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TITLE:

Development of elastic nanovesicles for transdermal drug delivery of Lisinopril Dihydrate

Aparanjitha.R* , Sunitha Reddy.M[1], , Sarangapani.M[1a]

*, 1a University College of Pharmaceutical Sciences, Warangal, Telangana 506092.
aparanjitharajpur@gmail.com, s*arangapanimanda*@yahoo.com
1- Center for Pharmaceutical Sciences , Hyderabad - 500 085 .
dr.baddam.sunitha@gmail.com.

Abstract

Angiotensin-converting enzyme inhibitor lisinopril dihydrate The drug's oral bioavailability ranges from 6 to 60%, thus transferosomal patches for transdermal application were designed to overcome the drug's low bioavailability. Lisinopril was encapsulated into vesicles using a film hydration process and studied for transferosome morphology, particle size, entrapment efficiency, and drug release. The nanocarriers were prepared by rotary evaporation method using various concentrations of edge activators. It was found that tween-80 as edge activator with soya lecithin is suitable for the preparation of nanocarriers for lisinopril dihydrate. The prepared nanocarriers were evaluated for Size, entrapment efficiency, Zeta potential and PDI. The selected formulations were optimized using DOE (central composite design) design expert software. The optimized formulation TL-4 (58%w/v tween 80 with 30% soya lecithin) have shown particle size 144±4.5nm, Zeta- -39±8.5, %Entrapment efficiency with 92%.

The optimized formulation was incorporated into transdermal patches prepared using various concentrations of HPMC E5 & HPMC E15, along with plasticizer PEG-400. The prepared patches were evaluated for invitro, Ex-vivo and in vivo drug release studies. The invitro drug release studies indicate that the prepared patches have better drug release when compared to normal transdermal patches. The Ex-vivo permeation studies of optimized transfersomal patch(HPMC E5 2%w/v, 1.5%w/v PEG-400, 2% nano suspension i.e., 58%w/v tween 80 with 30% soya lecithin) have flux- 44± 0.87 (µg/cm^2/hr), Permeation Coefficient (cm/hr)(Kpx10^3)-9, Enhancement Ratio- 3, Lag time(hr)-0.5 and Q_{24}(µg/cm^2)- 736± 0.38. The optimized patches follow zero order kinetics type of drug release, having non-fickian diffusion in Korsemeyer peppas model with n value < 0.8.

The invivo studies were performed in rats for optimized patches, comparing with Drug solution, marketed oral formulation and normal transdermal patch. The C_{max}, T_{max}, AUC were studied. The transfersomal patches have better pharmacokinetic parameters having prolonged C_{max} and T_{max} indicating sustainable drug release and higher AUC indicating better availability of the drug. The transfersomal patches have not shown any significant skin irritation and they found to have good stability.

Abstract:

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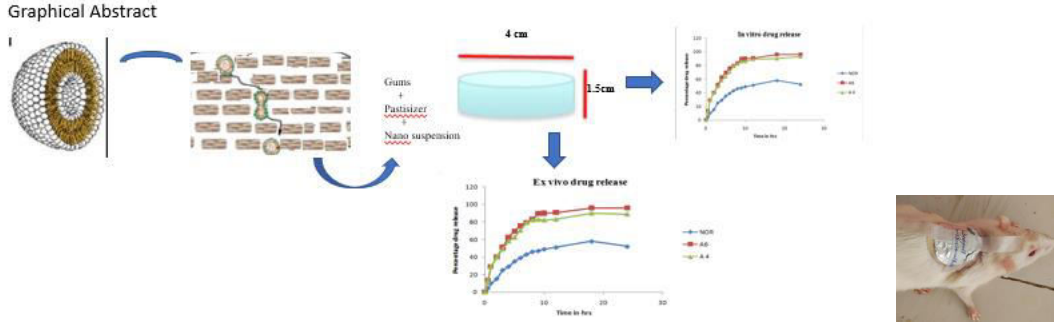
Graphical Abstract



Key Words: Transferosomes, transdermal patches, transdermal drug delivery.

Introduction

Transdermal route offers several potential advantages over conventional routes like avoidance of first pass metabolism, predictable and extended duration of activity, minimizing undesirable side effects, utility of short half-life drugs, improving physiological and pharmacological response, avoiding the fluctuation in drug levels, inter-and intra-patient variations, and most importantly, it provides patients convenience. In the last few years, the vesicular systems have been promoted as a mean of sustained or controlled release of drugs. (Ahlam Zaid et al., 2015).

Lisinopril dihydrate is a lysine derivative of enalaprilat, inhibits angiotensin converting enzyme. It has an extensive hepatic first pass metabolism resulting in an oral bioavailability of 6-60%. To enhance bioavailability and to overcome the drawbacks of oral drug delivery transfersomal transdermal patches were formulated.

Materials and method

Lisinopril dihydrate was from Hetero Drugs Ltd, Hyderabad. Tween 80were purchased from Gattefosse, Mumbai. All the reagents employed were of analytical grade. Soyalecithin, Span 80 and Tween 80, HPMC E 15 and HPMC E 5 were bought from SD fine Chemical, Mumbai, India. Other reagents and compounds were of analytical grade.

Preparation of nanovesicles:

The rotary evaporation sonication method was used to create the nanovesicles. In a hygienic, dry round bottom flask, lipid has been merged with Edge activator (EA) and solubilized in organic solvent until completely dissolved. Organic solvent was removed from the sample by rotating the container at temperatures above the lipid phase transition. Continual vacuum extraction of the remaining solvent over night. At the specified temperature, the lipid film was filled with water to PBS (pH 7.4) containing the drug and spun for 60 minutes. The eventually results vesicles were allowed to swell for 2 h at room temperature before being used (LMLVs). It took 30 minutes at 40 degrees Celsius and a frequency of 53 kHz to sonicate the thick suspension in order to achieve the desired vesicle size (Anubruj J et al., 2011).

Characterization of nanovesicles:

Globule size, PDI, zeta potential (Ghanbarzadeh S et al., 2013)

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Nano dispersion had been dissolved 100 times in double distilled water, the vesicle size, PDI, and Surface Charge of the microemulsion were measured with a zeta sizer (Nano - ZS 90) to obtain the results (Malvern Instruments, Ltd., UK) When the globules were measured at room temperature, it was able to ascertain their size by measuring them at a 90 ° angle.

Drug content

The drug content of the microemulsion was determined by diluting it appropriately in methanol and using the HPLC method described previously.

Entrapment Efficiency

Using centrifugation, the amount of drug in transfersomes could be determined. The prepared transfersomes had to be centrifuged at 14000 rpm for 30 minutes at room temperature.

%Entrapment Efficiency = (Total Drug-Unentrapped drug)/Total Drug X 100

Thickness Variation Test: Films were measured at five points on the patch (in the centre and at each corner) using a digital screw gauge. The five measurements yielded the mean thickness.

Weight Variation Test: To ensure quality, the films were made in triplicate. For each batch, three films were weighed separately, and their average weights were calculated.

Folding Endurance : Patches were tested for folding endurance by repeatedly folding a two cm by two cm strip of film until it broke. To calculate folding endurance, the number of times the film can be folded at the same location without breaking.

Content uniformity : In order to ensure that each film depicts the appropriate amount of drug this test has been conducted on a sample of patches. The patches were made into three pieces each trying to measure 4cm2 in space, and each part was evaluated for the presence of drug. The patch as a whole was used in this test

In vitro diffusion studies

The drug diffusion from the patch was studied using a Franz permeation cell. A patch formulation with a surface area of 2 cm^2 was applied evenly to the filter membrane, and the segment was clamped after the patch formulation was applied. The receptor compartment consists of 7.4 pH phosphate buffered saline along with a magnetic bead. Throughout the experiment, the specimens were withdrawn and replaced to buffer at regular intervals, and the results were recorded. Spectrophotometric analysis was performed on the samples in order to determine their drug concentration.

Ex vivo permeation study

The permeability of 3.14 cm^2 films exvivo using an altered Keshary–Chien cell (capacity, 100 mL). The experiment was carried out on 6 male guinea pigs (Hartley strain) weighing 250–300 g and weighing 250–300 g. During their stay, they have been managed to keep in an animal house with good lighting and temperature 27 degrees Celsius. A standard laboratory diet, as well as unrestricted access to drinking water, were provided to the subjects. Guinea pigs have been killed by the process of cervical dislocation and dorsal skin removal is done. (Lei W, Yu C et al., 2013)

Cumulative amount of drug (Q_{24})

Q_{24} is during a 24-hour period, the cumulative amount of drug released for ex-vivo drug diffusion studies.

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Flux (Jss)

Flux is usually used to assess molecule mass transport in solutions or molecular transport across physical barriers. For this definition, flux is the mass or number of molecules passing through a given cross-sectional region in a given moment.

$$Jss = \frac{dM}{s}dt$$

dM-amount of drug permeated

S-unit cross-section area

t -time (t).

Permeability coefficient

It is calculated by dividing the flux by the donor concentration over a given period of time in a given space of time. (Jain S.et al., 2005)

$$K_P = \frac{Jss}{Co}$$

Jss=steady state flux (µg/cm²/min)

Co=drug donor concentration (mg/cm³)

Lag Time (hrs)

The value of the x-intercept is used to calculate the lag time.

Pharmacokinetic study (2014)

All animal research protocols were approved by the CPCSEA and the IAEC in a joint letter. No , all the experiments were done in accordance with the CPCSEA's guidelines.

Male Wistar rats with weight 200-250g were selected for the study. The animals were fed with rodent diet and were given unlimited access to fresh water. The animals received 7 days to adjust to their new environment. The animals were fasted and granted free water access for the duration of the study.

Skin irritation studies

The skin sensitization test was performed in compliance with the Guidelines provided, with some adjustments made in connection with the Banerjee method.

To determine the effectiveness of various patch formulations, the Guinea pig will be exposed to an irritancy test on to its skin. The Guinea pigs have been categorized into 3 groups, which each contained six animals, and were then placed in a cage. The hair on the dorsal body surface was removed with the help of a depilatory device.(Omar M, Hasan O et al., 2019).

Stability Studies

For six months, the formulation was subjected to accelerated stability studies in accordance with the International Conference on Harmonization (ICH) guidelines (40°C/2°C/75 percent

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RH/5 percent). A variety of parameters, including morphology, drug leakage, and drug entrapment, were assessed (Marwah H, Garg T et al., 2016).

Results and discussion

Screening of edge activators:

Based on the deposition of thin film in RBF a preliminary screening of edge activators were done. Edge activators like Span 20, Span 40, Span 80, Tween 40, Tween 80 and Labrosol were taken for study. Span 80, Tween 80 and Labrosol were selected and proceeded for further screening depending on the vesicle Size and entrapment efficiency.

The nano vesicles were prepared based on the method mentioned and evaluated for Size and entrapment efficiency

Table 1 Formulation of Lisinopril Dihydrate transferosomes using various edge activators

Formulation code	S:S-80	S: T-80	S: L	Drug (mg)	Solvent (C:M)	Buffer Solution (ml)	% Entrapment efficiency	Size (nm)
LT1	1:1	-	-	50	02:01	5ml	74± 0.05	240±9.2
LT2	2:1	-	-	50	02:01	5ml	69± 0.12	220±6.3
LT3	3:1	-	-	50	02:01	5ml	65± 0.29	198±3.2
LT4	-	1:1	-	50	02:01	5ml	80 ± 0.17	146±4.2
LT5	-	2:1	-	50	02:01	5ml	86± 0.18	138±8.2
LT6	-	3:1	-	50	02:01	5ml	82± 0.26	169±8.2
LT7	-	-	1:1	50	02:01	5ml	80± 0.24	186±6.4
LT8	-	-	2:1	50	02:01	5ml	78± 0.23	193±7.2
LT9	-	-	3:1	50	02:01	5ml	75± 0.09	198±8.2

Where n = 3, Mean ± SD, S – Soya lecithin, S-80 = Span 80, T-80= Tween 80, L= Labrosol, C- Chloroform, M- Methanol.

From the table no.1 it is evident that the nanovesicles formed with the help of Tween 80 (LT4, LT5 & LT6) have shown better entrapment efficiency and better size in comparison with other formulation. From the data it is evident that with increase in the concentration of Phospholipid there is a decrease in entrapment efficiency of the drug. The edge activator Tween80 was found to be more suitable for the transfersomal formulation of the selected drug based on results Hence for further optimization of transfersomal formulation Tween80 is selected.

Optimization of the formulation by Central Composite Design:

The central composite design having two independent variables (X1 andX2) was used to study the effect on dependent variables (Y1, Y2). As per the design (table no.- 2) total 13 runs were generated out of which 9 with unique combination were observed. The formulations were prepared (table No.-3) according to the generated combinations of the design and evaluated for the responses like %entrapment efficiency (Y1) and particle size (Y2) mentioned in table no.- 2. The formulations have shown wide range in dependent

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variables, particle size having range of 140nm-190nm and %entrapment efficiency having 40-92% as shown in table No. 2

Table 2: Optimization of lisinopril dihydrate nanocarriers by central composite design

Formulation code	Coded value		Actual value	
	X1	X2	X1	X2
T-L1	-1	1	10	50
T-L2	0	0	30	30
T-L3	0	+1.41421	30	58
T-L4	+1.41421	0	58	30
T-L5	-1	-1	10	10
T-L6	0	0	30	30
T-L7	0	0	30	30
T-L8	-1.41421	0	2	30
T-L9	0	0	30	30
T-L10	0	0	30	30
T-L11	1	-1	50	10
T-L12	1	1	50	50
T-L13	0	-1.41421	30	2
Independent variables	**Levels**			
	Low (-1)	**High (1)**		
X1- Tween 80 (%w/v)	10	50		
X2-Soya lecithin (%w/v)	20	60		
Dependent variables	Y1- mean size (nm) Y2- percentage entrapment efficiency (%)			

The experimental design has total 9 runs which consists of 4star points, 4 factorial points and 1 centre point, design is analysed by the statistical software Design Expert® 8.0.7.1 (Stat-Ease Inc., USA).

Table 3: Formulation of lisinopril dihydrate transferosomes using central composite design

Formulation code	Tween 80 (%w/v)	Soya lecithin (%w/v)	Solvent (C:M)
T-L1	10	50	2:1
T-L2	30	30	2:1

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T-L3	30	58	2:1
T-L4	**58**	**30**	**2:1**
T-L5	10	10	2:1
T-L6	30	30	2:1
T-L7	30	30	2:1
T-L8	2	30	2:1
T-L9	30	30	2:1
T-L10	30	30	2:1
T-L11	50	10	2:1
T-L12	50	50	2:1
T-L13	30	2	2:1

Table 4: Evaluation of lisinopril dihydrate transferosomes using central composite design

Formulation code	Size (nm)	Zeta potential	PDI	% Entrapment Efficiency
T-L1	173±7.8	-32±6.2	0.1±0.25	60± 0.04
T-L2	168±6.2	-47±7.3	0.3±0.86	40± 0.13
T-L3	168±5.3	-41±8.2	0.2±0.83	58± 0.16
T-L4	**144±4.5**	**-39±8.5**	**0.3±0.51**	**92± 0.24**
T-L5	181±4.8	-51±9.2	0.1±0.09	72± 0.26
T-L6	161±5.6	-43±7.4	0.2±0.6	75± 0.15
T-L7	168±6.8	-40±7.5	0.3±0.45	75± 0.18
T-L8	243±6.7	-34±8.4	0.4±0.82	30± 0.29
T-L9	169±8.3	-34±9.2	0.2±0.84	75± 0.07
T-L10	169±5.7	-40±10.2	0.1±0.45	75± 0.16
T-L11	158±6.8	-37±11	0.1±0.86	53± 0.21
T-L12	158±7.7	-31±8.4	0.2±0.83	53± 0.18
T-L13	153±8.6	-39±6.8	0.2±0.45	64± 0.04

Where n=3

The fitted polynomial equations for the responsesY1 and Y2 to the independent factors

The **Predicted R^2** of 0.8290 is in reasonable agreement with the **Adjusted R^2** of 0.9588; i.e. the difference is less than 0.2. **Adeq Precision** measures the signal to noise ratio. A ratio

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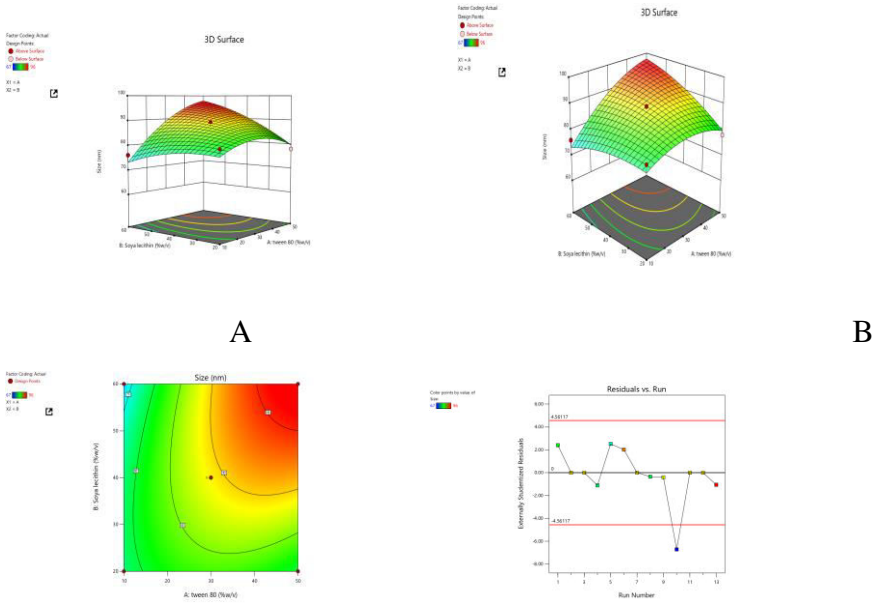
greater than 4 is desirable. Your ratio of 24.841 indicates an adequate signal. This model can be used to navigate the design space.

Equation : R1 = +151.00 -13.30 A -16.99 B +25.75 AB +3.31 A^2 +11.31 B^2

The **Model F-value** of 56.83 implies the model is significant. There is only a 0.01% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case A, B, AB, B^2 are significant model terms. Values greater than 0.1000 indicate the model terms.



A B

C D

Fig-1: Response surface plots showing the effect of edge activators: lecithin on Particle size (A&B) 3D plot (C) Contour plot (D)Residual plot

Equation: R2 = 87.00 +0.7071A-0.2929B+3.00AB+3.25A^2-2.75B^2

The **Model F-value** of 8.86 implies the model is significant. There is only a 0.62% chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case AB, A^2, B^2 are significant model terms.

A B

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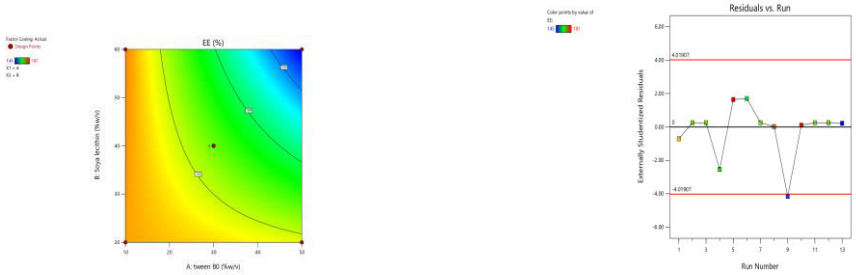


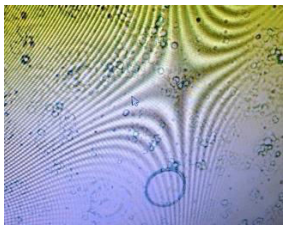
C D

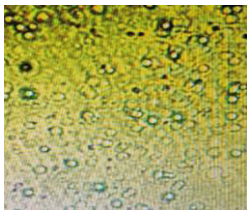
Fig-2: Response surface plots showing the effect of edge activators: lecithin on % Entrapment efficiency (A&B) 3D plot (C) Contour plot (D)Residual plot

The relationship between the dependent and independent variables was further enlisted using response surface plots Fig.1&2. These plots show the interaction between the edge activator and lipid on particle size and %entrapment efficiency. From the graphs it concluded that with increase in the amount of edge activator, the entrapment efficiency has increased. As the drug is hydrophilic in nature more amount of drug is entrapped in edge activator. The particle size of the vesicles formed are also optimum with increase in the edge activator. But after a particular point the increase in the edge activator concentration resulted in decrease in the entrapment efficiency, which is possible due to the coexistence of mixed micelles and vesicles at higher concentration of edge activator.

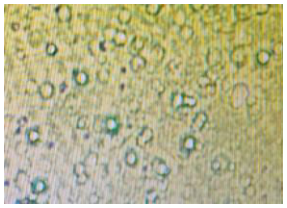
The ANOVA results are shown in table no.15, the f value 56 and 8.6, which is less than the table value (128 and 132) for Y1 and Y2 respectively.

From the data obtained by Central composite design the nano vesicular formulation is optimised and formulation with Tween 80 54(%w/v), lipid i.e Soya lecithin 30%w/v is selected for the further study.





A **B**



C D

Fig-3(A, B, C&D): Fluorescence microscopic evaluation of nanovesicle formulation with Tween 80 54%w/v, lipid 30%w/v

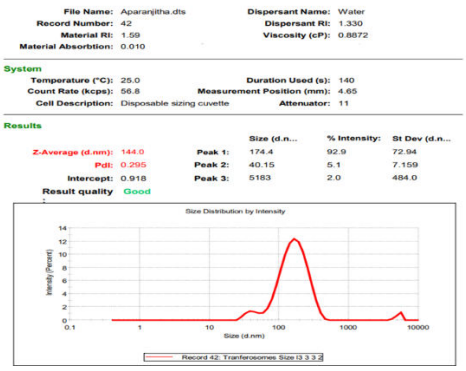


Fig-4: Particle Size determination of Lisinopril Dihydrate nano vesicles with the help of Malvern zetasizer

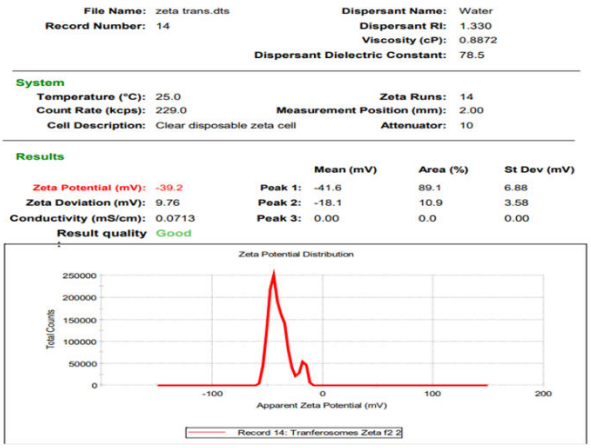


Fig-5: Zeta potential determination of Lisinopril Dihydrate nano vesicles with the help of Malvern zetasizer

Preparation of transdermal patches:

The transdermal patches were prepared using HPMC E15& HPMC E5 of various concentrations. Polyethelenglycol (PEG)- 400 is used as plasticizer and water is used as solvent. Transdermal patches with drug are prepared first shown in Table No. 15.

Table 5: Formulation of transdermal patch with lisinopril dihydrate

Formulation code	HPMC E5 (%w/v)	HPMC E 15 (%w/v)	PEG-400 (%w/v)	Solvent
PL1	1	-	1	water
PL2	2.5	-	1	water
PL3	3	-	1	water
PL4	3.5	-	1	water

PL5	-	1	1	water
PL6	-	1.5	1	water
PL7	-	2	1	water
PL8	-	2.5	1	water

The prepared formulations were evaluated for Weight variation, thickness, folding endurance, moisture absorption and percentage drug release as shown in table no.15. The formulations were prepared using solvent casting method using polymers HPMC E 15&5, along with plastizicer PEG-400 (%w/v) water is used as solvent.

Table 6: Evaluation of transdermal patch with lisinopril dihydrate

Formulation code	Weight(mg) ± SD	Thickness(mm) ± SD	Folding endurance ± SD	Diameter(cm) ± SD	% Drug Release	Moisture absorption (%)
PL1	186± 2.45	0.053 ± 0.01	450 ± 2.52	4.03 ± 0.03	60± 0.13	6.2± 0.52
PL2	168.1± 3.2	0.050 ± 0.02	302± 1.73	4.06 ± 0.02	64± 0.03	6± 0.29
PL3	201.10 ± 2.48	0.048 ± 0.01	304± 2.51	4.10 ± 0.05	67± 0.04	4.8± 0.49
PL4	160.70 ± 2.32	0.047 ± 0.01	300± 1.73	4.03 ± 0.02	72± 0.16	5.1± 0.43
PL5	240.80 ± 2.74	0.054 ± 0.01	308 ± 2.08	4.06 ± 0.02	74± 0.16	4.6± 0.56
PL6	184.00 ± 2.16	0.048 ± 0.01	278 ± 2.64	4.06 ± 0.02	67± 0.28	3.9± 0.65
PL7	178.70 ± 1.34	0.050 ± 0.02	266 ± 2.08	4.08 ± 0.02	62± 0.21	4.9± 0.32
PL8	161.80 ± 2.21	0.050 ± 0.01	253 ± 1.53	4.05 ± 0.04	60± 0.27	4.2± 0.51

Where n=3

From the table No. 6 it can be depicted that the formulations prepared with pure drug has drug release in the range 60-70% invitro through dialysis membrane. The folding endurance is in acceptable range for all the prepared formulations. All the prepared formulations were found to maintain appropriate weight, thickness and diameter. The moisture absorption of the transdermal patches is optimum.

Preparation of transfersomal patches:

The formulations with nanocarriers are prepared using optimised nano formulation i.e., The nano formulation having drug equivalent to 10mg is added to the mixture having HPMCE15/HPMC E5, Plasticizer (PEG-400), Solvent (water) as shown in the table no.4.18. The prepared formulations are subjected to evaluation.

Table 7: Formulation of transdermal patch with lisinopril dihydrate nanocarriers

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Formulation code	HPMC E5 (%w/v)	HPMC E 15 (%w/v)	PEG-400 %w/v	Nano suspension %w/v
TPL1	1	-	1.5	2
TPL2	2.5	-	1.5	2
TPL3	3	-	1.5	2
TPL4	3.5	-	1.5	2
TPL5	-	1	1.5	2
TPL6	-	1.5	1.5	2
TPL7	-	2	1.5	2
TPL8	-	2.5	1.5	2

Evaluation of transfersomal patches:

The evaluation of transfersomal patches were carried out for Folding endurance, weight variation, thickness, moisture content and percentage drug percentage.

Table 8: Evaluation of transdermal patch with lisinopril dihydrate nanocarriers

Formulation code	Weight(mg) ± SD	Thickness(mm) ± SD	Folding endurance ± SD	Diameter(cm) ± SD	% Drug Release (24hrs)	Moisture absorption (%)
TPL1	207± 3.48	0.053 ± 0.5	106 ± 2.52	4.03 ± 0.03	86± 0.05	8.8± 0.52
TPL2	206± 2.48	0.050 ± 0.2	102 ± 1.73	4.06 ± 0.02	89± 0.13	8.4± 0.29
TPL3	**204± 2.48**	**0.048 ± 0.1**	**104± 2.51**	**4.10 ± 0.05**	**93± 0.05**	**6.8± 0.49**
TPL4	195 ± 2.32	0.047 ± 0.2	107 ± 1.73	4.03 ± 0.02	90± 0.12	3.1± 0.43
TPL5	194± 2.74	0.054 ± 0.3	108± 2.08	4.06 ± 0.02	88± 0.27	5.6± 0.56
TPL6	186± 2.16	0.048 ± 0.4	108 ± 2.64	4.06 ± 0.02	82± 0.21	4.6± 0.65
TPL7	193± 1.34	0.050 ± 0.2	106 ± 2.08	4.08 ± 0.02	87± 0.24	4.1± 0.32
TPL8	182± 2.21	0.050 ± 0.4	103± 3.53	4.05 ± 0.04	80± 0.2	4± 0.51

Where n=3

From the evaluation of the transfersomal patches it is evident that the patches prepared with HPMC E5 have shown better drug release (24hrs) when compared to other formulations. The transfersomal patches with increase in the concentration of HPMC E5 the invitro drug release have shown enhancement drug release.

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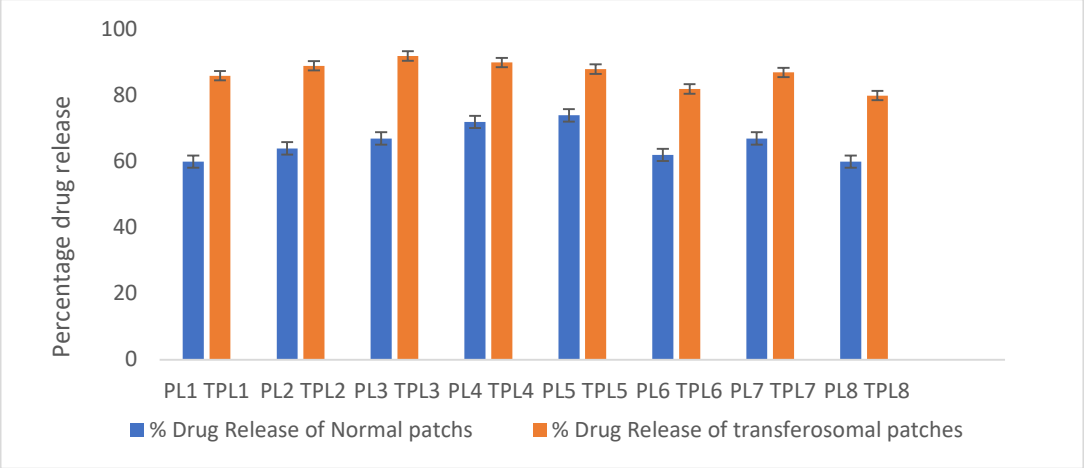

Fig-9 Comparative drug release study of transdermal patches loaded with drug and transfersomes.

When a comparative drug release bar graph as shown in Fig-13 is drawn between the drug release studies of transdermal patches with drug and transfersomal patches. It is evident that there is enhancement in the drug release of transfersomal patches due to the presence of flexible nano vesicles that enhances diffusion.

SKIN PERMEATION STUDY OF TRANSDERMAL PATCH WITH NANOCARRIERS USING PIG SKIN

Transfersomal patches were evaluated for exvivo skin permeation studies using pig skin. The flux, permeation coefficient, cumulative amount of drug permeated for 24hrs, enhancement ratio and lag time (hrs) were calculated.

Table 9: Skin permeation study of transdermal patch with Lisinopril Dihydrate nanocarriers using pig skin

Formulation code	Q_{24}(µg/cm^2)	Flux(µg/cm^2/hr)	Permeation Coefficient (cm/hr) Kpx10^3	Enhancement Ratio	Lag time(hr)
CONTROL	432±0.43	12±0.65	3±0.5	-	0.7±0.64
TPL1	722± 0.82	39± 0.68	9.5± 1.4	2.4	0.2± 0.6
TPL2	718± 0.87	38± 0.87	9± 2.2	2.2	0.2± 0.6
TPL3	**736± 0.38**	**40± 0.87**	**9± 2.8**	3	**0.2± 0.4**
TPL4	720± 0.47	39± 0.76	8.2± 2.4	2.3	0.3± 0.6
TPL5	657± 0.56	32± 0.39	8± 2.6	1.7	0.3± 0.4
TPL6	657± 0.6	33± 0.34	9± 4.2	1.7	0.3± 0.3
TPL7	697± 0.9	31± 0.67	7± 3.4	1.6	0.3± 0.8

TPL8	640± 0.48	36± 0.3	7± 3.8	1.9	0.4± 0.4

Where n=3, Control patch - HPMC E15 1%W/V, 1% PEG-400 & pure drug

The results were analysed, from the table no. 4.19 it can inferred that all the transfersomal formulations have shown better permeation properties, when compared with all the formulations the patches with HPMC E5 of 2.5% with plasticizer 1.5% along with nano formulation have shown better permeation results having flux- 39± 0.87 (µg/cm^2/hr), Permeation Coefficient (cm/hr)(Kpx10^3)-9, Enhancement Ratio- 3, Lag time(hr)-0.5 and Q_{24}(µg/cm^2)- 736± 0.38.

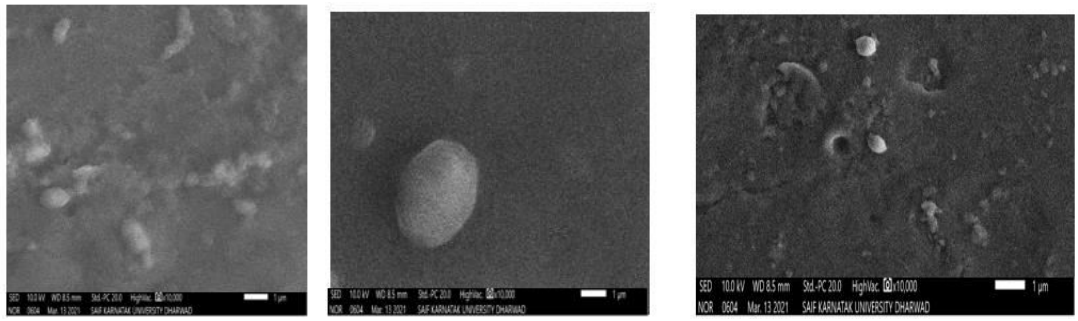

Fig – 5 Scanning electron microscopic analysis of Optimized patch

Table 10: model dependent kinetics of Lisinopril Dihydrate transferosomal patches

Formulation code	Zero order	First order	Higuchi	Korsemeyer-Peppa's equation		Diffusion mechanism
	R^2	R^2	R^2	R^2	n	
Control	0.73	0.88	0.78	0.85	0.4	Fickian diffusion
TPL1	0.84	0.765	0.889	0.889	0.7	Non-Fickian diffusion
TPL2	0.86	0.78	0.881	0.887	0.5	Non-Fickian diffusion
TPL3	**0.92**	**0.75**	**0.903**	**0.95**	**0.6**	**Non-Fickian diffusion**
TPL4	0.91	0.82	0.833	0.93	0.6	Non-Fickian diffusion
TPL5	0.903	0.828	0.852	0.91	0.6	Non-Fickian diffusion
TPL6	0.86	0.864	0.83	0.94	0.7	Non-Fickian diffusion
TPL7	0.89	0.85	0.865	0.89	0.6	Non-Fickian diffusion

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TPL8	0.87	0.86	0.87	0.89	0.5	Non-Fickian diffusion

The model release kinetics were calculated for all the transfersomal formulations. Table no. 20 shows that the formulations are follow zero order kinetics which shows the controlled release of the drug, and n value range from 0.5-0.8 conclude that the release is non-fickian diffusion.

Pharmacokinetic study

The optimised transdermal patches were compared with pharmacokinetic parameters of marketed conventional formulation oral and transdermal patch with drug (normal transdermal patches) in table 11.

Table 11: Pharmacokinetic parameters of optimized Lisinopril Dihydrate transfersomal patch and conventional marketed formulation in rat model

Parameter	Control	Normal transdermal patch (NP)	Transferosomal patch (TP)
T_{max} (h)	1.5	2	4
C_{max} (ng/ml)	64±36.4	78 ±36.4	118 ±33
$AUC_{0\text{-}24h}$ (ng·h·ml^{-1})	125±48.9	132±48.9	288 ±42.5

Where n=6, , Control- Pure drug suspension oral.

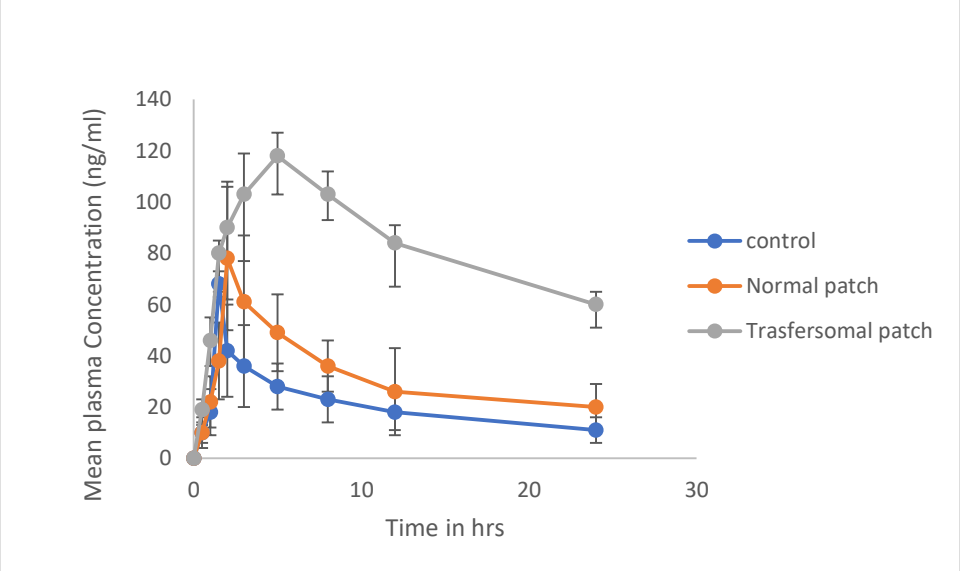

Fig-7 Pharmacokinetic profiles of optimized transfersomal patch with control & Normal transdermal formulation in rabbit plasma, *error bars represent standard deviations of three replicates

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From the table No. 11 it is confirmed that the optimized transfersomal patch has prolonged T_{max}, and C_{max} representing sustained drug release, Fig 4.24 represents that AUC of transfersomal patch is higher than normal patch which indicates the enhancement of bioavailability of drug. This may be due to the presence of flexible nanocarriers that help in better passage of drug through the skin.

it is evident that the drug release when compared with transfersomal patch gave a significant result when compared with the control, marketed and normal patch.

The table 25 reflects the significant C_{max} (ng/ml) and $AUC_{0\text{-}24h}$ ($ng \cdot h \cdot ml^{-1}$) of transfersomal formulation in comparison with control, marketed and normal patch.

(AUC_{TP}/ AUC_{Oral}) AUC of transfersomal formulation was 1.6 folds than oral marketed formulation and 2.1 folds higher than normal transdermal patch (AUC_{TP}/ AUC_{NP}).

SKIN IRRITATION STUDY

Guinea pigs were selected for the study of skin irritation. skin irritation studies were carried out for 14 days and tabulated accordingly (Anroop Nair et al., 2011)

Results revealed that skin irritant produced irritation with minimal erythema and definite erythema, readily visible edema was produced after 12 days. Compared with this both the placebo and optimized batch did not show any type of irritation up to 11 days after that there was less.

Table 14: Stability studies of lisinopril dihydrate transfersomal patch

Stability conditions	Time in days	Folding endurance	Drug content uniformity (%)	(%) drug release through skin	Moisture absorption (%)
Accelerated condition (40 ± 2 C and 75 ± 5% RH)	0	106 ± 2.52	99± 0.26	92± 0.2	4.1± 0.52
	15	102 ± 1.73	97± 0.39	91.6± 0.2	4.2± 0.29
	30	104± 2.51	96± 0.82	91± 0.4	4± 0.49
	60	104 ± 1.73	96± 0.45	91± 0.4	5.4± 0.43
	180	104 ± 2.52	96± 0.48	90± 0.82	6.6± 0.56

Where n=3

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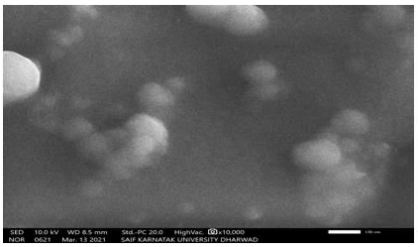

Fig- 6 representing the SEM imaging of optimized transfersomal patch after stability studies.

The stability of the optimized transfersomal patch was conducted using stability chamber following ICH guidelines for accelerated stability studies. The selected patches were evaluated for 0,15,30,60 and 180 days' time points, at each time point the patches were evaluated for folding endurance, drug uniformity, drug release through skin and moisture absorption. The patches have shown very minute changes after 6months which are at acceptable range.

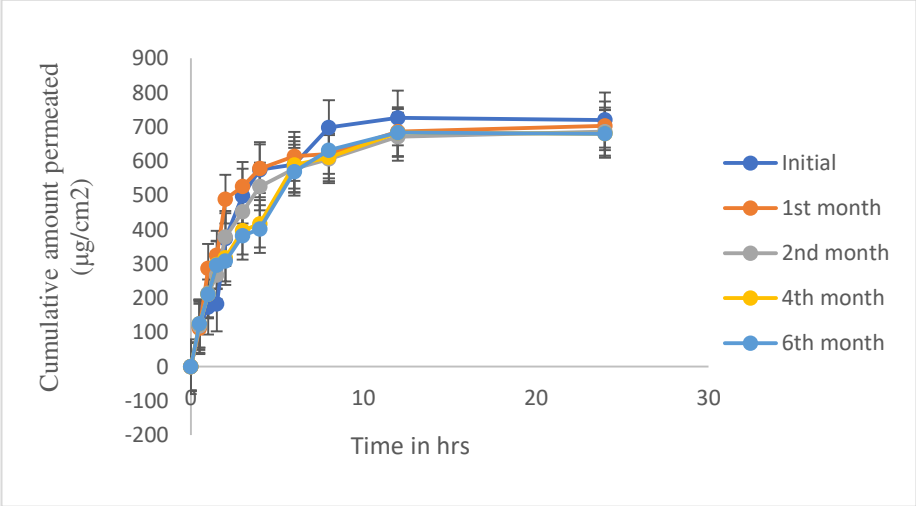

Fig-7 Graphical representation of cumulative amount of drug permeated at different time point in stability studies *error bars represent standard deviations of three replicates

The cumulative amount of drug permeated is tabulated in table 30, the results obtained are satisfactory and the optimized transdermal patches have shown better stability.

Conclusion:

The transfersomal lisinopril transdermal patches were prepared and evaluated. The nanocarriers were prepared by rotary evaporation method using various concentrations of edge activators. It was found that tween-80 as edge activator with soya lecithin is suitable for the preparation of nanocarriers for lisinopril dihydrate. The prepared nanocarriers were evaluated for Size, entrapment efficiency, Zeta potential and PDI. The selected formulations were optimized using DOE (central composite design) design expert software. The optimized formulation TL-4 (58%w/v tween 80 with 30% soya lecithin) have shown particle size 144±4.5nm, Zeta- -39±8.5, %Entrapment efficiency with 92%.

The optimized formulation was incorporated into transdermal patches prepared using various concentrations of HPMC E5 & HPMC E15, along with plasticizer PEG-400. The

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prepared patches were evaluated for invitro, Ex-vivo and in vivo drug release studies. The invitro drug release studies indicate that the prepared patches have better drug release when compared to normal transdermal patches. The Ex-vivo permeation studies of optimized transfersomal patch(HPMC E5 2%w/v, 1.5%w/v PEG-400, 2% nano suspension i.e., 58%w/v tween 80 with 30% soya lecithin) have flux- 44± 0.87 (µg/cm^2/hr), Permeation Coefficient (cm/hr)(Kpx10^3)-9, Enhancement Ratio- 3, Lag time(hr)-0.5 and Q_{24}(µg/cm^2)- 736± 0.38. The optimized patches follow zero order kinetics type of drug release, having non-fickian diffusion in Korsemeyer peppas model with n value < 0.8.

In rat trials, the improved patches were compared to a drug solution, a commercial oral formulation, and a standard transdermal patch. Cmax, Tmax, and AUC were investigated. The pharmacokinetic properties of the transfersomal patches are better, with longer Cmax and Tmax suggesting sustained drug release and a greater AUC indicating enhanced drug availability. In compared to commercial formulations and normal transfersomal patches, the relative bioavailability of the transfersomal patch has been improved. The skin irritation caused by the transfersomal patches was minimal, and they were proven to be stable.

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