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Characterization of Ciprofloxacin-Loaded Polymeric Fiber Mats Prepared by Melt Electrospinning

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Electrospun drug-loaded polymers are used to make formulations that slowly release medication. This study creates ciprofloxacin (Cip)-loaded fiber mats by melt electrospinning using polycaprolactone (PCL) and PEG4000 for controlled release of Cip. The increase in Cip concentration and PEG4000 percentages increases the mat thickness resulting in uniform morphology. The tensile strength of the PCL mat is significantly improved by adding higher concentrations of Cip while PEG inclusion reduced the tensile strength significantly. Differential Scanning Calorimetry (DSC) curves of PCL and PEG 4000 either as free components of after melt electrospinning are identical and both components shows a single endothermic peak at 63 and 61 °C respectively. Fourier transform infrared spectroscopy confirms the chemical stability of the raw materials, while X-ray diffraction shows the conversion of PEG and Cip from crystalline to amorphous structure following melt electrospinning. Cip is released gradually over 72 h, and the release is increased in the presence of PEG with a maximum Cip release ≈25% after 72 h. The study provides new insights into the development of controlled release fiber mats loaded with antibacterial agents. This can help to develop formulations for wound dressings that improve the clinical outcomes.

1. Introduction

Open wounds are characterized by deep cuts in the skin that are highly susceptible to bacterial infection. Infected wounds may cause prolonged and serious conditions that affect the surrounding healthy tissues.[1] Proper management of open wounds involves preventing bacterial infections at the wound site and using topical or oral antibiotics in addition to the wound dressing with the latter being considered as one of the best options in the management of infected open wounds. One approach is to design wound dressings that contain antibacterial agents that are released at slow and controlled rates. This approach could result in improved treatment outcomes, reduce the need for frequent drug administration, and lower the risk of developing bacterial resistance.[1,2]

Wound dressings have been extensively investigated with formulations loaded with various types of antibiotics. Polymeric materials are commonly used to prepare these dressings, and they are loaded with antibacterial agents and cast into specific shapes. Polymeric materials are preferred as they allow for slow and controlled drug release through drug diffusion or polymer degradation.[3] Additionally, the use of biodegradable and safe polymers has minimal toxic effects on the body. Recently, polymeric fibrous mats prepared by melt electrospinning have been widely used for wound dressings due to their high porosity, which facilitates drug diffusion. Melt electrospinning is a solvent-free, simple, and inexpensive process that has been employed for the fabrication of polymeric mats or meshes for several biological applications including wound dressings [4,5] tissue engineering [6,7], and controlled drug delivery.[8] The preparation of polymeric mats for wound dressings is based on mixing the polymer with the target drug and casting this formula into mats under specific electrospinning conditions such as temperature, voltage, and pressure.[9]

Melt electrospinning is characterized by being a solvent-free method and avoids the need for solvent evaporation as in the case of solution electrospinning, in which the build of 3D structures is difficult since the residual solvent evaporation from existing fibers would affect the deposition of the next fibers. In melt electrospinning, micron, and submicron fibers can be repeatedly deposited layer by layer using the melted polymer by controlling the pressure and the electrical current–voltage during the build-up of the 3D structures. Since no solvent is used in this process, no effect of any residual solvent will be seen, such as in the case of solution electrospinning.[10,11]

Several types of polymers can be used in melt electrospinning for the preparation of drug-loaded mats such as Poly-ϵ-caprolactone (PCL), and polyethylene glycol (PEG). PCL is an

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aliphatic polyester that is approved by the US Food and Drug Administration (FDA) and is a commonly used polymer in melt electrospinning. It has the advantages of being biocompatible, biodegradable, and resorbable, and its degradation product can be metabolized by the tricarboxylic acid cycle.[12] Several researchers have used PCL in the preparation of mats with electrospinning for various biological applications, such as the work of Zhang et al., in which PCL/Ranachensinensis skin collagen (RCSC) composites loaded with silver nanoparticles (AgNP) were prepared by electrospinning for wound dressing.[13] In the work of Zaiss et al., PCL was used by melt electrospinning in the preparation of tissue engineering scaffolds in a 3D environment with osteoblast cultures.[14] Moreover, PCL meshes prepared by electrospinning and loaded with resveratrol were prepared to induce a slow and controlled release of resveratrol, which could adjust the endothelial cells' and macrophages' behavior and improve vascular regeneration.[15]

The desired release profile of the loaded drug is influenced by several factors, such as the type of polymer used, the mat geometry, the diameter of the fibers and their porosity, the method of drug loading, and the hydrophilicity of the matrix.[16,17] The desired release profile can be achieved by using either a single type of polymer with the appropriate characteristics or a mixture of different types of polymers with different characteristics in order to achieve the intended dosage and duration of drug release.[16] In terms of fiber geometry, the release profile of the loaded drug will be affected by the number of layers of the prepared mats, where the drug release rate and duration will be different using multilayered mats, and this can be controlled by adjusting the morphological features of the electrospun mats.[18] PCL is a highly hydrophobic polymer, which might affect its biological application and the process of drug release after application to the target tissues. These hydrophobic properties of the prepared PCL mats can be adjusted by using a mixture of polymers through the incorporation of hydrophilic polymers with PCL, such as the nontoxic, biocompatible, and hydrophilic PEG. Polymeric fiber mats prepared by electrospinning using such type of polymeric combination have improved hydrophilicity, which can improve the overall compatibility and the way the loaded drug releases following the mats' application.[17]

The aim of this work is to prepare PCL-based mats by melt electrospinning loaded with the antibacterial agent ciprofloxacin (Cip) for possible wound dressing applications. PCL was chosen to prepare the fiber mats as its one of the most commonly studied polymers used for electrospinning and it was among the list of recommended polymers that are compatible with the instrument used in our lab for melt electrospinning. The attempt to increase the rate of drug release was done through the incorporation of a hydrophilic polymer such as PEG 4000. However, any compatible polymers could be investigated provided that they are stable at the electrospinning conditions such as high voltage and temperature and can result in a suitable viscosity for the electrospinning process. The effects of the drug percentage and the addition of a hydrophilic polymer on the properties of the prepared mats will be investigated. Various percentages of Cip will be used with PCL alone or in combination with PEG 4000 to fabricate the mats using melt electrospinning under controlled temperature, voltage, and pressure. This method will result in polymer melting and then casting this melted polymer into fibrous mats with a predesigned trajectory where the fibers can have a diameter ranging from micro to nanometres.[19] The prepared empty and Cip-loaded mats were evaluated in terms of their physicochemical properties, morphology, Cip release profiles, and antibacterial activities.

Table 1. The composition of the fiber mats prepared by melt electrospinning is expressed as weight percentages of PCL, PEG4000, and Cip. Each formulation is labeled according to the weight percentage of Cip.

Mat name	PCL:PEG ratio [w/w]	Ciprofloxacin concentration [% w/w]
F1	100:0	0
F1/Cip 0.5%	100:0	0.5
F1/Cip 1%	100:0	1
F1/Cip 2%	100:0	2
F2	95:5	0
F2/Cip 0.5%	95:5	0.5
F3	90:10	0
F3/Cip 0.5%	90:10	0.5

2. Experimental Section

2.1. Materials

PCL pellets (M.W 80 000 g mol^{-1}), polyethylene glycol 4000 (PEG4000), phosphate-buffered saline tablets (PBS, pH 7.4), Cip, and Mueller Hinton Broth (MHB) were purchased from Sigma–Aldrich (UK). Mueller Hinton Agar (MHA) was purchased from Oxoid Ltd, UK. The gram-negative Escherichia coli (*E. coli*) bacteria and the gram-positive Staphylococcus aureus (S. aureus) were purchased from the American Type Culture Collection (ATCC).

2.2. Fabrication of Fiber Mats by Melt Electrospinning

Both empty and Cip-loaded fiber mats were made using direct-writing melt electrospinning using Spraybase melt electrospinning (Spraybase Avectas Ltd., Ireland), The electrospinning featured a high-voltage power source, a temperature controller, a melting head with a movable polymer container along the Z-axis, an air compressor for polymer emission, and a movable X- and Y-axis metal flat plate collector for gathering the mats. The mats were prepared by weighing PCL, PEG, and Cip in varying mixing ratios, followed by mixing and loading the resulting mixture into the holder of the electrospinner. The mixture was subjected to a temperature of 90°C for a duration of 15 min to ensure the polymer melting. **Table 1** presents the composition specifications of the mats that were prepared. Mats were cast using a nozzle size of 0.3 mm at 90 °C. The voltage range used in this study was from …mV to mV and the pressure range from … pa to … pa. Each composition has its own ideal voltage and pressure. Single-layer mats with a rectangular shape were formed by depositing fibers onto the moving stage.

2.3. Morphology

The measurement of mat thickness was conducted by means of an electronic caliper featuring a precision of 0.01 mm at diverse

locations on the specimens. From several measurements, the mats' average thickness was determined. Scanning electron microscopy (SEM) (Quanta 250 from FEI in Oregon, USA) was used to analyze the morphological characteristics of the mats. Using a gaseous secondary electron detector, secondary electrons were gathered. Scanning evaluations of the mats' surface morphology, fiber diameter, and pore size distribution were done using SEM images.

2.4. Differential Scanning Calorimetry (DSC)

Using a differential scanning calorimeter (DSC) with a cooling accessory and nitrogen gas sweeping (a Mettler Toledo DSC analyzer), the thermal behavior of the electrospun fiber mats and each of their individual components—including PCL, PEG, and Cip were examined. Standard aluminum pans were loaded with 5 mg of each sample and heated at a rate of 10 °C min^{-1} to 320 °C while receiving a 20 mL min^{-1} flow of nitrogen. Samples were performed in duplicate, and the average values were calculated with their standard deviations.

2.5. Fourier-Transform Infrared Spectroscopy (FT-IR)

FT-IR spectra were captured using a SHIMADZU FT-IR spectrophotometer equipped with an attenuated total reflection (ATR) accessory that operates between 4000 and 400 cm^{-1}. The samples analysed included pure polymers (PCL and PEG 4000), Cip, as well as empty and drug-loaded electrospun mats. Prior to recording the sample spectrum, the ATR crystal was utilized to press the sample, and a background scan was conducted for each measurement. The experimental parameters were established in accordance with established protocols for ATR-FTIR examination, utilizing a resolution of 4 cm^{-1} and 32 scans per specimen to enhance the signal-to-noise ratio. The spectra obtained were subjected to processing using suitable software to detect and measure the distinctive peaks of the various constituents.

2.6. X-ray Diffraction (XRD)

The study utilized X-ray diffraction (XRD) methodology to examine the influence of electrospinning on the level of crystallinity of the drug and polymers, as well as the impact of the drug on the crystalline configuration of polymer nanofibers. X-ray diffraction (XRD) patterns were acquired for Cip, PCL, PEG, and composite nanofiber mats containing drugs and those without drugs. A small amount (10–50 mg) of each sample was examined to determine the crystalline form using transmission XRD and data obtained on a Bruker D8 Discover Multi-well transmission diffractometer outfitted with geometry, Cu radiation (Cu $_{\lambda}$ = 1.54056 Å), an SSD136 PSD, and an automated multi-position x–y sample stage. The samples were mounted on a sample plate with 40 positions that was supported by a film made of polyamide (Kapton, 7.5 m thick). Information was recorded over a span of 4–35 ° in each direction, with a step size of 0.017 ° and a count time of 0.5 s for each step. During the data collection process, the samples underwent oscillation in the x-y plane to optimize particle sampling and reduce the impact of preferred orientation effects. Prior to data acquisition, the instrument underwent calibration utilizing a standard reference material. The XRD patterns were analysed utilizing the Bruker Diffrac. EVA software.

2.7. Tensile Strength

The maximum stress of the mats was determined using a TA-XT2i texture analyzer (Stable Micro Systems, UK) coupled with Exponent software. mat samples were chopped into rectangular shapes of 2 × 5 cm, and both ends of the texture analyzer were clamped using clamps. A crosshead speed of 5 mm min^{-1} was maintained throughout the test, which was run until the sample was totally broken. When a sample broke under the most pressure, that was recorded as the maximum stress value. The tests were carried out in triplicate, and the results were presented as the average and standard deviation.

2.8. Drug Release

The method employed for the evaluation of the Cip release rate from the mats that were prepared is as follows. The mats were submerged in 3 ml of PBS (pH 7.4) within a 15-ml Falcon tube and subjected to rotation at ambient temperature for a duration of 72 h. Samples of the release medium were collected at predetermined intervals of time (5 min, 15 min, 30 min, 1 h, 2 h, 3 h, 6 h, 12 h, 24 h, 36 h, 48 h, 72 h) by withdrawing 500 µl of the medium and replenishing it with an equal volume of fresh PBS to maintain sink conditions. High-performance liquid chromatography (HPLC) was used to measure the amount of Cip released into the solution from each mesh under the following chromatographic conditions: column YMC basic C18, 250 × 3.0 mm, injection volume of 20 µl, the flow rate of 1 ml min^{-1}, UV detection at 278 nm, a mobile phase of PBS: acetonitrile (70:30), pH adjusted to 2.8 using HCl. The Agilent 1260 Infinity HPLC system (Agilent Technologies, CA, USA) was utilized for the HPLC analysis.

The accumulated release percentage concentration (C) of Cip was calculated according to the following equation after determining the Cip concentration at each time point using a constructed linear calibration curve for Cip:

$$C = \frac{CnV + V1\left(\sum_{i=1}^{n} Cn - 1\right)}{q} \times 100\% \quad (1)$$

Where C_n is the ciprofloxacin concentration at the nth measurement, V is the total volume of the sustained release system, V_1 is the volume of the sample withdrawn at each time point, and q is the theoretical content of the ciprofloxacin in the mesh. Standard Cip solutions were generated and evaluated by HPLC to create a calibration curve for the HPLC. To make the standards, a known quantity of ciprofloxacin was dissolved in PBS: acetonitrile (70:30) at a pH of 2.8 (using HCl), yielding concentrations of 1 to 100 mg Ml^{-1}.

2.9. Antibacterial Activity

Staphylococcus aureus (*S. aureus*) and *Escherichia coli* (*E. coli*) were used as model bacteria in assessing the antibacterial activity of

the manufactured fiber mats. One colony of *S. aureus* or *E. coli* was cultured in 10 ml of Mueller-Hinton broth (MHB) at 37 °C to get ready for use. The following day, after proper dilution, sterile cotton swabs were used to distribute each bacterial strain onto Mueller-Hinton agar (MHA) plates in Petri dishes. The fiber mats were sliced into 1 cm square discs so that the inhibition zone could be studied. After placing the fiber mats on top of the MHA, it was incubated for 24 h at 37 °C. Electronic calipers (Mitutoyo Corporation, Japan, Model 500-196-30) were used to determine the diameters of the inhibition zones. The sizes of the inhibitory zones were measured in triplicate for each experiment and represented as means SDs.

2.10. Statistical Analysis

One-way analysis of variance (ANOVA) was used to assess statistical significance. A Tukey's multiple comparison test and *t*-test were performed for paired comparisons. The statistical analysis was performed using Minitab software version 17. A p-value <0.05 was considered statistically significant.

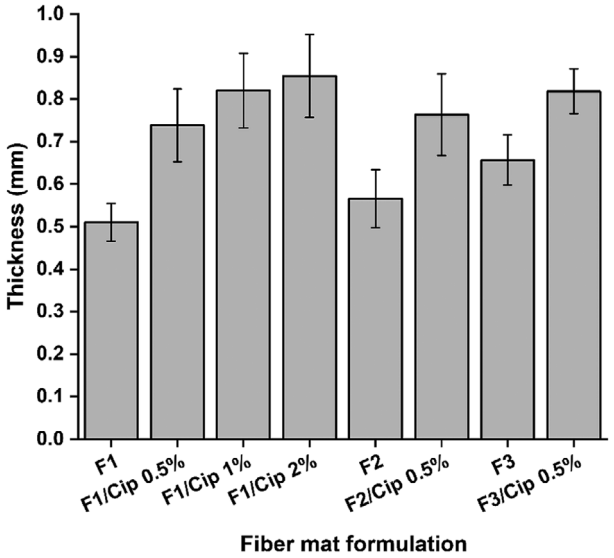

Figure 1. The thickness of melt electrospun fiber mats, both without and with Cip loading, was measured in millimeters. The data is shown as the mean standard deviation ($n = 3$).

3. Results and Discussions

To create the patterned composite fiber mats, we used a solvent-free direct-writing melt electrospinning method. To obtain controlled release profiles, Cip was successfully incorporated into the fibers using this method. We were able to modify the drug release kinetics to suit various applications by manipulating the fibbers' composition and morphology. Mechanically, the resulting composite mats were superior, and the drug release was controlled over long periods of time.

3.1. Fiber Mats Thickness

Figure 1 displays the thicknesses of PCL with and without PEG 4000 melt-electrospun mats that were empty and drug-loaded, respectively. The loading Cip concentration and the PCL/PEG ratio both influenced the mat thickness. Loaded mats were consistently thicker than unloaded mats across all formulations. The mat's thickness also grew as the Cip concentration increased from 0.5 to 1% to 2%. Because Cip can cause swelling of the polymer matrix, its presence in the fibers may account for the observed increase in mat thickness for loaded mats. At larger drug concentrations, this action is magnified, resulting to a more substantial rise in mat thickness. Mat thickness was raised by PEG 4000 when compared to mats made with PCL alone for both unloaded and loaded conditions. Since PEG 4000 acts as a plasticizer and enhances the processability of the polymer solution during electrospinning, it can also be used to increase mat thickness. Overall, the data support the idea that drug-loaded mats of tuneable thickness can be prepared using the melt-electrospinning method for use in drug delivery. [20]

One crucial factor that can alter the release kinetics and mechanical qualities of drug-loaded mats is their thickness. Since more medicine may be put into a thicker mat, it may offer a more gradual release profile when used for drug delivery. [17] The drug diffusion out of the mat may also be affected by changes in pore size as the mat's thickness increases. To provide optimal release kinetics and mechanical qualities, the mat thickness must be carefully optimized for each drug delivery application. [21] The electrospinning process may be being influenced by Cip, which may explain the observed tendency of greater mat thickness at higher drug concentrations. Drugs added to polymer melts can alter melts properties like viscosity, surface tension, and conductivity, which in turn can alter fiber diameter and mat thickness.[22] However, it is plausible that the physical qualities of the medication are influencing the mat structure. Adding nanoparticles to polymer solutions has been shown to alter the solution's rheological properties, which in turn affects the electrospinning process and modifies the resulting fiber diameter and mat thickness.[23] This pattern of increasing mat thickness with increasing Cip concentration necessitates additional research to discover the precise mechanism responsible for this phenomenon.

3.2. Morphology

In **Figure 2**, SEM images of the finished fiber mats are presented at different magnifications. Images show that fiber surfaces are consistent and smooth over a range of PEG and Cip concentrations. The resulting set of the SEM pictures demonstrates that the fiber surfaces of all formulations, regardless of PEG and Cip concentration, are uniform and smooth. This shows that the fibers produced in this investigation were consistently of high quality using the melt electrospinning method. In drug delivery applications, the homogeneity of the fiber mats is preferred because it enables predictable release profiles and prevents localized drug concentrations, both of which can have undesirable side effects. But it's important to keep in mind that the arrangement and form of the fibers can have a big effect on the rate at which the drug is released and on the additional characteristics of the mats. Different mats can have different mechanical qualities and drug

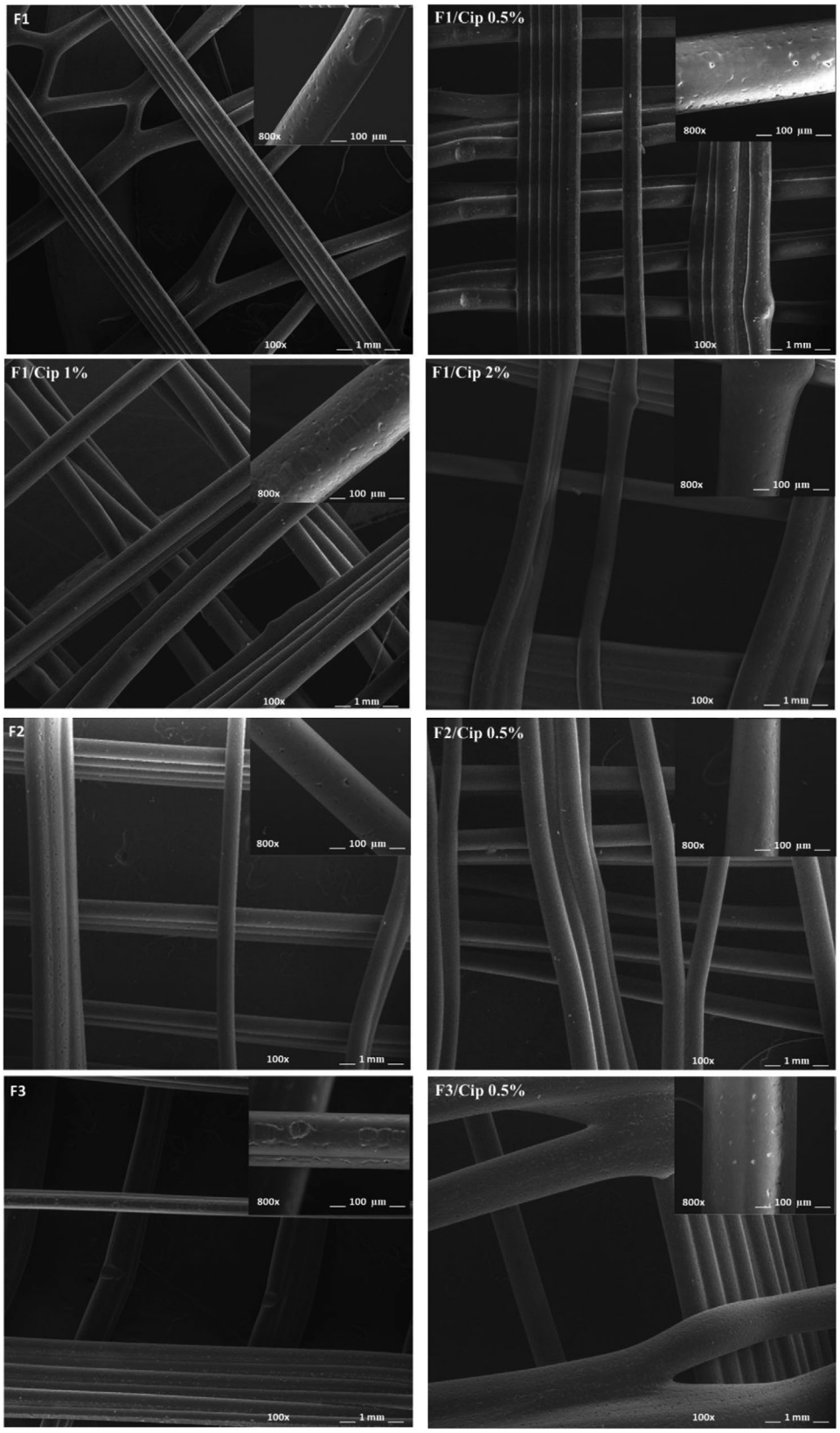

Figure 2. SEM images of fiber mats generated by melt electrospinning using PCL with and without PEG and Cip.

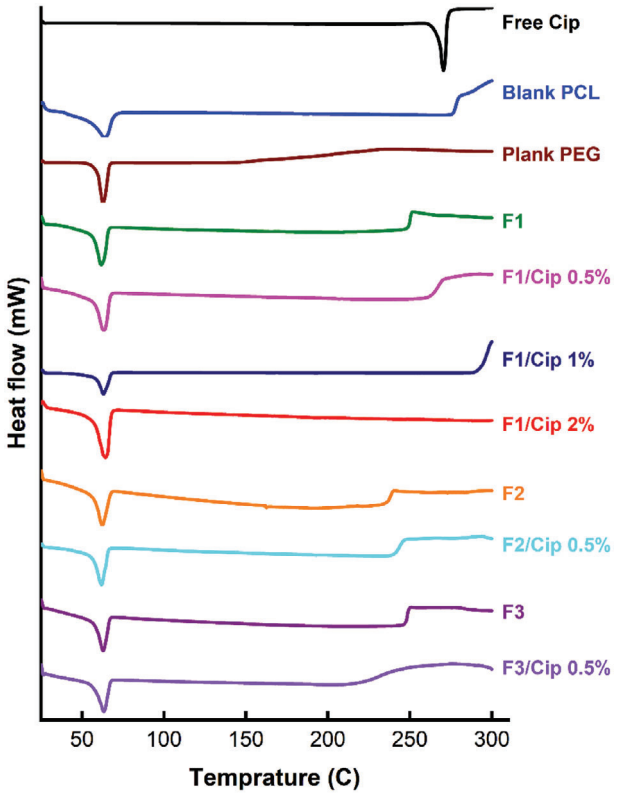

Figure 3. DSC profiles of PCL pellets, PEG4000, and Cip, as well as electrospun fiber mats F1, F2, and F3 loaded with varied percentages of Cip.

release rates due to differences in fiber diameter, pore size, and alignment.[24] Furthermore, the SEM images showed no visible particle deposition on the surface of the generated fibers strongly indicating that the Cip might be well dispersed within the mats.

3.3. Thermal Analysis

To evaluate the thermal behavior of the components used in the preparation of the fiber mats before and after melt electrospinning, DSC was performed for each raw material as a free component and compared with the thermal behavior of the fiber mats (**figure 3**).

The DSC curves of pure PCL and PEG 4000 were identical and both components showed a single endothermic peak within the same region at 63 and 61 °C respectively. These results are consistent with what has been reported previously.[25,26] For the free Cip, the DSC curve showed an endothermic peak indicating that the Cip melting point is ≈268 °C. The DSC curves of F1, F2, and F3 were almost identical to their free PCL and/or PEG in which the endothermic peaks were all ≈61–62 °C. However, the DSC analysis for all the electrospun mats incorporating Cip (F1/Cip 0.5%, F1/Cip 1%, F1/Cip 2%, F2/Cip 0.5%, F3/Cip 0.5%) showed a single endothermic peak at a temperature ≈63 °C and this peak represents the melting point of PCL and PEG 4000 since both components showed an endothermic peak at this temperature as free components before electrospinning. Obviously, the endothermic peak that represents the melting point of Cip at 268 °C disappeared in these mats loaded with various percentages of Cip. It is noteworthy that a consistent heating and cooling rate of 10 °C min^{-1} was adhered to throughout the experiment to ensure that reliable and comparable results were obtained.

During the process of melt electrospinning in our protocol, the polymers were heated to a temperature of 90 °C. At this temperature, both the PCL and PEG were in the melted state but not the Cip. Therefore, we manually stirred the Cip to increase its distribution within the melted polymers and the drug might be dissolved in the melted PCL with/without PEG and this was previously reported in the literature.[27,28] The resulting mixture following the polymer melting and the drug stirring has a suitable viscosity to be cast as mats through the melt electrospinning process.

In reference to the SEM images presented in Figure 2 for the Cip-containing mats, the fibers of the mats looked clear and smooth with no particles deposited on the surface observed. This means that Cip might be dissolved in the PCL with/without PEG during the process of polymer heating and electrospinning and mat casting resulting in the disappearance of the melting curve of the Cip in the DSC results.

Another explanation for the disappearance of the Cip endothermic peak might be the concentration of Cip in all the melt electrospinning mats was too low to be detected by the thermal analysis and presented as an endothermic peak.

3.4. Fourier Transform Infrared Spectroscopy (FT-IR)

FTIR analysis reveals important details about the presence and interaction of the various components in the fiber mats. By identifying their signature peaks, we know that Cip, PCL, and PEG are all present in the fiber mats, and it's likely that they didn't suffer any major chemical changes throughout the melt electrospinning process. The characterization of the mixture and the potential for the interactions between all the fiber mats polymeric components, PCL and PEG 4000, with each other and with the Cip was evaluated by FT-IR and presented in **Figure 4**.

With regard to Cip, the distinct peaks shown at ≈1589, 1541, and 1496 cm^{-1} resulted from the stretching vibrations of the benzene ring of the Cip. Specific peaks corresponding to the stretching of O—H and C═O showed at 3043 and 1618 cm^{-1} respectively.[29] PCL beads showed characteristic peaks between 2965 and 2991 cm^{-1} that are related to the C—H stretching vibration. Moreover, there was another distinct peak for PCL representing the stretching vibration of the C═O in the ester bond that appeared at 1723 cm^{-1}. The vibration stretching peaks of PCL presented at 1160 and 1240 cm^{-1} related to the presence of C—O—C.[30]

For PEG4000, there were stretching vibrating peaks presented in the PEG4000 chain at 1097, and 2882 cm^{-1} that could be assigned to C—O—C and CH_2 stretching vibration respectively.

The abovementioned characteristic peaks for Cip, PCL, and PEG were found in the fiber mats prepared from any of these components by melt electrospinning. This confirms the presence of PCL in F1, the presence of PCL and Cip in F1 mats loaded with Cip, and the presence of PCL, PEG, and Cip in F2 and F3 fiber mats loaded with Cip (Figure 4).

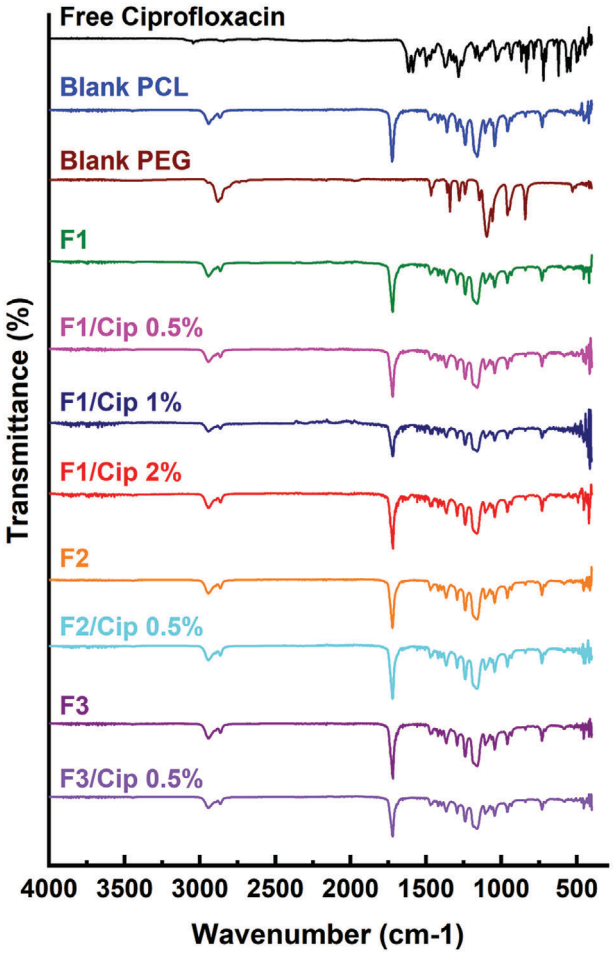

Figure 4. FTIR spectra of fiber mats F1, F2, and F3 loaded with varied proportions of Cip, as well as the spectra free PCL pellets, PEG4000, and Cip.

These FT-IR results confirm no significant changes in the peaks of the fiber mats raw materials following the casting with melt electrospinning in which the chemical bonds of these raw materials were preserved after the process of melt electrospinning. This means that the application of high temperature and voltage during the melt electrospinning will not induce any chemical reactions between the fiber mat components. FTIR analysis verifies the presence of Cip, PCL, and PEG 4000 in the fiber mats and hints at possible interactions between the components due to hydrogen bonding or other types of bonding.

3.5. X-ray Diffraction

XRD analysis was performed on all components of the fiber mats (PCL pellets, PEG4000 powder, Cip powder) was tested and compared to the XRD pattern of the electrospun mats composed of PCL/Cip and PCL/PEG/Cip (**figure 5**). Cip powder has a crystalline nature and exhibited sharp characteristic peaks at $2\theta = 14.48°$, 20.79°, and 25.40°. PEG4000 showed its characteristic peaks at $2\theta = 19.21$ ° and 23.36 ° and the PCL pellets showed their peaks at $2\theta = 21.45$ ° and 23.90 ° demonstrating the crystalline nature of PEG and PCL respectively.

The XRD pattern of F1 fiber mats had similar sharp peaks as the PCL pellets before electrospinning which means that the crystallinity of the PCL didn't change during the electrospinning process. XRD analysis of PCL mats incorporated with different percentages of Cip and/or PEG (F1/Cip 0.5%, F1/Cip 1%, F1/Cip 2%, F2, F2/Cip 0.5%, F3, and F3/0.5%) showed a disappearance of the characteristic peaks of both Cip and PEG (**Figure 5**A,B). The observed disappearance of these characteristic peaks of PEG and Cip can be attributed to the conversion of PEG and Cip from crystalline to amorphous structure following the melt electrospinning either due to the high temperature and/or the high voltage used during the melt electrospinning process. Similar observations were reported by He et. al. and Li et. al. were the characteristic peaks of PEG and Cip disappeared following the melt electrospinning.[17,29] The electrospinning process does not alter the crystalline structure of PCL, and the XRD results back up the crystalline nature of the constituent components. The melt electrospinning process likely converted the PEG and Cip in the electrospun mats to an amorphous state, as their distinctive peaks disappeared. Such findings have implications for the fiber mats' architectural characteristics, and more investigations are required to fully comprehend the influence of the method of electrospinning on the mats' chemical and physical characteristics.

3.6. Tensile Strength

The electrospun mats' tensile strength versus Cip and PEG concentration is displayed in **Figure 6**. According to the findings, the tensile strength of F1 was significantly improved by adding higher concentrations of Cip to the material. Mats made of F1/Cip 0.5% had significantly greater tensile strength than those made of pure F1 ($p < 0.05$) but adding more Cip did not significantly improve the mats' strength. However, when PEG was included in the PCL mats, the tensile strength dropped. Tensile strength was significantly lower in F2/Cip 0.5% mats with 5% PEG and F3/Cip 0.5% mats with 10% PEG than in F1/Cip 0.5% mats ($p < 0.05$). The tendency toward lower tensile strength in fiber mats can be explained by the fact that the addition of PEG to melted PCL decreased the PCL's viscosity. The results show that the ideal composition is F1/Cip 0.5% and that Cip and PEG content have a significant impact on the tensile strength of the electrospun mats. The results of this study have significant implications for the development of electrospun mats for use in the medical field.

3.7. Drug Release

To understand the influence of the fiber mat compositions on the drug release profile, the release of Cip from the mats was studied over the period of 72 h (**Figure 7**). For mats prepared with the hydrophobic polymer PCL only, the release was slow with a gradual increase over time. The accumulated release rate of Cip from F1 loaded with 0.5% Cip was higher than F1 mats loaded with 2% Cip and the release rate from F1 loaded with

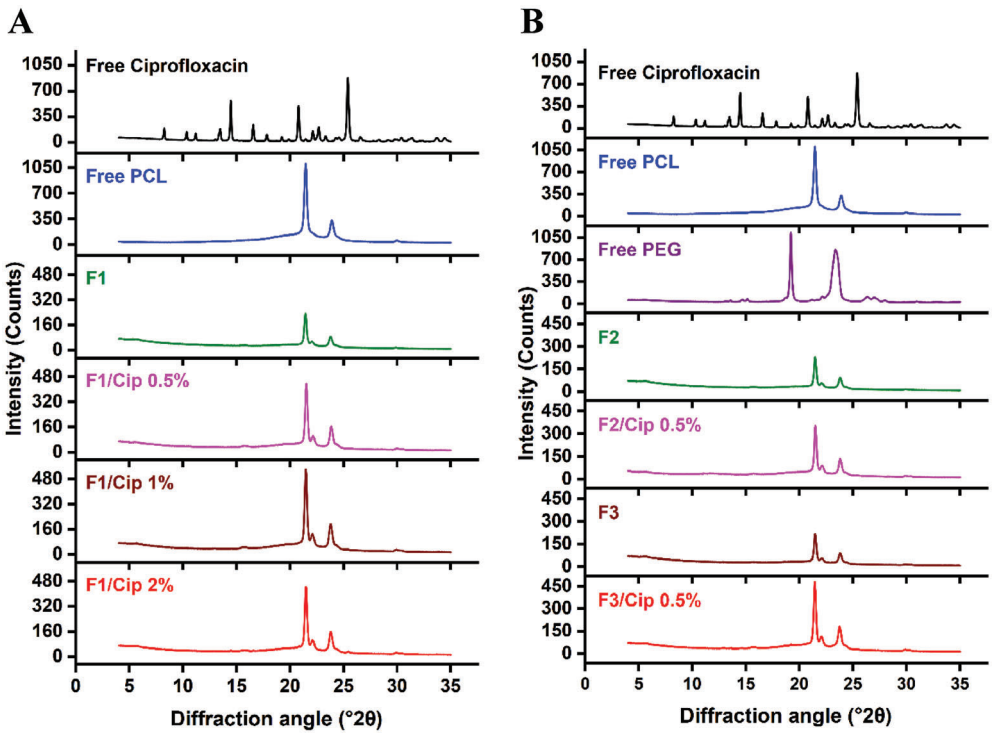

Figure 5. A) XRD patterns of electrospun fiber mat (F1) prepared with PCL and loaded with varied proportions of Cip, and (B) XRD patterns of electrospun fiber mats F2 and F3 that are composed of PCL and PEG4000 and loaded with Cip.

1% Cip was the slowest. The maximum release at 72 h was ≈8.40 ± 0.18% from F1/Cip 0.5%, 5.36 ± 0.04% from F1/Cip 2%, and 2.23 ± 0.03% from F1/Cip 1%. It is notable that the incorporation of the hydrophilic polymer PEG4000 into the fiber mats with PCL significantly increases the rate of Cip release in which the increase of PEG concentrations from F2 to F3 resulted in a higher rate of drug release. ≈12% of Cip was released from both F2/Cip 0.5% and F3/Cip 0.5% fiber mats during the first 12 h.

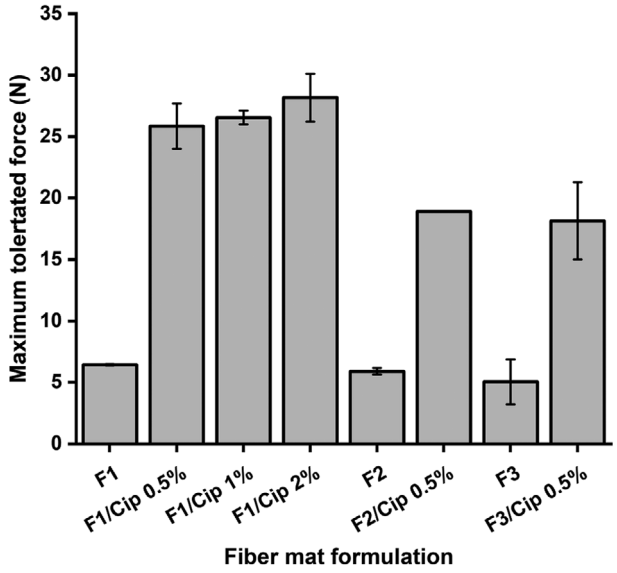

Figure 6. Melt electrospinning fiber mats' tensile strength. Using weight ratios, the graphs show the average tensile strength of fiber mats made from PCL alone or in conjunction with PEG4000. Standard deviations are shown as error bars (n = 3).

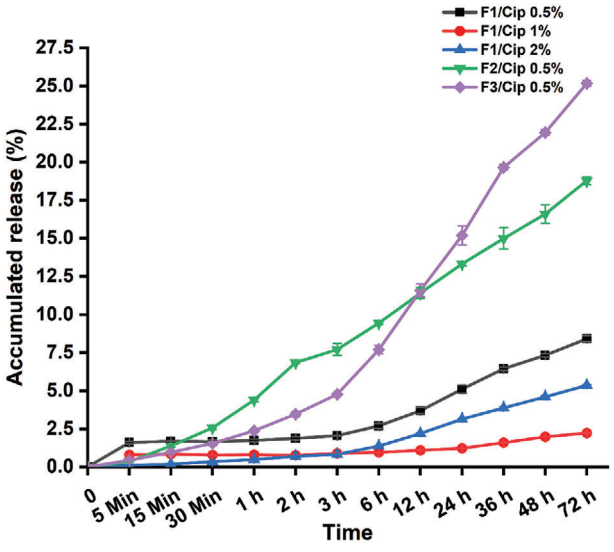

Figure 7. Cumulative Cip release patterns from melt electrospun fiber mats with varying PCL/PEG/Cip concentrations in phosphate buffer saline (PBS) at 37 °C. Higher Cip release rates were seen in the fiber mats made with PEG4000 compared to those prepared with PCL alone. Data represents average ±SD.

For F2/Cip 0.5%, the maximum accumulated release at 72 h was 18.78 ± 0.26% while the maximum accumulated release at 72 h from F3/Cip 0.5% fiber mats was 25.18 ± 0.19%. In all the prepared fiber mats, there was no initial burst release as the release was slow and gradual even in F2 and F3 which contain 5 and 10% of PEG4000 respectively although the incorporation of the PEG4000 increased the release rate of Cip compared to F1 formulations. These differences in the Cip release profiles are related to the differences in the water absorption capabilities of the different fiber mats. The presence of the hydrophilic polymer PEG4000 resulted in higher rates of Cip release as a result of higher water absorption and the dissolution of PEG4000 which facilitated the Cip release. These results were consistent with what has been reported previously where the incorporation of a hydrophilic polymer tends to increase the rate of the loaded drug release.[29,31]

The Cip release from the polymer fibers was caused by diffusion, polymer erosion, or a combination of both mechanisms. [32] Typically, Cip diffusion was very slow in the fiber mats composed only of PCL (F1 formulations) and thus the polymer erosion is a more important mechanism of drug release. The addition of PEG4000 in F2 and F3 formulations improves the hydrophilicity of the fiber mats and thus the release of Cip by diffusion improved.

At the end of 72 h, all the prepared fiber mats still have unreleased Cip with the highest percentages remaining in the fibers prepared with PCL only. This remnants of unreleased Cip can be explained by this unreleased Cip was incorporated deep inside the polymer fibers when the polymers were melted and these Cip molecules diffuse slowly in the lipophilic PCL fibers. Again, in the formulations with PEG4000, the dissolution of PEG4000 facilitated the drug release and resulted in less percentages of the unreleased Cip. In the work of He et. al., other factors were reported to affect the rate of drug release from the fiber mats prepared by melt electrospinning such as the fiber diameter and the geometric structures of the fiber mats.[17]

Several parameters, such as fiber mat composition, water absorption capacity, and drug release mechanism, affect Cip release from the various fiber mat formulations investigated. The hydrophilicity of PCL-containing fiber mats is improved by the incorporation of PEG4000. PEG4000 is a hydrophilic polymer that can dissolve in water and absorb a lot of it. Adding PEG4000 to PCL fibers allows for easier Cip release due to the PEG's ability to absorb water. Small channels or pores are formed in the fiber mats as PEG4000 dissolves during release, which can enhance the surface area available for drug release. This, in turn, can hasten the fiber mats' drug delivery. The mechanism of medication release also plays a role in how much Cip is released from the fiber mats. medication release from PCL-only fiber mats occurs primarily via polymer erosion, with the medication being released during the degradation of the polymer. Because these fiber mats are hydrophobic, Cip diffuses very slowly through them. However, PEG4000 added to the fiber mats increases their hydrophilicity, making diffusional drug release easier. Fiber diameter and the geometric structure of the fiber mats are other crucial parameters that affect the release of Cip from the mats. The accessible surface area of the fiber mats for drug release can be affected by several variables, which in turn can affect the rate of drug release. The hydrophilicity of the fiber mats and the method of drug release are both altered by PEG4000's insertion into PCL fiber mats, which has a major effect on Cip release.

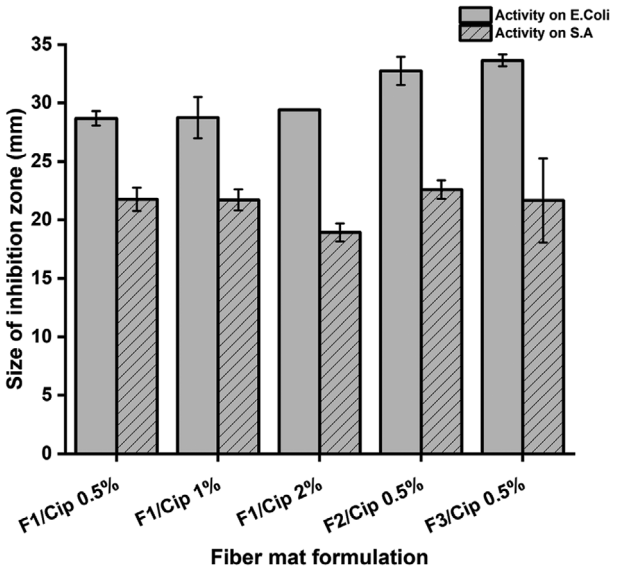

Figure 8. The size of the inhibition zones on agar plates of fiber mats prepared with melt electrospinning using different component ratios against two distinct bacterial strains, namely gram-negative *E. Coli* and gram-positive *S. aureus*. Results represent average ±SD.

3.8. Antibacterial Activity

The present study assessed the antibacterial efficacy of fiber mats that were loaded with Cip against two distinct bacterial strains, namely gram-negative *E. Coli* and gram-*positive S. aureus*. The measurement and comparison of the inhibition zones of the bacterial growth on the fiber mats were conducted. The bacteria were spread on agar plates and then a piece of each fiber mat was placed on the agar and then the bacteria inhibition zone were measured and presented in **Figure** 8. The F1 formulations containing 0.25%, 0.5%, and 1% Cip exhibited comparable inhibition zones against both *E. Coli* (≈28 mm) and *S. aureus* (≈20 mm) bacteria. However, a slightly greater efficacy was observed against the gram-negative *E. Coli*.

The introduction of PEG4000 into the fiber mats yielded greater inhibition zones on both bacterial strains in comparison to the fibers that were solely composed of PCL. The study reports that F2/0.5% fiber mats, which were produced using a 95:5 wt/wt ratio of PCL: PEG, exhibited an inhibition zone of 32.73 ± 1.20 mm against *E. Coli* and 22.60 ± 0.79 mm against S. aureus. The F3/0.5% formulation, which contained 10% wt/wt PEG4000, exhibited inhibition zones of 33.63 ± 0.50 mm and 21.67 ± 3.59 mm against *E. Coli* and *S. aureus*, respectively. The findings suggest that the inclusion of PEG4000 in the fiber mats amplifies the antibacterial efficacy of the fiber mats against both bacterial strains. The enhanced antibacterial activity observed with the addition of PEG4000 may be attributed to its ability to increase the hydrophilicity of the fiber mats. This increased hydrophilicity may facilitate the diffusion of the drug from the fiber mats, resulting in higher rates of drug release and subsequently

larger inhibition zones on the bacteria. These results are consistent with the release rate data obtained in the study, which showed that the incorporation of the hydrophilic PEG4000 polymer resulted in F2 and F3 formulations with higher rates of drug release compared to the PCL-only fiber mats. Overall, these findings suggest that the incorporation of PEG4000 in the fiber mats can enhance the antibacterial activity of the mats against both gram-negative and gram-positive bacteria.

Our study provides valuable insights into the design and optimization of fiber mats loaded with antibiotic agents for wound dressing applications, highlighting the importance of polymer composition and drug release profiles in achieving effective antibacterial activity.

4. Conclusions

The present investigation involved the successful fabrication of fiber mats through solvent-free melt electrospinning methods utilizing PCL alone or in conjunction with PEG4000. The primary objective of this study was to attain a regulated release profile of Cip for the purpose of wound dressing applications. The morphology of the fiber mats was observed to be smooth, and the release profile of the loaded Cip was found to be slow. The results of our study indicate that the incorporation of PEG4000 led to an increase in the hydrophilicity of the fiber mat. This, in turn, resulted in a higher rate of Cip release and greater antibacterial efficacy against *E. coli* and *S. aureus* bacterial strains. The findings of our study indicate that modulation of the constituents and their hydrophilic properties can regulate the release pattern of Cip from melt electrospun fiber mats, thereby facilitating tailored release profiles for diverse wound dressing uses. In summary, the results of our study indicate that the fiber mats possess the capability to serve as a substrate for regulated drug delivery in the context of wound healing treatments. The integration of PEG4000 presents a potentially effective approach for attaining accelerated release rates and enhanced antibacterial efficacy. Moreover, the capacity to customize the release profile by modifying the fiber mats' composition confers adaptability for diverse applications. Our future work will focus on investigating the incorporation of other therapeutic molecules such as anticancer agents for topical anticancer therapy along with investigating more parameters regarding the fiber mats prepared by melt electrospinning such as mats modulus, breakage stress, strain, and other parameters.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by M.O. The first draft of the manuscript was written by M.O., L.A., A.A., and I.K. and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Keywords

Ciprofloxacin, Controlled drug release, Melt electrospinning, PEG4000, polycaprolactone, Wound dressing

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