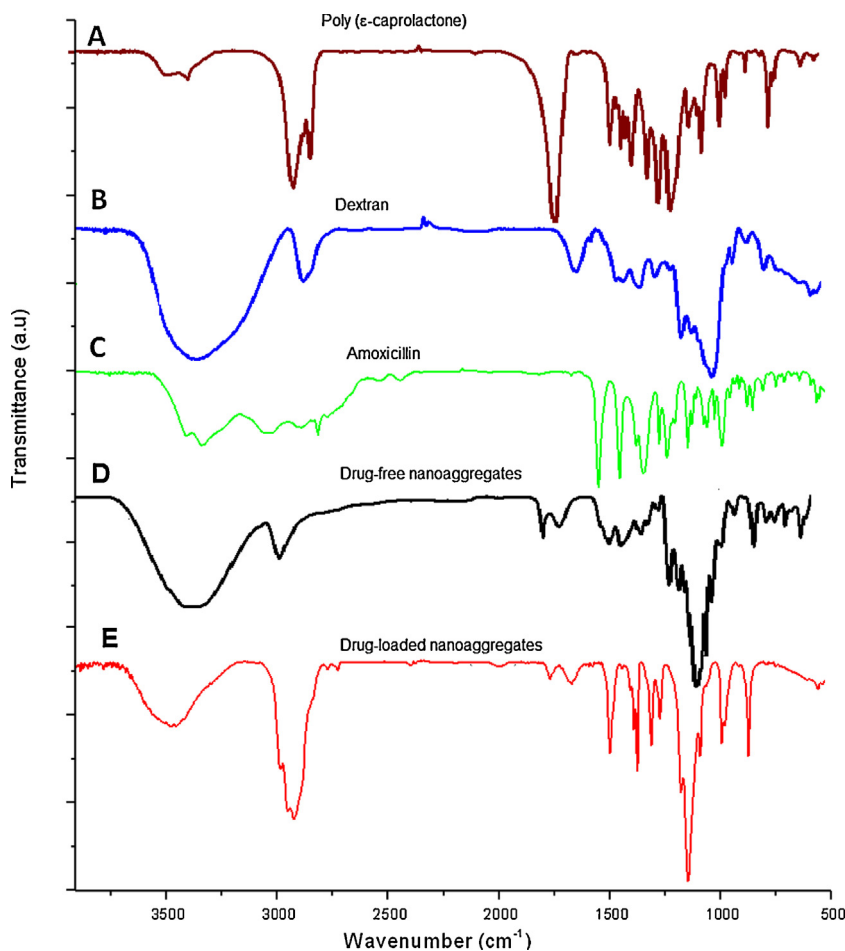
the medium surrounding the Pyrene molecules (Kalyanasundaram & Thomas, 1977). In Fig. 3, the plot corresponding to the ratio  $I_1/I_3$  is shown as a function of the Dextran-g-Poly ( $\epsilon$ -caprolactone) copolymer logarithmic concentration. It is worth noting the dependence on the fluorescent behavior of Pyrene with the variation of the copolymer concentration, indicating changes in the polarity of the environment due to the formation of micellar aggregates. This fact

can be attributed to the presence of the PCL-grafted chains, which should be understood as an increase in the local hydrophobicity.

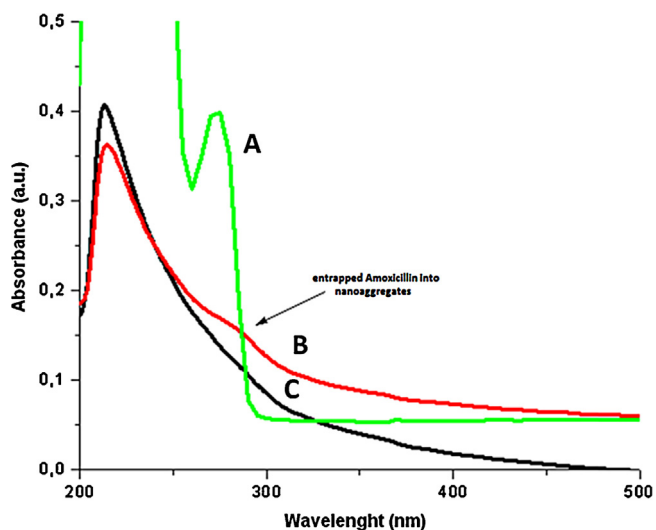
Finally, the CAC for the copolymer was determined to be approximately  $6.5 \times 10^{-4}$  M, as indicated by the interception of two straight lines displayed in the figure. Apparently, the CAC is lower than for other micellar systems under similar conditions, indicating a suitable stability of the Dx-g-PCL micellar nanoaggregates in aqueous medium (Francis, Lavoie, Winnik, & Leroux, 2003; Lukyanov & Torchillin, 2004; Nasongkla et al., 2006).

### 3.3. IR and UV–vis spectroscopy studies

Spectroscopic techniques were used as tools to analyze the copolymer micellar nanoaggregates ability to host Amoxicillin molecules in their nanoreservoirs. It is expected that the spectrum of drug-loaded nanoaggregates should show frequency shifts and intensity changes of specific signals compared to the precursor spectra. Fig. 4 shows the normalized IR spectra for PCL and Dextran precursor polymers, drug-free nanoaggregates, bare Amoxicillin and Amoxicillin-loaded nanoaggregates. A moderate intensity signal for the drug-free Dx-g-PCL micellar nanoaggregates is observed around  $1740 \text{ cm}^{-1}$  corresponding to the carbonyl stretching vibrations of the PCL-grafted chains (Ydens et al., 2000). The broad band near  $3500 \text{ cm}^{-1}$  is assigned to the hydroxyl groups of the Dextran backbone. Additionally, the bands from the IR spectrum of Amoxicillin at  $3350 \text{ cm}^{-1}$  and  $1570 \text{ cm}^{-1}$  are assigned to the N–H and C=O stretching vibrations (García-Reiriz, Damiani, & Olivieri, 2007).



**Fig. 4.** FT-IR spectra of (A) Poly ( $\epsilon$ -caprolactone) = 2000 (brown line), (B) Dextran = 10,000 (blue line), (C) Amoxicillin (green line), (D) Amoxicillin-free nanoaggregates (black line) and (E) Amoxicillin-loaded nanoaggregates (red line). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)



**Fig. 5.** Absorption spectra for solution of (A) Amoxicillin 0.5 g/L (green line), (B) Amoxicillin-free nanoaggregates 1.5 g/L (black line) and (C) Amoxicillin-loaded nanoaggregates 1.5 g/L (red line). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Spectra of the precursors show remarkable differences from the spectrum of the drug-loaded nanoaggregates. This is evidenced in the initial broad signals becoming sharp ones between  $1500\text{ cm}^{-1}$  and  $700\text{ cm}^{-1}$ , which could be attributed to drug entrapment in the nanoaggregates. In addition, the intensity of the  $-\text{OH}$  signals for the drug-loaded nanoaggregates is diminished in comparison with the drug-free ones. Similar behavior was observed for the signal of the carbonyl groups in the PCL chains. As discussed earlier, it is likely that these functional groups contribute significantly to the formation and stability of the drug–copolymer nanoaggregates, playing a key role in the processes of drug entrapment and release behavior.

The UV–vis spectra for drug-free and drug-loaded Dx-g-PCL micellar nanoaggregates, as well as for Amoxicillin, are plotted in Fig. 5. All spectra measurements were carried out in a phosphate buffer medium (0.1 M, pH 7.2). The analysis of the Amoxicillin absorbance shows two peaks at 226 nm and 274 nm. Several authors attribute these peaks to the chromophore groups, such as, amides, carbonyls, phenyl rings and so on (Fuentes, Gonzalez-Gaitano, & García-Mina, 2006; van Thor, Gensch, Hellingwerf, & Johnson, 2001). Hence, the drug–copolymer interactions should generate absorbance changes and a slight red shift (bathochromic effect) corresponding to the chromophore groups. Indeed, a very slight red shift nm of the absorbance bands for the drug-free Dx-g-PCL micellar nanoaggregates from 210 nm to 215 nm helps to confirm that the micellar nanoaggregates of the copolymer contain host nanoreservoirs of the drug. In addition, from the Amoxicillin spectrum, it is observed that the band initially at 274 nm becomes broader and is red-shifted to 290 nm as the drug is entrapped in the Dx-g-PCL nanoaggregates.

#### 3.4. Drug entrapment efficiency (EE%)

Because the Dx-g-PCL micellar nanoaggregates are considered to have properties suitable for potential nanocarriers, allowing for the incorporation of a wide range of drugs, the nanoaggregate entrapment efficiency (EE%) of Amoxicillin was assessed. Initially, a calibration curve was performed by recording the UV–vis spectra, following the 274 nm band at different concentrations of Amoxicillin in phosphate buffer. Based on the formulas detailed above, the EE% was determined to be approximately 78% (w/w). Apparently, considering other polymer–drug formulations previously reported by different authors, moderate values were obtained (Kaygusuz

**Table 1**

Values of size distributions and zeta potentials for drug-free and drug-loaded nanoaggregates at different times.

| Dx-g-PCL micellar nanoaggregates | Diameter (nm)    | $\zeta$ (mV)    |
|----------------------------------|------------------|-----------------|
| Drug-free                        | $86.3 \pm 7.2$   | $-38.1 \pm 1.7$ |
| Drug-loaded for 5 h              | $94.4 \pm 13.1$  | $-31.4 \pm 1.2$ |
| Drug-loaded for 30 h             | $117.0 \pm 21.6$ | $-18.4 \pm 2.4$ |

& Erim, 2013; Pongjanyakul & Rongthong, 2010; Xiong, Zhang, Xue, & Huang, 2013). A likely explanation is based on the fact that in an aqueous suspension, Dx-g-PCL nanoaggregates should be similar to traditional micellar aggregate organization; that is, a shell and core are formed by Dextran and PCL chains, respectively, using Poly (ethylene glycol) as a stabilizing agent to prevent agglomeration phenomena of micellar nanoaggregates. Depending on the predominant drug–polymer interaction types and the hydrophilic–hydrophobic balance, it is expected that Amoxicillin be mainly hosted in micellar nanoreservoirs formed by PCL tails. In this context, the amphiphilic features of Amoxicillin play an important role in the entrapment process by the PCL nanoreservoirs.

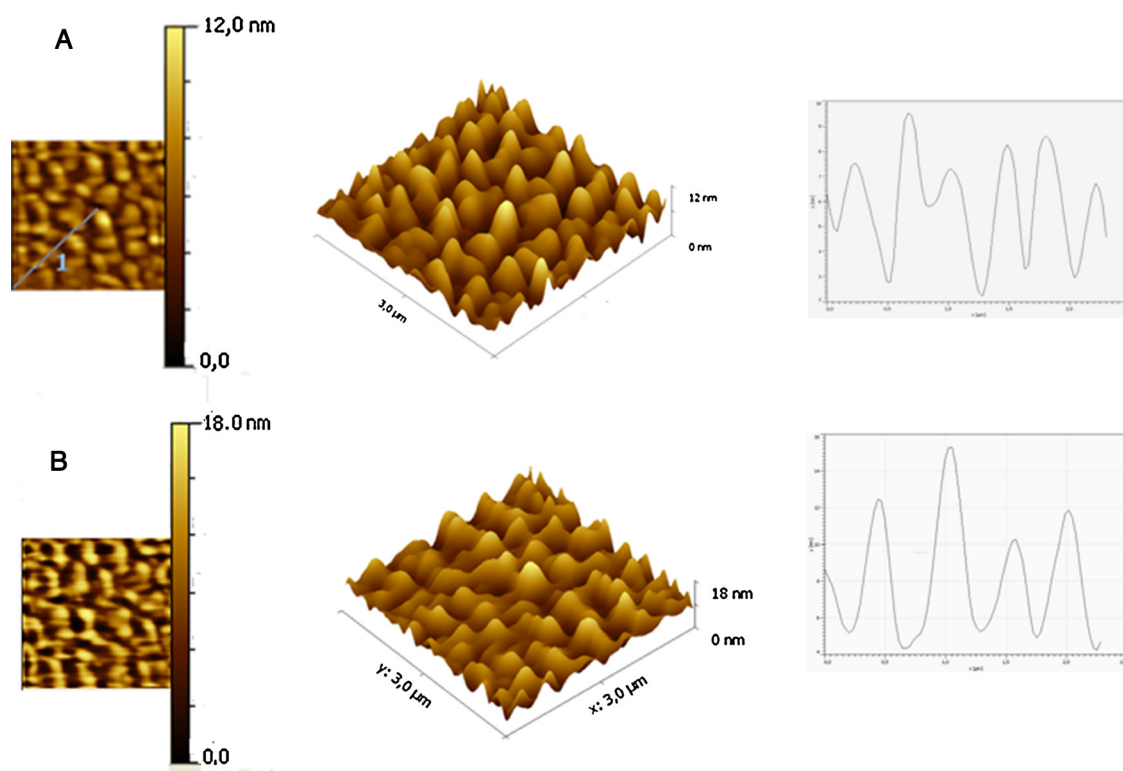
#### 3.5. Size distribution and zeta potential ( $\zeta$ )

It is well known that the encapsulation of drugs into polymeric nanoaggregates could improve the solubility and subsequent release at a specific action site or organ tissue. Keeping in mind these considerations, small particles should penetrate easier through different biological membranes, thereby contributing to a controlled release process. Considering these reasons, both the size distribution and stability of the micellar nanoaggregates were also characterized. Table 1 summarizes both the drug-free and drug-loaded size distribution and zeta potential ( $\zeta$ ) values at different release times for the micellar nanoaggregates.

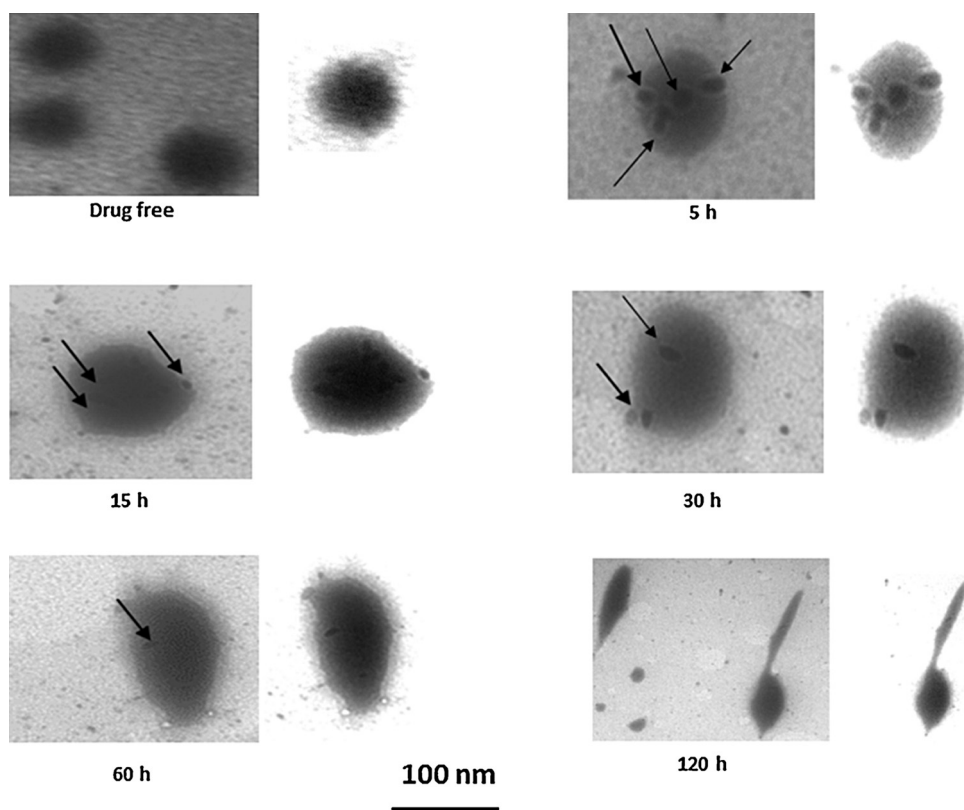
Both the drug-free and drug-loaded nanoaggregates were previously lyophilized and resuspended in a phosphate buffer (PBS 0.1 M, pH 7.2) for measuring the size distribution. The results showed that drug-free nanoaggregates have an average size of approximately 86 nm with a narrow size distribution (less than 10%) (Bretler, Pellach, Fridman, & Margel, 2014; Nie, Liu, Shen, Chen, & Jiang, 2007). Hence, it was demonstrated that the double emulsion method was a procedure quite suitable for the preparation of Dx-g-PCL micellar nanoaggregates in an aqueous solution using PEG as a steric stabilizing agent.

As for the drug-loaded nanoaggregates, greater average sizes than for the drug-free nanoaggregates at different release times were observed. Additionally, the polydispersity also increased, which is attributed to the drug release mechanism, producing a loss of the spherical shapes in comparison to drug-free nanoaggregates in similar phosphate buffer conditions.

Zeta potential is an important parameter used to establish a criterion about the stability of the nanoaggregates in a determined environment. The potential is calculated from electrophoretic mobility measurements. In simple terms, high absolute values of zeta potential are related to high stability of the nanoaggregates; hence, aggregation phenomena would be prevented. All measurements were carried out by applying a voltage of 150 mV. Negative surface charges were found for both the drug-free and drug-loaded nanoaggregates. From Table 1, it was observed that the absolute value of  $\zeta$  for drug-free nanoaggregates is approximately 38 mV, greater than the drug-loaded values (approximately 31 and 18 mV for drug-loaded nanoaggregates after 5 h and 30 h, respectively). Additionally, a diminishing stability in the drug-loaded nanoaggregates as the release time increases in a phosphate medium was also observed. This behavior could be related to factors such as ionic



**Fig. 6.** AFM images and respective topographic profiles obtained for (A) drug-free and (B) drug-loaded Dx-g-PCL nanoaggregates.



**Fig. 7.** TEM micrographs of drug-free and drug-loaded Dx-g-PCL micellar nanoaggregates at different times of release. On right side, bright field micrographs of the nanoaggregates are shown.

strength, pH, the quantity of drug entrapped in the nanoaggregates and the release mechanism, among others.

### 3.6. Topography and morphology of copolymer micellar nanoaggregates

The use of AFM techniques allowed for the analysis of the topography for both the drug-free and drug-loaded Dx-g-PCL micellar nanoaggregates to gain insight into the processes of entrapment and release behavior based on surface morphology, roughness parameters and nanostructure. The results of both the scans and surface-roughness profiles obtained from the AFM image analyses are shown in Fig. 6. All samples were prepared from an aqueous suspension, which was deposited by dripping onto a silicon wafer substrate assisted by a spin-coater. Then, the substrates were dried under vacuum for 48 h to remove any residual solvent. In general, it is noted that drug-free nanoaggregates are displayed as isolated structures with remarkable spherical shapes and an average maximum height profile of  $R_z = 4.7$  nm. This value is smaller than the drug-loaded ones ( $R_z = 7.7$  nm), helping to confirm the presence of drug-entrapped nanoaggregates. Moreover, the surface roughness was also analyzed by the root mean square (RMS) roughness, providing information about the deviations of the actual surface from an ideal one that is composed of atoms. The values of RMS roughness obtained for drug-free and drug-loaded nanoaggregates were 1.6 and 2.9 nm, respectively. Hence, the drug-loaded nanoaggregates showed a RMS roughness greater than the drug-free ones, suggesting that the drug-entrapped nanoaggregates would yield a deviation from spherical shapes as the drug is hosted in the nanoreservoirs.

The morphology of both the drug-free and drug-loaded copolymer micellar nanoaggregates was analyzed by Transmission Electronic Microscopy. The micrographs are shown in Fig. 7. In the case of the drug-free nanoaggregates, spherical morphologies were also observed, which is in good agreement with the AFM measurements. This observation confirms that the micellization process takes place because of self-assembling of the Dx-g-PCL macromolecules. In contrast, drug-loaded nanoaggregates undergo an appreciable deviation from the spherical morphologies as release takes place. Drug release mechanisms were responsible for increasing both the size distribution and polydispersity of the drug-loaded nanoaggregates at different release times. The micrographs of the drug-loaded nanoaggregates show the presence of small agglomerates (indicated by arrows). This is attributed to the phenomenon of drug agglomeration into hydrophobic core microdomains formed by PCL. In addition, bright field micrographs were also analyzed with the aim of achieving a better assessment of the Dextran (shell) and PCL (core) polymeric microdomains and drug agglomerates. The results indicate that drug agglomerates entrapped in micellar nanoaggregates diminish in size as release takes place because of drug diffusion from the nanoaggregates toward the bulk solution. Additionally, it can be noted that the morphologies of micellar nanoaggregates are turned into irregular shapes. This fact is attributed to the PCL block degradation in the phosphate medium. Based on the above-mentioned phenomena, the micrograph sequence shows that the size of the drug agglomerates gradually diminish until the fully released state observed after 120 h in a phosphate medium.

### 3.7. In vitro drug release

Because the drug release mechanism is a very important issue, a better understanding of the processes involved is required. For this reason, it is necessary to focus on achieving a steady concentration of the drug in the human body, avoiding the use of high doses in medical therapies. In this context, *in vitro* assays

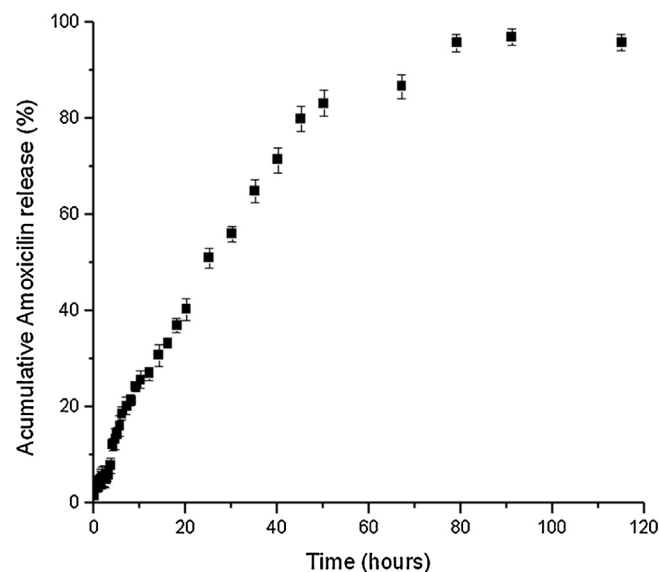


Fig. 8. In-vitro drug release profile of Amoxicillin into Dx-g-PCL micellar nanoaggregates. The data ( $N = 3$ ) present respective mean standard deviation.

are a useful way to gain insight into the release behavior of the drug–copolymer nanoaggregates. In general terms, drug release mechanism occurs by either drug diffusion or eroding of the polymer matrix, which influences the rate of release. The rate of release mainly depends on the copolymer characteristics, such as molecular weight, hydrophilic–hydrophobic composition, size distribution and polydispersity of the nanoaggregates, among others.

Fig. 8 shows the accumulative release profile of Amoxicillin as a function of exposure time. From the curve, two stages can be observed. The first stage is characterized by an initial quick release up to 50 h, and the second stage is followed by a remarkably slower release stage (50 h onwards). Likely, some drug agglomerates are closer to the micellar nanoaggregate surface, whereby a quick release should be expected to occur. These observations were confirmed by TEM analyses (Fig. 8), in which it is observed that after approximately 5 h, drug agglomerates appear close to the micellar shell. Likewise, it was also found that drugs were entrapped in the core of the micellar nanoaggregates. The release profile shows that after the first 50 h, the release rate reaches a plateau, which remains relatively steady until the release assay is fully carried out.

A very useful empirical exponential relation was developed by Ritger and coworkers. This relation can appropriately describe the first 60% of fractional drug release from a polymeric matrix considering different mechanisms and geometries (Ritger & Peppas, 1987a,b):

$$\frac{M_t}{M_0} = kt^n \quad (1)$$

where  $M_t/M_0$  is the ratio between the cumulative amount of drug released at time  $t$  and the initial amount of drug loaded into the nanoaggregates;  $t$  is the release time,  $k$  is a constant that depends on the drug type, geometry and structure of the polymeric matrix, and  $n$  is an exponent related to the drug release mechanism. It should be noted that an in-depth discussion of Eq. (1) can be found elsewhere (Ritger & Peppas, 1987a,b).

For polymeric systems with a spherical geometry, a value of  $n = 0.43$  is related to a drug release process controlled by diffusional transport. A value of  $n = 0.85$  means that polymeric matrix eroding dominates the drug release process. In our case, a correlation coefficient,  $R^2$ , value of 0.9946 indicates that the data fits well with this empirical relation. From Eq. (1), a value of  $n = 0.73$  for Dx-g-PCL nanoaggregates was calculated. An intermediate value should



be understood as a release process controlled by a combination of both drug diffusion and a polymeric eroding matrix mechanism (Miao, Cheng, Zhang, Wang, & Zhuo, 2007). As discussed earlier, spherical-shaped drug-free nanoaggregates were observed by TEM. Eroding processes would be induced by water imbibition of the micellar nanoaggregates, which leads to their swelling and subsequent chain degradation of the PCL segments (Ferrari et al., 2013; Persenaire, Alexandre, Degee, & Dubois, 2001). This is supported by TEM micrographs showing highly irregular micellar nanoaggregate shapes corresponding to the last hours of the drug release process.

#### 4. Conclusions

Biodegradable and biocompatible Poly( $\epsilon$ -caprolactone)-grafted Dextran copolymers were successfully synthesized. Micellar nanoaggregates formed by simple emulsion (W/O) in aqueous medium were successfully prepared and assessed as a potential drug nanocarrier system. The characterization of the nanoaggregates by TEM and DLS techniques was carried out. Spherical morphologies with an average diameter of approximately 83 nm for the drug-free nanoaggregates were observed. In addition, the zeta potential measurements show that the stability for drug-free nanoaggregates was greater than those of drug-loaded nanoaggregates in a phosphate medium. Likely, this effect is due to the presence of Amoxicillin in the nanoaggregates. In this respect, Dx-g-PCL nanoaggregates showed moderate Amoxicillin entrapment efficiency and uninterrupted *in vitro* drug release during the whole duration of the experiment. The release mechanism consists of a combination of both diffusion and eroding of the polymer matrix. Based on these results, Dx-g-PCL micellar nanoaggregates are ideal entrapment systems with attributes suitable for use as nanocarriers in drug delivery.

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