Electrospinning of Drug-Loaded Polymer Systems: Preparation and Drug Release

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ABSTRACT: In this study, biomedical devices for tissue regeneration loaded with anti-inflammatory drugs were formulated and characterized. We realized these systems by homogenously dispersing an interclay, a lamellar hydrotalcite loaded with diclofenac sodium (HTlc-DIC), in a polymeric matrix made of poly(ε-caprolactone) to produce a controlled release of the drug. These biomedical devices were obtained with the electrospinning technique, which has proven to be very efficient. In particular, in this study, microfibers loaded with HTlc-DIC were obtained, and the drug delivery of diclofenac sodium from these systems was studied and compared with the release from biomedical devices loaded with the free drug. We analyzed these results by evaluating the diffusivity coefficient by means of the pure diffusive mathematical model. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 122: 3551–3556, 2011

Key words: diclofenac sodium/hydrotalcite (HTlc-DIC); poly(ε-caprolactone) (PCL); electrospinning; drug release

INTRODUCTION

Biomedical devices are the results of tissue engineering, a branch of science that has the potential to create tissues and organs *ex novo*.[1,2] In particular, because of bone tissue repair, the autogenous cell/tissue transplantation would eliminate problems of donor scarcity, supply limitation, pathogen transfer, and immune rejection.[3,4] Biodegradable polymers are potential material candidates and are under investigation for scaffolds and tissue repair.[5,6] They can be of use also as drug-delivery systems because they are able to release, in a controlled manner, the active principles that could be of aid in the tissue rebuilding process.

The main advantage of the use of biodegradable polymers as drug-delivery systems is the degradability of the dosage form and the elimination of the material from the body once the device is no longer needed.[7] Polymer-based controlled drug-delivery systems have indeed gained great attention because of the improvements they bring to therapeutic efficacy and their reduction of toxic effects.[8] The study and development of controlled drug-delivery systems has allowed a qualitative change in the approach to the development of new drugs.[7] However, the incorporation of low-molecular-weight antinflammatory molecules into polymeric matrices has led to two important disadvantages: (1) the migration is too rapid, and (2) the release of the drug cannot be easily predicted.[9–12]

To face these problems, a method for fixing active molecules (antinflammatory, antibiotic, antimicrobial) into an inorganic compound able to hold them that allows a very slow and controlled release in selected conditions has been recently proposed.[9–12]

These compounds, also known as *anionic clays*, have the general formula $[M(II)_{1-x}M(III)_x(OH)_2](A_{x/n})\cdot mH_2O$, where M(II) is a divalent cation, such as Mg, Ni, Zn, Cu, or Co; M(III) is a trivalent cation, such as Al, Cr, Fe, or Ga; and A^{n-} is an anion of charge n, such as CO_3^{2-}, Cl^-, NO_3^-, or organic anion.[13–17] These clays are generally indicated with the acronym of LDH (layered double hydroxides), and they can be modified with a simple procedure to obtain a high level of purity, with the drugs introduced in the form of anionic organics species that are more numerous than the cationic ones.

For this reason, layered compounds can be considered a very attractive class of lamellar solids: the release of active molecules in molecule-intercalated layered materials is potentially controllable.

Biomedical devices based on a modified clay with commercial antinflammatory drugs [e.g., diclofenac sodium (DIC), a nonsteroidal anti-inflammatory drug] have been obtained. These are called *hydrotalcite loaded with diclofenac sodium* (HTlc-DIC or HDik),[18–20] and they can be dispersed in an aliphatic polyester [poly(ε-caprolactone) (PCL)].[21–23] The active component may be released via a deintercalation process,

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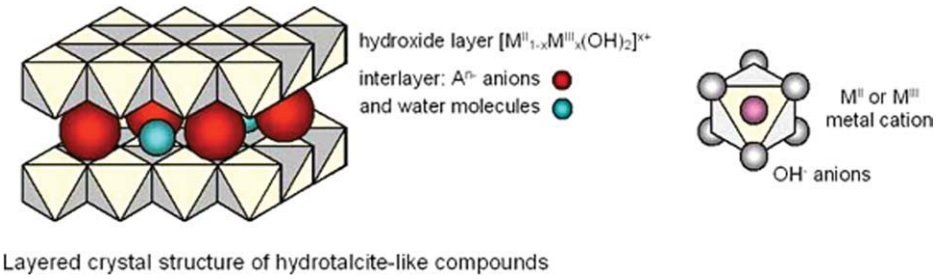

Figure 1 HTlc-DIC chemical structure. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

which occurs because of the ion exchange or displacement reactions. The rate of release is dependent on the diffusion trough the matrix. Because of the balance between the ion exchange and the diffusion process, the use of layered compounds can give a controlled release of the active ingredient.

Furthermore, the electrospinning process can be used to produce biomedical devices in the shape of membranes on the basis of a layered compound loaded with anti-inflammatory drugs. This technique allows one to create polymeric fibers with diameters in the range of the nanoscale–microscale.[24–28] In particular, the nanoscale–microscale diameter of electrospun fibers introduces properties such as an increased surface-to-volume ratio and modifications of the release rate.[29]

The aim of this work was then to study the preparation of such membranes made of PCL with HDik via electrospinning and to study the release process of diclofenac, with the final goal being a controlled release system that was able to release the drug over a long time.

EXPERIMENTAL

Materials

The materials used to realize the biomedical devices were biopolymers and fillers compatible with the human body; in particular, the polymeric matrix chosen was the aliphatic polyester PCL (60,000 Da, Sigma Aldrich srl, Milan, Italy).

The fillers chosen to design active devices had an antinflammatory effect and were (1) DIC and (2) HTlc-DIC.

DIC belongs to the category of nonsteroidal anti-inflammatory drugs. The drug used in this work was produced from Sigma Aldrich, and its chemical formula was $C_{14}H_{10}Cl_2NNaO_2$.

The clay used was HTlc-DIC. This structure was obtained according to a previously reported procedure.[18–20] The HTlc-DIC chemical formula was $[Mg_{0.65}Al_{0.35}(OH)_2]DIC_{0.35}{\cdot}0.86H_2O$; therefore, approximately one half of the mass was due to the drug, and the remaining mass was due to the clay and to the hydration water. A schematic of the structure is reproduced in Figure 1.

This nanohybrid/clay was chosen to obtain a retarded release of the drug; in fact, the peculiarity of HTlc-DIC is that the release process of the drug is triggered by the ion-exchange phenomenon occurring between the dissolution medium and the clay; this delays drug delivery.

Methods

Sample preparation and characterization

The method used in this work to realize a membrane with an antinflammatory effect was the electrospinning technique. With this technique, it was possible to obtain samples with a fibrous structure from a polymeric solution. In particular, the apparatus used in this work was based on an vertical support for the capillary (the syringe), whereas an aluminum plate (15×15 cm^2) was used as a second collector.

The solutions were prepared accordingly to previous findings:[30] the first step consisted of the dissolution of the polymer (PCL) in acetone (Sigma Aldrich; we obtained a solution with 15 wt % PCL). Then, the fillers were slowly added to the polymeric solution, and it was vigorously stirred at 50°C for 3 h.

To determine the optimal conditions for the release, the filler concentration was changed between 5 and 10 wt % (with respect to the PCL content) for samples loaded with the HTlc-DIC (the two samples were named PCL + 5% HTlc-DIC and PCL + 10% HTlc-DIC, respectively) and 2.5 and 5 wt % (with respect to the PCL content) for samples loaded with the free drug used for comparison (the two samples were named PCL + 2.5% DIC and PCL + 5.0% DIC, respectively). This last choice (to use amounts of free drug that were half of the loaded clay amount) was made because, as previously noted, half of the HTlc-DIC structure was DIC.

Once we prepared the starting solution, electrospinning in a typical run was carried out as follows. PCL, PCL/HTlc-DIC, or PCL/DIC solutions were placed in a glass syringe (5 mL) with a capillary tip diameter of 0.6 mm, and a flow rate of 10 μL/min was used (this value was chosen after different preliminary tests because it gave the best electrospinning

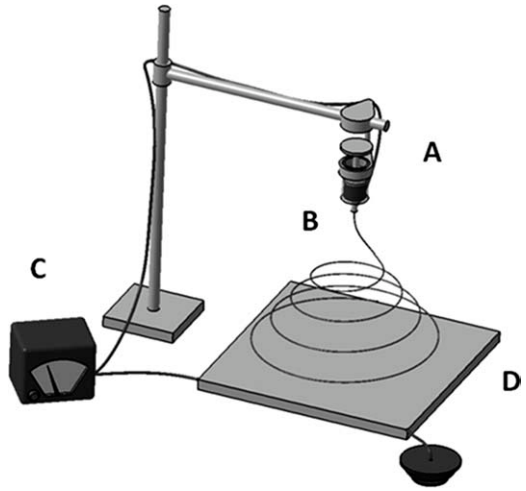

Figure 2 Schematic representation of the electrospinning process: (A) syringe (loaded with polymeric solution), (B) metal needle (the capillary), (C) voltage supply, (D) fiber generation, and (E) collector.

process). This flow rate was controlled by an infusion pump (HARVARD PHD 2000, Crisel Instruments Srl, Rome, Italy). The solution was fed to the pump after a heating step [temperature (T) = 40°C] to reduce the solution viscosity and help the spinning process. To maintain this temperature throughout the system, the syringe, and the tube, in which the polymeric solution moved, a heating system was realized.

A copper wire was mounted in the spinneret and used as the positive electrode. Between the tip of the syringe and the second collector we applied a constant voltage of 30 kV (direct-current power supply, HCP 35-35000, FUG, Rosenheim, Germany), and a constant distance (17 cm) was used for all of the tests. The device is schematically reproduced in Figure 2.

Samples obtained with these operative conditions were nonwoven membranes. These membranes were placed *in vacuo* for 72 h to remove any residual solvents. The morphology of the membranes was observed by scanning electron microscopy (scanning electroscope microscope (SEM), Leo 1430, Carl Zeiss NTS GmbH, Oberkochen, Germany). The drug-loading ratio was tested by thermogravimetric analysis (TGA; Mettler-Toledo TGA/SDTA851). TGA confirmed the expected loading ratio, and because it was already shown in a previous study,[30] no further TGA data are reported here.

Drug-delivery evaluation

The drug-delivery process was studied to evaluate the benefit for the drug release of the use of a nanohybrid polymeric material instead of a polymeric material loaded with the free drug. To evaluate the effect of the temperature on the release kinetics, the release tests were performed at two different temperatures [T = room temperature (T_{room}) and T = 37°C) for samples with 5 and 10% HTlc-DIC (PCL + 10% HTlc-DIC and PCL + 5% HTlc-DIC). For comparison, two samples in which the drug DIC was directly dispersed in the polymeric matrix were tested. The percentages of DIC used for these last samples were 2.5 and 5.0% (PCL + 2.5% DIC and PCL + 5.0% DIC). All of the samples loaded with HTlc-DIC (5 and 10%) were used for both experiments at $T = T_{room}$ and T = 37°C. Instead, all of the samples loaded with DIC (2.5 and 5%) were used at $T = T_{room}$, and only PCL + 5.0% DIC was used for the analysis at T = 37°C.

The runs performed at T = 37°C required the building of an *ad hoc* apparatus, which allowed the use of a dissolution volume of 25 mL, which was stirred and temperature controlled. This was mandatory because the USP dissolution testing apparatus (Sotax, Bergamo, Italy) required a minimum of 500 mL of dissolution volume, which caused a very low drug concentration in the dissolution medium. Indeed, the samples were very small (40/50 mg), and they contained drug in the amount 5–10%; therefore, the use of a large volume would have resulted in a very low drug concentration (10^{-3} to 10^{-4} mg/mL). The apparatus built consisted of a series of small beakers located into a isothermal bath and placed on a multiplace magnetic stirrer. On the other side, the experiments at $T = T_{room}$ were carried out with an orbital shaker KS 130 (IKA) to obtain constant mixing for the system. A physiological solution (9 g of NaCl in 1 L of distilled water) was selected as the dissolution medium.

For each sample, the dissolution medium was sampled, assayed spectroscopically (Shimadzu UV–VIS 2550 (Shimadzu UV-VIS 2550, Milan, Italy), accuracy = ±0.3 nm, resolution = 0.1 nm, and wavelength range = 190–900 nm; the Diclofenac was assayed working at λ = 275 nm), and replaced with fresh medium at different times for 1 month. Therefore, the release was evaluated by the summation of all of the single contributions obtained along the full experiment.

RESULTS AND DISCUSSION

To test the drug-delivery phenomena, the electrospun membranes were realized with different drug and clay percentages. They were characterized with SEM studies, and we evaluated the drug-release phenomena at two different temperatures ($T = T_{room}$, T = 37°C). The body temperature (37°C) was investigated because most of the release phenomena would take place at this temperature, whereas T_{room} was investigated to test the dependence of the diffusion phenomena on the temperature. Attention was

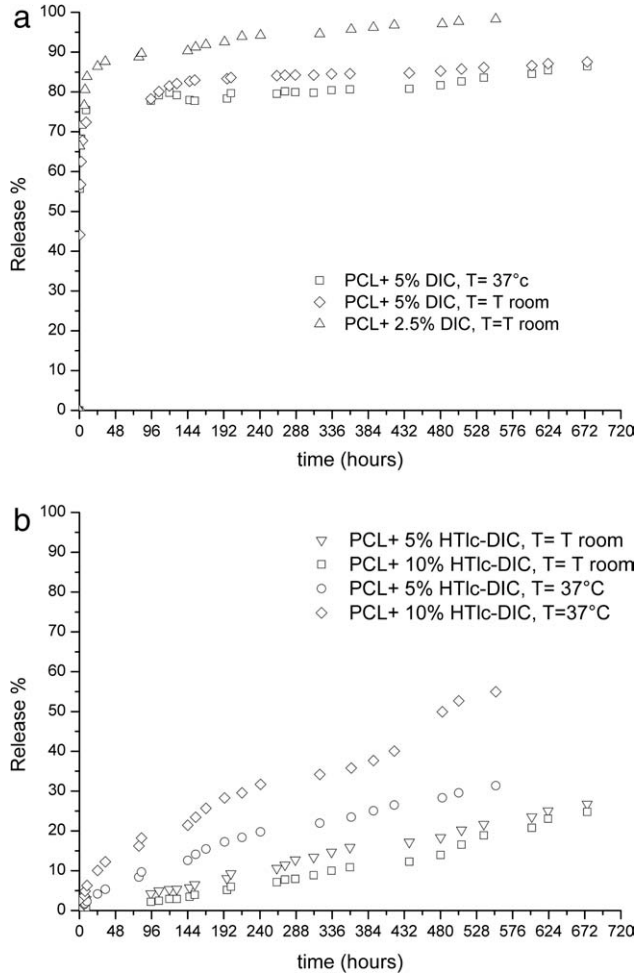

Figure 3 Drug-release curve samples loaded with: (a) DIC and (b) HTlc-DIC.

focused also on the morphology of the membranes before and after the drug-delivery tests.

Drug-release phenomena evaluation

In this section, the experimental drug release from the samples results are reported and used as source data to be analyzed with the proposed mathematical model; in particular, in Figure 3, the drug-release curves obtained with the tested samples are shown.

The curves of the samples where the DIC was directly dispersed in the polymeric matrix showed an initial rapid increase of concentration followed by a plateau. The active species exited by diffusion, and this phenomenon was very rapid and weak, depending on the temperature. A very different behavior was obtained with the samples loaded with HTlc-DIC. In this case, the release phenomenon from the matrix was governed not only by the diffusion of the molecules going out of the membrane but also by the diffusion of water going in and bringing the counterions and the ionic exchange reaction. As for the effect of the temperature on the drug-release phenomena, it could be observed that it influenced, as expected, the kinetics of the process, which become faster with increasing temperature and did not change the shape of the drug-release curves. These were rapid at beginning and soon became linear with time.

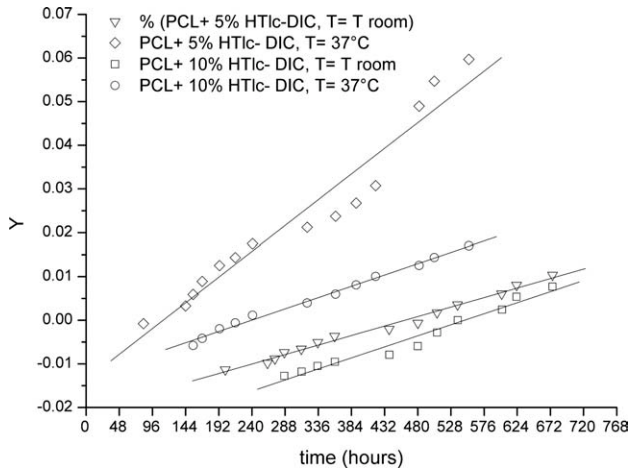

Figure 4 Diffusivity coefficient evaluation.

Even if the process was much more complicated than the pure diffusion, an attempt to describe the full process by a pseudo-diffusion phenomenon was carried out here. Therefore, the drug-release experimental results were used to evaluate the drug-release pseudo-diffusivity coefficients (D's).

In this evaluation, the system (membrane in the dissolution medium) was considered as a one-dimensional diffusion in medium bound by two parallel planes in a nonsteady state.[31] $C(t, x)$ is the actual drug concentration in the membranes, b is the thickness of the membranes ($b = 350$ μm), and the initial drug concentration inside the membrane was considered constant.

Solving the mass variation equation and using the experimental data, one can calculate the diffusivity coefficient using the concentration variation law for a long time, which is well approximated by the following equation:[31]

$$Y = -\frac{1}{\pi^2}\ln\left[\frac{\pi^2}{8}(1 - R(t))\right] = X = \frac{Dt}{b^2} \qquad (1)$$

where Y is the logarithmic ratio between the actual and the initial drug concentration, $Y = \ln(C(t)/C_0)$; instead $R(t)$ is the ratio between the total amount of

TABLE I
Diffusivity Coefficients Results (PCL + DIC)

Sample	Slope (D/b^2)	D (m^2/s)	Temperature (°C)
PCL + 2.5% DIC	1.4×10^{-2}	3.4×10^{-9}	T_{room}
PCL + 5% DIC	0.8×10^{-2}	1.6×10^{-9}	T_{room}
PCL + 5% DIC	1.7×10^{-2}	3.4×10^{-9}	37

TABLE II
Diffusivity Coefficient Results (PCL + HTlc-DIC)

Sample	Slope (D/b^2)	D (m^2/s)	Temperature (°C)
PCL + 5% HTlc-DIC	4.6×10^{-5}	2.5×10^{-14}	T_{room}
PCL + 10% HTlc-DIC	4.0×10^{-5}	2.4×10^{-14}	T_{room}
PCL + 5% HTlc-DIC	1.0×10^{-4}	4.0×10^{-14}	37
PCL + 10% HTlc-DIC	6.0×10^{-5}	3.1×10^{-14}	37

the diffusing substances which entered the sheet at time t, Mo is the initial mass of the drug inside the samples, and X is the dimensionless time. In Figure 4, the diffusivity coefficient evaluation, based on Eq. (1), is shown for each of the two samples based on HTlc-DIC and for the two temperatures investigated.

For comparison, the diffusivity coefficient for the membrane loaded with DIC was estimated, and they are shown in Table I. The results obtained for samples loaded with the free drug shown that the diffusivity coefficients were not influenced by the temperature or drug concentration. In particular, the diffusivity coefficients could be considered constant for the three samples and were around a value of 10^{-9} m^2/s.

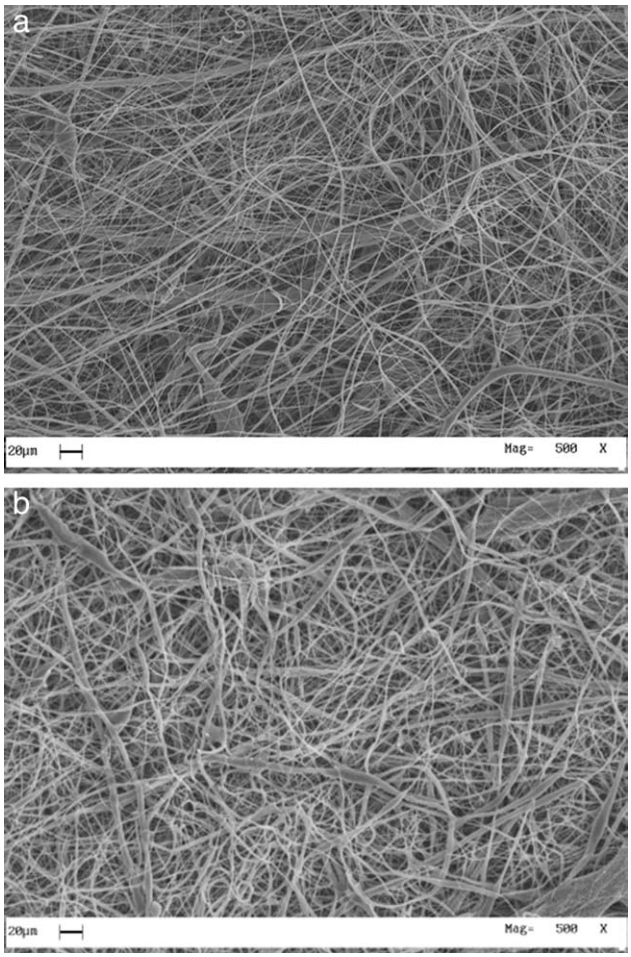

Figure 5 SEM of PCL + 10% HTlc-DIC: (a) before and (b) after drug delivery ($T = T_{room}$).

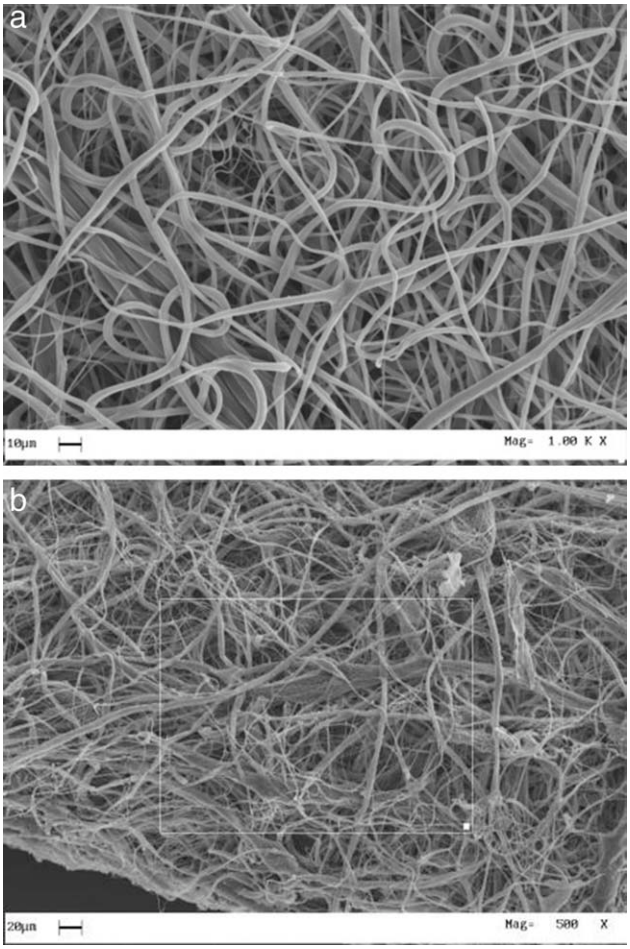

Figure 6 SEM of PCL + 5% DIC: (a) before and (b) after drug delivery ($T = T_{room}$).

In Table II, the diffusivity coefficients estimated for the membrane loaded with HTlc-DIC are summarized. The results indicate that for samples loaded with HTlc-DIC and analyzed at $T = T_{room}$, the diffusivity coefficients are not influenced by the percentage of the clay drug in the samples. Instead, for the same samples analyzed at $T = 37$°C, this parameter showed a similar value for the two different HTlc-DIC concentrations. This phenomenon could be summed at the effect of the temperature on the ionic interchange between the sample and the dissolution medium. The results obtained confirm also that the release of the drug was obviously faster for the devices loaded with the free drug.

Morphological analysis

Samples were analyzed by SEM before and after the drug-delivery tests to investigate if the shape of the

fibers changes before and after the dissolution test. Figures 5 and 6 show the micrographs of the membranes with 10% hydrotalcite (Fig. 5) and 5% DIC (Fig. 6) before and after the drug-delivery experiments.

The images show that the membrane morphology did not significantly change after drug release. Indeed, the mean fiber diameters (obtained from image analysis) were 1.72 ± 0.98 μm for PCL + 10 HTlc-DIC before drug release, 1.82 ± 0.79 μm after drug release, 1.40 ± 0.52 μm for PCL + 5% DIC before drug release, and 1.64 ± 0.46 μm for PCL + 5% DIC after drug release. Similar results were also obtained for samples used for the experimental drug-release evaluation at $T = 37°C$; this confirmed that the drug release did not affect the sample morphology.

CONCLUSIONS

Two kinds of biomedical devices based on PCL loaded with DIC and an innovative filler (HTlc-DIC) were prepared with the electrospinning technique. In the first case, the anti-inflammatory drug was free inside the membrane fibers, whereas in the second case, the drug molecules were bound to the inorganic lamellae with ionic bonds. This difference determined a very different release, which was found to be faster in the first case than in the second.

The diffusivity coefficients were evaluated in different operative conditions ($T = T_{room}$ and $T = 37°C$) and with different carrier percentages with the aid of a mathematical model based on Fick's law. The results show that diffusivity was not significantly influenced by the HTlc-DIC percentage, but it was strongly affected by the type of dispersion into the polymeric matrix.

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