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Formulation and Evaluation of Nano-Fiber Based Topical Patch of Linezolid

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ABSTRACT

In the present work polymeric nano-fiber patches were developed for the effective treatment of wound infection using Linezolid as the model drug. The nano-fibers were prepared by electrospinning technique by using Eudragit RL-100 as a polymer and nano-fibers were characterized based on fiber diameter, scanning electron microscopy, x-ray diffraction analysis, entrapment efficiency, drug release behavior and *in-vitro* antimicrobial test. The entrapment efficiency of the optimized formulation was 99.87%. The cumulative release of the nanofiber patch across the dialysis membrane was 94.62% at the end of 24hr. The *Ex-vivo* release of the nano-fiber patch across the goatskin was 81.44% at the end of 24hr. The nanofiber patch showed antibacterial activity in the *in-vitro* microbial study.

INTRODUCTION

Drug delivery refers to approaches, formulations, technologies, and systems for transporting a pharmaceutical compound in the body as needed to safely achieve its desired therapeutic effect.

Topical drug delivery systems involve the introduction of a drug to the surface of the body, from a formulation. Skin patches are an example of topical drug delivery systems. These systems are often very easy for patients to use, which makes them appealing. In all cases, the goal of a drug delivery system is to get the right dosage to the right place[1,2].

A recent approach in patch type of formulation is to deliver a drug through a nanofiber patch. They are prepared by the electrospinning technique. Electrospinning is considered as an electrostatically-driven process mostly conducted under controlled temperature and humidity conditions. Electrospinning is the process of using electrostatic forces to form a fine filament of the polymer solution. Fibers obtained from electrospinning have gained popularity in the field of drug delivery and are considered as ideal dressing materials for non-healing wounds since the method is versatile and can deliver various biological agents long-term to local tissues at the wound site [3,4].

Wound infections are the growth and spread of microbes, usually bacteria, within the skin or a break or wound in the skin. These infections trigger the body's immune system and cause inflammation and tissue damage within the skin or wound and slow the healing process. The skin is the body's first line of defense, the surface of which is protected by a thin, acid film produced by the sebaceous glands called the acid mantle. This acid mantle is a dynamic barrier that regulates the skin's pH and maintains microorganisms called the normal flora that helps prevent pathogens from entering the body. Skin wound infections can be bacterial, viral, fungal, or caused by parasites. Topical administration allows transport of drugs to the targeted site directly in a perfectly required ratio and dose, thus providing a better option to target drugs for wound healing activity[5,6]

Linezolid is an antibacterial drug, the first of the oxazolidinone class, used for the treatment of infections caused by multi-resistant bacteria like gram-positive and gram-negative bacteria.

There is no topical formulation available in the market so the nano-fiber patch is beneficial to avoid the side effects associated with the oral dosage form such as tongue discoloration, low blood platelet count, low white blood cell count, damage to the nerve of the eye and many more.

MATERIALS AND METHODS

MATERIALS

Linezolid was procured as a gift sample from USV Pvt. Ltd, Mumbai. Methanol was purchased from Molychem, Mumbai. All other chemicals and solvents used during the experiments were of analytical grade and procured from SD Fine Chemicals, Mumbai, India.

Fabrication of the electrospun nanofibers patch

Eudragit RL-100 nano-fibers were produced by an electrospinning technique. Eudragit RL-100 (25% w/v) solution was prepared in methanol (6.66ml) and N, N-Dimethylacetamide (3.34ml). Linezolid (1% w/v) was added to the homogeneous polymeric solution with constant stirring. The final solution was subjected to electrospinning using a rotating cylindrical drum as a collector placed at a distance of 20 cm from the needle under an applied voltage of 25 KV and a flow rate of 0.5ml/h. The nano-fiber sheets were cut into of $1cm^2$ patches and used for further characterization[7].

Optimization of formulation using 2^3 factorial design

2 level 3 factor was designed to optimize the formulation using design expert software version 11. Polymer concentration (X1), voltage (X2), electrode distance (X3) were selected as independent variables and entrapment efficiency of the formulation was selected as dependent variable[7]. The different levels for the independent variables for the 2^3 factorial design are given in Table **1**, Table **2** shows 2^3 factorial design for optimization of the developed formulation.

Table No. 1: Factors and factor levels investigated in 2^3 factorial design

FACTORS	LEVEL	
	-1	+1
(X1), POLYMER CONCENTRATION (% w/v)	20	25
(X2), VOLTAGE (Kv)	20	25
(X3), ELECTRODE DISTANCE (cm)	15	20

Table No. 2: 2^3 factorial design for optimization of developed formulation:

Experiment Number	(X1)	(X2)	(X3)
1	20	20	15
2	25	20	15
3	20	25	15
4	25	25	15
5	20	20	20
6	25	20	20
7	20	25	20
8	25	25	20

Characterization of nano-fiber patches

Nano-fiber patches were characterized based on various parameters such as surface morphology, entrapment efficiency, x-ray diffraction analysis, *in-vitro* drug release, *ex-vivo* drug release and *in-vitro* antimicrobial study.

Entrapment efficiency

Electrospun nanofibers are expected to possess high drug entrapment efficiency (EE) due to the presence of high surface area. EE describes the efficiency of the preparation technique to incorporate the drug into carrier system [8]. The drug-loaded nano-fiber patch was weighed and dissolved in phosphate buffer pH 7.4. The solution was assayed in triplicate for entrapped drug concentration by UV spectrophotometer at 257nm.

The percentage of drug EE was calculated as follows:

$$EE\ \% = \frac{\text{Entrapped drug}}{\text{The total amount of drug}} \times 100$$

Surface morphology and fiber diameter

Surface characteristics such as uniformity in diameter, smoothness of surface are important parameters to evaluate the quality of the developed nano-fibers sheet. Surface morphology and fiber diameter of the optimized nanofibers was analyzed by using a scanning electron microscope (SEM) [8].

***In-vitro* drug release**

The *in-vitro* diffusion study was carried out using Franz diffusion cell. The receptor compartment was filled with 20 ml of phosphate buffer pH 7.4 and maintained at 37^0C±0.5^0C. An optimized nanofiber sample was kept in the donor compartment over a dialysis membrane. The aliquot of 1ml was taken in a suitable interval of time and replaced with fresh buffer solution maintained at the same temperature. The sample was analyzed using a UV spectrophotometer in triplicate at 257nm [9].

X-ray diffraction (XRD)

X-ray diffraction (XRD) analysis was performed to illustrate the crystalline structures of Linezolid powder and drug present in the optimized nano-fiber patch using a Philips X'Pert-Pro MPD with a 3 KW ceramic tube as the X-ray source (Cu-K_) and an X'Celerator detector. Cu-K radiation was used with a diffraction angle range of 20–50^0 at 45 kV and 40 mA at a scanning rate of 10^0/min [9].

***Ex-vivo* drug release**

In the present study, hairless goatskin was used. Hairs were removed with an animal hair clippers. The skin was washed with phosphate buffer pH 7.4 and used immediately. *Ex-vivo* release studies on the optimized nano-fiber patch were performed using Franz-diffusion cell. The goatskin was mounted between the donor and receptor compartment. The same procedure was followed as discussed under *in-vitro* drug release study [7].

Microbial study

The microbial study of the formulation was carried out by zone inhibition method by using sterilized agar medium on three different organisms namely *Staphylococcus aureus*, *Klebsiella aerogenes*, *Escherichia coli*[9]. The bacterial suspension was streaked aseptically

over the plate containing agar medium and was spread uniformly. A blank polymeric nano-fiber and drug solution were used as a positive and negative control respectively. Blank nano-fibers and drug-loaded optimized nano-fibers were gently placed at the center of the solidified agar gel in different Petri dishes. Aqueous drug solution (1%) was poured in the well-formed using 12 mm borer. The plates were incubated at 37°C for 24 h. The bacterial growth was compared with the controls [8,9]. The experiment was carried out in triplicate.

RESULTS AND DISCUSSION:

Optimization of formulation using 2^3 factorial design

Excipient selection was made based on information available in the literature, physicochemical properties of drug and compatibility studies. Nanofiber patch was prepared by the electrospinning method. Table 3 shows the EE of experimental batches.

Table No. 3: 2^3 factorial design showing results for the response of entrapment efficiency

Experiment number	(X1)	(X2)	(X3)	Entrapment efficiency (%)
1	20	20	15	70.6
2	25	20	15	74.5
3	20	25	15	94.68
4	25	25	15	85.25
5	20	20	20	62.70
6	25	20	20	76.72
7	10	25	20	83.38
8	20	25	20	99.87

Selection of the optimized batch

A 2^3 full factorial design was employed to evaluate the individual and combined effects of three formulation variables on nano-fiber patch performance and characteristics. In this design, three factors were evaluated, each at two levels and experimental trials was performed at all eight possible combinations. The effects of selected independent variables namely polymer concentration (X1), voltage (X2), electrode distance (X3) was observed on EEas a dependent variable.

The optimized batch was selected based on the highest EE in Table 3. The high value (0.94) of the correlation coefficient (R2) indicates a good fit. The model *F* value of (13.57) implies that the model is significant. The values of Prob was <0.05.

The software provides 8 new batches (Table 4), batch with highest entrapment value was selected and performed experimentally. Contour plots (Fig.1) were further applied to explore the effects of the independent factors on the responses.

Table No. 4: Software provided batches

Number	Polymer concentration	Voltage	Electrode distance	Entrapment efficiency	Desirability	
1	*25.000*	*25.000*	*20.000*	98.127	1.000	**Selected**
2	*20.425*	*23.138*	*15.394*	84.280	1.000	
3	*20.000*	*25.000*	*20.000*	82.873	1.000	
4	*22.884*	*22.431*	*17.479*	81.289	1.000	
5	*25.000*	*20.000*	*20.000*	78.463	1.000	
6	*21.221*	*22.664*	*19.079*	78.368	1.000	
7	*21.853*	*21.431*	*19.978*	74.503	1.000	
8	*25.000*	*20.000*	*15.000*	70.043	1.000	

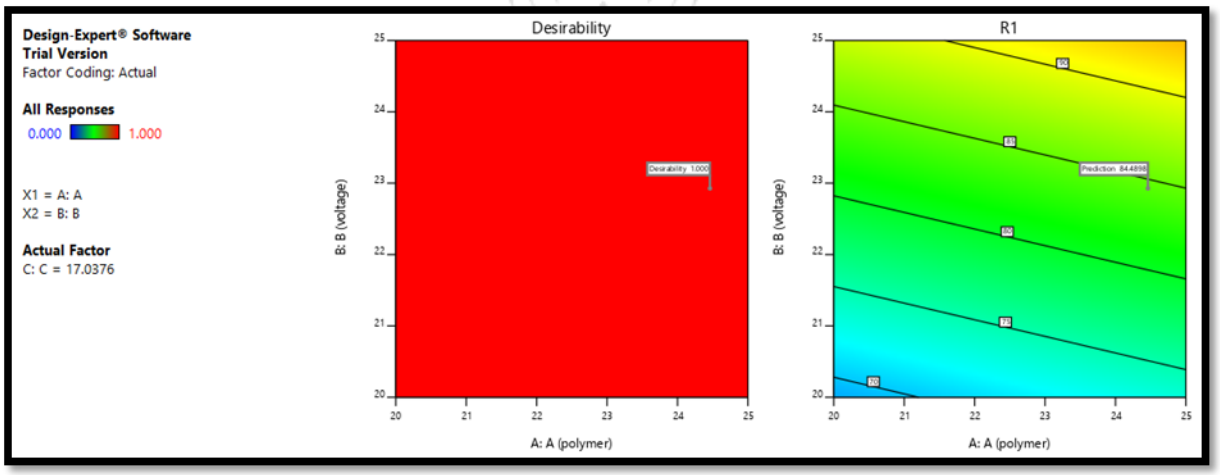

Figure No. 1: Contour Plot

Entrapment efficiency

The entrapment efficiency of the optimized batch was found to be 99% which is nearly the same as that of Entrapment efficiency of software predicted batch (Table 4) indicating the accuracy of the method adopted.

Surface morphology

The diameter and shape of the nano-fibers were determined using SEM. The diameter of optimized nano-fibers was found to be in the range of 200–600 nm. Fig. 2shows the SEM images of optimized formulation which confirms the presence of smooth round-shaped fibers.

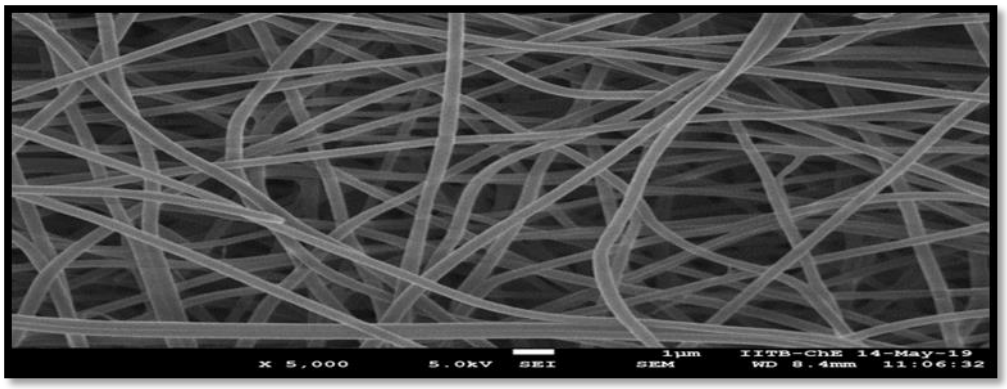

Figure No. 2: SEM image of Linezolid nanofiber

***In-vitro* drug release**

In-vitro release studies showed that the developed nano-fibers are capable of sustained-release drug delivery up to 24 hr. Fig. 3 shows a release of 94.6% at the end of 24hrs.

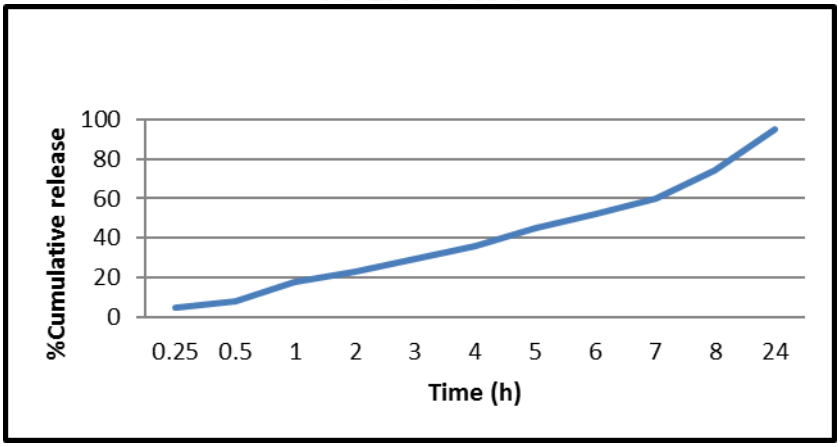

Figure No. 3: *In-vitro* drug release

XRD analysis

XRD patterns with distinctive crystalline peaks of Linezolid are shown in Fig. 4. As seen in the figure the XRD spectrum of Linezolid displayed sharp and intense peaks of crystallinity, which suggested a highly crystalline nature.

The XRD spectra of the nanofiber patch containing Linezolid showed a reduction of peak intensity, as compared to the Linezolid, which indicated decreased crystallinity or conversion into an amorphous phase of the drug.

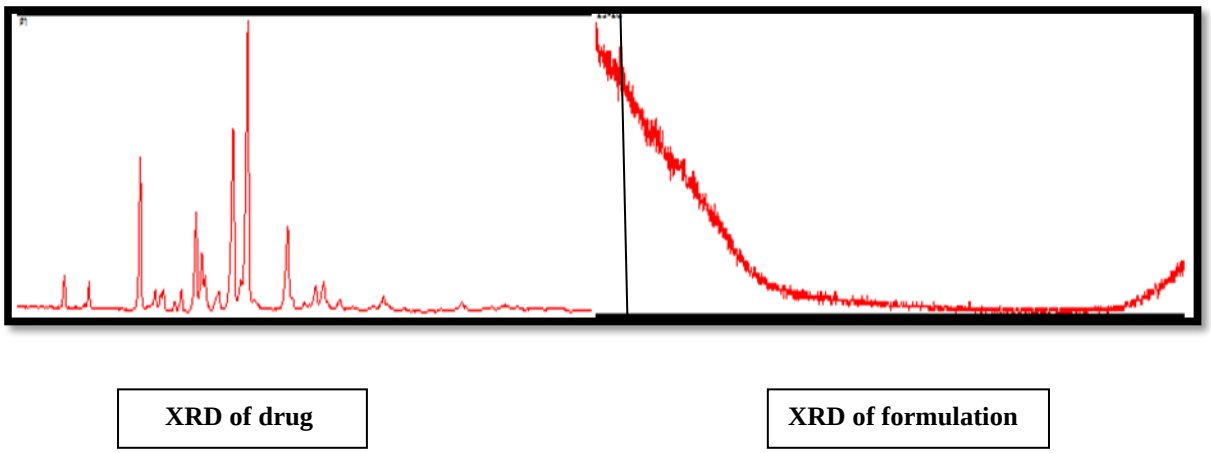

Figure No. 4: XRD of drug

***Ex-vivo* drug release**

Ex-vivo release studies showed that the developed nano-fibers are capable of sustained-release drug delivery up to 24 h (Figure. **5**) shows a release of 81.44% at the end of 24hrs. The decrease in the release compared to *in-vivo* study can be due to difficulty that drug might have faced while penetrating to thick skin of a goat.

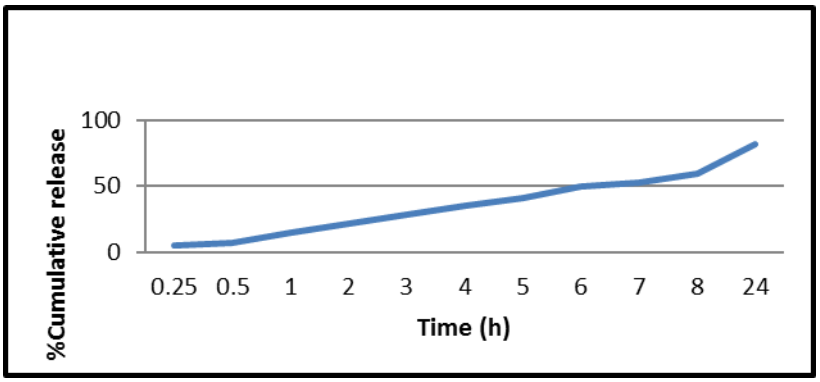

Figure No. 5: *Ex-vivo* release study

Microbial study

The microbial study on the organism shows a zone of inhibition as mentioned in Table 5. The blank nano-fiber patch did not show any zone of inhibition. Fig.6 shows the images of the

zone of inhibition. Increased zone of inhibition with drug solution might be because of ease of release of drugs through solution compared to nano-fibers.

Table No. 5: Zone of inhibition

Microorganisms	Zone of inhibition (mm)	
	Formulation	Drug solution
Staphylococcus aureus	10	20
Klebsiella aerogenes	14	17
Escherichia coli	13	18

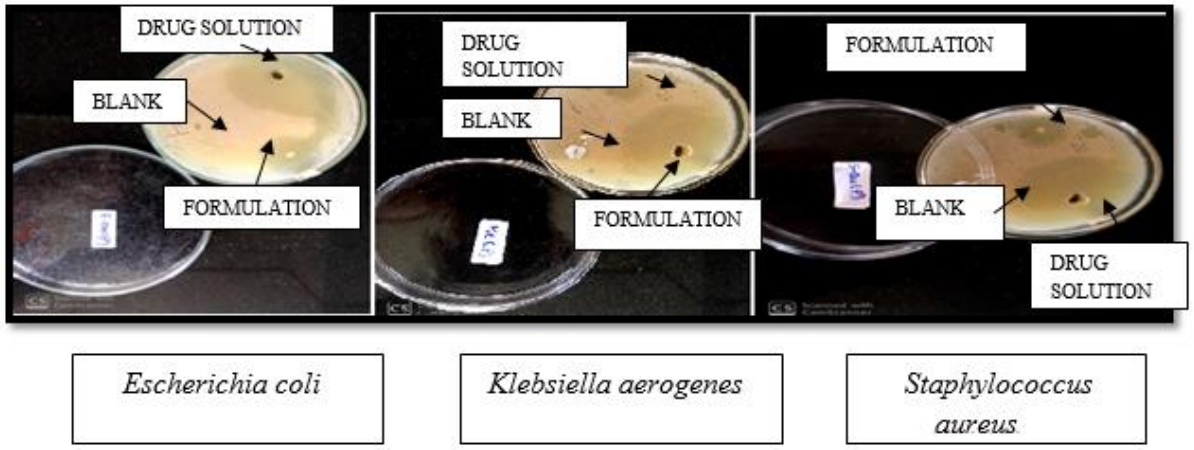

Figure No. 6: Microbial study

CONCLUSION

In the present work, biodegradable polymeric nano-fibers were successfully developed by using the electrospinning technique. The microscopic study indicated that the nano-fibers were uniform in diameter with a smooth surface. We achieved very high (99%) entrapment efficiency. Hence, the nano-fibers patch can be effectively utilized for topical drug delivery in various Skin conditions.

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