Nanostructured Polymeric Membranes by Solution Blow Spinning for Drug Delivery

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Abstract

The regeneration of skin injuries can be aided by Tissue Engineering strategies, which allow the recovery of the structural and functional integrity of the damaged tissue. The Solution Blow Spinning (SBS) technique has attracted the attention of researchers due to the production, in a continuous process, of nanofiber mats, which exhibit high porosity, similarity with the tissues' extracellular matrix, and the ability to drugs local delivery. Thus, in this work polycaprolactone (PCL) / polyethylene glycol (PEG) fibers loaded with Ibuprofen, a fast-acting analgesic, were developed for application as wound dressings, aiming to assist in the skin healing process while providing comfort to the patient. Initially, fibers were produced from PCL solutions in chloroform at 8% (w/v) and PCL/PEG solutions in mass ratios of 2:1 and 1:1, where the influence of the concentration of PEG, the gas pressure (compressed air), and the solution injection rate on fiber morphology was analyzed by Scanning Electron Microscopy. Then, the best condition for the formation of PCL/PEG fibers was selected (1:1, 137,90 kPa and 7.2 ml/h), for the fabrication of membranes containing ibuprofen at proportions of 10, 30, and 60% by mass of PCL. It was possible to obtain ibuprofen-loaded PCL/PEG membranes composed of submicron fibers. However, the addition of ibuprofen destabilized the system, causing defects in the structure. The controlled drug delivery profile and the high swelling capacity of the films were verified, indicating the potential of the SBS technique in the manufacture of drug delivery systems. Nevertheless, further studies must be conducted to optimize the fiber morphology.

Keywords: SBS, Nanofibers, Wound Dressings, PCL, PEG, Ibuprofen, Drug Delivery

1. Introduction

Skin plays a crucial role in the human body, acting as a protective barrier for other organs against possible external threats. Skin wounds can compromise its structure and function, which, in turn, makes its repair crucial to the body. The natural healing process, however, is usually slow, taking from 8 to 12 weeks, in the case of acute wounds. It can also generate complications such as bacterial infections, whose proliferation is favored by the humid, warm and nutritive environment provided by the wound. Thus, tissue engineering aims to assist the regeneration of wounds, developing dressings that act as support for the tissue healing process. [1][2][3][4]

The production of dressings aims to manufacture biomaterials that mimic the extracellular matrix, that do not present toxicity, and that provide a favorable environment for cell proliferation. Among the different types of wound dressings, nanostructured membranes showcase a structure with high porosity, high surface area, and high resemblance to the extracellular matrix. In addition, the use of membranes as dressings enables the implementation of loading drugs and their controlled local delivery. [4][5]

The Solution Blow Spinning (SBS) technique is a potential wound dressing manufacture process, standing out due to its high production rate. In this technique, fibers are formed with the help of a pressurized gas, which surrounds the injected solution, molding and solidifying the fiber. By this process, it is possible to

manufacture fibers more safely, when compared to other commonly used techniques, such as electrospinning, without the presence of an electric field or the need to use conductive solvents and targets. [6-8]

Polycaprolactone (PCL) is a synthetic polyester used in the production of dressings due to its biocompatibility, biodegradability, and non-cytotoxicity. Due to its hydrophobic nature, which makes it impossible to absorb exudates and control wound moisture, PCL is normally used in conjunction with other polymers such as polyethylene glycol (PEG). As PCL, PEG also presents biocompatibility and non-cytotoxicity. However, it has a hydrophilic nature and a high capacity for liquid absorption. [9][10]

Among the drugs used for drug delivery in wound dressings, analgesics are implemented to provide comfort to the patient, while aiding in the healing process. Ibuprofen is an analgesic that has antipyretic and anti-inflammatory action, part of the Non-Steroidal Anti-Inflammatory drugs (NSAIDs), that act by suppressing enzymes that inhibit the expression of chemical signals involved in inflammation. Furthermore, its use causes a reduction in scar formation. [11]

The objective of the present work was the production by SBS technique of PCL/PEG fibers loaded with ibuprofen at 10, 30, and 60% of the mass of PCL, aiming for their application as wound dressings. For this, morphological analyzes were performed of PCL/PEG fibers, produced from PCL 8% (wt/v) solutions and PCL/PEG solutions in mass ratios of 2:1 and 1:1, spun with varied values of gas pressure and injection rate.

2. Experimental Methods

2.1. Materials

Polycaprolactone (PCL) (M_w = 93,416 g/mol, M_n = 59,920 g/mol) was obtained from Perstorp (UK) in the form of pellets. Polyethylene Glycol (PEG) (V001254, batch 0503226) was obtained from Vetec Quimica Fina (BR). Ibuprofen (IBU) (batch 336/21, 99.95% purity), used as the drug model, was imported from Delaware and fabricated in Alka Laboratories (IN). Chloroform (batch SHBG9949V), used as a solvent, was obtained from Sigma Aldrich.

2.2. Preparation of the nanofiber's films

Table 1.
Composition of the solutions used.

Sample	PCL (wt%)	PEG (wt%)	IBU (wt/wt% of PCL)
PCL	8	-	-
PCL/PEG_4	8	4	-
PCL/PEG_8	8	8	-
PCL/PEG_8-10	8	8	10
PCL/PEG_8-30	8	8	30
PCL/PEG_8-60	8	8	60

The films were fabricated by the Solution Blow Spinning method. Accordingly, 8% (w/v) PCL solution was prepared by dissolving PCL pellets in chloroform, likewise, the solutions of 4% (w/v) and 8% (w/v) PEG were made by dissolving PEG in chloroform. The PCL and PEG solutions were mixed to obtain the PCL/PEG solutions in mass ratios of 2:1 and 1:1. The solution containing Ibuprofen was prepared by dissolving Ibuprofen at 10, 30, and 60% of the dry weight of PCL on the PCL/PEG 1:1 solution. The concentration of the used solutions is displayed in Table 1.

2.3. Film Characterization

2.3.1. Fiber morphology

The morphologies of the PCL and PCL/PEG fibers were analyzed by Scanning Electron Microscopy (Versa 3D Dual Beam – FEI) using an acceleration of 10kV and a magnification of 200x, 500x, and 1000x.

2.3.2. Swelling tests

The water uptake capacity of PCL/PEG-IBU fibers was evaluated by the immersion of the fiber films of approximately 2x2 cm in 20 ml of saline solution at temperatures of 37ºC. Following that the films were removed from the containers, removed of their excess of liquid, and measured their weight in time frames of 45 minutes, 2, 3, 4, 5, 24, 48, and 72 hours. The swelling ratios of the films in percentage were calculated based on Eq. (1).

$$GI = 100\ x\ \frac{M_{ti} - M_0}{M_0} \qquad \text{Eq. (1)}$$

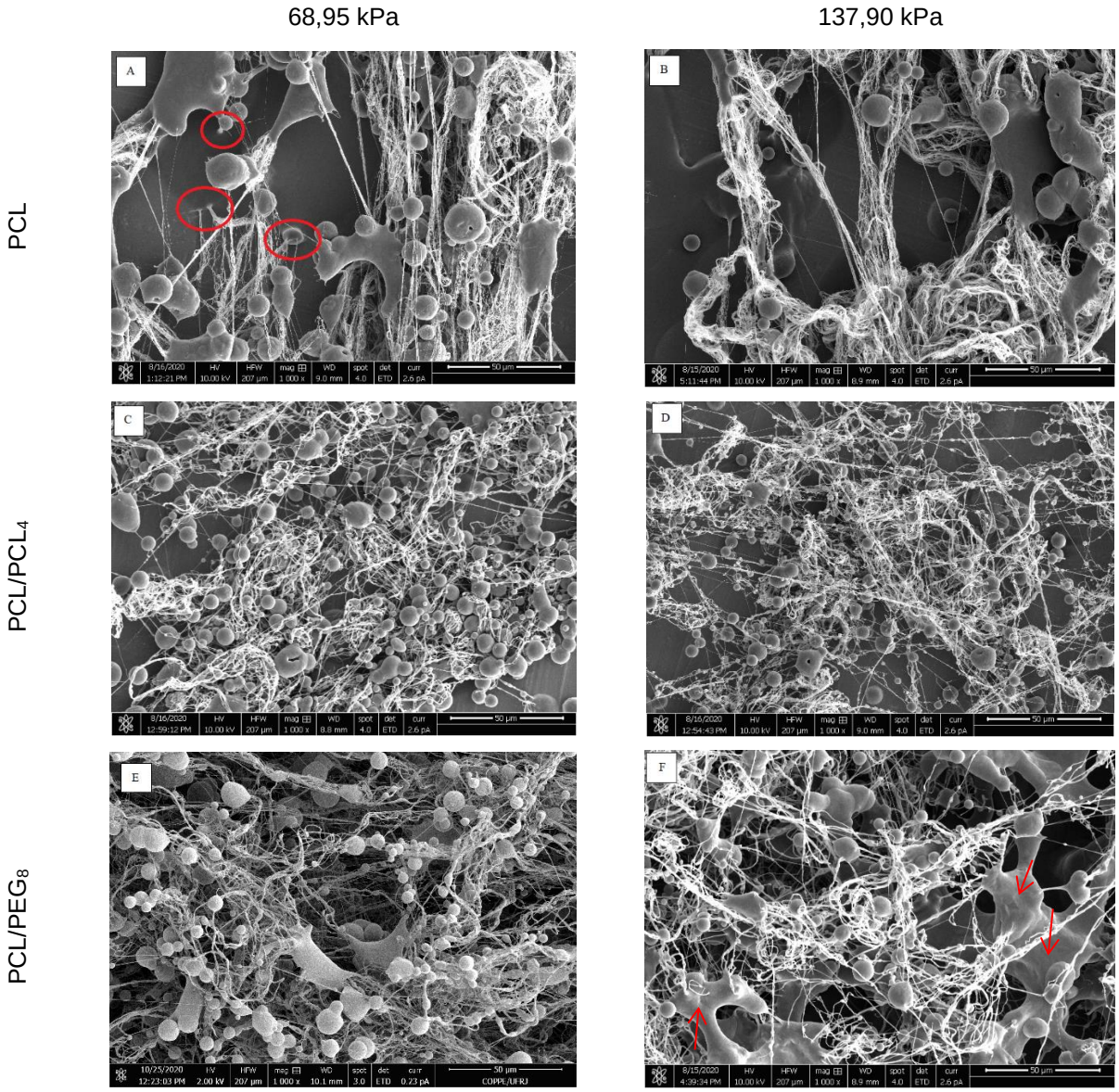

Figure 1.
The influence of the gas pressure and the composition of PEG in the fiber morphology at a fixed injection rate of 6ml/h.

Where GI is the swelling rate, M_0 is the initial mass of film, and M_{ti} is the mass of the film at the specified time frames.

2.3.3 *In vitro* drug release studies

The drug release studies of the PCL/PEG-IBU fibers were evaluated by the immersion of the fiber films of approximately 2x2 cm in falcon tubes containing 20 ml of saline solution at a temperature of 37ºC. At time intervals of 30 minutes, 1, 2, 3, 4, 5, 24, 48, and 72 hours, 2 ml of the solution was withdrawn and replaced by 2 ml of saline solution. All absorption measurements were made through UV-VIS spectroscopy. Due to the withdrawn and the replacement of the solution, for the correct reading of the results, a Correction Factor was used according to Eq. (2).

$$FC = \left(\frac{V_i}{V_i - V_r}\right)^{n-1} \qquad \text{Eq. (2)}$$

Where FC is the correction factor, V_i is the volume for the release medium, V_r is the withdrawn volume and n is the number of withdrawn times.

3. Results & discussion

3.1. Fiber morphology and configuration selection

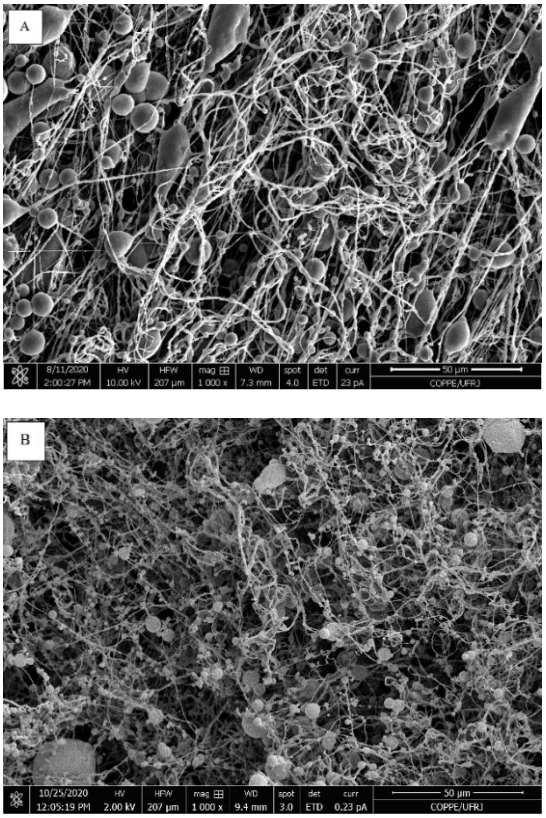

Figure 2.
SEM images for the fibers spined with a injection rate of 7.2 ml/h for (a) PCL/PEG$_8$ at 68,95 kPa and (b) PCL/PEG$_8$ at 137,90 kPa.

Initially, the fibers were produced for the selection of the best configuration for the following spinning process containing Ibuprofen. Therefore, it was produced PCL, PCL/PEG$_4$, and PCL/PEG$_8$ fibers with a fixed working distance of 30 cm and a fixed injection rate of 6 ml/h. Regarding the gas pressure, fibers were made utilizing 68,95 and 137,90 kPa. The morphology of the produced fibers was evaluated by Scanning Electron Microscopy analysis (Figure 1).

For all studied configurations, it was observed the formation of a morphology composed of fibers and beads. In particular, the PCL/PEG$_4$ fibers presented a considerable amount of beads, as seen in Figures 1C and 1D. The PCL/PEG$_8$ fibers produced from the use of 137,90 kPa, presented in Figure 1F, showed the formation of a film between the fibers (indicated by arrows). The PCL and PCL/PEG$_4$ fibers, on the other hand, appeared to have been deposited on a continuous plate, which can be more easily observed from the PCL fibers, in Figure 1A, which shows fibers and beads connected to the background of the images, as can be seen in the highlighted regions of Figure 1A.

Although the PCL/PEG$_8$ have large defects, where, in the case of Figure 1F, the beads tend to percolate, this structure does not show the formation of a plate, as all other conditions. Therefore, this PCL/PEG$_8$ composition was selected to further continue this work.

The presence of a plate deposited at the bottom of the A-D samples suggests that there was no fiber formation in the process initially, but rather a deposition of solvent-rich material still in solution on the target. The presence of the plate may also have been due to the difficulty in maintaining continuous stretching of the solution, which forms droplets instead of fiber formation, as observed by DENEFF et al. [12] The low amount of fiber is also an indication of an inadequate injection rate. The increase in the injection rate in the spinning may be favorable for the formation of more homogeneous fibers with fewer defects, even though it might cause slight increases in the fiber diameter, as observed by HOFMAN et al. [6], VASIREDDI et al. [13] noted that the increase in the injection rate made the effect of the influence gas pressure on the fiber diameter more drastic. In addition, increasing the injection rate will increase the amount of polymer being injected, possibly increasing the polymer's ability to maintain its shape during spinning, which could hinder, or even prevent, the formation of droplets.

Regarding the new batch of fibers, displayed in Figure 2, all analyzed configurations obtained a morphology consisting of fibers and beads, similarly to the previous batch of fibers. However, it can be noted the predominance of fibers, and not beads. The images show that with the increase of the injection rate there was an increase in the number of fibers, indicating a more continuous fiber formation and not the formation of droplets, as proposed. Nonetheless, the fibers in Figure 2B, which were produced with a gas pressure of 137,90 kPa, seem to be more homogeneous and visibly smaller.

As observed in Table 2, the increase in the concentration of PEG led to thicker fibers, displaying the smallest diameter in fibers spun

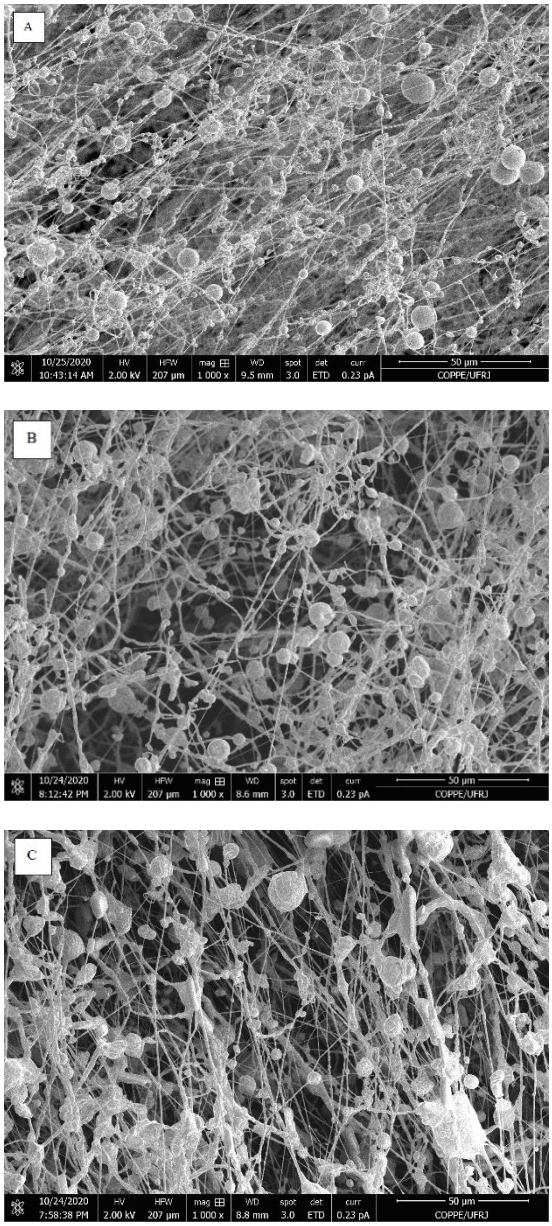

Figure 3.
Sem Images of the PCL/PEG8 fibers containing (A) 10%, (B) 30% and (C) 60% ibuprofen.

from solutions without PEG. These results are in agreement with the literature, the increase in solution concentration leads to an increase in the solution viscosity, which hinders the molding of the fibers by the pressurized gas, increasing its diameter.[12] It can also be observed that with the increase in gas pressure there was a decrease in the mean diameter, except for PCL/PEG$_4$ and PCL/PEG$_8$ at injection rates of 6ml/h, which appeared to increase in diameter with the increase in pressure. However, this phenomenon might be associated with the high fiber diameter dispersion, as shown in Figures 1D and 1E, showcasing a higher standard deviation than its counterpart with the same composition. The relation between the diameter and gas pressure is given by the stretching provided by the pressurized gas on the solution, molding it, thus, by increasing the pressure, this phenomenon will become more efficient and finer fibers will be obtained.[12]

Table 2.
The fiber diameter observed in all tested configurations.

Sample		Fiber Diameter (nm)
PCL	68,95 kPa 6ml/h	(4,8 ± 1,5)x10^2
	137,90 kPa 6ml/h	(4,8 ± 1,2)x10^2
PCL/PEG$_4$	68,95 kPa 6ml/h	(5,0 ± 1,2)x10^2
	137,90 kPa 6ml/h	(5,6 ± 1,6)x10^2
PCL/PEG$_8$	68,95 kPa 6ml/h	(5,5 ± 2,5)x10^2
	137,90 kPa 6ml/h	(5,8 ± 1,9)x10^2
	68,95 kPa ′.2ml/h	(6,4 ± 1,9)x10^2
	137,90 kPa ′.2ml/h	(5,1 ± 1,5)x10^2

Afterward, the selected configuration for the production of the PCL/PEG-Ibuprofen fibers was chosen, being utilized the PCL/PEG$_8$ at 137,90 kPa and 7.2 ml/h configurations for the remaining spun fibers.

3.3 The morphology of ibuprofen carrying fibers.

With the selection of the process configuration, it was then produced the PCL/PEG fibers carrying ibuprofen.

In Figure 3 it is possible to notice the influence of the ibuprofen concentration on the fibers, in which the diameters appear to be visibly larger as the drug concentration increases. Defects concentration also appears to be affected by the presence of the drug, showing an increase in the number of defects as the concentration of ibuprofen increases, also influencing the shape of these defects, which is more easily observed in Figure 3D, which non-spherical defects are predominantly seen, displaying a “slab-like” form. From these images, we can observe a large presence of junctions and the formation of less

homogeneous fibers with increasing drug concentration.

In Table 3, it can be noted that the most homogeneous fibers were obtained by the solutions lacking ibuprofen. We can observe that the addition of the drug causes an increase in fiber diameter, as well as an increase in the fiber size dispersion. Although the influence on the fibers' mean average size was not very significant at low ibuprofen concentrations, the effect of the drug's presence on the standard deviation can be observed at all analyzed concentrations.

Table 3.
The fiber diameter for the ibuprofen carrying fibers.

Sample	Fiber Diameter (nm)
PCL/PEG$_8$	(5,1 ± 1,5)x10^2
PCL/PEG$_8$-10	(5,1 ± 2,6)x10^2
PCL/PEG$_8$-30	(5,2 ± 2,2)x10^2
PCL/PEG$_8$-60	(6,3 ± 3,4)x10^2

3.3. Fiber swelling capacity

Considering the swelling behavior presented in Figure 4, we can see that PCL/PEG$_8$-Ibuprofen fibers have a high capacity for liquid absorption, obtaining little variation in swelling during the entire test run. The absorption peak of saline solution was observed in the first 5 hours of immersion for all samples, followed by an equilibrium profile. From the data obtained, it was possible to observe that the fibers containing ibuprofen had a lower swelling rate when compared to the PCL/PEG$_8$ fibers, possibly due to the mass loss related to the drug release profile, since the liquid absorption process and the drug release process occur simultaneously. This absorption can stretch the polymer chains to a relaxed state, favoring the drug's output.

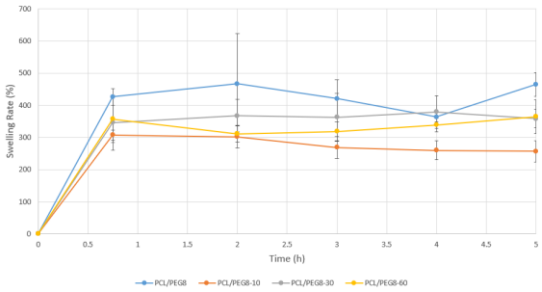

Figure 4.
The swelling profiles for the PCL/PEG$_8$-Ibuprofen fibers in the first five hours.

Therefore, with the results of the swelling test, we observed the improved liquid absorption capacity of the fibers. This ability is very important for wound dressings since it relates to the film's ability to absorb exudates, which is a very important characteristic to aiding the wound healing process.

3.4 Drug delivery behavior analysis

Considering Figure 5, we can see that for all samples the release profile follows an accelerated release followed by a more controlled release phase. The PCL/PEG$_8$-10 curve shows that most of the release occurred in the first 5 hours, with a very low variation in the other times analyzed. The burst release, commonly seen on drug delivery profiles, was responsible for releasing a large part of the drug contained in the film. The PCL/PEG$_8$-60 fibers, in turn, also showed a release mainly in the first 5 hours. Figure 4 shows that the films experienced an accelerated swelling in the first 5 hours, which might have facilitated the ibuprofen release during that period. However, in the following phase, it is possible to observe a significant difference between the concentrations released by PCL/PEG$_8$-60 and PCL/PEG$_8$-30 fibers, we can assume that the difference between the mean average weights of the films is one of the possible reasons for the occurrence of this phenomena, since the mean weight of the PCL/PEG8-60 fiber is close to 3 times smaller than the other ibuprofen carrying fibers.

Considering the PCL/PEG$_8$-30 curve, we can observe that the burst release is less pronounced than in the other compositions, taking longer to reach a level of stability. This release profile might have also occurred due to the morphology of the fibers, shown in Figure 3. The PCL/PEG$_8$-30 fibers exhibited a considerable amount of defects such as beads, that may have acted as ibuprofen reservoirs that hindered the drug diffusion, unlike PCL/PEG$_8$-10 fibers, with a smaller distribution of defects. Even though the PCL/PEG$_8$-60 fiber displayed a large number of defects, they are remarkably different, having plates (regions with low solvent evaporation rate) along with beads. These structures could have facilitated the drug diffusion, rather than limited it.

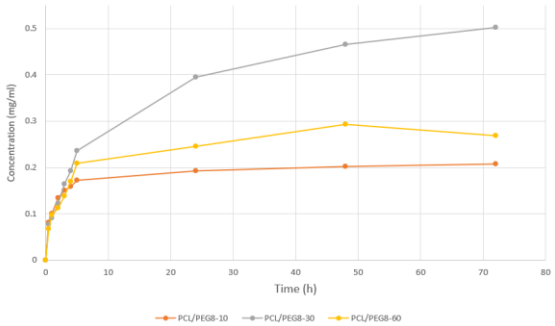

Figure 5.
The Ibuprofen release profile for the PCL/PEG$_8$-Ibuprofen fibers

From the release test, it was possible to demonstrate the potential of these films for the controlled release of ibuprofen. In their study, FERNÁNDEZ-CARBALLIDO et al [14] calculated the theoretical therapeutic concentration of 8 µg/ml from the ibuprofen pharmacokinetic parameters, that is, the study of the body's effect on a drug. Thus, we can observe that the theoretical therapeutic concentration of ibuprofen was reached by PCL/PEG-Ibuprofen fibers in the first 30 minutes, therefore, showcasing their potential to act in the inflammatory process initial stages. In the case of the PCL/PEG$_8$-10 and PCL/PEG$_8$-60 fibers, it was possible to maintain the delivery above the therapeutic concentration for up to 5 hours. The PCL/PEG$_8$-30 fibers, however, exhibited a more prolonged release, maintaining the concentration throughout the whole experiment (72 h).

4. Conclusions

In this study, we tried to produce PCL/PEG-Ibuprofen fibers for their applications in wound dressing, providing patient ease through the local delivery of ibuprofen, an analgesic. For this, PCL/PEG fibers were produced, varying solution and process parameters to find the optimal condition for the production of fibers for these biomaterials. With the morphology analysis of the produced fibers, it was possible to identify the presence of fibers and beads for all examined conditions, with varying quantities and dimensions. In some cases, it was observed the formation of plates along with the fibers. it was observed that the PCL/PEG$_8$ fibers, produced with a 7.2 ml/h solution injection rate, did not show the formation of plates and, for the 137,90 kPa pressurized gas configuration, it was observed more homogeneous fibers with fewer defects. Using the selected parameters, it was possible to manufacture PCL/PEG-Ibuprofen fibers containing drug concentrations of up to 60% of PCL mass. However, it was observed that the addition of the drug destabilized the system, increasing the regions with low solvent evaporation rates. With the swelling test, it was possible to analyze the capacity of the membranes to absorb liquids, where a high degree of fiber swelling was observed, showing a swelling rate in the range of 250 to 380%. The ibuprofen release profile showcased a pronounced release in the first 5 hours for all membranes. For PCL/PEG$_8$-30 the burst release consisted of about 47% of the maximum concentration observed during the test run, followed by a more gradual release throughout the 72 h of analysis.

This preliminary study demonstrated the potential of the Solution Blow Spinning technique in the production of PCL/PEG-Ibuprofen fibers as drug delivery systems in wound dressings. Future studies may be carried out, taking as a starting point supplementary characterizations such as contact angle; Fourier-Transform Infrared Spectroscopy (FTIR); Differential Scanning Calorimetry (DSC); Thermogravimetric analysis (TGA); and X-Ray Diffraction (XRD). In addition, the investigation of the influence of the PEG concentration on the degree of swelling and the release profile of PCL/PEG fibers, as well as analyzing the influence of working distance and solvent choice on the production of PCL/PEG fibers by Solution Blow Spinning.

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