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FORMULATION, OPTIMIZATION AND EVALUATION OF NORFLOXACIN TRANSDERMAL PATCH FOR ANTI-BACTERIAL ACTIVITY

Rajni [1], Dinesh Kumar [*2], Sonakshi Antal [2] and Praveen Nasa [3]

Starex University [1], Gurugram, Gurugram - 122413, Haryana, India.
Atam Institute of Pharmacy [2], Hisar - 125033, Haryana, India.
Department of Pharmaceutical Education and Research [3], BPSMV, Bhainswal Kalan, Sonipat - 131409, Haryana, India.

Keywords:

Fluoroquinolone, Norfloxacin, Transdermal drug delivery system, Tulsi Oil, Tea Tree Oil

Correspondence to Author:
Dinesh Kumar

Assistant Professor,
Atam Institute of Pharmacy,
Om Sterling Global University, Hisar - 125033, Haryana, India.

E-mail: dineshpotlia123@gmail.com

ABSTRACT: Objective: This investigation aimed to formulate, optimize and evaluate the matrix-type transdermal patch of norfloxacin for antibacterial activity by incorporating different polymer concentrations and penetration enhancers. **Methods:** Nine formulations were prepared by a solvent evaporation method using ethyl cellulose dibutyl phthalate, tea tree oil, and tulsi oil at concentrations (0.25%, 0.5%, 1%) as penetration enhancers. Characterization done FTIR, DSC, SEM, skin permeation study and antibacterial activity. The patch has uniform weight and low moisture content based on physicochemical evaluation and in vitro characterization. The FTIR shows no or very minor changes in main drug peaks in the IR spectra of a mixture of drug and pure drug. SEM shows smooth surface and uniform drug distribution. In skin permeation study, a combination of tea tree oil and tulsi oil showeaug two-fold increase in drug relea, indicatinged Higuchi release kinetics. **Result:** 2-fold increase in permeation of transdermal patch as compared to pure drug. **Conclusion:** Norfloxacin could be delivered through the transdermal patches by incorporating natural permeation enhancers and combining hydrophobic and hydrophilic polymers.

INTRODUCTION: Norfloxacin (1-ethyl-6-fluoro-4-oxo-7-piperazin-1-ylquinoline-3-carboxylic acid) is synthetic first generation broad spectrum antibacterial agent of fluoroquinolone class. It acts by inhibiting DNA gyrase, type II topoisomerase and topoisomerase IV, enzymes necessary to separate bacterial DNA, thereby inhibiting cell division. It is mainly effective against some selected sensitive bacilli, mostly Enterobacteriaceae (*E. coli*, *Klebsiellas*, *etc*.), *Pseudomonas aeruginosa* and many pathogenic enteric bacteria (*Salmonella*, *Shigella, etc*.), but also *Neisseria* (especially gonococci). Streptococci are partially resistant, whereas anaerobic bacteria are completely resistant [1].

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Transdermal drug delivery systems (TDDS) are rate-controlled drug delivery systems that deliver a specific medication dose through the skin into the bloodstream. It promotes the soothing of injured areas of the body [2]. The main objective of designing transdermal dosage is to enhance the flux through the skin into the systemic circulation and

simultaneously minimize the drug's retention and metabolism in the skin [3]. The transdermal dosage offers more advantages over injectables and oral routes due to its non-invasiveness, increased patient compliance, and avoids first-pass metabolism, respectively [4].

Penetration enhancers were employed in transdermal drug delivery to improve the drug's penetration rate and therapeutic efficiency; absorption of unabsorbable drugs can be increased [5]. They are also known as accelerants or sorption promoters. These include water, pyrrolidone's, fatty acids and alcohols, azone and its derivatives, alcohols and glycols, essential oils, terpenes and derivatives, sulfoxides like dimethylsulfoximide (DMSO) and their derivatives, urea and surfactants [6]. The literature review suggested that essential oils and vegetable oils are most widely used as permeation enhancers because they are non-toxic, less allergic, easily available, and compatible with drugs and excipients. Natural oils for a greater period used in cosmetics, and medicine so safe to use, and then metabolized in the body also showed higher permeability because they contain unsaturated fatty acids which alleviates the lipid and protein structure of stratum corneum and increases penetration of drug through the skin [7].

Transdermal patches products were first approved by FDA in 1981. Nitroglycerin patches were approved in 1981, and nowadays, various patches of drugs are available such as clonidine, fentanyl, lidocaine, nicotine, nitroglycerin, oestradiol, oxybutynin, selegiline, scopolamine, and testosterone. Combination patches are also applicable for contraception and hormone replacement [8]. TDDS has significantly predisposed the delivery of different therapeutic agents, especially in pain management, hormonal therapy, and treatment of diseases of the cardiovascular and central nervous systems.

TDDS does not involve passage through the gastrointestinal tract; therefore, there is no loss due to first-pass metabolism. Drugs can be delivered without interfering with pH, enzymes, and intestinal bacteria. Additionally, TDDS can be used to control drug release according to usage limits [21]. It is now well-known that penetration enhancers improve the permeation of drugs across the skin. However, the study of the mechanism of action of many such compounds proposes that refining the penetrability of drugs via skin disruption may cause toxicity [22]. Olanzapine drug products are administered via oral or intramuscular routes. Many complications are associated with oral and intramuscular routes of drug administration. Olanzapine drug-in-adhesive transdermal patch release drug for 3 days [23].

Phytosome nanotechnology can enhance the external therapeutic potential to a clinical context is their extremely low absorption rate and limited penetration through biological barriers. Phytosomes, as lipid-based vesicular nanocarriers, achieve an essential role in improving the pharmacokinetic and pharmacodynamic characteristics of herbal-derived polyphenolic chemicals, making this nanotechnology a prospective tool for creating of novel topical formulations [24].

Polymers are employed in skin preparation, marking the basis of TDDS. The Surface and bulk properties of the polymer can give the desired chemical, interfacial, mechanical, and biological functions. The choice of polymer and its physicochemical properties depend on the need for extensive biochemical characterization and specific preclinical tests to prove its safety.

Surface properties such as hydrophilicity, lubricity, smoothness, and surface energy directs the biocompatibility with tissues and blood, in addition to influencing the physical properties such as durability, permeability, and degradability [25].

MATERIAL AND METHODS:

Materials: Norfloxacin was obtained as a gift sample from Laborate Pharmaceuticals India Ltd., Ponta sahib (H.P). Ethyl cellulose and PVP K30 were procured from Hi Media Laboratories and Sisco Laboratories.

Tulsi oil and tea tree oil were obtained from Grasse International and Siva Sri Retail. All the chemicals used were of analytical grade.

Methods:

Preformulation Studies:

Solubility Profile: The solubility of norfloxacin was tested in various solvents like acetic acid,

acetone, ethanol, methanol, dilute HCl and sodium hydroxide. A sufficient quantity (10mg) of drug dissolved in each investigated solvent at room temperature. The solubility was only determined by visual inspection [9].

Melting Point: The melting point of the drug was determined by the digital melting point apparatus by using a small amount of the drug in a capillary tube closed at one end and placed in the decibel melting point apparatus, and the temperature at which the drug melts was noted. An average of triplicate readings was taken.

Partition Coefficient: The partition coefficient of norfloxacin was obtained by preparing a drug solution of 1mg/ml in n-octanol. 25 ml of this solution was taken in a separating funnel and shaken with an equal volume of phosphate buffer of pH 7.4 (aqueous phase) for 10 minutes and allowed to stand for two hrs.

Then aqueous and organic phases were collected separately and analyzed for the drug concentration using a U.V. spectrophotometer. The partition coefficient was calculated by taking the ratio of the drug concentration in n-octanol to the concentration in aqueous phase [10].

Permeability Coefficient: The permeability coefficient of the drug was calculated by using "Potts and Guy equation",

LogKp = -2.7 + 0.71 x log Ko/w – 0.0061 x Molecular weight

Where, LogKp=Permeability coefficient, Ko/w = Partition coefficient [11]

Analytical Method for Estimation of Norfloxacin [9]:

Preparation of 0.1 N HCl: 8.5 ml of conc. HCl was diluted with distilled water to produce 1000 ml.

Preparation of Standard Stock Solution of Norfloxacin: 100 mg of norfloxacin was accurately weighed and transferred to a 100 ml volumetric flask containing 0.1 N HCl.

The flask was gently shaken to dissolve its contents. Volume was finally made up to 100 ml using volume with 0.1 N HCl and labeled as stock solution A. 10 ml of this solution was taken into a volumetric flask and diluted up to 100ml with 0.1N HCl to obtain the resulting solution of 100 µg/ml and was labeled as stock solution B.

Preparation of Dilutions: By using stock solution B, solutions of various concentrations like 10, 20, 30, 40, 50 and 60µg/ml were prepared by taking 1, 2, 3, 4, 5 and 6ml of stock solution A and diluting with 0.1 N HCl up to 100ml respectively.

These solutions were then made to contact with UV-Visible spectrophotometric studies, and absorbance was measured at 278 nm against 0.1 N HCl as blank.

Preparation of Transdermal Patch: Matrix-type transdermal patches containing norfloxacin were prepared using 2 polymers in combinations EC with PVP K30 in ratio (1:2, 1:4, 1:6) w/w by solvent evaporation technique using petridishes. The polymers like EC, and PVP were selected as rate controlling polymers as they are biodegradable, easily affordable, economic, and non-toxic.

The purpose of taking a mixture of two polymers, one polymer is hydrophobic (EC) and another is hydrophilic, which may release the drug in a controlled manner with a definite rate. The bottom of the Petri dish was wrapped with aluminum foil. The backing membrane was cast by pouring 4% w/v PVA solution in distilled water, followed by drying at 60°C for 6 h in a hot air oven.

The polymers of each combination were dissolved in dichloromethane: acetic acid (6:1). Dibutyl phthalate (15% v/w of polymer weight) was added as a plasticizer and tea tree oil and tulsi oil were taken as permeation enhancer. Norfloxacin (80 mg) was added and stirred with a mechanical stirrer to get a homogeneous dispersion.

The dispersion (2 mL) was cast on each mold's prepared PVA backing membrane. The rate of evaporation was controlled by inverting a funnel over the mold and dried at 40°C for 6 h in hot air oven and the films were cut into small patches ($1cm^2$) containing 3.25 mg of norfloxacin and stored between sheets of wax paper in a desiccators [12].

TABLE 1: COMPOSITION OF TRANSDERMAL PATCH

Norfloxacin (mg)	Ethyl cellulose: PVP K30	Dichloromethane: Acetic acid	Dibutyl phthalate	Tea tree Oil	Tulsi Oil
80	1:2	6:1	15%v/w	0.5%	0.25%
80	1:4	6:1	15%v/w	0.5%	0.25%
80	1:6	6:1	15%v/w	0.5%	0.25%
80	1:2	6:1	15%v/w	0.5%	0.5%
80	1:4	6:1	15%v/w	0.5%	0.5%
80	1:6	6:1	15%v/w	0.5%	0.5%
80	1:2	6:1	15%v/w	0.5%	1%
80	1:4	6:1	15%v/w	0.5%	1%
80	1:6	6:1	15%v/w	0.5%	1%

Optimization using Central Composite Design: Design Expert Software, version 11.0 (Stat-Ease, Inc. Minneapolis, MN, USA) was employed to fit polynomial equations with attached interaction terms for the inter-relation of studied responses with chosen variables.

Drug content and % elongation were selected as response variables for systematic optimization. Optimized formulation was found by locating feasible space, and exhaustive grid search was done to trace the possible solution. An optimum solution was also provided by the software using the overlay plots. The optimized formulations were utilized for all the in vitro studies.

Evaluation Parameters of Transdermal Patch:

Weight Uniformity: The patches are dried at 60°C before weighing. The weight uniformity of the patch is measured by cutting and weighing the 1 cm^2 piece of 3 patches and then measuring the weight variation. The mean of the 3 is taken as the weight of the patch. The individual weight should not deviate significantly from average weight [13].

Drug Content Uniformity: The patch (1 cm^2) was cut and added to a beaker containing 100 ml of phosphate-buffered saline pH 7.4 (PBS). The medium was stirred (500 rpm) with magnetic bead for 5 h.

The contents were filtered using Whatman filter paper and the filtrate was analyzed by U.V. Spectrophotometer at 278 nm for the drug content against the reference solution consisting of placebo films [14].

Folding Endurance: It is calculated by cutting the patch in particular size by using sharp blade. Folding endurance was determined repeatedly until it broke by following a small patch strip at the same place. The folding endurance is the no. of time the patch could be folded at the same place without breaking [15].

Moisture Content: The prepared patches are cut into strips of specific size. The strips are then weighed individually and kept in a desiccator containing activated silica at 300°C for 12 hours. The films are reweighed individually until a constant weight is achieved [16].

Percentage (%) of moisture content = Loss in wt. / Initial wt. × 100

Swelling Property: The patch is applied on a pre-weighed cover slip, and weight is taken. Now it is placed in a Petri dish containing 50 ml 7.4 pH phosphate buffer. At an interval of 5 minute the cover slip is removed from Petri dish, washed and weighed for 30 minutes. The change in the weight shows the patch's swelling due to water uptake. Percentage swelling (S) is given by:

$$\% S = (Xt-X0 / X0) \times 100$$

Where Xt = weight at time‘t’ after swelling and X0 = original weight of the patch [17].

Water Vapor Transmission Studies (WVT): Glass vials of equal diameter were used as transmission cells. The transmission cells were washed thoroughly and dried in an oven at 100°C. About 1g anhydrous calcium chloride was placed in the cells and respective polymer film (1sqcm) was fixed over brim. The cells were accurately weighed and maintained a relative humidity of 84% by stored in a closed desiccator containing saturated potassium chloride (200 ml). The cells were taken out after 24h and weighed after storage. The amount of water vapor transmitted was found using the following formula.

$$WVT = WL / S$$

Where W = water vapor transmitted in gm, L = thickness of the film in cm, S = exposed surface area in square cm.

It is expressed as the number of grams of moisture gained/h/cm^2 [18].

Percentage Elongation Break Test: The percentage elongation break is the length just before the break point, the percentage elongation can be determined from the below-mentioned formula.

$$\text{Elongation percentage} = L1\text{-}L2/\ L2 \times 100$$

Where, L1 is the final length of each strip and L2 is the initial length of each strip [19].

Characterization of Transdermal Patch [18]:

Fourier Transform Infrared (FT-IR) Spectroscopy: The identification of the obtained sample was confirmed by presence of functional groups by utilizing FT-IR technique.

Firstly the background was scanned and then crystal window closed. Samples were finely ground with infra-red grade KBr then pressed into pellet and IR spectra were taken in transmission over the range of 4000- 500 cm-1at ambient temperature. The sample was pressed and scanned. In the spectra, that was appeared on the screen, the baseline was corrected. The drug was fixed by infrared spectroscopy and characteristic peak obtained compared with standard spectra of pure drug reported in official monograph.

Scanning Electron Microscopy (SEM): Scanning electron microscopy has been hugely employed to study the morphology and surface topography of the patch. The SEM (JEOL-JSM-6100) used SEM grids which were prepared by placing a small amount of transdermal patch on a gold coated grid and drying under lamp. The accelerator voltage was set at 30.0 KV during scanning. Magnification is of order 10,000 X and resolution 10nm [21].

Skin Permeation Study: The *in-vitro* permeation of transdermal patch was studied using Franz diffusion cell. The cell was mounted with dialysis membrane for drug permeation study. The drug diffuses into receptor compartment through effective permeation area. The receptor fluid was composed of acidic buffer (0.1 N HCl). The capacity of receptor compartment was 20 ml. The temperature of donor compartment was maintained at 100rpm and 37± 1°C. The membrane was kept between donor and receptor compartment such that dermis had contact with buffer solution. The sample was withdrawn at determined time intervals and same volume was replaced with fresh buffer solution. The samples were analyzed using UV spectrophotometer /HPLC assay at respective λ max for estimation of drug concentration [22].

Statistical Analysis: Statistical analysis of all data was carried out using Graph Pad in Stat Software. One-way Analysis of variance (Turkey's multiple comparison test) was used for statistical comparison of the data with a significance level fixed at $p<0.01$. Results are expressed as mean ± standard deviation (mean ± SD).

RESULT AND DISCUSSION:

Solubility and Melting Point: Norfloxacin is fully soluble in dilute HCl, acetic acid and sodium hydroxide. The melting point was found to be 220-221^oC.

The Partition Coefficient ($K_{o/w}$): The value of the partition coefficient of norfloxacin was found to be 0.457. This indicates the drug possesses sufficient lipophilicity, suggested to formulate into a transdermal patch.

Analysis of Drug:

Calibration Curve of Norfloxacin in 0.1 N HCl: Calibration curve of norfloxacin in 0.1 N HCl was analyze in the range of 10 - 60µg/ml. The regression coefficient (R^2) at 278 nm was found to be 0.999. It showed that the drug follows Beer's Lambert Law. The absorbance and calibration curve of norfloxacin in 0.1 N HCl are shown in **Table 2** and **Fig. 1** respectively

TABLE 2: ABSORBANCE OF NORFLOXACIN IN 0.1 N HCL AT 278nm

Sr. no.	Concentration(µg/ml)	Absorbance in 0.1 N HCl
1.	10	0.14±0.004
2.	20	0.26±0.001
3.	30	0.38±0.002
4.	40	0.50±0.004
5.	50	0.61±0.009
6.	60	0.73±0.008

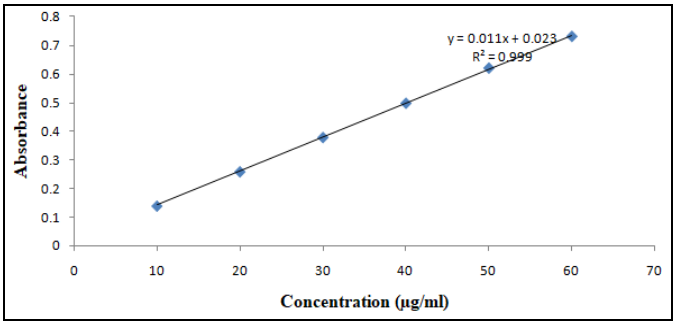

FIG. 1: CALIBRATION CURVE OF NORFLOXACIN IN 0.1 N HCL

Appearance: The prepared transdermal patches were transparent, smooth, linear, and flexible. The method adopted for the preparation of system was found satisfactory.

Optimization using Central Composite Design: For finding the effect of factors on response variables, optimization was performed for 13 trial batches.

Amount of EC: PVP K30 ratio (X_1) and concentration of tulsi oil (X_2) were selected as factors and drug content (Y_1) and % elongation (Y_2) as response variables that were given in **Table 3**. The model F- value (4.78) for Y_1 and (6.02) for Y_2 implies the model is significant. The positive sign in front of the factors indicates a synergistic effect, and the negative sign indicates an antagonistic effect of the factors on responses Y_1 and Y_2.

TABLE 3: RESPONSE VARIABLES RESULT FOR TRIAL BATCHES F1 TO F13

Formulation Code	Variables		Response Variable	
	X_1	X_2	Drug content (%)	% Elongation
F1	1	1	90.2	33
F2	0	1	86.5	28.6
F3	0	0	82.1	24.2
F4	0	0	74.5	20.8
F5	1	0	92.02	26.5
F6	0	0	88	20.93
F7	0	0	84.5	29.4
F8	0	-1	80.3	27.3
F9	-1	0	76.7	36.5
F10	-1	1	72.06	22.8
F11	1	-1	68.6	20.1
F12	0	0	82	21.5
F13	-1	-1	76.1	35.2

X1: EC: PVP K30 ratio 1:2, 1:4, 1:6, **X2:** Concentration of Tulsi oil 0.25%, 0.5% 1%. The polynomial equation shows the effects of both the independent variables on the response variables of batches F1-F13. The polynomial equation derived for the drug content and % elongation was mentioned in equations 1 and 2.

Drug content (Y_1) = 81.1062+ 4.032822 A+3.29102 B+6.14AB ……. [1]

% Elongation (Y_2) = +23.366 – 2.38027A+ 0.29231B+6.325AB + 3.5795A^2 + 1.8045B^2….[2]

Drug content and percentage elongation of the formulations were in range of 72.06±0.01 to 90.20±0.03 and 20.1 ±0.02 % to 36.5± 0.05%. The prepared patches were also found to be strong enough & provide good mechanical properties. In the case of drug content (Y_1), factor X_1X2 was found to be significant (< 0.05) i.e., as the concentration of tulsi oil increased, the % drug content of the patches also increased. But less effect was observed by increasing the amount of EC: PVP K30. In case of % elongation (Y_2), factor X_1 X_2 was found to be significant *i.e.* as the amount of EC: PVP K30 and concentration of tulsi oil increased, the % elongation was found to be increased. Further, the interaction between factors X1 and X2 can be elucidated by using a response surface plot as illustrated in **Fig. 2** and **3.**

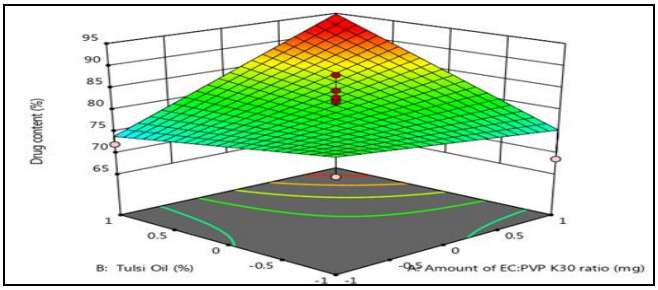

FIG. 2: 3D SURFACE PLOT SHOWING EFFECT OF EC: PVP K30 RATIO AND CONCENTRATION OF TULSI OIL ON DRUG CONTENT

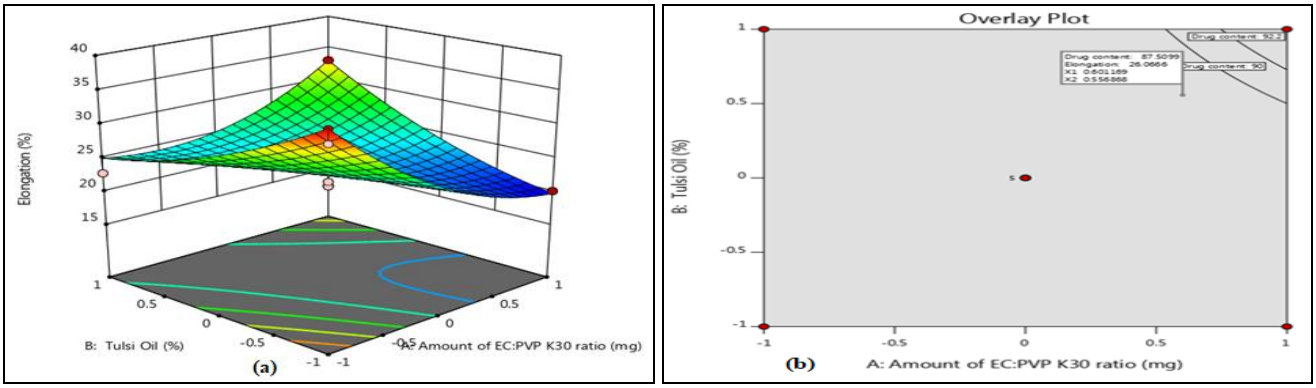

FIG. 3: (A) 3D SURFACE PLOT SHOWING THE EFFECT OF EC:PVP K30 RATIO AND CONCENTRATION OF TULSI OIL ON % ELONGATION BREAK AND (B) OVERLAY PLOT SHOWING OPTIMUM SOLUTION FOR THE FORMULATION

Evaluation of Transdermal Patch: Transparent, flat, flexible, and uniform norfloxacin transdermal patch was obtained using ethyl cellulose and PVP K30. Dibutyl phthalate 20% w/w of dry weight of polymer added as plasticizer for a good flexibility and elasticity. Tea tree oil and tulsi oil were used as penetration enhancers. The literature review found that natural oils as a permeation enhancer gave good elasticity to the patch.

Weight Uniformity: The average weight of batches F1 to F9 range between 16.8±0.20 to 20.76±0.15mg shown in **Fig. 4**. The results indicate the physical uniformity of the prepared patches. The minimal SD values show that the process used for preparing the patches can formulate patches with minimum intra-batch variability.

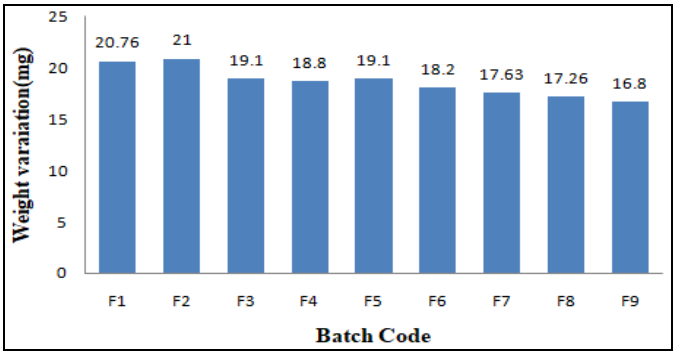

FIG. 4: WEIGHT VARIATION FOR BATCHES F1 TO F9

Drug Content Uniformity: The drug content of the entire batches lies between 73.4±0.01 to 87.93±0.02 %, shown in **Fig. 5**. The test indicates that the drug is distributed uniformly in the patches developed by a solvent evaporation method, and the method was found to be suitable and reproducible. F7 has maximum drug content due to the higher amount of polymer and maximum concentration of combination of tea tree and tulsi oil.

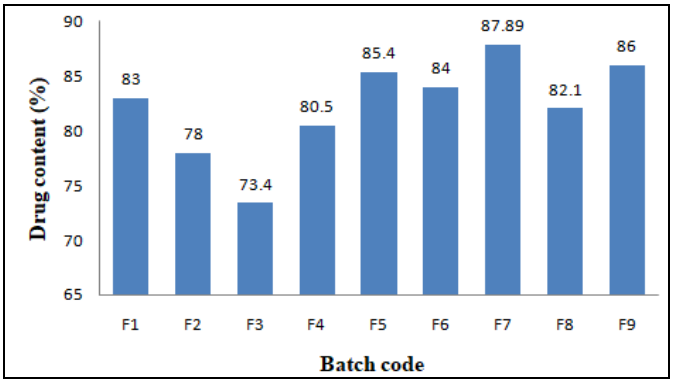

FIG. 5: DRUG CONTENT FOR BATCHES F1 TO F9

Folding Endurance: The folding endurance measures the ability of patch to withstand rupture. The folding endurance was measured manually, and results indicated that the patches would not break and would maintain their integrity with general skin folding when used. The results of folding endurance were shown in **Fig. 6**. It was found to be high in patches F1 and F7 containing EC: PVP K30 (1:2) and a higher amount of tea tree and tulsi oil.

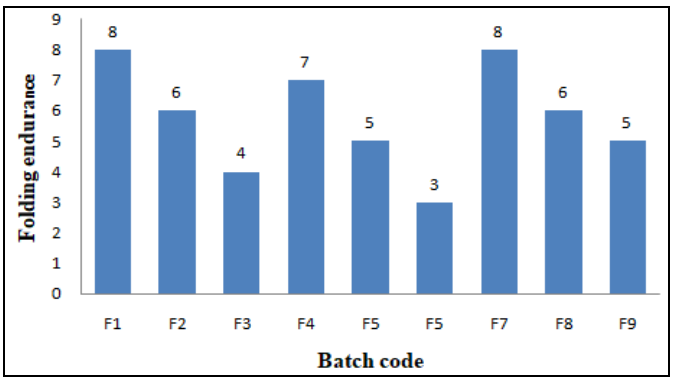

FIG. 6: FOLDING ENDURANCE FOR BATCHES F1 TO F9

Moisture Content: The batches F1 to F9 were obtained between 0.94±0.02 to 4.73±0.07. The moisture content of the prepared transdermal film was low in F5, which could help the formulation remain stable and from being completely dried and reduce brittleness during storage. The low moisture content protects the patches from microbial attack and avoids the bulkiness of the patches.

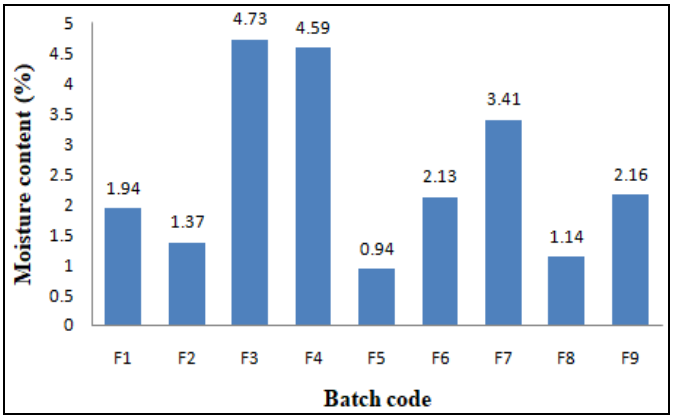

FIG. 7: MOISTURE CONTENT FOR BATCHES F1 TO F9

Swelling Index: The swelling index of F1 to F9 batches was found to be 10.61±0.02 to 59.06±0.02.F9 has high swelling index achieves maximum drug release due to increased ratio of EC: PVP K30 (1:6) and increased concentration of tea tree and tulsi oil.

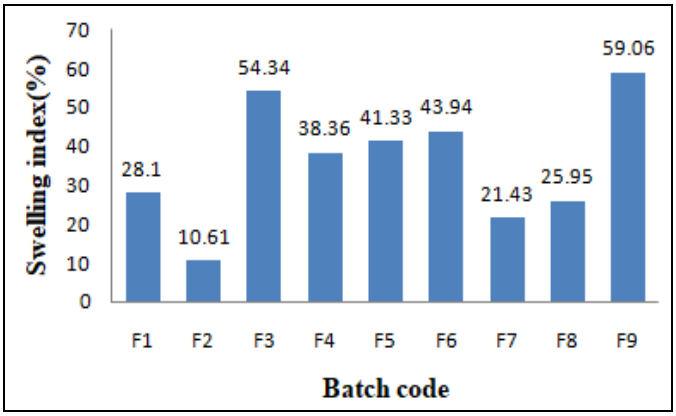

FIG. 8: SWELLING INDEX FOR BATCHES F1 TO F9

Water Vapor Transmission Studies: The results for water vapor transmission are depicted graphically in **Fig. 9**.

The prepared patches showed minimal moisture absorption rates ranging from 0.19% ± 0.10 to 0.55% ±0.15 thus ensuring general stability and protection from microbial contamination.

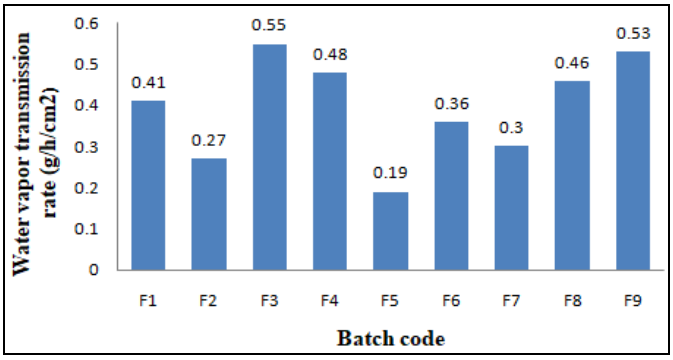

FIG. 9: WATER VAPOR TRANSMISSION FOR BATCHES F1 TO F9

Percentage Elongation Break Test: The % elongation of batches F1-F9 was in the range 20.1±0.15 to 26.4±0.20 showed that as amount of EC: PVP K30 and oil concentration was increased % elongation was also increased.

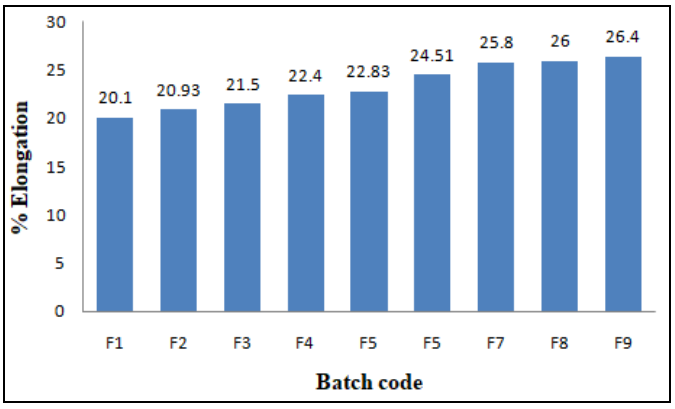

FIG. 10: % ELONGATION BREAK FOR BATCHES F1-F9

Characterization of Transdermal Patch:

FTIR Study: FTIR techniques have been used here to study the physical and chemical interaction between drugs and excipients. Infrared (IR) spectra of norfloxacin (A) and optimized formulation are shown in **Fig. 11** and 1**2,** respectively.

The pure drug peaks at 3590, 3480, 2749, 1619, 1481.74, 1032.54, 929.07 and 739.44 for OH, NH, CH_3, (C-O-C), O-C-O group of acid, (C-F), NH_3, aromatic. The optimized formulation shows sharp peaks at 3525, 3394.6, 2721.43, 1758, 1487.80, 1021.32, 931.82 and 800 cm^{-1}. From the figure, it was observed that there were no changes in these main peaks in IR spectra of a mixture of drugs and polymers. Which show there were no physical interactions because of some bond formation between drug and polymers

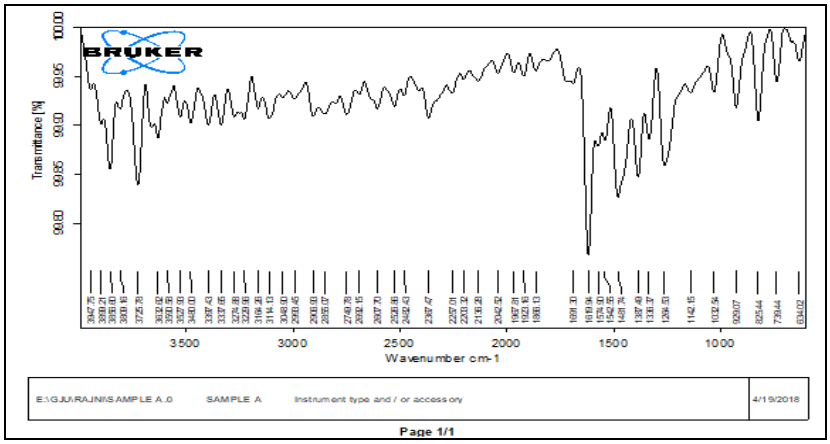

FIG. 11: FTIR SPECTRA OF NORFLOXACIN

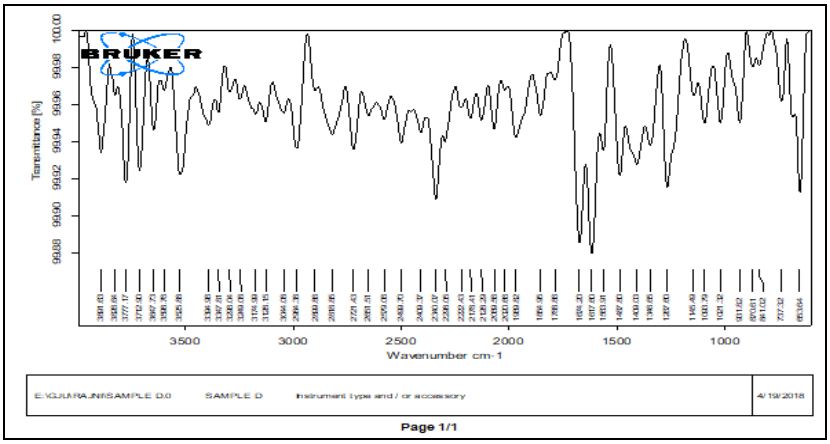

FIG. 12: FTIR SPECTRA OF FORMULATION

SEM Study: The SEM (JEOL-JSM-6100) used SEM grids which were prepared by placing a small amount of transdermal patch on a gold-coated grid and drying under lamp SEM photomicrographs of norfloxacin patch are shown in **Fig. 13**. SEM photomicrograph of norfloxacin patch indicated the patch was smooth and homogeneous distribution of polymer without crystallization of drug.

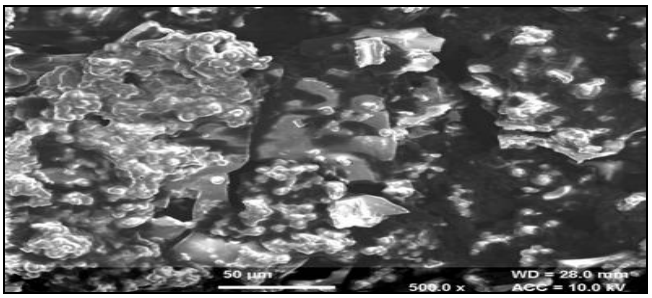

FIG. 13: SEM PHOTOGRAPH OF OPTIMIZED TRANSDERMAL PATCH

Skin Permeation Study: The main purpose of *in-vitro* permeation studies was to screen the formulations further and to correlate the permeation pattern of norfloxacin from the norfloxacin-loaded transdermal systems with the observed release patterns. The study was performed for the optimized batch and clearly showed that the amount of drug release was increased with the concentration of tulsi oil. This confirms that the presence of permeation enhancer and combination of hydrophilic and hydrophobic polymer is crucial in permeating norfloxacin to control drug permeation across the membrane. The values were obtained in triplicates as Mean±S.D. with $p<0.05$.

TABLE 4: PERCENTAGE OF DRUG RELEASED OF PURE DRUG AND TRANSDERMAL PATCH

Time (hr.)	Pure drug	Transdermal patch
0.08	2.44±0.08	3.35±0.23
0.25	7.11±0.15	8.65±0.15
0.5	13.70±0.23	11.39±0.08
0.75	18.24±0.15	13.19±0.08
1	24.43±0.40	28.75±0.81
2	27.78±0.08	49.68±0.08
4	38.29±0.08	60.30±0.08
6	43.08±0.08	77.87±0.17
8	44.53±0.15	80.04±0.08
10		86.12±0.15
12		89.16±0.17
24		91.07±0.15

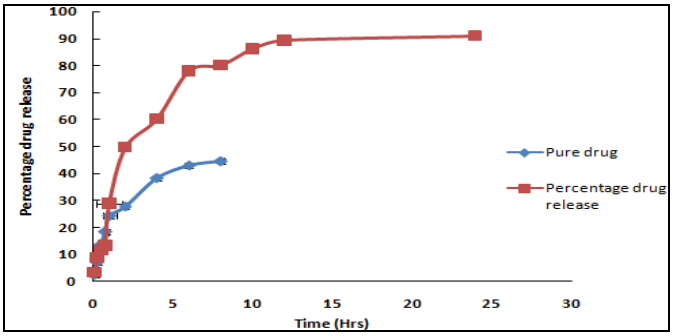

FIG. 14: PERCENTAGE OF DRUG RELEASED OF PURE NORFLOXACIN AND TRANSDERMAL PATCH

Kinetic models were applied to the permeation study graphically represented in **Fig. 15, 16, 17, 18** with their regression coefficients. The zero-order rate describes the system where the drug is released independent of its concentration and shows the cumulative amount of drug release *vs* time for zero-order kinetics. The first-order rate describes the release from systems where the release of drugs from a matrix as a square root of a time-dependent process based on Fickian diffusion. The data obtained for *in-vitro* release shown in **Table 5** were fitted into the equation for the zero order, first order, and Higuchi and Korsmeyer-Peppas release models. The interpretation of data was based on the value of the resulting regression coefficients.

Zero Order:

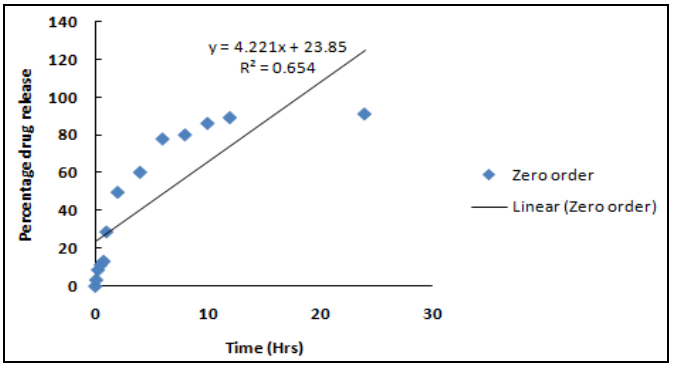

FIG. 15: ZEROORDER GRAPH OF TRANSDERMAL PATCH

First Order:

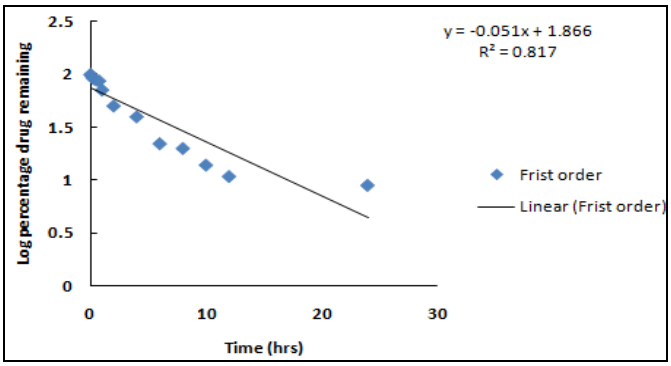

FIG. 16: FIRST-ORDER GRAPH OF TRANSDERMAL PATCH

Higuchi Model:

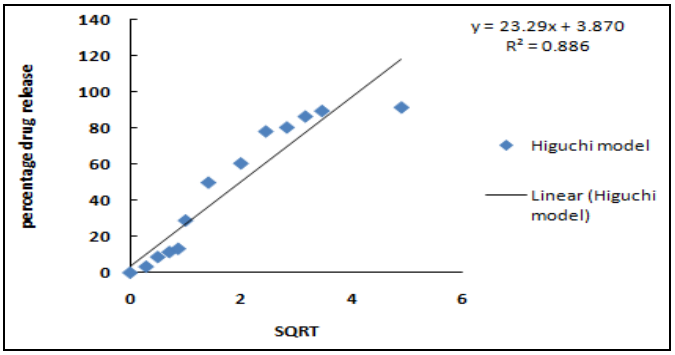

FIG. 17: HIGUCHI MODEL OF TRANSDERMAL PATCH

Korshmeyar- Peppas Model:

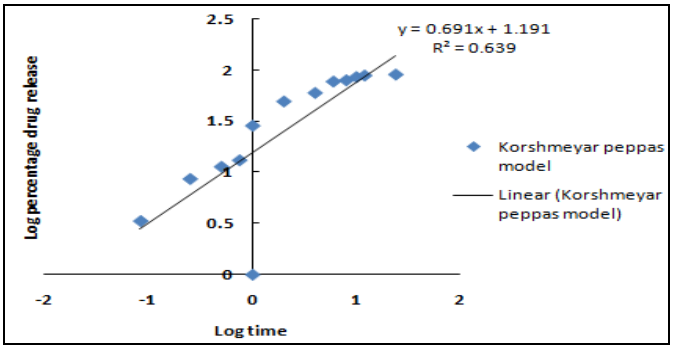

FIG. 18: KORSHMEYER-PEPPAS MODEL OF TRANSDERMAL PATCH

TABLE 5: KINETIC EQUATION PARAMETER OF TRANSDERMAL PATCH

Formulation Code	Zeroorder		Firstorder		Higuchi		Peppas	
	R^2	K_0	R^2	K_0	R^2	K_0	R^2	K_0
Transdermal patch	0.654	4.221	0.817	-0.051	0.886	23.29	0.639	0.691

The calculated regression coefficients for zero order, first order and Higuchi models and Korshmeyer were shown in **Table 5**, it was found that the first-order model best explained the *in-vitro* drug release of the optimized transdermal patch as the plot showed the highest linearity. The value of R^2 was found to be 0.886 highest for the Higuchi model.

DISCUSSION: Transdermal drug delivery system is the most suitable for long-term or This system also increases the bioavailability of the drug by

avoiding the first-pass metabolism and increases the therapeutic efficacy of drug by reaching into the systemic circulation. Transdermal patches are designed to deliver control drug release over a specific period. Transdermal patches prepared by incorporating a combination of hydrophilic and hydrophobic polymers releases drug at a controlled rate. Using natural penetration enhancers (essential oils) also maximizes drug permeation through the skin. Nowadays, novel approaches have been developed that enhance permeation through the skin. This includes prodrugs, iontophoresis, microneedles, ultrasound, and targeted drug delivery systems like noisomes, nano emulsion, transferosomes, liposomes.

Norfloxacin is flouroquinolone class of drug currently available in the market for treatment of urinary tract infections, prostatitis *etc*. Ethylcellulose and PVP K30 were selected as polymersbased on their adhering property and non-toxicity. The result of the finding showed excellent adhering property and controlled release. In this study nine matrix type patches were prepared different polymer ratios and varying concentration of oils. Plasticizers like di-butyl phthalate and permeation enhancers like tea tree oil and tulsi oil were added by solvent evaporation.

In present study solubility of drug was determined in different solvents, it was found that norfloxacin was soluble in acetic acid, 0.1 N HCl, NaOH. The standard curve for norfloxacin was determined λmax 278nm and the regression was found to be 0.999 with slope of 0.017 and Y-intercept of 0.109. The formulation was optimized by central composite design to study the effect of EC: PVP K30 polymer ratio and oil concentration on drug content and % elongation as response variables. The surface plot for drug content and % elongation showed that EC: PVP K30 polymer ratio has less effect on drug content and increases % elongation. The concentration of tulsi oil also shows the maximum effect on drug content and % elongation. The prepared patches were subjected to weight variation, folding endurance, drug content uniformity, percent moisture content, % swelling, % elongation and water vapor transmission studies. Weight variation ranges from 16.8±0.20 to 20.76±0.15mg. Folding endurance and % elongation, results indicated that the patches would not break and would maintain their skin integrity with general skin folding when applied. The moisture loss of the prepared formulations was low (0.94±0.02 to% to 4.73±0.07 %), which could help the formulations remain stable and reduce brittleness during long-term storage. Good drug content uniformity was observed in all the patches ranging from (73.4±0.18 to 87.93±0.24 %). The low water vapor transmission (WVT) rates shows emphasize the stability of the patch on long-term storage. Fourier transform infrared spectroscopy (FTIR) was done to study the compatibility of norfloxacin with formulation excipients.

FTIR studies were performed on pure drug samples (Norfloxacin) and the transdermal patch. The frequency of principle peaks in FTIR spectra of a physical drug mixture with other excipients was nearly similar to the frequency of principle peaks present in FTIR spectra of pure drugs. Therefore, these results revealed that the drug was compatible with excipients, and neither did the drug decomposition non-drug-excipients and excipients-excipients interactions in the formulation. SEM reveals that the formulated patch was a smooth, flexible, and uniform drug distribution. The skin permeation study was carried out for 24 hrs through Franz diffusion cell by using a dialysis membrane for pure drug and transdermal patch. The study results in a two-fold increase in patch permeation compared to pure drugs.

CONCLUSION: The present study aimed to prepare and evaluate the transdermal patch using different penetration enhancers. This patch was also evaluated for anti-bacterial activity. In this study, matrix-type patches we redeveloped by a solvent evaporation method using a combination of hydrophilic and hydrophobic polymers (ethyl cellulose and PVP K30) and tulsi oil and tea tree oil as penetration enhancers at varying concentrations. All the prepared formulations showed the best results. Based on *in-vitro* evaluation characterization and *ex-vivo* studies, the drug release was maximized because of the increase in the amount of hydrophilic polymer to hydrophobic polymer maximize drug release and the increased concentration of oils produced reproducible results. Hence, it was concluded that norfloxacin could be delivered through the transdermal patches by

incorporating natural permeation enhancers and combining hydrophobic and hydrophilic polymers.

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