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Optimization and characterization of azadirachta indica loaded transdermal patches preparation

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***Abstract*---**The Transdermal patches of Neem (methanolic extract) (F1, F2, F3, F4 and F5) were successfully prepared by solvent casting method using HPMC K100M in 1:1.1:2, 1:2, 1:3 and 1:4 ratios. The FTIR studies did not reveal any significant drug interactions. The prepared patches showed good results for physicochemical evaluations, in-vitro diffusion studies and anti-bacterial screening studies. On comparing major in evaluation criteria, formulation F4 was selected as best formulation. The release kinetics of all the patches showed zero-order kinetics and followed non-Fickian diffusion mechanism. The anti-bacterial screening study showed good anti-bacterial activity against Bascillus subtilis and Pseudomonas aeruginosa and zone of Inhibition (ZOI) was compared against standard anti-biotic drugs. It can be concluded from this project work that, from this novel approach, herbal drugs in the extract/ oil of the product can transformed into Transdermal Patches for the bio active properties pertaining to a stable formulation in accordance with its appropriate formulations. Although Transdermal systems provide a promising route of delivery for new age drugs, conventional and new dosage forms are equally essential for other drugs to increase their therapeutic efficacy. The patented innovations of TDDS focus on these parameters to make dosage form more patients complied and site specific delivery of the drug. Moreover in vitro performance of the dosage form specifies the ultimate test for the therapeutic efficacy of the drug.

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Introduction

Any drug delivery system aim is to provide a therapeutic amount of drug to the proper site in the body and then maintain desired drug concentration. Drugs are administered by various routes such as oral, parental, nasal, transdermal, rectal, intravaginal, ocular etc. Among all of them, oral route is most common and popular but this route of administration have some drawback like first pass metabolism, drug degradation in gastrointestinal tract due to pH, enzyme etc. To overcome these drawback, a novel drug delivery system (controlled drug delivery system)was developed in which a polymer(natural or synthetic)combined with a drug in such a way that drug is released from the material in a predesigned manner.

The discovery of Transdermal drug delivery system (TDDS) is a breakthrough in the field of controlled drug delivery system. It becomes a great field of interest. TDDS are self-contained, discrete dosage forms which when applied to the intact skin; deliver the drug, through the skin at control rate to the systemic circulation. In 1965 Stoughton first conceived of the percutaneous absorption of drug substances. FDA approved the first Transdermal system Transdermal-SCOP in 1979 FDA approved this for the prevention of nausea and vomiting.1In TDDS, the drug is mainly delivered through the skin with the aid of transdermal patch which is a medicament adhesive patch that is placed on the skin to deliver a specific dose of medication through the skin and to the blood stream. Now a day TDD is a well-accepted means of delivering many drugs to the systemic circulation in order to achieve a desired pharmacological outcome.

Materials and Methods

Materials

The Leaves (Azadirachta Indica*) is purchase from* Doha Organic Fertilizers Corporation Meerut, Glycerol is a purchase Sarvodaya Enclave New Delhi, Ethyl Cellulose, Propylene glycol, HPMC K100M, Methanol purchase form Sigma Aldrich and PEG-400 form Himedia bio science.

Pre-formulation Studies
Methods

Extraction of Leaves of Azadirachta Indica Methanolic Extract

The shade dried leaves were subjected to size reduction and passed in to sieve no 20 and then 40. About 500g of the dried powder was extracted continuously in Soxhlet apparatus with methanol for 72 hours to obtain the crude extract. The extract was dried under vacuum oven [42].

Phytochemical Studies
The Methanolic extract was subjected to phyto-chemical studies to find out the presence and absence of constituents [43, 44].

FTIR Studies

The application of infrared spectroscopy lies more in the qualitative identification of substances either in pure form or in the mixture and as a tool in establishment of the structure. Since I.R. is related to covalent bonds, the spectra can provide detailed information about the structure of molecular compounds. In order to establish this point, comparisons were made between the spectrum of the substance and the pure compound. The infrared data is helpful to confirm the identity of the drug and to detect the interaction of the drug with the polymer. Infrared spectra of drug and polymer, alone and in physical mixtures were taken. Then it was investigated for any possible interaction between polymer and the drug.

Determination of λmax

For assurance of λmax stock solution of Azadirachta Indica (Conc. 1000µg/ml) in methanol were readied. 1ml of the readied stock arrangement was additionally weakened to 100ml. Coming about arrangements were examined in the range of 540 to 556nm utilizing methanol as a clear with the assistance of a UV-visible spectrophotometer. Normal triplicate readings were taken [45, 46].

Calibration curve of Azadirachta Indica in pH-6.8 phosphate buffer

The above stock arrangement filtered for the most extreme absorbance utilizing UV max of Azadirachta Indica in phosphate buffer pH 6.8 was seen as 543nm. The above stock arrangement (100g/ml) was additionally weakened to get focus in the range of 10-50g/ml. The absorbance of every arrangement was estimated utilizing a UV-Visible double beam spectrophotometer by putting reference standards of a particular medium. The standard bend produced for a whole range of conc. and the tests acted in triplicate [47].

Preparation of Azadirachta Indica Transdermal Patch

The methanolic extract of leaves of Azadirachta Indica the four (F1, F2, F3 and F4) transdermal patches were prepared using drug with polymer in different ratios (1:2,1:3,1:5 ,1:7). Weighed quantity of polymer was dissolved in calculated quantity of methanol and kept for stirring. Calculated amount of extract was added to the above mixture and stirred well until a homogenous mixture was formed. Then calculated amount of permeation enhancer and glycerol were added. The resultant mixture was poured into a Petri dish and air dried at room temperature for 24hr.The patches were then peeled off from the Petri dish with the help of a knife and kept in desiccators [48].

Table. Formulation of transdermal patches of Azadirachta Indica A.

Formulation Code					
Ingredients	F1 (1:1)	F2 (1:2)	F3 (1:2)	F4 (1:3)	F5 (1:4)
Drugs (Azadirachta Indica)	50	50	50	50	50
HPMC K100M	50	100	100	150	200
Glycerol	Q.S	Q.S	Q.S	Q.S	Q.S
Ethyl Cellulose	10	10	10	10	10
Propylene Glycol	5	10	15	20	25
PEG-400	10	12	15	20	25
Methanol	Q.S	Q.S	Q.S	Q.S	Q.S

Evaluation of Azadirachta Indica Transdermal Patch

Physical Appearance:
All the transdermal patches were visually inspected for colour, clarity, flexibility, and smoothness.

Weight Variation:
This was done by weighing five different patches of individual batch taking the uniform size at random and calculating the average weight. The tests were performed on patch which was dried at 60˚C for 4 hr prior to testing.

Thickness of the Patch:
The thicknesses of the patches were assessed by using digital Vernier calliper at different points of the patch. From each formulation three randomly selected patches were used. The average value for thickness of a single patch was determined.

% Drug Content:
The patches (2×1cm2) were taken and cut into small pieces, added to a beaker containing 100 ml of distilled water the medium was stirred magnetic bead for 5hrs. The solution was filtered and analysed for drug content with proper dilution at 543nm U.V spectrophotometrically.

Folding Endurance:
This was determined by repeatedly folding one patch at the same place till it broke. The number of times the patch could be folded at the same place without breaking gave the value of folding endurance.

% Moisture Uptake:
The patches were weighed accurately and placed in desiccators containing aluminium chloride. After 24 hr, the patches were taken out and weighed. The percentage moisture uptake was calculated as the difference between final and initial weight. It was calculated by using following formula.

$$\% \text{ moisture uptake} = [\text{Final weight} - \text{Initial weight} / \text{Initial weight}] \times 100$$

Determination of Surface pH:
The patches were allowed to swell by keeping them in contact with 5ml of distilled water for 2hr at room temperature and pH was noted down by bringing the electrode in contact with the surface of the patches, allowing it to equilibrate for 1 min.

Tensile Strength and % Elongation:
Tensile strength of the patches was determined with Universal strength Testing Machine (Hounsfield, Slinford, Horshan, U.K). It consisted of two load cell grips. The lower one was fixed and upper one was movable. The patches of size (2×1cm2) were fixed between these cell grips and force was gradually applied till the patches broke. The tensile strength of the patches were taken directly from the dial range reading in kg [49].

In-vitro Diffusion Studies:
In-vitro diffusion study was performed by using a Franz diffusion cell with a receptor compartment of 250ml. The cellophane membrane was mounted between the donor and the receptor compartment of the diffusion cell. The formulated patches were cut in size of (2×1cm2) and placed over the cellophane membrane and the receptor compartment of the diffusion cell was filled with phosphate buffer pH 7.4.The whole assembly was fixed on a magnetic stirrer and the solution in the receptor compartment was constantly and continuously stirred using magnetic beads at 100 rpm; The temperature was maintained at 37±0.50c.Tha samples of 5ml were withdrawn at the time interval of 1hr up to 21 hr and analysed for drug content U.V spectrophotometrically at 310nm against blank. The receptor medium was replaced with an equal volume of phosphate buffer pH 7.4 at each time of sample withdrawal. The cumulative amounts of drug permitted were plotted against time [50].

Diffusion Kinetics:
To analyse the mechanism for the release and release rate kinetics of the dosage form, the data obtained was fitted in to Zero order, First, Higuchi matrix, Korsmeyer's Peppa's model. By comparing the r2-values obtained, the best model was selected [51].

Scanning Electron Microscopy (SEM):
The exterior surface characteristics of received sample (semi solid; herbal constituents along with presence of polymer) were examined by Scanning electron microscope (Model JSM - 6390LV, Jeol, USA). The sample was placed on plain glass stub and sputter coating of gold was done to make surface of particles electroconductive. Images were recorded using SEM equipped with SEM digital camera [52].

Screening of Antimicrobial activity:
Organisms used: Bacillus subtilis (Gram+ ve),
Pseudomonas aeruginosa (Gram- ve)
Media used: Nutrient Agar
Test used: ME patch.

Preparation of Nutrient Agar:
8.2gm of agar powder was dissolved in 25ml of water. The medium was steamed in boiler to precipitate any heat coagulable material. Then the medium was filtered. The filtrate was distributed in 5ml quantity into culture tubes. The tubes were plugged with non-absorbent cotton. The medium in the tubes were sterilized by autoclave not less than 15 minutes at 15 pounds per sq. inch at 121oC.

Preparation of Paper Disc:
By using standard punching machine Whatman filter paper was cut and standard paper of 6.0mm diameter was prepared. The paper discs were sterilized in a hot air oven at 160oC for 1hour. The paper discs were then impregnated with the test solution.

Inoculation of Organism to Petri dishes for the Anti-bacterial Activity:
The marketed standard drug solution (Penicillin, Streptomycin) paper disc (1mg/ml), extract solution paper disc (1mg/ml) (1:10) (1:20), test optimized formulation solution paper disc (40mg) were prepared by proper dilutions using sterilized distilled water. The sterilized boiling tubes, agar media, Petri plates, sterilized formulation paper disc's, sterilized standard drug paper disc's, sterilized extract paper discs were taken under laminar air flow chamber. The All the inoculation procedures should be performed only in laminar air flow chamber to aseptic conditions. The nutrient media was first poured into boiling tube and then test culture is inoculated to the tubes and mixed properly. After uniform distribution the nutrient culture media is poured in to the petri plates and set aside for uniform distribution. The prepared standard paper discs, formulation paper discs, extract paper discs were added slowly on to the settled agar petri plate with the help of forceps. Markings are made for identification of respective readings and after inoculation Petri plates were transferred to the incubator. The plates were incubated at 370C for 24 hours, after that plates were observed for anti-bacterial activity by measuring zone of inhibition and reported [53, 54, 55].

Stability Studies:
Optimised formulation F4 was subjected to accelerated stability study at 40±2°C and 75±5 % RH for 1, 2 & 3-months. The Azadirachta Indica Loaded Transdermal Patch were evaluated for description, Color and Folding Endurance and% Drug Content and In-Vitro Drug.

Results and Discussion

Preformulation Studies

Table. Photochemical Screening

S.No.	Metabolites	Result
1.	Alkaloids	+
2.	Glycoside	+
3.	Flavonoids	+
4.	Tannins	+
5.	Reducing sugar	-
6.	Terpenoids	-

7.	Monoterpenoids	-
8.	Sesquiterpenoids	-
9.	Quinines	-
10.	Saponins Glycosides	+
11.	Steroids	+

Note:- (+) Presence and (-) Absence

Table. FTIR Spectrum

Functional Groups	Reported frequency cm^{-1}	Observed Frequencies cm^{-1}
O-H str	3400-2400	2400
C- H str	3150-3050	2854.24
CH2 stretching	3409-3389	3043.43
NH2 stretching	3270-3043	3193.34
C-H stretching	3032-2980	3267.56
R-O-CH3 stretching	3645-3590	3589.23

Determination of λmax:

Table. Data of concentrations and absorbance in phosphate buffer pH 6.8

S. No.	Concentration (µg/ml)	Absorbance
1	5	0.32
2	10	0.510
3	20	0.751
4	30	1.001
5	40	1.283
6	50	1.291

Fig. Calibration curve of Neem Extract in phosphate buffer pH 6.8

Evaluation of Azadirachta Indica Transdermal Patch:

Table. Organoleptic Property Analysis of Developed Transdermal Patches

S.No	Physical Appearance	Result
1	Appearance	Jellified Preparation
2	Color	Pale Yellow
3	Clarity	Opaque
4	Flexibility	Good
5	Smoothness	Fair

Table. All Evaluation Parameters

Formulation	Weight Variation	Thickness	% Drug Content	Folding Endurance	% Moisture Uptake	pH	Tensile Strength	% Elongation
F1	0.643±0.013	0.108±0.005	99.02±1.032	142±2.5	1.90±0.05	6.4±0.11	0.380±0.010	32.33±1.312
F2	0.812±0.030	0.114±0.034	98.84±0.690	198 ±1.6	2.78±0.06	5.8±0.14	0.404±0.008	30.51±0.422
3	0.787±0.041	0.130±0.021	98.16±0.332	110±6.0	2.64±0.014	5.6±0.10	0.217±0.012	28.51±1.562
F4	0.870±0.095	0.242±0.012	96.56±0.903	66±2.7	1.90±0.012	5.2±0.15	0.235±0.011	24.51±1.206
F5	0.982±0.092	0.290±0.014	95.63±0.922	78±2.2	1.80±0.016	5.1±0.14	0.215±0.014	22.51±1.160

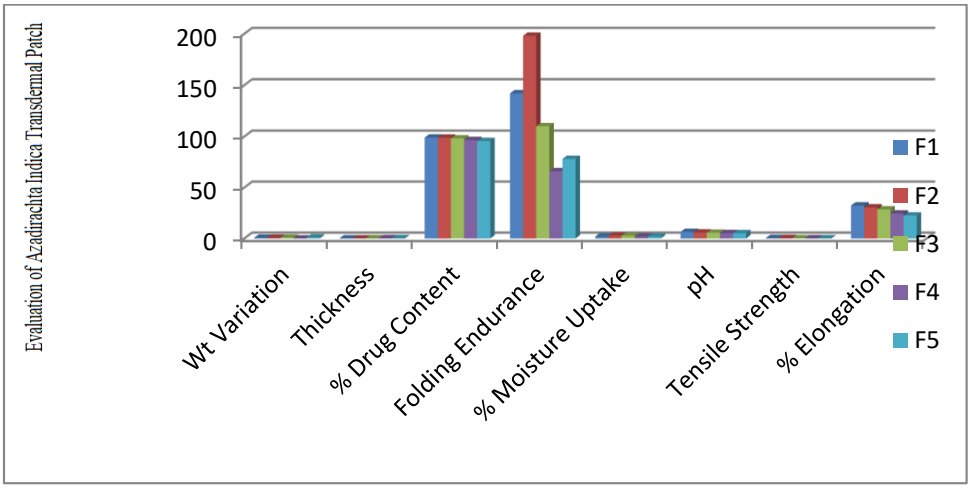

Fig. Evaluation of Azadirachta Indica Transdermal Patch

In-vitro Diffusion Studies

The in-vitro diffusion drug studies of patches F1 to F5 were performed by using cellophane membrane in Franz diffusion cell for 21hrs. The percentage of drug release across cellophane membrane for the formulations F1 to F5 were found to be F1 (78.65±0.27%), F2 (80.83±0.18%), F3 (90.10±0.04%), F4 (98.1±0.04%) and F5 (96.4±0.02%), at the end of 21 hrs, but F5 showed maximum drug release i.e. 96.4±0.2% at the end of 10hrs. It was revealed from the below results that with increasing in the concentration of HPMC K100M, the drug release from the patch increased. It might be attributed due to the hydrophilic nature of HPMC K100M.The formulation F4 showed highest drug release 98.1±0.04% at the end of 21hrs due to hydrophilic nature of HPMC K100M.The results are shown in Fig.3.8.

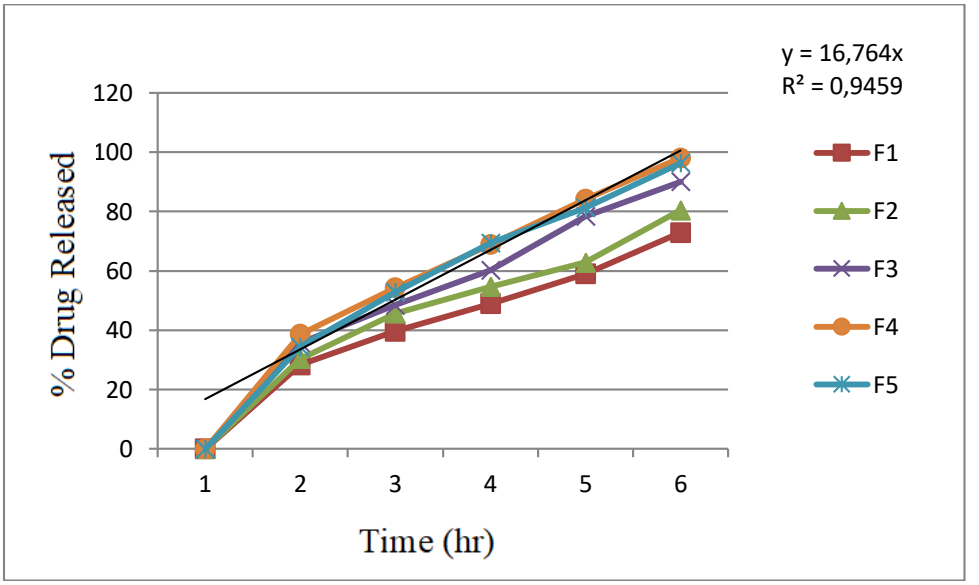

Fig. In-vitro Diffusion Drug Studies of Patches F1 to F5

Diffusion Kinetics

To know the mechanism of drug release, the data were fitted to models representing Zero-order, First-order, Higuchi and Korsmeyer-Peppas. It was found that the release of patches followed zero order kinetics. The coefficient of determination (F4) was found to be much closer to 1 for the Korsmeyer-Peppas equation. Slope values (n>1.0) suggest that the drug permeation from transdermal patches followed the non-Fickian diffusion mechanism, possibly owing to chain disentanglement and swelling of hydrophilic polymer.

Anti-Bacterial Screening

Table. Anti-bacterial screening test for optimized Transdermal patch of Neem, F4

S.No	Name of the microorganism	A 1:10	B 1:20	C (Pure Extract)	D (Test Formulation)	E (Std. Drug)
1	Bacillus Subtilis	Plate 2 (F4)	-	23mm	25mm	26mm
2	Pseudomonas Aeruginosa	Plate 2 (F4)	-	24mm	24mm	22mm

The anti-bacterial activity of test formulation Zone of Inhibition (ZOI) was compared with standard antibiotic drugs like Penicillin and streptomycin. The results are shown in Table.5

Scanning Electron Microscopy (SEM):

The morphological features acquired from optimized formulation was photographed using SEM. Images shown presence of irregular shaped crystals

with presence of aggregations. Rationales may be attributed to precipitation of the sample.

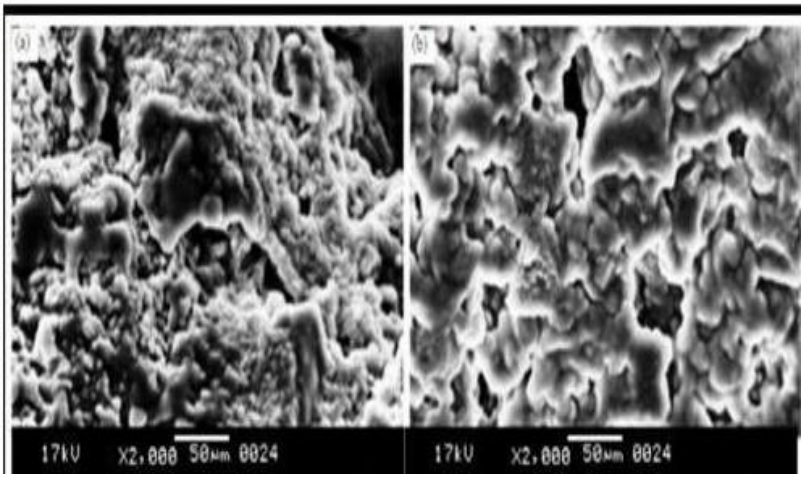

Fig. Morphology of Azadirachta Indica Loaded Transdermal Patch, as viewed by SEM

Stability Studies Results of Optimized Batch F4

Table. Stability Studies of Selected Formulation F4

Evaluation Parameters	Initial	15-Days	1-Month	3 Month
Color	Pale Yellow	Pale Yellow	Pale Yellow	Pale Yellow
Folding Endurance	66±2.7	66±2.5	66±2.0	65±2.5
% Drug Content	96.56±0.903	96.45±0.900	96.32±0.897	95.68±0.858
In-Vitro Drug Release Study	98.1±0.02	98.0±0.01	97.90±0.02	97.10±0.03

Conclusion

The Transdermal patches of Neem (methanolic extract) (F1, F2, F3, F4 and F5) were successfully prepared by solvent casting method using HPMC K100M in 1:1.1:2, 1:2, 1:3 and 1:4 ratios. The FTIR studies did not reveal any significant drug interactions. The prepared patches showed good results for physicochemical evaluations, in-vitro diffusion studies and anti-bacterial screening studies. On comparing major in evaluation criteria, formulation F4 was selected as best formulation. The release kinetics of all the patches showed zero-order kinetics and followed non-Fickian diffusion mechanism. The anti-bacterial screening study showed good anti-bacterial activity against Bascillus subtilis and Pseudomonas aeruginosa and zone of Inhibition (ZOI) was compared against standard anti-biotic drugs. It can be concluded from this project work that, from this novel approach, herbal drugs in the extract/ oil of the product can transformed into Transdermal Patches for the bio active properties pertaining to a

stable formulation in accordance with its appropriate formulations. Although Transdermal systems provide a promising route of delivery for new age drugs, conventional and new dosage forms are equally essential for other drugs to increase their therapeutic efficacy. The patented innovations of TDDS focus on these parameters to make dosage form more patients complied and site specific delivery of the drug. Moreover in vitro performance of the dosage form specifies the ultimate test for the therapeutic efficacy of the drug.

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