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Development of Poly(vinyl alcohol)−Chitosan Composite Nanofibers for Dual Drug Therapy of Wounds

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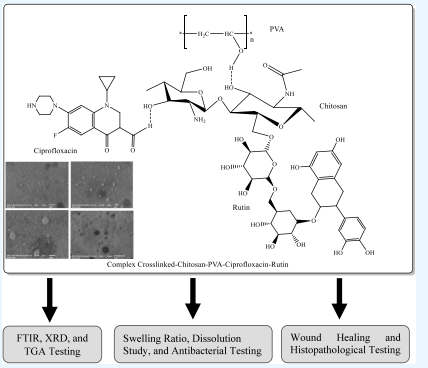
ABSTRACT: Current trends in localized drug delivery are emphasizing the development of dual drug-loaded electrospun nanofibers (NFs) for an improved therapeutic effect on wounds, especially infected skin wounds. The objective of this study was to formulate a new healing therapy for an infected skin wound. To achieve this goal, this study involved the development and characterization of poly(vinyl alcohol) (PVA)/chitosan nanofibers loaded with ciprofloxacin and rutin hydrate. Polymers and drugs were used in different ratios. Nanofiber morphology was studied by scanning electron microscopy, thermal stability by thermogravimetric analysis, structural determination by the X-ray diffraction method, and integrity by Fourier transform infrared spectroscopy. Dissolution studies were performed to check the drug release behavior of the formulations. Antibacterial studies were performed against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The wound healing efficiency of dual drug-loaded nanofibers was measured by a full-thickness excisional wound model of rabbits. The fabricated nanofibers were smooth in morphology. According to FTIR findings, the drugs remained intact in the nanofibers. The results of swelling ratio and porosity revealed that the pore size was increased as the amount of chitosan was increased up to 30% but a further increase in chitosan concentration reduced the swelling ratio and porosity. Drug release studies of nanofibers depicted an initial burst effect and afterward controlled drug release behavior. Drug-loaded nanofibers showed better activity against *S. aureus* than *P. aeruginosa*. The antibacterial efficacy of rutin hydrate with ciprofloxacin was improved compared to that of the formulation having rutin hydrate only, likely due to the additive effect in activity. Based on wound healing studies, nanofibrous membranes acted as a promising wound dressing material as compared to the commercial wound healing formulation. Drug-loaded polymeric nanofibers were successfully fabricated by using an electrospinning method. These nanofibers showed an efficient ability to deliver drugs and treat infected wounds.



1. INTRODUCTION

With the advancements in nanotechnology, polymeric nanofibers have a variety of applications in biomedical fields, including wound dressing, drug delivery systems (DDS), and tissue engineering. Because of their small size ranging from 5 to 100 nm in diameter, large surface area, high porosity, and high drug loading capacity, they are widely used for the development of antimicrobial products.[1] Both natural and synthetic polymers can be used for nanofiber production.[2] To make a nanofibrous mat with good mechanical properties and high immunogenicity, it must be combined with other synthetic or natural polymers like PVA (poly(vinyl alcohol)), PCL (polycaprolactone), and PLA (poly(lactic acid).[3] The composition, porosity, and morphology of electrospun nanofibers have a vital role in the prolonged drug release and prevention of the burst effect.[4] Drug encapsulation into a polymer increases its efficiency for sustained release and for a longer period of time.[2]

Burn wounds are the most periodically occurring traumas, leading to more than 250,000 deaths worldwide.[5] Burn wounds can be classified based on depth and penetration into the skin, generally classified as first-, second-, and third-degree wounds. Third-degree wound (full-thickness wound) is the most complex condition and is prone to growth of microorganisms, leading to delay in the wound healing process.[6] Polymeric nanofibers loaded with antibacterial compounds play their vital role in inhibiting the growth of bacteria.[7] Use of chitosan in the pharmaceutical field is increasing day by day because of its antibacterial, wound healing, and bioadhesion properties.[8] A transdermal drug delivery system (TDDS) is the most

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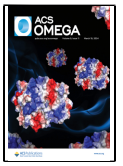

efficacious and novel technology in recent times because of its least side effects on the liver and gastrointestinal tract.[9] Transdermal drug delivery can be achieved with the help of patches, which are widely used for targeted drug release. It allows sustained drug release across the skin, while intramuscular and intravenous infusions are the invasive approaches of drug delivery that require administration of drug under observation of medical staff.[10] Both natural (such as rutin hydrate) and synthetic (ciprofloxacin) drugs can be loaded onto a TDDS. On coming in contact with wounds, the loaded drugs interact with tissues and accelerate the process of wound healing.[11] It allows a limited and controlled absorption of the drug. Thus, a transdermal route is sometimes preferred over oral and injectables.[12]

Ciprofloxacin hydrochloride is a fluoroquinoline antibiotic used for treating wound infections. Ciprofloxacin carries a very short half-life, so it is very difficult to achieve slow and prolonged drug release.[13] It is used to treat several types of infections including joint and bone infections, typhoid, chronic bacterial prostatitis, skin infections, diarrhea, chronic sinusitis,[14] and outer ocular infection.[15] Mostly, it is used for treating urinary tract infection and for bacteria that is transmitted sexually.[16] Ciprofloxacin is widely used for treating Gram-positive and Gram-negative bacterial infections.[13] Rutin hydrate (MW 610 g/mol), also known as phytomelin, is a naturally occurring flavonoid that is present in more than 70 fruits and vegetables. Rutin is a glycoside that chemically can be mixed with natural and synthetic polymers. The major drawback of rutin is its bioavailability and poor solubility, which were overcome by PVA and chitosan polymers. The incorporation of a biologically active compound in a polymer matrix composite in an aqueous environment is an efficient method to improve the limited water solubility and bioavailability.[2,17] Nowadays, one of the cutting-edge techniques for developing polymer-based drug delivery carriers is electrospinning. This is due to the fact that the average diameter of polymer fibers decreases to micro- or nanometers, which increases the surface area, thereby increasing localized drug delivery.[10]

The use of nanofibers for biomedical applications is increasing day by day due to their unique properties, which include bioavailability, renewability, inexpensiveness, biodegradability, and biocompatibility.[18] Along with these properties, their structural resemblance with the structure of the extracellular matrix to promote cell adhesion and proliferation makes them a suitable candidate for wound healing and other biomedical applications.[19]

PVA is a synthetic hydrophilic polymer extensively studied in biomedical applications owing to its nontoxicity, hydrophilicity, and biocompatibility, which made it a useful candidate in different forms including nanofibers and scaffolds.[20−22] Water-permeable characteristics of PVA make it a suitable candidate for use with many drugs, and its dissolution with other natural polymers is quite easy.[23] Moreover, PVA is mostly used in electrospinning due to its high mechanical resistance, fiber-forming properties, and biocompatibility.[24] It supports cell adhesion, proliferation, and migration. Nonetheless, PVA, due to its inert bioactive nature, cannot be administered for full-thickness wounds. Thereafter, PVA is blended with other natural or synthetic polymers. Chitosan (CS) is a chitin derivative that is biocompatible, and biofunctional aminopolysaccharides have an efficient role in wound healing.[19] Its structure is mostly based on D-glucosamine units, and these rigid units' intermolecular hydrogen bonding and high crystallinity lead to its poor solubility in organic solvents. Positively charged polysaccharides carry aliphatic amine groups in their structure. Under acidic conditions, protonation of aliphatic amines takes place and gives a cationic polyelectrolyte. Cationic behavior, hydrogen bonding, and rigid D-glucosamine in the structure of chitosan make it highly viscous, which is the major reason for its poor electrospinning because it is not possible for the electrostatic field to overcome the surface tension of solution. To reduce this problem, chitosan must be dissolved in dilute acetic acid solution. So, by increasing the concentration of chitosan, there will be an increase in its viscosity and decrease in shear thinning effect, which will make its electrospinnability possible up to some extent.[25] To deal with all these problems, chitosan must be used with any other natural or synthetic polymer that is easily electrospinnable, *e.g.*, PVA, silk fibroin, and poly(ethylene oxide) (PEO). Mostly, PVA is used with chitosan in drug delivery. The incorporation of chitosan in PVA nanofibers improve its surface hydrophilicity.[26,27] In addition, chitosan nanofibers can mimic the natural extracellular matrix (ECM), which attracted extensive attention in tissue engineering and wound dressing applications.[28]

Cross-linking is an important phenomenon for increasing the stability and wet resisting capability of synthesized electrospun nanofibers. A variety of cross-linkers and cross-linking methods are employed in the fabrication of ciprofloxacin−rutin hydrate-loaded electrospun PVA/chitosan nanofibers.[29] The polymers used in this study, PVA and chitosan, are hydrophilic in nature. On forming nanofibrous mats of these polymers alone, they can be easily destroyed in aqueous media due to poor wet stability and least mechanical properties in aqueous media.[30] These hydrophilic polymers are indeed effectively used for wound dressing because they have an excellent ability of absorbing the exudates secreted from the wounds, but loading of hydrophilic drugs on to hydrophilic polymers leads to a burst release of drugs.[6] So, cross-linking could be carried out to achieve sustained drug release from the nanofibrous mat. Chemical cross-linking was performed by exposing the nanofibrous membrane to glutaraldehyde solution.[15,22] Other methods of cross-linking involve physical methods, which include UV irradiation, dehydrothermal treatment, and simple heating.[31] A chemical cross-linker interconnects polymer molecules by increasing mechanical properties that may lead to decreased degradability and least availability of functional groups in fabricated nanofibers. Montmorillonite (MMT) is a nanoclay that is used as a reinforcement material for increasing mechanical characteristics and stiffness of the polymer chains.[32] Due to its nontoxicity and slow drug release behavior, it has an efficient role in drug delivery.[33]

Therefore, the aim and novelty of this study constitute nanofibers impregnated with two compounds ciprofloxacin and rutin using an electrospinning method. Nanofibers offer a high surface area and porosity, enhancing drug release kinetics and bioavailability.[8] The controlled release of ciprofloxacin and rutin from the nanofibers can contribute to improved therapeutic outcomes by maintaining an effective drug concentration at the target site while minimizing systemic side effects. Additionally, the nanofibers provide a versatile platform for developing advanced drug delivery systems, paving the way for innovative approaches in pharmaceutical and medical applications.

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2. MATERIALS AND METHODS

2.1. Materials. PVA (MW 72,000 g/mol) was obtained from Deajung, Korea. Chitosan (MW range 50,000−190,000 g/mol) and montmorillonite (MMT) were purchased from Sigma-Aldrich, Korea. Glutaraldehyde was obtained from Panreac Applichem, Spain. Rutin hydrate was obtained from Crown Chemicals, Pakistan. Ethanol was obtained from Lab-Scan, Lahore, Pakistan. Quench cream was purchased from market sources.

2.2. Preparation of Nanofibers. Chitosan (CS) (2% w/w) was dissolved in distilled water with a few drops of acetic acid by continuous stirring at 340 rpm at 50 °C for about 12 h (Table 1, Figure 1). PVA was dissolved in distilled water under continuous magnetic stirring at 80 °C for about 7−8 h. Then, 1% (in weight) of MMT solution was added as a reinforcement in PVA solution to get high mechanical strength and thermal resistance. Both polymers were mixed with continuous stirring for about 10 h at 360 rpm for complete dissolution and stability of the polymer blend. Rutin hydrate solution was prepared separately by dissolving it in ethanol, while ciprofloxacin was dissolved in distilled water in addition to 10−12 drops of acetic acid. The drugs were loaded on polymer blend solution under continuous magnetic stirring for about 24 h at 380 rpm at room temperature to get a homogenized solution. To achieve a high level of uniform diameter of nanofibers, electrospinning was processed under controlled atmospheric conditions. Henceforth, the rutin−ciprofloxacin-loaded PVA-MMT/CS nanocomposite solution was transferred into a 5 mL syringe having 11.99 mm diameter. The distance of the needle tip and collector was kept 14 cm. The solution was ejected from the needle at a flow rate of 400 μL/h, and the voltage was adjusted between 14 and 15 kV. After solvent evaporation, the nanofibrous mats were collected from the collector.

Table 1. Formulations of Nanocomposite Samples upon Adding a Constant Amount of MMT (0.01 g)

	concentrations (g)				
formulation codes	rutin hydrate	ciprofloxacin	PVA	chitosan (CS)	PVA:CS
R1	0.10	0.10	1.50 (90%)	1.65 (10%)	90:10
R2	0.10	0.10	1.50 (80%)	3.75 (20%)	80:20
R3	0.10	0.10	1.50 (70%)	6.42 (30%)	70:30
R4	0.10	0.10	1.50 (60%)	10.00 (40%)	60:40
R5	0.00	0.20	1.50 (90%)	1.65 (10%)	90:10
R6	0.20	0.00	1.50 (90%)	1.65 (10%)	90:10
R7	0.00	0.00	1.50 (90%)	1.65 (10%)	90:10

2.3. X-ray Diffraction Spectroscopy (XRD). This characterization technique was performed to analyze the crystallinity of the fabricated nanofibers. Pure drug and the nanofibers were tested using an X-ray diffractometer (Phillips XPERT PRO 3040/60).

2.4. Thermogravimetric Analysis (TGA). Thermal stability of rutin−ciprofloxacin-loaded CPNFs was analyzed using a Q600 SDT TGA analyzer. The thermal decomposition of drug-loaded nanofibers was measured at 10 °C/min in a temperature range of 20−700 °C. The weight loss of the

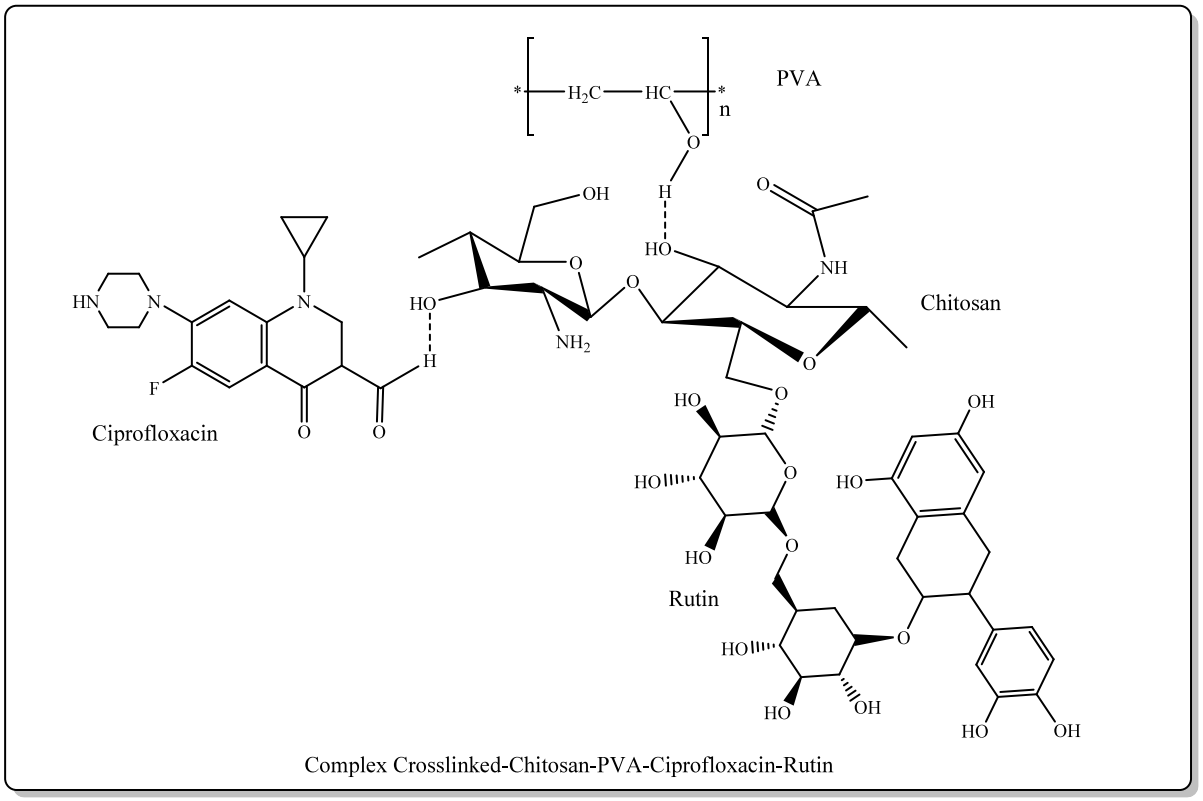

Figure 1. Complex cross-linked chitosan−PVA−ciprofloxacin−rutin.

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sample was observed as a function of temperature at the controlled temperature supply in a nitrogen atmosphere.

2.5. FTIR Characterization of Rutin−Ciprofloxacin-Loaded PVA/CS Nanofibers. By using FTIR, both qualitative and quantitative analyses can be carried out for determining the functional site or reacting site. This analysis was carried out on KBr pellets ranging from 4000 to 500 cm^{-1}. Through FTIR, we can determine the nature of bond, reaction kinetics, presence of impurities, presence of covalent bond, and presence of cis/trans configuration in the compound mixture.

2.6. SEM of Rutin−Ciprofloxacin-Loaded PVA/CS Nanofibers. SEM characterization of the synthesized nanofibrous mats was performed on a Joel, JSM 6400f. The morphology, topology, and diameter of the drug-loaded nanofibrous mat can be determined by this technique. SEM images determined the changing properties of the nanofibrous sheet after the addition of drugs and their exposure to glutaraldehyde solution.

2.7. Solubility Study. Distilled water and organic solvents including DMF, DMSO, methanol, and ethanol were used to check the solubility of drugs. A total of 10 mg of drug was added into each solvent and stirred continuously at 50 rpm until a clear solution was obtained. The absorbance of both drugs was determined using a UV spectrophotometer.

2.8. Calibration Curve. Stock solutions of both drugs were prepared by dissolving 10 mg of drug in 10 mL of methanol:distilled water mixture. From this stock solution, various dilutions in a range of 0.1−1 μg/mL were prepared by taking a required amount of stock solution and further diluting it with the help of 10 mL of fresh solvent. The absorbance of different dilutions was measured using a UV spectrophotometer at 278 nm for ciprofloxacin hydrochloride and 360 nm for rutin hydrate. Finally, calibration curves were drawn using MS Excel.

2.9. Swelling Ratio and Porosity Studies. Swelling ratio and porosity studies were also performed to assess the fluid absorption and permeation capabilities. Porosity study was performed to check how porous the nanofiber formulations were. It was calculated using the following formula:

$$W_2 - W_1/\rho V_1 - \rho V_2 \times 100 \quad (1)$$

where W_2 is the weight of the wet sample, W_1 is the weight of dry fiber, V_1 is the volume of solvent before adding fiber, V_2 is the volume of solvent after removing fiber, and ρ is the density of solvent in g/mL at 25 °C (for water, ρ = 0.9970 g/mL).

2.10. Drug Release Studies. Using a type II dissolution apparatus, drug release studies were performed in a dissolution medium containing 10% methanol having a pH of 6.8, maintained at 37 ± 0.05 °C, and stirred at 50 rpm. Sampling was done at the predetermined time points and tested using a UV spectrophotometer at 278 nm for ciprofloxacin and 360 nm for rutin. Dissolution studies were carried out in triplicate. Drugs were quantified by using the calibration curves.

2.11. *In Vitro* Antibacterial Assay. The antibacterial activity of the fabricated nanofibers was checked by an agar well diffusion test, and the zone of inhibition (ZOI) was studied against *Staphylococcus aureus* and *Pseudomonas aeruginosa* isolates. These isolates were acquired from a microbiology lab. Agar medium was prepared and poured into Petri plates, and samples were placed on the medium. Finally, the plates were incubated over standard conditions.

2.12. *In Vivo* Wound Healing Studies. Research Ethics Committee of COMSATS University Islamabad (CUI), Lahore Campus, approved all protocols for this animal study (approval number: 897/CUI/PHM-2021). All the animal experiments were conducted in accordance with the ARRIVE Guidelines and U.K. Animals (Scientific Procedures) Act 1986 and associated guidelines for the care and use of laboratory animals.

Antibacterial and wound healing studies were performed at the Department of Pharmacy, COMSATS University Islamabad, Lahore Campus. The wound healing property of nanofibers was checked against the marketed formulation (Quench). For this purpose, 12 rabbits weighing 1−2 kg were selected. The posterior dorsal region of rabbits was trimmed and shaved to get clear skin and washed with ethanol to minimize the chances of rashes. The rabbits were anesthetized with xylene and ketamine hydrochloride injections prior to wound creation. Four wounds were created on the four legs of each rabbit with the help of a red-hot iron having diameter 1−2 cm^2, and then, bacterial strains were seeded on each wound. On one wound, drug-loaded nanofibers were applied. The second wound received drug-free nanofibers. On the third wound, the marketed formulation (Quench) was applied, and the fourth wound was kept open and untreated. The following formula was used to check the healing of wounds:

$$\text{wound area}(\%) = \frac{A}{A_o} \times 100 \quad (2)$$

A_o is the original area of the wound, and A is the wound area after a fixed time.

2.13. Statistical Analysis. The paired t test was applied to compare the findings using SPSS version 22.0, keeping the level of significance set at 0.05.

3. RESULTS AND DISCUSSION

3.1. X-ray Diffraction Spectroscopy (XRD). This characterization technique was performed to check the crystallinity of the fabricated nanofibers. XRD results in Figure 2 clearly depict the semicrystalline nature of drug-loaded nanofibers. XRD of R1 was investigated to check whether the nanofibrous sheet is crystalline, amorphous, or semicrystalline in nature. XRD data in Figure 3 shows clearly the semicrystalline nature of nanofibers because the peaks are neither too sharp nor too broad. Although PVA and chitosan are crystalline in nature, the peak is not sharp due to the adsorbent nature of chitosan and cross-linking of nanofibrous

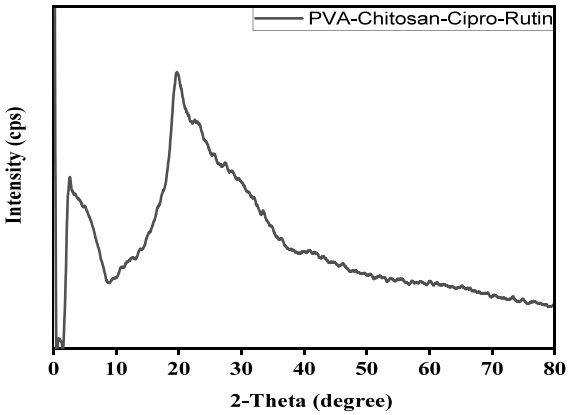

Figure 2. XRD graph of the R1 formulation in which both drugs are present in nanofibers.

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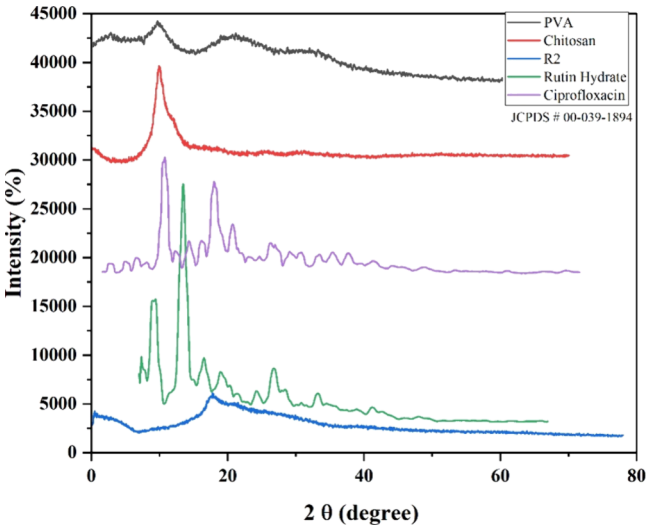

Figure 3. XRD diffractograms of PVA, chitosan compared with JCPDS #00-039-1894, R2 formulation, rutin hydrate, and ciprofloxacin hydrochloride.

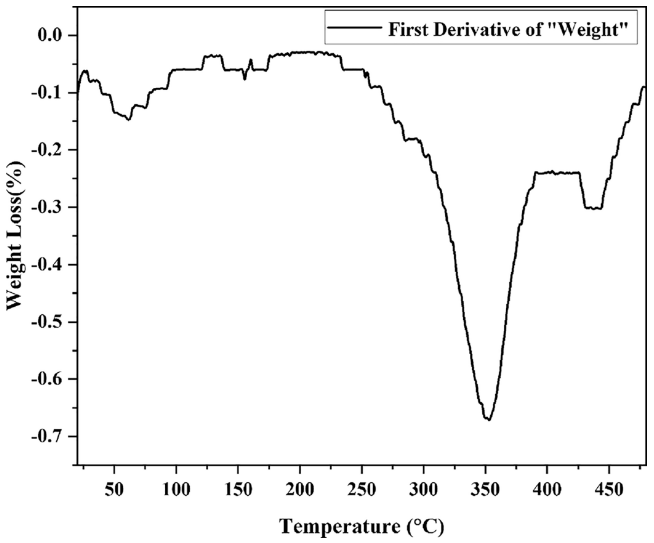

Figure 5. First derivative of weight loss in TGA showed a weight loss around 350 °C.

sheet with glutaraldehyde that changes the peak intensity. The peak of chitosan indicated the presence of inter- and intramolecular hydrogen bonding between the hydroxyl and amino groups, forming hydrated and anhydrous crystals.[34] The nanofibers R2 showed no sharp peak, and the intensity of peaks diminished, which could be due to obstruction of hydrogen bonding of cationic chitosan with ciprofloxacin and rutin hydrate.

3.2. Thermal Stability of Rutin−Ciprofloxacin Nanofibers by TGA. Thermal stability of drug-loaded nanofibers was estimated by TGA analysis performed for the R1 sample. Depending upon the shape of the curve, we can easily determine how stable our material is. TGA results of PVA/chitosan nanofibers revealed that weight loss occurs at three different points, but that weight loss is very low, which gives another hint about the sustained release of drug. The TGA graph of drug-loaded nanofibers for formulation R1 (rutin−ciprofloxacin) was investigated. The weight loss was measured at three different points that shows a slow decomposition of polymers and drugs (Figures 4 and 5). The first weight loss occurred at about 50−120 °C, which was due to loss of

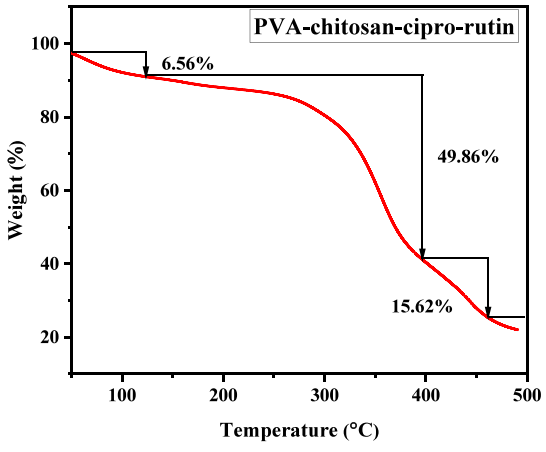

Figure 4. TGA of rutin−ciprofloxacin-loaded chitosan/PVA nanofibers.

bounded water present in all the formulation of nanofibers. The second weight loss started at 125−130 °C at which the decomposition of polysaccharide chains of both polymers PVA and chitosan started and the drugs started melting.

3.3. FTIR Analysis of Drug-Loaded CPNFs. The synthesized nanofibers were further subjected to FTIR analysis. In the IR spectra of drug-loaded nanofibers with both drugs (ciprofloxacin HCl and rutin hydrate), both showed peaks of OH at 3376.06 cm^{-1}, while for chitosan, it was observed at 3346.49 cm^{-1}. In the case of chitosan, other peaks appear including the C−H stretching at 2937 cm^{-1}, C=O at 1652 cm^{-1}, C−H bend at 1356 cm^{-1}, and C−O at 1059.72 cm^{-1}. Different peaks for ciprofloxacin were observed at different wavelengths; the NH peak appeared at 3340.57 cm^{-1}, OH at 2935.20 cm^{-1}, and C=O at 1705.52 cm^{-1}, C−F within the range of 1400−1000 cm^{-1}, and C−O at 1270.87 cm^{-1}. The hydroxyl group of rutin hydrate showed a peak at 3334.66 cm^{-1}, C=O at 1656.05 cm^{-1}, and C−O at 1296.29 cm^{-1}. The FTIR results of both polymers, drugs, and R1 formulation, which contains both polymers and drugs with MMT, are shown in Figure 6.

3.4. Physical Characteristics of Rutin−Ciprofloxacin Nanofibers by SEM. SEM micrographs of R1 formulations at different resolutions were carried out at 10 kV. SEM images at different resolutions at high magnifications revealed the smooth morphology of nanofibers having diameter in the nanorange (Figure 7), but they also showed bead formation, which is due to chitosan. Chitosan has gelling properties, so it blocks the needle tip of the electrospinning machine frequently and shows spitting on the sheet. The increase in the amount of chitosan increases this problem, which automatically affects the fiber diameter. The SEM results indicated that these electrospun nanofibers are placed in a crisscross manner, showing spaces among the nanofibers. In addition, the image scale shows the nanorange of these nanofibers' diameter.

3.5. Pharmaceutical Studies. *3.5.1. Calibration Curves of Rutin Hydrate and Ciprofloxacin Hydrochloride.* The calibration curves of rutin hydrate and ciprofloxacin hydrochloride were plotted between the drug concentration in different dilutions and its absorbance. The findings showed

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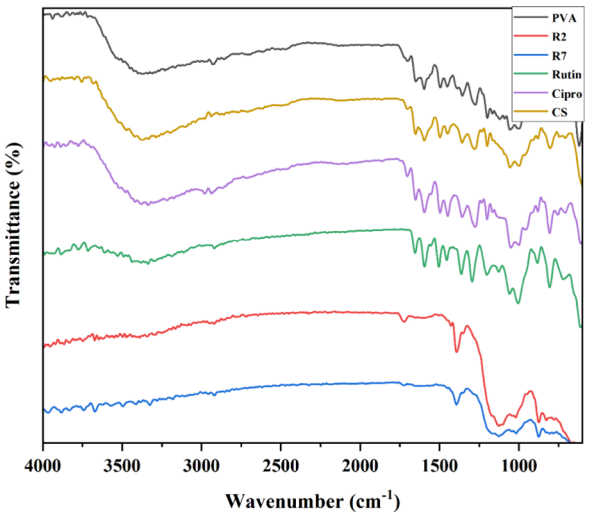

Figure 6. Comparison of FTIR spectra of PVA, chitosan, ciprofloxacin hydrochloride, and rutin hydrate with formulations R2 and R7 indicating loading of drugs completely in the nanofibers.

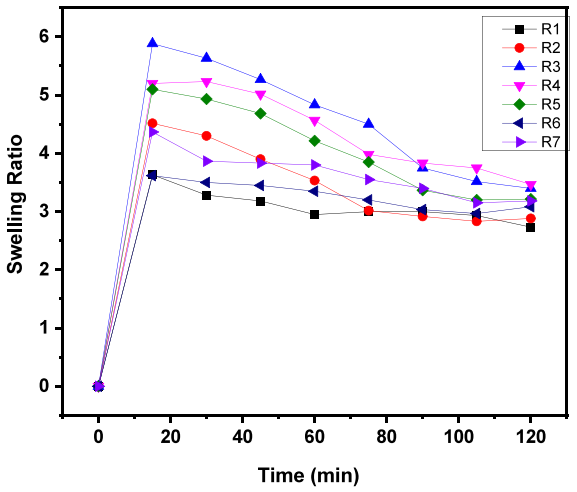

Figure 8. Swelling ratio of rutin−ciprofloxacin CPNFs.

that the linear regression coefficients were R^2 = 0.99462 for ciprofloxacin hydrochloride and R^2 = 0.9938 for rutin hydrate.

3.5.2. Swelling Ratio. This study was used to check the water uptake capacity of all nanofiber formulations. According to Figure 8, as the amount of chitosan increases in formulations R1 to R3, its swelling ratio also increases; however, this trend is not seen in formulation R4, likely due to bead formation. Chitosan used in higher amounts (such as 40%) leads to bead formation, which reduces the number of pores on the nanofiber surface, resulting in a reduced swelling ratio. PVA is permeable for water and chitosan is also a good absorbent. So, mixing of both polymers increases water uptake because of the increase in hydrophilic groups in the mixture. Thus, the swelling ratio first increases and then decreases when chitosan is increased more than 30%. The swelling ratio of R5, R6, and R7 is comparable to one another because these formulations have the same amount of chitosan. R7 is a blank formulation and shows more water uptake than R6, which

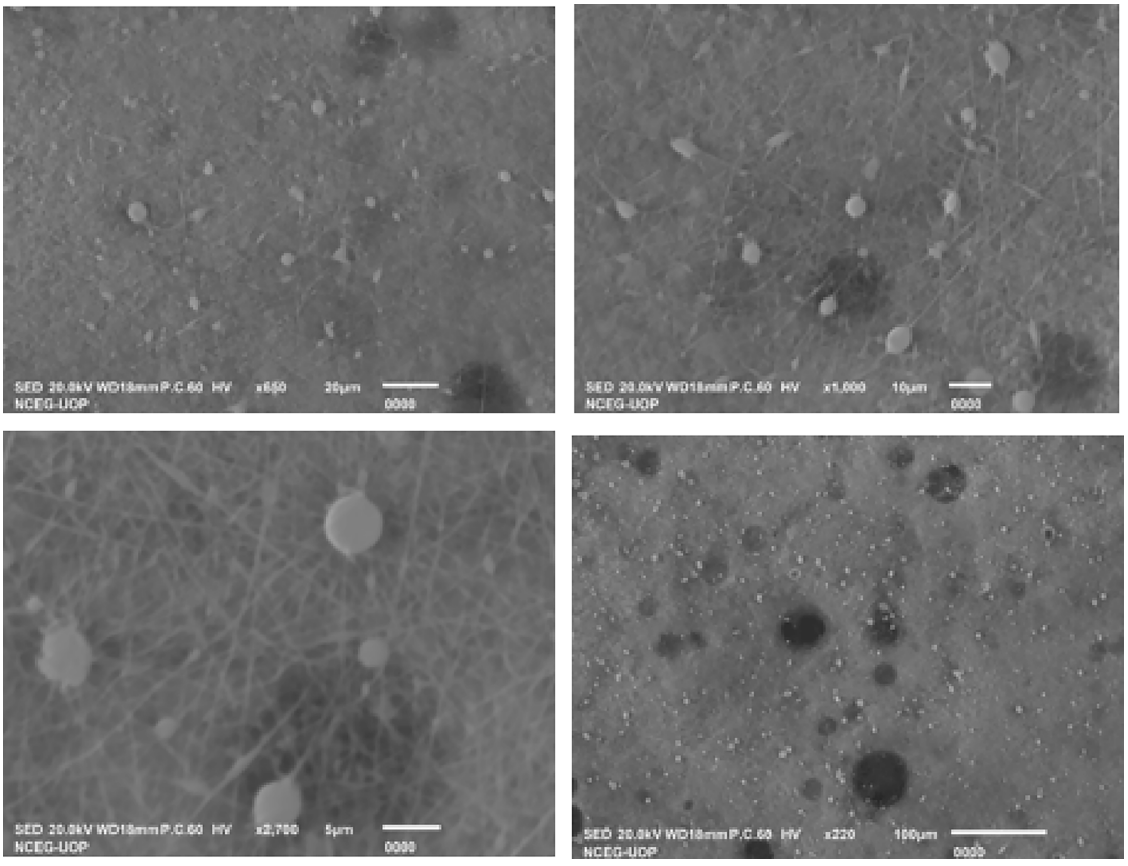

Figure 7. SEM analysis of rutin−ciprofloxacin-loaded CPNFs at magnifications of ×220, ×650, ×1000, and ×2700.

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could be due to the presence of 20% rutin hydrate in R6, which is insoluble in water.

3.5.3. Determination of Porosity. The porosity study is shown in Figure 9. The increase in swelling promotes porosity.

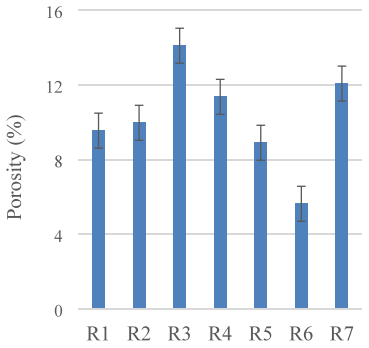

Figure 9. Determination of the porosity of different formulations of nanofibers.

Like the swelling behavior, R3 has the highest ($P < 0.05$) porosity. There was an increase in porosity from formulations R1 to R3, while the porosity was decreased in other formulations (Figure 9). Formulation R4 contained a maximum amount of chitosan, but the use of more than 30% of chitosan in the formulations led to bead formation in nanofibers, which resulted in a decreased number of pores. Formulation R5 containing 20% ciprofloxacin showed more porosity than R6 having 20% rutin hydrate. This behavior is likely because of the miscible and immiscible behavior of both drugs. Blank formulation R7 showed more porosity than R1, R5, and R6, although all of them had the same amount of PVA and chitosan; this difference could be due to the presence of drugs having different solubilities.

3.5.4. Drug Release Studies. Formulation R1 permitted the fastest release of ciprofloxacin followed by R2, R3, and so on. This decrease in the ciprofloxacin release rate was associated with a proportional increase in chitosan concentration. However, this increase in the amount of chitosan did not exert a significant retarding effect on the release of rutin. This difference in the release behavior of both drugs is due to their solubility of dissolution. Ciprofloxacin is more water-soluble than rutin,[4] and thus, it was released at a faster rate than rutin. In addition, a burst release of both drugs occurred in the first 15 min followed by a very slow-release phase. Various formulations allowed for a release of about 25−79% during the initial 15 min. This swift release could be attributed to the hydrophilic nature of the polymers (chitosan and PVA) used in the fabrications of nanofibers (Figure 10). Similar findings have been reported in previous publications.[5,6]

3.5.5. In Vitro Antibacterial Assay. The antibacterial activity of drug-loaded NFs was evaluated by a dynamic contact assay and agar well diffusion method (Figure 11A,B) at the MIC (6 μg/mL) against different strains of *S. aureus* and *P. aeruginosa*. In the dynamic contact assay method, both Gram-positive and Gram-negative bacteria were incubated with different membrane species for about 12 to 24 h. All the formulations showed an appreciable activity against *S. aureus* than *P. aeruginosa* (Figure 12). The antibacterial activity of PVA/chitosan nanofibers against *S. aureus* is more pronounced than that against *P. aeruginosa*. This is because the outer membrane structure of Gram-negative bacteria limits the permeability of both drugs. Formulation R3 (cipro−rutin ratio

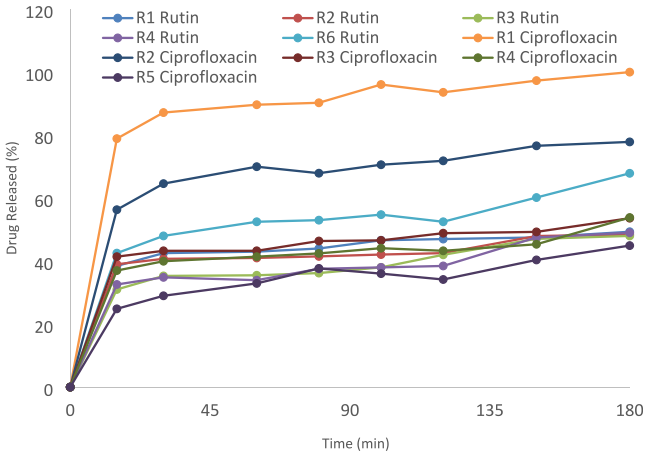

Figure 10. Drug release behavior of various formulations.

1:1) showed the highest ZOI against *S. aureus*. Although there is a nonspecific trend on the ZOI findings, the increased concentration of chitosan, as in formulations F3 and F4, showed a significantly ($P < 0.05$) improved activity against both bacterial strains, as compared to formulations R1 and R2. As chitosan acts as a natural antibiotic, so increasing the amount of chitosan increases the antibacterial activity. Chitosan is polycationic in nature, so its main function is to destroy the cell wall of bacteria, which hinders the delivery of nutrients into the cell and promotes the leakage of intracellular components. In the case of *P. aeruginosa*, nanofibers did not show promising results, and the change in the amount of chitosan did not show any impact on it. It is just because of bacterial resistance. The results depict that the antibacterial efficacy of rutin hydrate with ciprofloxacin is much better than that formulation having only 20% rutin hydrate. As expected, the pure PVA scaffold is completely dissolved at the end of the 24 h incubation and a nonsignificant ($P > 0.05$) inhibition zone formation was observed.[35] In contrast, a neat exhibition zone is observed for drug-loaded PVA/chitosan nanofibers. In the case of nondrug-loaded PVA/chitosan nanofibers, the narrow inhibitory zone is observed, which is due to the frequently reported antibacterial activity of chitosan.[36]

This study evaluated the antibacterial activity of dual drug-loaded nanofibers against *P. aeruginosa* and *S. aureus*, revealing that the antibacterial activity of drug-loaded nanofibers was greater (4 times more) than that of free drug.

3.6. Wound Healing Studies. The wound healing process is a combination of four stages, namely, cell homeostasis, cell inflammation, proliferation, and remodeling.[37] The process of homeostasis is initiated by vascular constriction followed by coagulation of blood to slow the flow of blood from the injured tissue. The second phase is the inflammation phase in which nutrient-rich blood is supplied at the site of injury, which results in swelling of tissues. This phase also includes the angiogenesis process, which involves proteins and cytokines, the signaling cells involved to prevent infection.[38] The proliferation phase involves formation of new cells, leading to granulation of new cells and tissues and formation of collagen by fibroblast cells at the site of injury.[4] As new cells formed around the wound, a scar is formed, while collagen is involved in remodeling and closure of wound.[35] This is known as the remodeling phase.[39,40]

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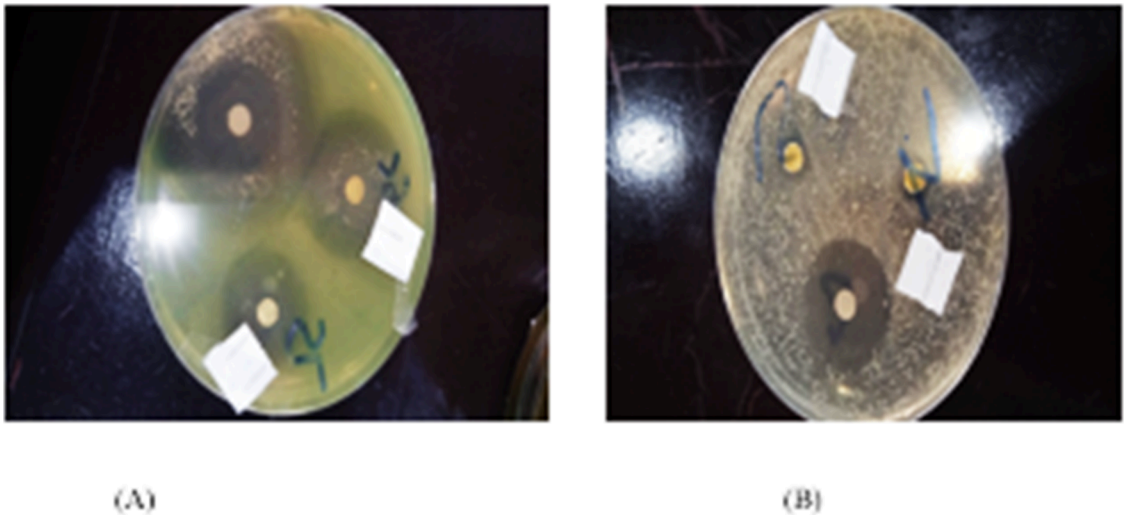

Figure 11. Optical images of antibacterial activity of NFs against (A) *S. aureus* and (B) *P. aeruginosa*.

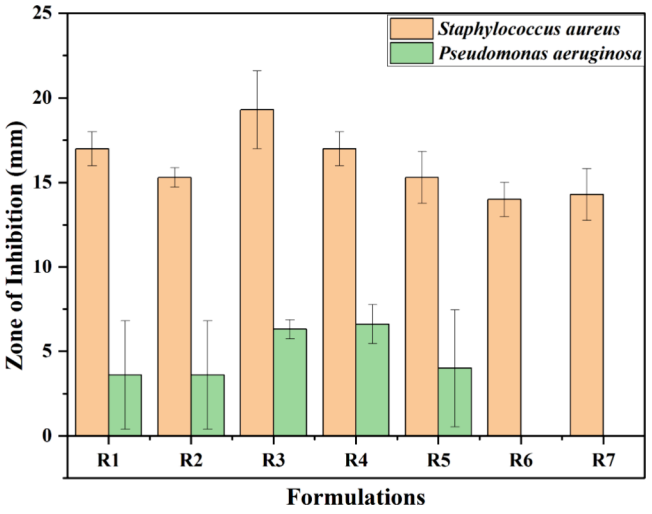

Figure 12. Antibacterial activity (ZOI) of PVA/CS, nondrug-loaded, and drug-loaded nanofibers against different strains of *S. aureus* and *P. aeruginosa*.

The wound healing performance was checked with the help of a scale after different time periods (Table 2, Figures 13 and 14).

The percentage area of wound closure was measured for 17 days. Wounds were treated with the antibacterial cream and drug-loaded and blank NFs. Healing was faster in wounds treated with drug-loaded and blank NFs than Quench-treated wounds and open wounds because chitosan itself has antibacterial properties and addition of drugs increases the healing properties of NFs.[37] The incision was fully closed after 17 days in the drug-loaded formulation. Drug-loaded NFs inhibit bacterial growth by killing them; however, blank NFs also show good results after drug-loaded NFs, which is likely due to the presence of chitosan, which acts as a wound healing accelerator.[38] The process of wound healing increases steadily

Table 2. Measurement of the Wound Area of Rutin–Ciprofloxacin-Loaded CS/PVA Nanofibers

time (days)	Quench	drug-loaded NFs	open (untreated) wound	blank NFs
0	100 ± 0.0	100 ± 0.0	100 ± 0.0	100 ± 0.0
3	87.5 ± 2.0	92.5 ± 2.0	100 ± 0.0	100 ± 0.0
5	83.0 ± 2.0	75.0 ± 4.0	95.0 ± 2.0	95.0 ± 1.0
7	80.0 ± 3.0	62.5 ± 3.0	90.0 ± 1.0	75.0 ± 2.0
9	77.5 ± 3.0	60.0 ± 3.0	85.0 ± 2.0	65.0 ± 2.0
10	72.5 ± 3.0	52.5 ± 4.0	85.0 ± 2.0	60.0 ± 4.0
13	65.0 ± 2.0	50.0 ± 5.0	80.0 ± 3.0	60.0 ± 5.0
15	65.0 ± 2.0	25.0 ± 4.0	60.0 ± 4.0	50.0 ± 3.0
16	55.0 ± 3.0	20.0 ± 4.0	50.0 ± 3.0	25.0 ± 4.0
17	30.0 ± 4.0	5.0 ± 4.0	25.0 ± 3.0	15.0 ± 4.0

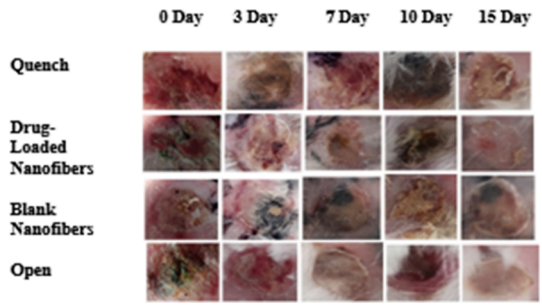

Figure 13. Wound healing studies using (A) Quench, (B) dual drug-loaded nanofibers, (C) blank nanofibers, and (D) open (untreated) wound.

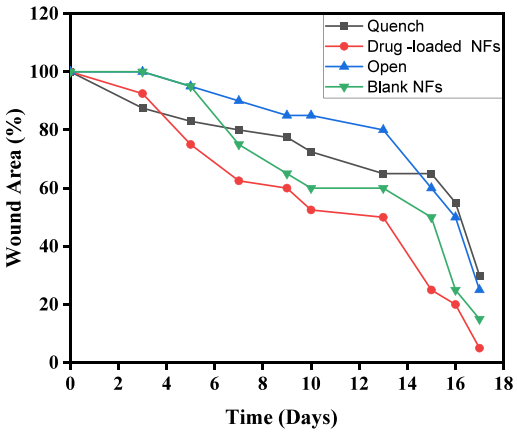

Figure 14. Relationship between wound closure and treatment days of wound healing studies.

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when the drug is incorporated into the nanofibers. So, PVA−chitosan NFs along with the addition of ciprofloxacin HCl and rutin hydrate act as an excellent wound dressing.

4. CONCLUSIONS AND PERSPECTIVE

In summary, the research study provided insights into the potential applications of ciprofloxacin- and rutin-loaded nanofibers. The study highlighted the successful synthesis of dual drug-loaded nanofibers using the electrospinning method and emphasized the optimal conditions for electrospinning. The cost-effectiveness of rutin−ciprofloxacin PVA−CS nanofibers was demonstrated as an excellent dressing material with efficacious antibacterial activity compared to the standard. The cell viability of the synthesized nanofibers is higher, as evaluated in cell culturing. Moreover, the dissolution studies revealed the burst release of drugs within 15 min, which depends upon the solubility of dissolution of ciprofloxacin and rutin hydrate. The dissolution of rutin is proportional to the increase in the amount of chitosan. Finally, *in vivo* animal studies revealed the progression of wound healing in 17 days. Overall, rutin−ciprofloxacin dual drug-loaded PVA−chitosan nanofibrous films were evaluated to have great potential for biomedical applications because of their antibacterial, effective biocompatibility, and full-thickness burn wound healing.

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Notes

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