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Formulation development and evaluation of Loratadine (LOR) loaded electrospun nanofiber film for buccal delivery

Prashant PATIL [1] *, Kisan JADHAV [1], Vilasrao KADAM [1]

[1] Department of Pharmacy, Bharati Vidyapeeth College of Pharmacy, Sector 8, CBD Belapur, Navi Mumbai, 400614 India.

* Corresponding Author. E-mail: prashupatil2297@gmail.com (P.P.); Contact: +91-721-893 46 98.

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ABSTRACT: Loratadine, a BCS class II drug, is used as an antihistaminic in the treatment of allergies. It has a poor water solubility and a low bioavailability as a result of significant first-pass hepatic metabolism after oral administration. Nanofibers, which are strings of polymeric fibres with diameters smaller than 1 micron, are one such strategy. They are superior to other varieties of the substance in a number of ways, including surface area to volume ratio, elasticity, and mechanical properties. Typically, electrospinning is used to make nanofibers. These properties could be utilized for overcoming the problems associated with the loratadine. Thus, the aim of this study was to formulate loratadine nanofiber film for buccal application. The formulation of loratadine nanofiber films involved electrospinning a solution containing a definite ratio of Polyvinylpyrrolidone (PVP) and Ethyl Cellulose (EC). The optimized film was characterized by SEM, DSC and FTIR. Mucoadhesion strength, ex-vivo permeation tests, and in-vitro drug release were all assessed for the end formulation. The results showed that the drug was evenly dispersed and enclosed within the nanofiber matrix. The film made of nanofibers showed consistent morphology and enhanced drug penetration and release. The developed Loratadine-loaded electrospun nanofiber film may therefore be used as an improvement over conventional dosage forms by increasing patient compliance.

KEYWORDS: Loratadine; nanofiber; electrospinning; buccal delivery; PVP; ethyl cellulose.

1. INTRODUCTION

Product development is increasingly using technology to reduce the size of pharmaceutical particles to the sub-micrometer range. Novel drug delivery systems (NDDS) are being developed with the intention of reducing drug loss or degradation, avoiding negative side effects, increasing drug bioavailability, and promoting and encouraging the accumulation and release of therapeutically effective amounts of medication at the site of action[1]. Nanofibers are used to create novel drug delivery systems, such as nanofiber films, multi-layered nanofiber-loaded mashes, and surface-applied nanofibers, which are fibres with a size range of 50-1000 nm that possess enormous surface areas, high levels of porosity, small pore sizes, and low densities. Electrospinning combines electrostatic repulsion forces with medicinal polymers to create a revolutionary medication delivery method. Nanofibers have been produced using a variety of techniques, including molecular assembly, thermally induced phase separation, electrospinning, etc. [2] Of all the practises currently in use, one of the most well-known and commonly used techniques is electrospinning. An electric field generator, a pump, a counter electrode or grounded target, and a syringe with a nozzle make up the typical electrospinning setup. The electrostatics theory, which forms the basis of the electrospinning process, states that the electrostatic repulsion forces generated by a powerful electrical field are used to create nanofibers.[3].

LOR, a BCS class II medication, is a peripheral H1-receptor antagonist that is nonclassical selective and has structural resemblances to azatadine and cycloheptadiene. LOR's primary pharmaceutical action is to block peripheral H1-receptor sites. Due to its poor water solubility and low bioavailability as a result of its high hepatic first-pass metabolism after oral administration, it must be dosed once or twice daily. Only liquid formulations of LOR, such as syrup or suspension (5 mg/5 mL), are accessible for children under six years old

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in Europe, whereas chewable tablets have just been released in the USA. Solid dose forms, such as tablets and Oro dispersible tablets (ODT), are freely available on the market (Claritin chewable 5 mg). Redi Tabs, which are orodipersible tablets with 5 mg or 10 mg of LOR, are only approved for use in children older than six years old [4].

Buccal mucosa has a higher surface area than sublingual mucosa, provides an alternative platform for drugs with severe first-pass metabolism, prevents stomach and other fluids from degrading the drug, and increases bioavailability[5,6].

Nanofibers are well suited for use in drug delivery via buccal absorption because of their large surface to volume ratio, tiny diameter, adaptable pore topologies, and high flexibility. With the rising demand for them, buccal nanofiber film can be a useful alternative to oral dispersible formulations, especially if it allows for dose management by splitting the film into smaller pieces[7]. Children under 30 kg in weight should take 5 mg of LOR once day. The goal was to include at least 5 mg of LOR in buccal film of this size because the maximum size of the buccal film recommended for small children is 4 cm^2.[4]

In our study, it was aimed to develop nanofiber film formulation of LOR in order to improve drug solubility and, as a result, improve dissolution and transbuccal permeation profile by encorporating the hydrophobic polymer for a slow, consistent release over a longer time, as well as improved mucoadhesive strength, flexibility, and application simplicity. The optimized film was characterized by SEM, DSC and FTIR. This formulation was also evaluated ex vivo permeation test, mucoadhesion strength, and in vitro release trials.

2. RESULTS AND DISCUSSION

The goal of this research was to create nanofiber film formulations because nanofibers having large surface to volume ratio, tiny diameter, adaptable pore topologies, and high flexibility that would increase the loratadine's solubility as a result, improve dissolution and transbuccal permeation.

For effective development of formulation of the nanofiber film of LOR, one of the crucial elements in the production of smooth and beadless electrospun nanofiber is the solvent choice. Before choosing the solvent, it is typically important to keep in mind two aspects. Initially, the polymers used in the electrospinning process are entirely soluble in the selected solvents. The solvent ought to have a reasonable boiling point, second. The volatility of a solvent can be estimated from its boiling point. In general, volatile solvents are preferred because of their rapid evaporation rates, which facilitate the solvent's simple evaporation from nanofibers as they travel from the needle tip to the collection. However, highly volatile solvents are typically avoided because of the drying effect they have on the jet at the needle tip due to their low boiling temperatures and rapid evaporation rates. Different polymer solvent combinations were screened for parameters such as solubility, electro-spinnability, fiber film formation, volatility of solvents. Various combinations of solvents were used for getting optimum solubility of polymer as well drug into the solvent system (ethanol, distilled water, and DMF), viscosity and consequently the texture, peeling of drug loaded film in Table 1.

2.1 Authenticiation of drug

2.1.1 Ultraviolet (UV)-visible spectrophotometric characterization

By UV visible spectrophotometric characterisation, λmax of LOR was determined. It was found to be 248 nm which was confirmed with the reported λmax value.[4] The spectra of LOR in methanol are shown in Fig.1.

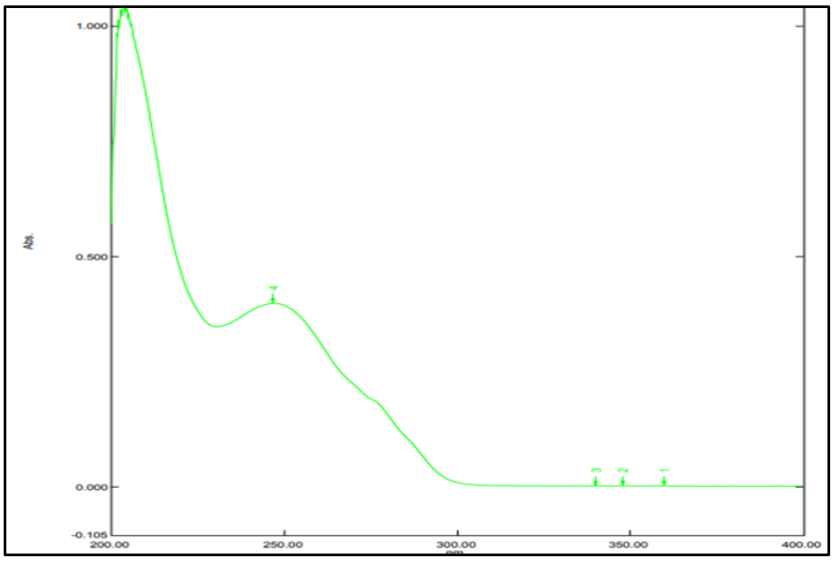


Figure 1. UV Spectroscopy of LOR

Table 1. Results for trails involving different polymer-solvent systems

Name of polymer used	Solvent used	Interpretation
PVP-K30	Ethanol	Soluble but film formed was brittle in nature.
PVP-K60		Soluble but film formed was brittle in nature.
PVP-K90		Thick fibres visible on the tip of needle.
PVP-K90	Distilled Water	Soluble, but during electrospinning thick fibres were seen on the tip of the needle.
PVA		Soluble and suitable for electrospinning.
PVA	Ethanol: Distilled water(50:50)	Slightly soluble, Opaque solution was formed.
Chitosan	Acetic Acid (1% W/V)	Highly viscous solution was formed as result it couldn't be electrospinned.
Eudragit-L100	Ethanol: DMF (80:20)	Drops were formed at the tip of needle which fell in the midway during the electrospinning process.
HPMC K100	Ethanol	Solution formed was not clear as polymer was slightly soluble in the solvent.
Ethyl Cellulose		Polymer was freely soluble in solvent but electrospinning of this solution didn't result in formation of film.

2.2. Formulation and development of nanofıber film for loratadine

2.2.1 Prelimanary batches

Different ratios of polymer: solvent concentrations of PVP and ethyl cellulose were taken to define the range of concentration that can be taken for optimization batches to determine highest drug loading.As seen in the Table 2, the nanofibers produced with PVP and ethyl cellulose in the ratio of 2:3 in the total polymer concentration of 12.5% respectively resulted in the highest drug release of 87.12% and entrapment efficiency of 92.24%. In order to investigate the impact of these variables on drug release and entrapment in nanofiber matrix, optimization was further conducted based on the findings of preliminary trial batches at total polymer concentrations of 10 to 15% and flow rates of 1.5 to 2.5 mL/hr.

Table 2. % EE, % drug release and drug content for different ratios of PVP:

Ratio of PVP:EC	Drug Release(%)	Entrapment Efficiency(%)	Drug Content(mg)
1:0	68.07	75.87	3±0.5
4:1	72.87	79.19	4±0.5
3:2	81.35	85.55	4±0.5
2:3	87.12	92.34	4±0.5
1:1	84.44	88.23	4±0.5
1:2	76.59	82.13	4±0.5
0:1	No film was formed	No film was formed	-

2.2.2 Experimental design

The combination of PVP and Ethyl cellulose was found to be effective for getting desired properties thereafter a two level two factor a single centric central composite design was used for the systematic analysis of the combined effects of independent variables [Total polymer concentration, Flow rate] on the dependent variables [% Drug release and % Entrapment efficiency] with the help of Design Expert® software (Stat-Ease, Minneapolis, MN). According to the design, experiments were conducted, and results were obtained. Analysis of Variance (ANOVA) was conducted on the response surfaces of variables within the experimental area using Design Expert® software. This central composite design is suitable for constructing models of second-order polynomials and exploring quadratic response surfaces. Multiple linear regression analysis was used to generate mathematical relationships among the variables mentioned as shown in Table 3. As shown in these equations, total polymer concentration (A), flow rate (B) and their interaction on percent drug release (R1) and entrapment efficiency (R2) can be derived quantitatively. The effects of these factors on the responses R1 and R2 are indicated by the values of the coefficients of A and B.

Table 3. Regression equations for the responses - % Drug release and % Entrapment efficiency

Response	Code	Equation
% Drug release	R1	$87.7578 + 0.838333\ A - 1.33333\ B + 0.1725\ AB - 5.79167\ A^2 - 0.576667\ B^2$
% Entrapment efficiency	R2	$89.21 + 1.42167\ A - 3.385\ B - 1.4325\ AB - 4.125\ A^2 + 0.425\ B^2$

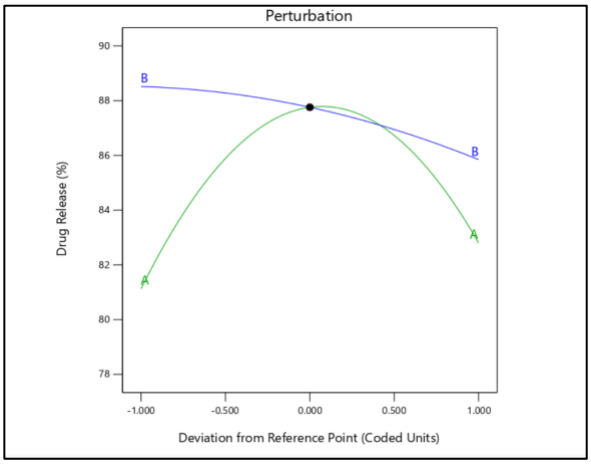
As shown in table 4. the statistical model created for percent drug release (R1) was found to be significant with an F-value of 105.21 and an R2 value of 0.9849. It can be inferred that as the quantity of total polymer concentration rises, nanofibers with more drug release are produced, followed by B, and that the independent factors A, B, and A^2 all have substantial impacts on the drug release, with A having the biggest and most direct impact on R1. Figure 2A clearly shows that A has a major and a direct effect on R1 followed factor B which has a little effect on R1. The relationship between the dependent and independent variables was further elucidated using the perturbation (Figure 2A) and 3D response surface plots (Figure 2B).

Additionally, as shown in Table 5. the regression equation for R2 showed a good correlation coefficient (0.9666) and a Model F-value of 17.36, which implies significance. The quadratic term of important model variables in this instance is B, A^2. Figure 3A illustrates the major indivisual effects of A and B on drug release

and it is discovered that factor B has the greatest and most exponential effect on R2, while factor A has a smaller effect than B on the drug release. Figure 3A shows that factor B has maximum and an exponential effect on R2, whereas factors A also influences the drug release but its less than B. The interactive effects of independent factors on the response R2 are shown in Figure 3B. 3D response surfaces plot of the response R2, where one variable was held fixed while the other fluctuated within a certain range.

Table 4. The ANOVA results of the Quadratic model for the response % Drug Release (R1)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	82.75	5	16.55	105.21	0.0014	significant
A-Total Polymer Concentration	4.22	1	4.22	26.80	0.0140	
B-Flow Rate	10.67	1	10.67	67.80	0.0037	
AB	0.1190	1	0.1190	0.7566	0.4484	
A^2	67.09	1	67.09	426.44	0.0002	
B^2	0.6651	1	0.6651	4.23	0.1320	
Residual	0.4720	3	0.1573			
Cor Total	83.23	8				



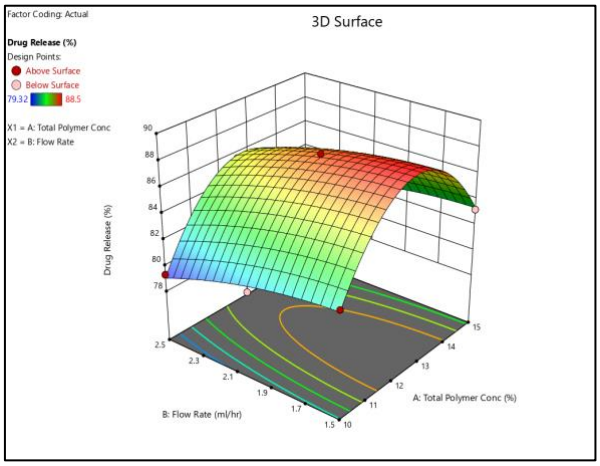
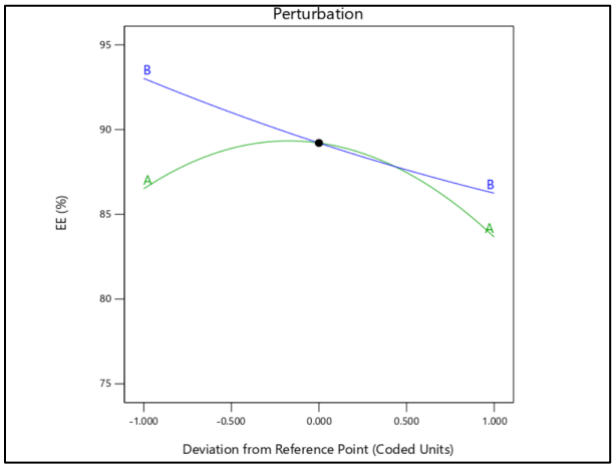


Figure 2. (A) The perturbation plot for R1 **2. (B)** The 3D response surface plot for R1

Table 5. The ANOVA results of the Quadratic model for the response % Entrapment efficiency (R2)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	123.48	5	24.70	17.36	0.0201	Significant
A-Total Polymer Conc	12.13	1	12.13	8.53	0.0615	
B-Flow Rate	68.75	1	68.75	48.34	0.0061	
AB	8.21	1	8.21	5.77	0.0957	
A^2	34.03	1	34.03	23.93	0.0163	

B^2	0.3613	1	0.3613	0.2540	0.6490
Residual	4.27	3	1.42		
Cor Total	127.74	8			



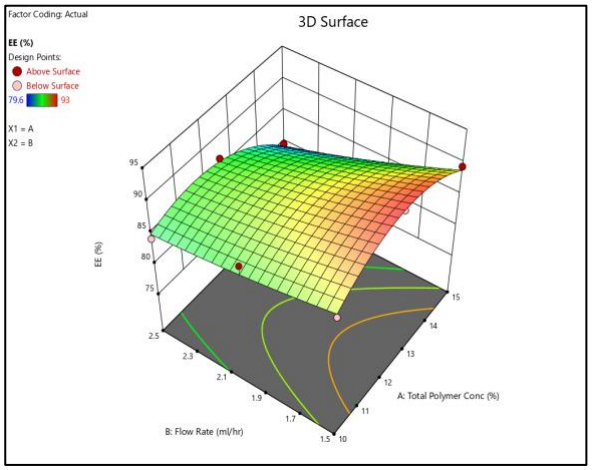


Figure 3. (A) The perturbation plot for R2 **3. (B)** The 3D response surface plot for R2

Using Design Expert® software, the desirability function was then used to carry out numerical optimization. In order to achieve the maximum possible % drug release and % entrapment efficiency in LOR-loaded nanofiber film, total polymer concentration and flow rate had to be within the study range. There were discovered to be 61 distinct solutions, each of which held a different value for each independent variable. On the basis of the overlay plot (Figure 4), the solution with a desirability value of 1 was chosen as the optimized processing condition. The final batch was obtained with total polymer concentration of 12.4947 and Flow rate of 1.62357. This predicted the formulation of a batch of LOR- loaded nanofiber film the % Drug release of 88.4327 % and the % entrapment efficiency to be 92.0018%.

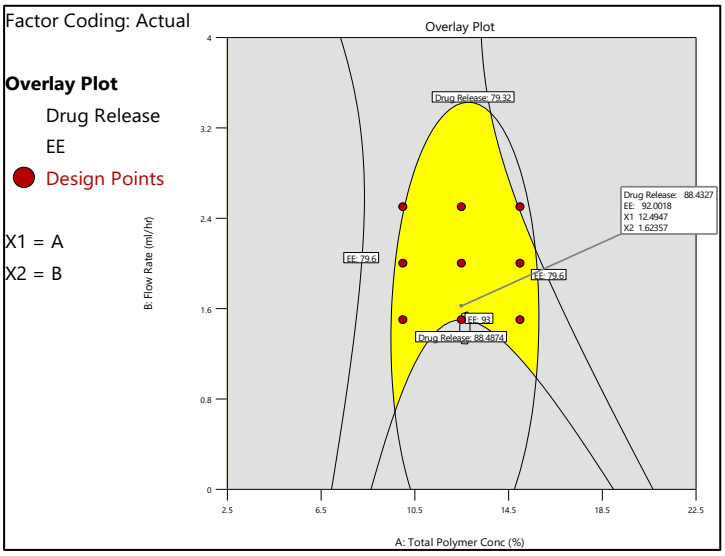


Figure 4. Overlay plot for final predicted batch

The LOR- loaded nanofiber film formulations using the desirability function to optimise the formulation, the combination showing desired responses, comprising of a optimum amount of excipients and desirability as 1 was finalized. The final optimized formulation consisted of total polymer concentration of 12.4947 and Flow rate of 1.62357. Optimized formulation was then scaled up and used for further evaluations.

2.3. % Entrapment efficiency (% EE)

By using a UV spectroscopic technique, the final optimized batch of LOR-loaded nanofiber scaffold's % entrapment effectiveness was investigated. The %EE of the LOR-loaded nanofiber scaffold was discovered to be 93.3561%, suggesting that the drug has been extensively trapped inside the fibers, as also indicated by the SEM studies.

2.4. Scanning Electron Microscopy (SEM)

The LOR powder had plate-shaped crystals with rough sides and an amorphous crystalline structure. While LOR-loaded nanofiber formulations show uniform fibers without drug crystals in SEM photos. The SEM images showed the creation of uniform, homogeneous fibers with random orientations that ranged in size from 268 to 434 nm (Figure 5). Since no drug crystals or aggregates were visible in the images, it is assumed that the drug was molecularly distributed and encapsulated within the electrospun fibers. There were no crystallized drugs found, indicating amorphous forms. Other prepared formulations reported have also yielded in similar conclusions [8],[9].

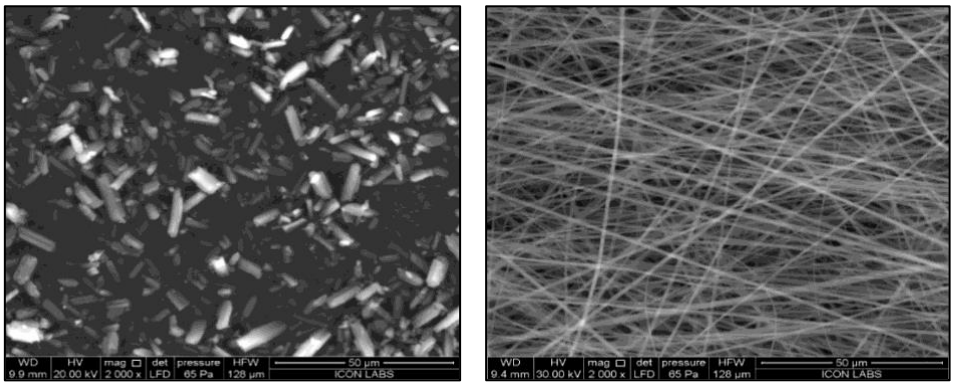


Figure 5.(A) SEM images of Pure LOR (2000x) **5.(B)** LOR loaded nanofiber film (2000x)

2.5. Thickness

Thickness of nanofiber film was measured using Mitutoyo Digital Vernier Calliper. The optimized batch showed the thickness of 105± 0.03 µm. The small standard deviation indicated the uniformity in the thickness of nanofiber film. The electrospun films in this study were found to be thicker than those previously described by researchers[10].

2.6. Folding endurance

By manually folding the film until it broke at a spot, folding endurance was measured. In spite of folding over 240 times, there were no cracks in the films. Hence it was taken as the end point. It was found that the folding endurance fell between 242 and 245. It was discovered that the values were optimum for revealing excellent film properties. The electrospun films in this study had greater folding endurance than those described by other researchers[11].

2.7. Weight variation

The average weight for the films cut into 2x2 cm^2 from different areas of the nanofiber scaffolds was found to be 25±0.7mg indicating the uniformity in the weight of the films.

2.8. FTIR analysis

To evaluate how the polymers interacted physically and chemically, FTIR spectroscopy was employed. All of the distinctive bands were retained in the spectra of the LOR-loaded nanofiber scaffold. (Figure 6) The finished LOR-loaded nanofiber formulation, Ethyl cellulose, PVP, and the overlay plot of the pure drug do not exhibit any significant interaction. Additionally, there was no significant shifting of the bands that were already present or the emergence of new bands, indicating compatibility of all nanofiber scaffold components. This is because there was no chemical interaction that would have been expected to affect the drug by allegedly degrading or altering its structural properties. The absence of the extremely intense drug peak in the film

formulation indicates that there has been proper encapsulation of the drug and that there has been no drug precipitation in the final formulation.

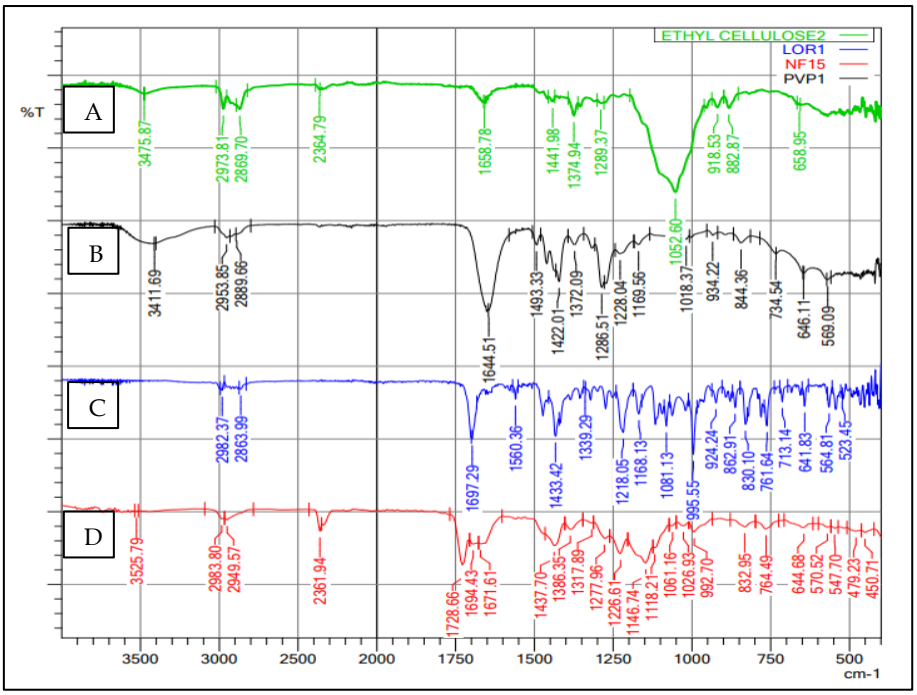


Figure 6. Overlay plot of FTIR spectrums of Ethyl cellulose(A), PVP(B), LOR (C) and LOR loaded nanofiber film(D)

2.9. DSC analysis

DSC thermogram depicted in Figure 6B with a sharp endothermic peak at 133.2°C attributed to drug melting. PVP thermogram shows a broad endotherm peak at 173.2°C. (Figure 7C). The ethyl cellulose thermogram shows two small peaks at 38.7.3°C and 78.7°C. The peak showing the melting of LOR at 133.4°C has disappeared in PVP-Ethyl cellulose nanofibers containing LOR (Figure 7A), indicating a loss of the crystal structure of LOR and its conversion to the amorphous form during the electrospinning process. (Figure 7D).

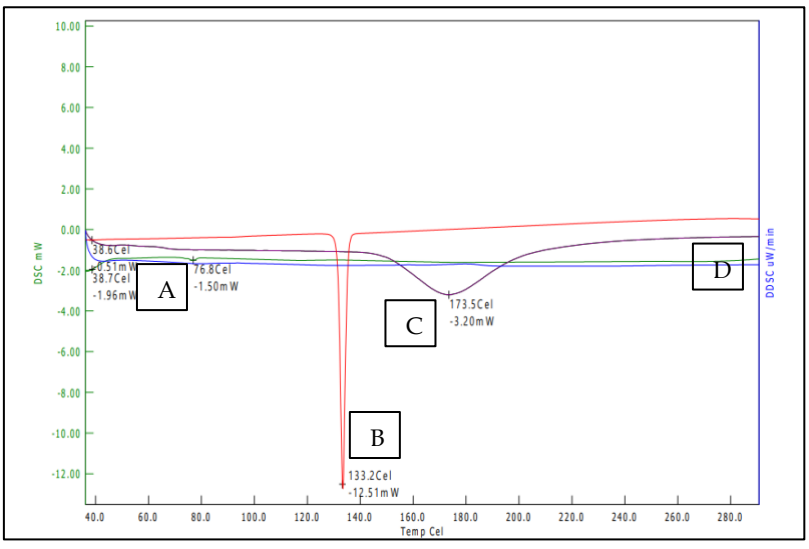


Figure 7. DSC overlay of LOR (A), PVP (B), Ethyl cellulose (C), LOR loaded nanofiber film (D)

2.10. *In vitro* drug release

In-vitro drug release of optimized LOR loaded nanofiber film formulation was compared with physical mixture and results were shown in Figure 8, Dissolution studies were performed for the LOR loaded film formulations in 6.8 pH phosphate buffer. As compare to drug release of Physical mixture (40.6879 %) and release of LOR loaded nanofiber film was more than 87.8904% at the end 8 hrs of dissolution studies. In case of LOR loaded nanofiber film the drug showed a initial slow release in first two hrs that is 26.1359% followed by sustained release of the remnant dose of drug (87.8904%) at the end of 8 hrs.

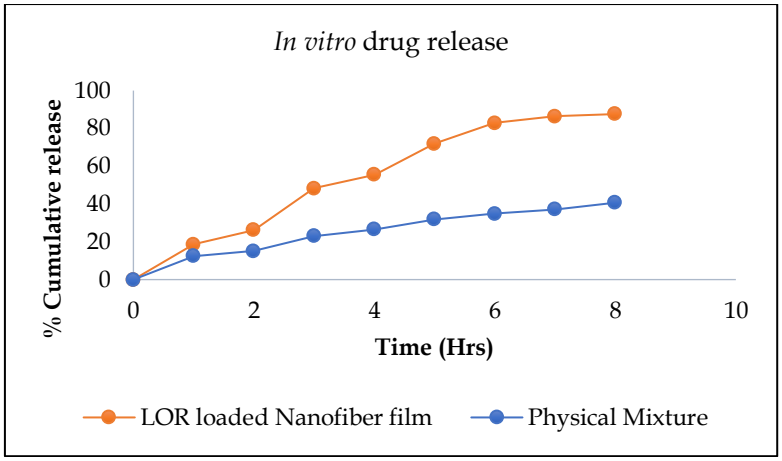


Figure 8. Comparative i*n vitro* drug release of LOR loaded nanofiber film for buccal application vs LOR physical mixture in pH buffer 6.8

This is due to the drug being encapsulated within the matrix of nanofiber which slowly erodes to release the drug in an uniform and sustained manner. The slow release can also be attributed to presence of ethyl cellulose in the matrix of nanofibers. Physical mixture shows release of only about 40.6879 % at the end of 8 hrs which can be attributed to the poor water solubility of drug in the aqueous phosphate buffer media (pH 6.8) and also due to any possible lack of chemical and physical interactions which could have affected the release of drug from physical mixture by any mechanism to increase the percent of drug getting released. The drug released from nanofiber film was found to be more and sustained as compared to the film made by solvent casting method reported by other researchers[12].

2.11. *Ex vivo* permeation study

Franz diffusion cells were used in this study to investigate the transbuccal permeability of the drug from the LOR loaded nanofiber film and physical mixture through freshly extracted goat buccal mucosa. Goat buccal mucosa was selected because of it's resemblance in thickness to human tissue and availability in large quantities from the slaughterhouse. The percent cumulative amount drug permeated in 8 hrs was calculated to study how the nanofibers affect the permeation of LOR for extended period. The protocol for the research project, BVCP/IAEC/05/2022, was approved by the Institutional Animal Ethics Committee (IAEC) of Bharati Vidyapeeth's College of Pharmacy, Navi Mumbai.

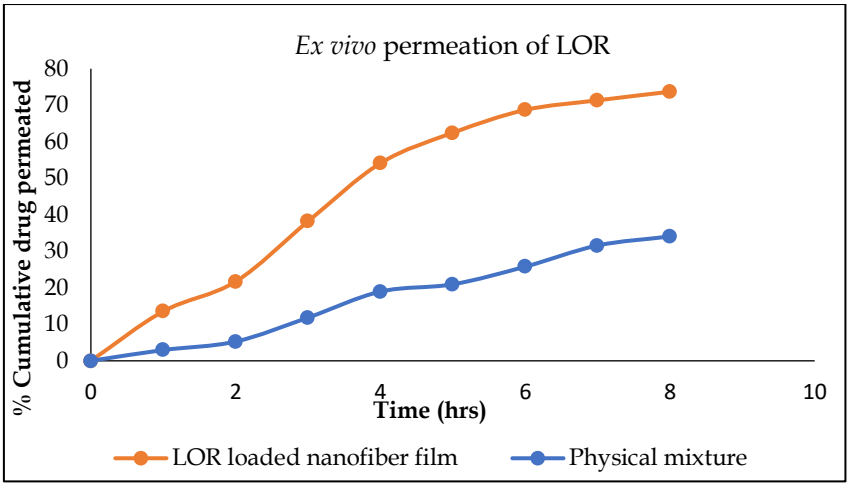


Figure 9. Comparative *ex vivo* permeation of LOR from LOR loaded nanofiber film and physical mixture through goat buccal mucosa in buffer pH 6.8

The percent cumulative drug permeated, flux and permeability coefficient for the LOR loaded nanofiber film are 73.69%, 98.9109 µg/cm²/hr and 5.17881E-06 cm/s respectively as compared to the physical mixture which has percent cumulative drug permeation of about cumulative 34.06 %, flux of 45.7489 µg/cm²/hr and permeability coefficient of 2.39401E-06 cm/s (Figure 9). The nanofiber film clearly increased the permeation characteristics of the LOR by releasing the drug in a steady and sustained fashion within the goat buccal mucosal membrane and might have penetrated through the human buccal membrane as well, based on the 2.1632-fold enhancement of permeation. The permeation curves of nanofiber film showed more promising permeation than other formulations[13,14].

2.12. Determination of Mucoadhesion strength

The adhesion of the film to the buccal mucosa is a prerequisite for maintaining drug release. The mucoadhesive strength values for LOR loaded nanofiber film was found to be 17.12 ±1.25 g. As per the result it can be interpreted that PVP contributes to the mucoadhesive properties of the film as the presence of a hydrophilic group in PVP binds to mucin via a hydrogen bond, resulting in a mucoadhesive interaction with the buccal cells and the retention of the film in the oral cavity. Force of adhesion was found to be 0.1696 N. The mucoadhesive strength was also found to be between 0.205 ± 0.035 and 0.790 ± 0.014 N[15].

3. CONCLUSION

Loratadine is a the 2nd generation (H1) antihistamine. It is commonly used to treat allergic reactions. LOR corresponds to the biopharmaceutical classification system (BCS) class II, which has high permeability and low water solubility. Typically delivered orally in the form of solid dosage forms like tablets and liquid dosage forms like suspensions, LOR undergoes considerable first-pass hepatic metabolism and has a bioavailability of roughly 40%. It is a weakly ionizable base with pH-dependent solubility; as pH rises, solubility falls off rapidly. As a result, there is little dosage proportionality and a considerable degree of intra- and inter-subject variability in LOR oral absorption as compared to conventional oral route, buccal route using mucoadhesive dosage forms offers a novel route of drug administration. Nanofiber systems as transbuccal carriers for drugs have highly mucoadhesive properties, fast disintegration at a specified pH, delayed release using enteric polymers, and formulations containing therapeutically sensitive macromolecules. Thus, encapsulating LOR in strings of electrospun nanofibers were synthesized for benefits such as consistent and sustained release of LOR into the buccal cavity.

The components for formulation i.e., polymer(PVP-EC)-solvent(Ethanol) system was selected using various parameters like film formation, solubility and electrospinnability polymers and volatility of solvent. Central Composite Design (CCD) created the LOR-loaded nanofiber film with Stat-Ease Design Expert® software V13.0, optimizing the total polymer concentration to 12.4947% and the flow rate to 1.62357 mL/hr. The DSC, FTIR and SEM studies showed encapsulation of drug without any precipitation.The *in vitro* release studies showed an initial slow release in first two hrs that is 26.1359% followed by sustained release of the remnant dose of drug(87.8904%)at the end of 8 hrs. The *Ex vivo* drug permeation study showed cumulative

drug permeation of 73.69%, flux of 98.9109µg/cm²/hr and permeability coefficient of 5.17881E-06 cm/s for after 8 h through goat buccal mucosa. The mucoadhesion strength was found to be 17.12±1.25 gm for the LOR loaded nanofiber film which renders it to be suitable and effective for buccal delivery of LOR. According to the findings, drug permeation was slow and steady, suggesting sustained release, as observed in *in-vitro* studies. As a result,LOR loaded nanofiber film formualtions were successfully developed and can be good option for LOR delivery through buccal mucosa compared to marketed formualtions.

4. MATERIALS AND METHODS

4.1. Materials

Loratadine was generaously provided by Vasudha Pharma Chem Ltd. All chemicals and solvents were analytical reagent grade.

4.2. Authentication of Drug

4.2.1 UV spectrum of LOR

Accurately weighed 100 mg of LOR dissolved in methanol and then poured it into 100 mL volumetric flask. Volume was made up to the mark using methanol in 100 mL volumetric flask. The standard stock solution of concentration 1000 µg/mL was further diluted with methanol to get a concentration of 10 µg/mL. This solution was analyzed under a range of 400 nm to 200 nm in UV spectrophotometer (Shimadzu UV-1800) using methanol as blank to determine the wavelength of maximum absorbance.

4.3. Screening of excipients

4.3.1. *Selection of polymer*

Different polymers were used to perform the screening experiment. Polymers used were PVP, PVA, Chitosan, Eudragit-L100, PEO, HPMC and Ethyl cellulose. In this experiment 5 mL of polymer solution each were made using appropriate solvent/ solvent system to check the spinnability of the polymer solutions after loading into the nanofiber machine.

4.3.2. *Selection of solvents*

Different solvents systems were studied for the selection of appropriate solvent system and ratio of polymer: solvent. Solvents used were Methanol, Ethanol, Acetone, Dichloromethane (DCM), Dimethyl formamide, Acetic acid, Acetonitrile etc. Selection of polymer was done according to solubility criteria for polymer and drug, electrospinning ability and volatility (Table 6).

Table 6. List of different polymers: solvent combinations taken for screening

Name of polymer used	Solvent used
PVP-K30	
PVP-K60	Ethanol
PVP-K90	
PVP-K90	
PVA	Distilled Water
PVA	Ethanol: Distilled water (50:50)
Chitosan	Acetic Acid (1% W/V)
Eudragit-L100	Ethanol: DMF (80:20)

HPMC K100	
Ethyl Cellulose(18-22cps)	Ethanol
PEO	

4.4. Formulation and development of nanofiber film

4.4.1. Preparation of electrospinning drug (LOR) loaded polymeric solution

The selected polymer-solvent combinations were used to prepare polymer solutions. The polymeric solutions had been stirred on a magnetic stirrer for 1 hr at temperatures ranging from 40°C to 50°C until a clear homogeneous solution was obtained; subsequently, LOR was added to this solution and mixed until the final dissolution of LOR.

4.4.2. Electrospinning procedure

The electrospinning instrument (E-spin Nanotech) used for the electrospinning process of this study, which consisted of an adjustable DC power supply, a syringe, a stainless-steel needle, and a syringe pump. The experimental parameters such as flow rate, supplied voltage, tip-to-collector distance, drum speed, was set based on previous literature as well as experimental conditions. Electrospinning processing was conducted at ambient temperature with a relative humidity of 55%. For the preparation of nanofiber buccal film, LOR loaded PVP:EC polymeric solution was deposited on aluminium foil. The fibers collected from aluminium foil on the collector were then placed in a vacuum desiccator for overnight. Various trial batches are taken according to optimization design to obtain the optimized nanofiber buccal film formulation.

4.4.3. Preliminary batches

Different ratios of polymer: solvent concentrations of PVP and EC were taken to define the range of concentration that can be taken for optimization batches to determine highest drug loading.

Table 7. Trial batches in different total polymer concentration of PVP:EC (1:1)

Batch	Total polymer concentration (%)	Drug (mg)	Voltage (kv)	Flow rate (mL/hr)
1	8	600	25	2
2	10	600	25	2
3	12	600	25	2
4	15	600	25	2

Table 8. Trial batches for LOR in different PVP: EC ratio

Batch	PVP:EC ratio	Drug (mg)	Total Polymer Concentration (%)	Voltage (kv)	Flow rate (mL/hr)
1	4:1	600	10	25	2
2	3:2	600	10	25	2
3	2:3	600	10	25	2
4	1:1	600	10	25	2

5	1:2	600	10	25	2

4.4 Experimental Design

In this research, central composite design (CCD) with two variables at two layers in Statease® Design Expert software was used to evaluate the effect of formulation factors on the characteristics of the final film (Table 9). The flow rate and total polymer concentration were the two independent variables investigated in this study. Table 7 displays the independent variables along with their corresponding levels. Nine formulations based on these variables and their levels were created. (Table 8). Responses (dependent factors) were the Percent drug release (R1) and Percent entrapment efficency(R2). The measured responses were subjected to multiple linear regression analysis to evaluate the impacts ofindependent components.

Table 9. Coded and actual values of central composite design

Factor	Levels		
	Low	Medium	High
Total Polymer concentration (%)	10	12.5	15
Flow rate (mL/hr)	1.5	2	2.5

Table 10. Formulation of nanofiber buccal film using central composite design

Runs	Total polymer concentration (%) [X1]	Flow rate (mL/hr) [X2]
1	10	1.5
2	10	2
3	10	2.5
4	12.5	1.5
5	12.5	2
6	12.5	2.5
7	15	1.5
8	15	2
9	15	2.5

Selected optimized batch was then formulated according to electrospinning procedure and evaluated for different parameters.

4.5. Evaluation And Characterization of Nanofiber Buccal Film

4.5.1. % Entrapment efficiency (% EE)

The amount of drug that is both contained within and adsorbed onto the nanofibers is referred to as entrapment efficiency. The concentration of unentrapped drug in the nanofibers was measured to calculate the entrapment effectiveness (% EE) using the formula below

$$\frac{Weight\ of\ initial\ drug - Weight\ of\ free\ drug}{Weight\ of\ initial\ drug} \times 100 \ldots (1)$$

Methanol was used to properly dilute the produced nanofiber mat. The nanofiber solution was then centrifuged for 30 minutes at 10,000 rpm using an appropriately attenuated version. A UV/VIS spectrophotometer operating at a wavelength of 248 nm was used to determine the amount of free drug present in the supernatant. The difference between the initial drug content and the free drug in the supernatant was used to calculate the quantity of drug that was incorporated. The experiment was conducted three times[16].

4.5.2. Scanning Electron Microscopy (SEM)

Using a scanning electron microscope (SEM), the fiber diameter and morphology of the electrospun nanofiber layer were examined. The nanofiber samples were sputter covered with gold prior to imaging, and micrographs were taken at various magnifications of 10X, 20X, and 50X. Measuring at least 100 distinct fibers at random allowed us to ascertain the diameter size distribution in the manufactured membranes[15].

4.5.3. Thickness

Digital vernier calipers were used to measure the thickness of the fibers at six distinct locations. All measurements were made in triplicate on 2x2 cm^2 films, and the data were provided as mean ± S.D[17].

4.5.4. Folding endurance

The electrospun sheets' resilience to folding contributed to their fragility. Using a sharp blade, three films (2x2 cm^2) of each mixture were cut. The test for folding endurance involved repeatedly folding a tiny piece of film in the same spot until it broke. The worth of folding endurance was determined by the number of folds the film could endure in the same spot without breaking. The tests were performed in duplicate.[18]

4.5.5. Weight variation

For each formulation, three randomly selected films with surface area 2x2cm^2 were used. Each patch was weighed individually on an analytical balance and the average weights calculated[19].

4.5.6. FTIR analysis

Incorporation of drugs and polymer-drug interactions were studied by differential scanning calorimetry (DSC). As a reference, an empty aluminium pan was used. DSC measurements were taken using an aluminium sealed pan and a heating rate of 10 °C/minute from 10 to 300 °C. For each measurement, the sample size was 5-10 mg. The sample cell was purged with nitrogen gas during the measurement[20].

4.5.7. DSC studies

Differential scanning calorimetry was used to study drug incorporation and interactions between medicines and polymers. (DSC). As a comparison, an empty aluminum pan was used. Using an aluminum sealed pan, DSC observations were carried out between 10 and 300 °C at a heating rate of 10 °C/minute. For each measurement, a sample quantity of 5–10 mg was used. The sample cell was gassed with nitrogen during the test[21].

4.5.8. In vitro drug release

Paddle-style USP type II dissolution equipment was used for the research. At a temperature of 37±1°C for eight hrs, films totalling 5 milligrams LOR were dissolved in 900 mL of phosphate buffer solution (pH 6.8). The sink condition was kept throughout the trial, and the dissolution apparatus was set to stir at 50 rpm. Fresh dissolution medium was added at predetermined intervals to replenish the 5 mL of solution were removed from the vessel, and all materials were examined for 8 hrs at 248 nm. Three tests were carried out, and the cumulative release rate was calculated as mean ± S.D[22].

4.5.9. Ex vivo permeation study

The protocol (BVCP/IAEC/05/2022) for the ex vivo drug permeation study was authorized by the IAEC of Bharati Vidyapeeth's College of Pharmacy, Navi Mumbai. A research on drug permeation in ex vivo was completed using the Franz diffusion apparatus to explore buccal permability of nanofiber film. The donor and receptor compartments were separated by a diffusion cell on which goat buccal mucosa was affixed. The mucosal membrane was covered with the mucoadhesive material. As dissolution fluid, 30 mL of phosphate

buffer with a pH of 6.8 was placed in the receiver compartment and 5 mL of phosphate buffer with a pH of 6.8 was placed in the donor compartment for mimicking the buccal environment as buccal mucosa having pH 6.8. The fluid was kept at 37±2°C and constantly stirred using a magnetic stirrer at a very low speed, or 50 RPM. The Franz diffusion cell's temperature was maintained by connecting the exterior jacket to a water bath. During the 8 hr period, 1 mL aliquots were collected at regular intervals and simultaneously, 1 mL fresh media was added to the franz diffusion cell for mentaining the sink condition, and the amount of drug was calculated by measuring the absorbance at 248 nm with a UV-visible spectrophotometer. After each sample withdrawal, pre-warmed (37±2°C) dissolution fluid was introduced to the diffusion cell. For the purpose of calculating ex vivo drug permeation, the procedure was performed in triplicate (n = 3)[23]. Absorption is a passive diffusion process that can be described using Fick's law equation. For LOR loaded film flux was calculated using the formula below:

$$Js = dQr/Adt \ldots (2)$$

J is the steady-state buccal mucosa flux in µg/cm² per h, dQr is the change in quantity of material passing through the membrane into the receptor compartment expressed in µg, A is the active diffusion area in cm² and dr is the change in time. The steady-state flux of LOR through the goat buccal mucosa was calculated from the slope of the linear portion of the cumulative amount permeated through the membrane per unit area vs. time plot. The Apparent Permeability coefficient (Papp) was also calculated by the formula,

$$Papp = Q/(A \times c \times t) \ldots (3)$$

Where is total amount permeated within the incubation time(µg),A is diffusion area of the chamber(cm^2),c is initial concentration of drug in donor chamber ($\mu g/cm^3$)t is total time of the experiment (s).The Enhancement ratio was calculated by,

$$\text{Enhancement ratio} = \text{Papp of nanofiber film} / \text{Papp of physical mixture} \ldots (4)$$

4.5.10. *Determination of Mucoadhesion strength*

With double-sided adhesive tape, the films were attached to the glass vial's bottom side. Under the glass vial, a clean 500 mL glass beaker was positioned, inside of which was a 100 mL glass beaker with the cap reversed, on which a portion of goat buccal mucosa was positioned. To prevent the beaker from moving, a suitable weight was applied. Phosphate buffer with a pH of 6.8 was put in the 500 mL container. The mucoadhesive strength was measured by the mass (in grams) needed to separate the film from the mucous surface[24].

$$\text{Force of adhesion (N)} = \frac{\text{Mucoadhesive strength (g)}}{1000} \times 9.81 \ldots (5)$$

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