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Blended Polymeric Nanofibres for Antimicrobial Activity

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KEYWORDS

Polymers, nanofibers, Electrospinning, antimicrobial material.

ABSTRACT:

Introduction : Polymeric nanofibers have gained significant due to their high surface area-to-volume ratio, tunable and excellent properties for functionalization. Among these, blended nanofibers created by combining different polymers offer improved mechanical strength, chemical stability, and enhanced bilogical activity. In this study, we synthesized and characterized novel blended polymeric nanofibers composed of Polyvinyl alcohol (PVA), Pectin, and sodium carboxymethyl cellulose (NaCMC), and evaluated their antimicrobial activity against harmful pathogens.

Objective : To Synthesize and Characterize blended PVA-NaCMC-Pectin nanofibers and assess their antimicrobial activity against Staphyloccus aureus and Escherichia coli, exploring their potential application as novel antimicrobial agents.

Methods : Blended polymeric solution were prepared by combining PVA, NaCMC, Pectin, and fumaric acid as a crosslinker agent. This blended solution was used to fabricate nanofibers through electrospinning. The synthesized nanofibers were analyzed using Fourier-transform infrared spectroscopy (FTIR). The surface morphology of nanofiber were examined by Scanning electron microscopy (SEM). The antimicrobial activity of nanofibers was evalated using disk diffusion method against Staphyloccus aureus and Escherichia coli, measuring zones of inhibtion to determine efficacy.

Results : FTIR confirmed successful polymer blending and crosslinking, while SEM showed uniform nanofiber Morphology. Disk diffusion tests revealed clear inhibition zones against Staphyloccus aureus and Escherichia coli, confirming good antimicrobial activity.

Conclusion : This study presents blended PVA-NaCMC-Pectin nanofibers with good antimicrobial activity against both gram-positive and gram negative bacteria. These nanofibers show promise for medical and wound care application, through further studies on mechanism and safety are needed.

1. Introduction

A wide range of products are labelled with the terms nanoscience and nanotechnology. They are frequently used in reports and commercials addressing novel discoveries and appealing new products. Since polymers have significantly improved the world, they are an important area of research in the field of nanotechnology. Polymers have a lot of highly desired qualities in addition to being inexpensive to manufacture, such as high strength or modulus to weight ratios, toughness, resilience, resistance to corrosion, and lack of conductivity [1]. Electrospun nanofibers, one of the various kinds of nanostructures, have been actively used in many different applications because of their high porosity, high surface area, high loading capacity, flexibility, and large surface-to-volume ratio [2]. Polymeric nanofibers have gained excellent attention as a versatile platform in various biomedical engineering fields including tissue engineering scaffolds, drug release systems, and wound dressings [3]. One of the most crucial challenges in most biomedical applications is to prevent bacterial infections, as they worsen or drastically slow down the healing process in all cases and can lead to very complicated situations. Hence, the development of polymeric nanofibers with either inherent or improved antibacterial characteristics has attracted increasing interest [4]. Blended polymeric nanofibers have emerged as promising materials for antimicrobial applications. Studies have shown that incorporating antimicrobial agents like essential oils into electrospun nanofibers can enhance their antibacterial properties [5]. The use of chitosan in nanoform blended with polyvinyl alcohol (PVA) has demonstrated increased antibacterial activity with higher chitosan concentrations, showcasing the potential for inhibiting bacterial growth [6]. Furthermore, the impregnation of nanofillers in polymeric nanocomposites has led to the development of nanorods, nanowires, and nanotubes with enhanced tensile strength, contributing to their antimicrobial applications [7]. Electrospinning techniques have been pivotal in fabricating composite polymer fibers with controlled release properties, making them effective in various fields such as biomedicine, pharmacy, and food industry for combating antibiotic-resistant pathogens [8]. These nanofibers exhibit controlled release properties, effective inhibition of bacterial growth, and biocompatibility, making them suitable for applications in active food packaging, biomedicine, pharmacy, and wound dressings. The incorporation of antimicrobial agents like chitosan, polyvinylpyrrolidone, and nerolidol into the polymeric matrix has shown promising results in enhancing the antimicrobial efficacy of the nanofibers, providing a potential solution to combat antibiotic resistance in various pathogens [9]. The continuous advancements in electrospinning techniques and the engineering of nanofibers highlight the promising future of blended polymeric nanofibers for antimicrobial applications, including active food packaging and wound dressings [10].

The goal of this study is to prepare blended polymeric nanofibers based on poly vinyl achohol (PVA), pectin, sodium carboxymethyl cellulose in aqueous medium. The as-synthesized blended polymeric was characterized their antimicrobial activity to be assed. The Scanning Electron Microscopy studies confirmed the successful fabrication of nanofibres. The antimicrobial efficacy of blended polymeric nanofibers represents a cutting-edge approach to combating antimicrobial resistance, offering a versatile platform for the development of innovative antimicrobial materials.

2. Materials and Methods

2.1 Materials

Polyvinyl alcohol Mw 89000-98000, Carboxymethyl Cellulose sodium purchased from Polychem Ltd. Mumbai. Pectin with Mw 30000-100000 was purchased from Bangalore Fine Chemicals, Bangalore. Fumaric acid with Mw 116.07 was purchased CDH fine chemicals Ltd. New Delhi. Double distilled water, magnetic stirrer, Electrospinning machine ESPIN-NANO, PECO, Chennai, India (JNU, New Delhi).

2.2 Synthesis of blended polymer

The blended polymer was synthesized by using PVA, NaCMC, pectin and the fumaric acid (Crosslinker). The polymer solution was prepared by mixing 0.5gm PVA, 0.4 gm pectin, 0.2 gm NaCMC, and 0.5 gm fumaric acid and then made up with distilled water to a final volume of 50

ml. The solution was stirred around 3-4 hrs continuously on magnetic stirrer. Firstly, the PVA is allowed to dissolve around 30 min. at 60-70ºC with constant stirring. After that 0.4gm pectin was mixed with PVA solution and allowed to stirr at same temperature or 20 min. And then later dissolving the pectin, 0.2 gm NaCMC is mixed in the PVA and pectin solution at same temperature around 20 min. At last, the fumaric acid was added to crosslink the polymer at same temperature for 60- 80 min. The final prepared polymeric solution was stored at for 24hrs.

2.3 Optimization of nanofibres

The electrospinning ESPIN-NANO consisted of an infusion pump, a high-voltage generator with a continuous current source, and a 200 mm in length and 100 mm in diameter stainless steel rotary cylinder that served as the nanofiber collector. To reduce the possibility of discharges during operation, the electrospinning process took place inside a completely grounded, electrically insulated compartment shown in **Figure 1**. The initial step involves setting the electrospining parameters, which include the flowrate, applied voltage, and distance between the collector and the needle. The syringe needle diameters (0.55 mm) and the distances (9, 10, 11 and 12 cm) between the needle

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and collector were measured in preliminary testing. The electrospinning formulations were fed using a 5 mL plastic syringe and a metallic needle with a 0.55 mm opening (**Table 2**). For every experimental run, the electrospinning parameters remained the same: 5 mL/hr, a 19 kV electrical field. The metallic drum collector was static at their position and the tailor cone was moving around, covered in aluminium foil. The polymeric solution was run at different flow rates of 0.1ml /hr, 0.15ml/hr and 0.2ml/hr.

Figure 1.ESPIN-NANO Electrospinning Assemby

Table 1 Optimization polymeric sample(PS) ratios for fibres formation.

Sample	PVA(gm)	Pectin(gm)	NaCMC(gm)	Fumaric acid(gm)	Total solution(ml)	Fibre formation
PS1	1.5	0.5	0.5	0.7	70 ml	NO
PS2	2	0.3	0.3	0.5	70 ml	NO Spray only
PS3	3.5	0.3	0.4	0.5	75 ml	Spray jet , fibres form
PS4	**4**	**0.3**	**0.3**	**0.5**	**75 ml**	**Fibres form , NO droplets**

Table 2 Different optimization of polymeric sample (PS4) for fibre formation.

Sample	Distance (cm) b/w Collector and Syringe	Flow rate (ml/hr)	Voltage applied (KV)	Results
PS4a	10	0.25	16	Spray Jet
PS4b	12	0.15	22	Preliminary formation of fibres

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PS4c	10	0.1	19	fibres formation with spray jet
PS4d	10	0.1	19	Fibres form with droplets
PS4e	12	0.1	19	Fibres with droplets
PS4f	9	0.1	19	Fibres formed
PS4g	9	0.15	19	Fibres formed
PS4h	**9**	**0.2**	**19**	**Fibres formed**

2.4 Characterization

FTIR is used infrared radiation to scan the test samples and observe the chemical properties of the same. The FTIR spectrum and the peaks gives the information about the molecular structure and chemical bonding. The prepared nanofibres were directly placed onto the ATR crystal and then different spectrum were obtained of every sample.

SEM was also carried by using the JSM-IT200 JEOL, to study the physical morphological studies of the sample. And the image J software was used to analyze the size of electrospun nanofibres.

2.5 Antimicrobial Studies

To test the antimicrobial efficacy of the prepared polymeric nanofibres a standard strain of stapylococcus aureus (S. aureus gram positive) and *E.coli* (gram negative) was used in this work.The antimicrobial properties of prepared nanofibres, polymeric solution was tested on tryptic soy plates inoculated with *S. aureus* strains and *E. coli* through disk diffusion method in which sample PS4 was tested for antimicrobial activity. And the Levofloxacin was used as control in this test with concentration around 0.06 mg/ml. After 24 h of incubation, inhibition zones around PS4 samplewas evaluated. And percentage of zone of inhibition was calculated by the following formula [11]

$$Zone\ of\ Inhibtion\ (\%) = \frac{Zone\ of\ Inhibtion}{Diameter\ of\ Petri\ Dish} \times 100 \quad (1)$$

3. Results and Discussion

3.1 Synthesis of polymeric nanofibres

Different parameters have been varied to optimise the formation of nanofibres using polymeric samples of various ratios as shown in **Table No. 1** In samples PS1 and PS2, there was no fibres formation observed might be due to decreased PVA dosage in the blended sample and the low viscosity. Next, a droplet formation was observed in the PS3, where the PVA dosage was increased to 3.5 g in the polymeric solution. Sample PS4, included 4 g of PVA in the polymeric solution, which improved the fibre formation, which was observed to be 200 nm - 700 nm of diamter. Best result or formation of the nanofibres was showed at 9 cm distance the needle and the collector, and the flow rate with 0.2 ml /hr with 19 kV voltage.

3.2 Characterization of nanofibres

The prepared nanofibres are characterized by FTIR which results are shown in the **Figure 2**. The obtained FTIR spectrum of the nanofibres, a clear indication of broad band at wave number 3289 cm^{-1} which was assigned for O-H stretching of PVA [12]. Peak appeared at wave number 1666.97 cm^{-1} indicate stretching of C=O vibration which ascribed to carbonyl stretching of pectin chain [13]. Appearance of peak at 1424.33 cm^{-1} indicates stretching of –C=O inorganic carbonate with symmetric vibrations of carboxyl group for sodium carboxymethyl cellulose [14]. Absorption band obtained in between 1015.47 cm^{-1} 1074.89 cm^{-1} indicate bending of –C=O inorganic carbonate and C-O carbohydrate for sodium carboxymethyl cellulose. Wave number at 1274.19 cm^{-1} indicates C-N amide stretching. Peaks at wave number 1232.80 cm^{-1} indicates C-O bond and Absorption spectra at 901.81 cm^{-1} bending of C=O for fumaric acid [15].

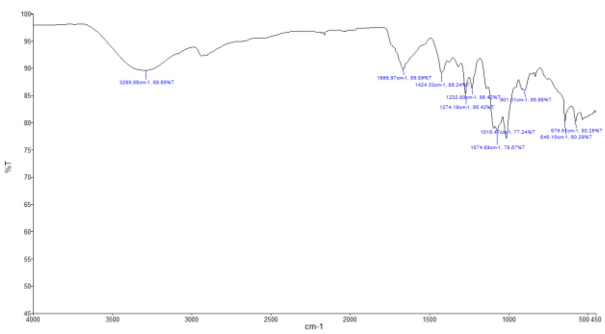

Figure 2. FTIR image of polymeric nanofibres.

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The morphology and size of the as-synthesized nanofibres were examined using SEM after the formation of polymeric electrospun nanofibres shown in **Figure 3.** The polymeric solution having concentration of 4g of PVA sample (PS4) showed formation of nanofibres. The SEM results of electrospun nanofibres were analyzed by the image J analysis. From this particular software it was observedthat the average size range of the nanofibre was apporoximately 200 nm- 700 nm

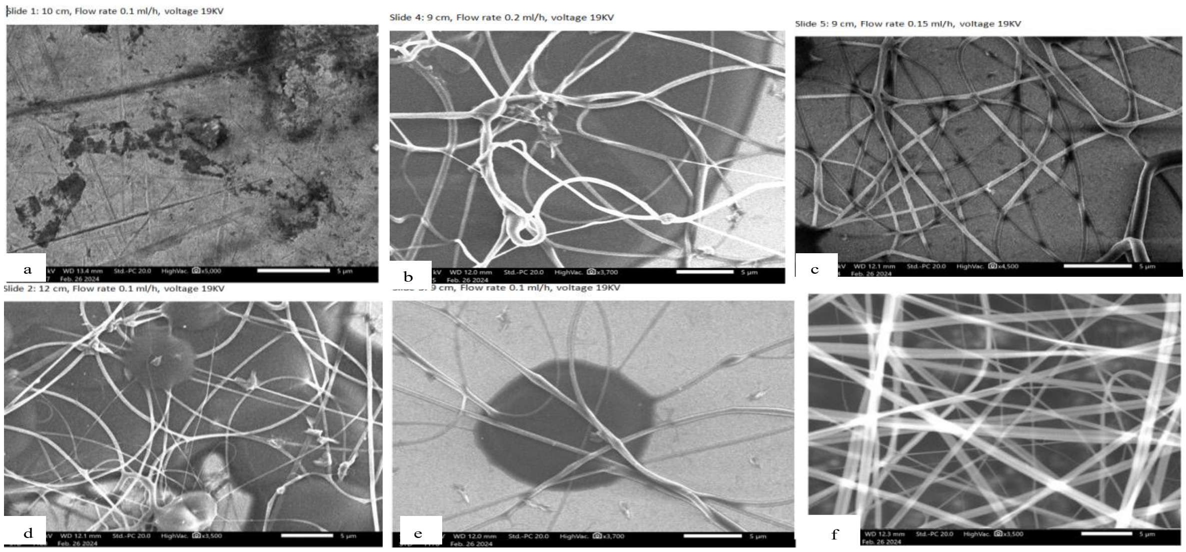

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Figure 3. SEM images of a) sample PSc, b) sample PSd, c) sample PSe, d) sample PSf, e) sample PSg, f) sample PSh.

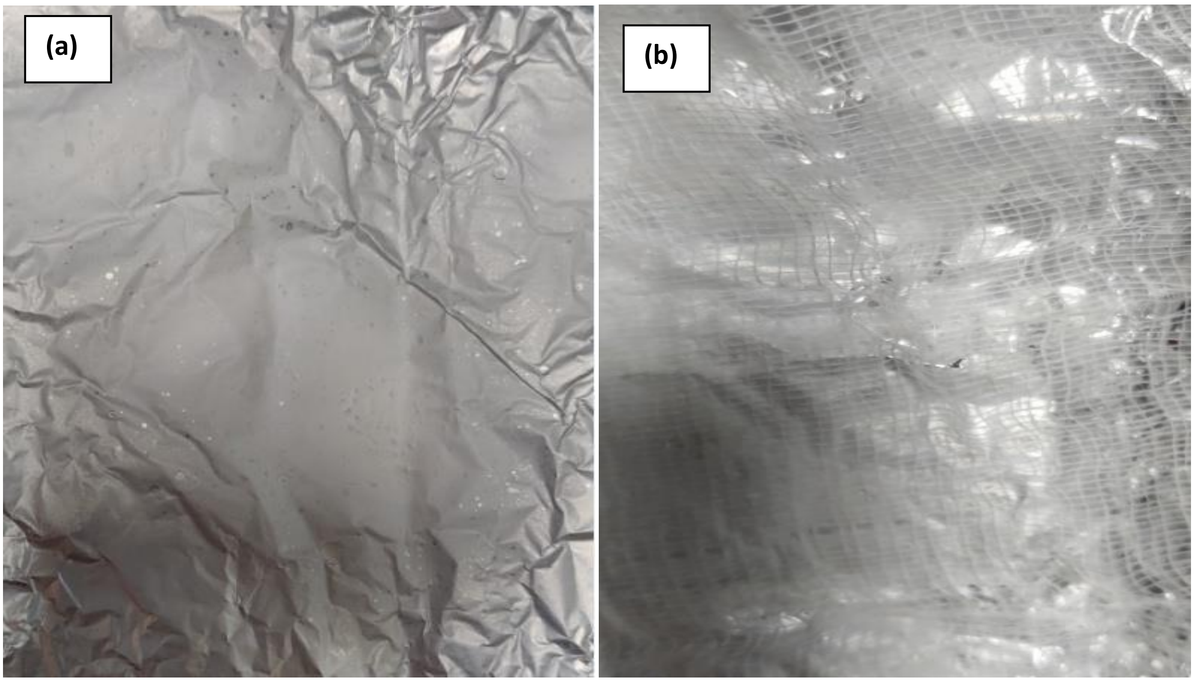

Figure 4. Nanofibres of sample PS4h on substrate a) aluminium foil and b) guaze

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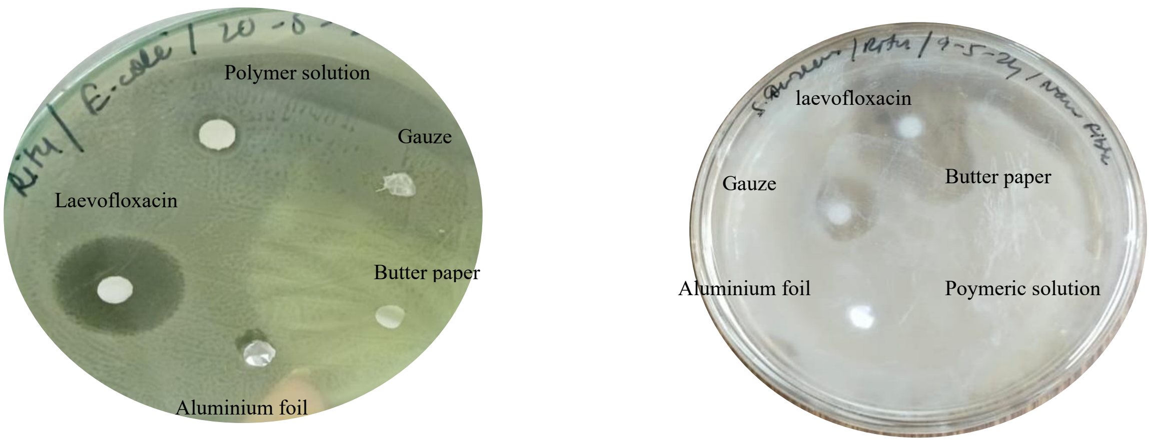
3.3 Antimicrobial studies discussion

The disk diffusion method was used to investigate the antibacterial activity of prepared samples and the nanofibres against *S. aureus* and *E.coli*. For polymeric gel, and the synthesized nanofibres the inhibition zone diameters for S. aureus were observed to be approximately 6 mm and 7 mm respectively. The petri dish with the respective inhibition zone is shown in **Figure 5.** The zone of inhibition percentage showed improved results for polymeric nanofibres as shown in **Table 3.** and **Table 4.**



a b

Figure 5. Disk diffusion test for sample the prepared nanofibres for a) *E. coli* and b) S. aureus.

Table 3 . Zone of inhibition of polmeric sample and the prepared nanofibres for S. aureus

Sample	Zone of inhibition (mm)	Diameter of Petri Dish (cm)	Percentage %(ZOI)	Efficiency
Laevofloxacin(control)	12	9.1	13.18	1
Polmeric solution	6	9.1	6.59	0.5
Polymeric nanoibers on aluminium foil	6	9.1	6.59	0.5
Polymeric nanoibres on butter paper	6	9.1	6.59	0.5
Polymeric nanofibre on gauze	7	9.1	7.69	0.58

Table 4. Zone of inhibition of polmeric sample and the prepared nanofibres for *E. coli.s*

Sample	Zone of inhibition (mm)	Diameter of Petri Dish (cm)	Percentage %(ZOI)	Efficiency
Laevofloxacin(control)	15	9.1	16.48	1

Polmeric solution	7	9.1	7.69	0.4
Polymeric nanofibres on gauze	9	9.1	9.89	0.6
Polymeric nanofibres on butter paper	5	9.1	5.49	0.3
Polymeric nanofibres on aluminium foil	5	9.1	5.49	0.3

4. Conclusion

In this present study, the polymeric nanofibres was formed by optimizing the concentration ratios of PVA, pectin, NaCMC and fumaric acid (crosslinker). Electrospun nanofibres synthesized in this study is in the nanorange approximately 200- 700nm (confirmed by SEM images). Mainly, the synthesized nanofibres exhibited significant antimicrobial activity which was performed by using the Disk Diffusion test against gram positive S. aureus and gram negative *E. coli* strains. The zone of inhibition was observed to be improved antimicrobial efficiency in presence of polymeric nanofibres on gauze for both S. aureus and *E. coli*. These findings of blended polymeric nanofibers provide a promising strategy and as preventive agents for antimicrobial applications.

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