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Formulation And In Vitro Evaluation of Transferosomal Patches for Enhanced Drug Delivery of Lisinopril Dihydrate

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Abstract: **Lisinopril dihydrate is an angiotensin converting enzyme inhibitor. The oral bioavailability of the drug is 6-60%, to overcome the destitute bioavailability of the drug, transferosomal patches for transdermal application were planned to prepared. Lisinopril was encapsulated into vesicle by film hydration technique using different surfactants and characterized for morphology of transferosomes, particle size, entrapment efficiency and drug release. The optimized transferosomal suspension was fabricated into transdermal patch by casting of solvent method and in vitro evaluation is performed. Based on the studies it was inferred that the P4 formulation having (HPMC E15: PVP – 2:1) with PEG which has transferosomal formulation having soya lecithin and tween 80 was found to be better formulation in comparison with the other preparations. The amount of drug released from the formulation was 84%. Based on the permeation studies the flux was found to be 26.72±0.23 (µg/cm^2/hr) and cumulative amount of the drug permeated was found to be (Q_{24}) 751±0.94 µg/cm^2. The formulation prepared was found to have better bioavailability.**

Index terms: **transferosomes, transdermal patch, Lisinopril dihydrate.**

I. INTRODUCTION

Majority of the disease like asthma, diabetes and hypertension are caused due to lifestyle, the concentration of drug in the body needs to be maintained within the therapeutic level for prolonged periods. To prolong the concentration of drug and to overcome the disadvantages of the oral drug delivery there is need for alternate route of administration. Skin has shown promising route for delivery of drugs in recent times. surface area and ease of administration makes it best alternate choice for drug delivery. The major drawback of transdermal drug delivery systems (TTDs) is it has less permeability for hydrophilic drugs and macromolecules fine difficult to permeate the skin. Many different types of methods have been developed to overcome this problem. The stratum corneum of the skin is the rate limiting membrane that allows only selected drug to permeate through the skin. Hence to overcome this problem trasferosomes were planned to formulate and to fabricate into a transdermal patch. (A. P. Devi et al, 2003)

Cevc's group introduced trasferosomes, they are prepared from phospholipids and surfactants (edge activators). Surfactant is mostly a single-chain surfactant with a greater radius of curvature which destabilizes the lipid bilayers and enhances the deformability of the Vesicle. Surfactants were used as edge activators. In comparison with subcutaneous administration, trasferosomes have shown enhanced skin permeation of many drugs and effectively transferred adequate amounts of drug. (Liu Y et al, 2018)

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 transferosomal transdermal patches were formulated. (Pandey S et al, 2009)

II. MATERIALS AND METHODS

Lisinopril dihydrate was supplied by Lupin Laboratories Ltd India. Soyalecithin, Span 80 and Tween 80, HPMC E 15 were

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procured from SD fine Chemical, Mumbai, India. Other reagents and chemicals were of analytical grade.

A. Preparation of lisinopril dihydrate loaded transfersomes

Soya lecithin along with surfactants tween80/span 80 was placed in a round bottomed flask. The solvent system is then added to the mixture and the ingredients were dissolved in the solvent (Chloroform: methanol) by hand shaking (Kumar A et al, 2012). The flask was attached to a rotary evaporator and immersed in water bath maintained at 60ºC, rotated with 100rpm for 45min. Formation of thin film at the bottom was observed. The thin film is hydrated using 6.8pH buffer. The resultant solution was sonicated in ultra sonicator for 10mins. (Omar M et al, 2019).

B. Characterization of trasferosomes

Characterization of transferosomal formulations:

Determination of vesicle size, poly dispersity index and zeta potential the vesicle size, PDI and zeta potential of the prepared trasferosomes were determined based on laser diffraction using the Malvern Master seizer by diluting the sample using water as dispersant.

*Entrapment efficiency***:** The percentage of drug entrapped in the transferosomal suspension was determined by disrupting the vesicles

Trasferosomes containing drug was separated from entrapped drug by centrifugation at 14,000 rpm for 30 min. The supernatant was filtered and assayed. (Nastiti CMRR et al, 2020)

C. Drug content determination

The amount of drug contained in the transferosomal suspension was determined by dissolving 100 ml of the formulation in 10ml of ethanol. Analysis of the mixture was done. (Guyot M et al, 2000)

D. Preparation of transdermal patch

The prepared transferosomal formulations were incorporated into transdermal patch by solvent casting method using aluminium foil as a backing membrane. The optimum ratio of HPMC and PVA (1:1) were taken (Lei W, et al 2013). Glycerol was added as plasticizer to the formulation.

E. Characterizations of transfersomal patch:

All the transdermal patches were visually inspected for flexibility, clarity and smoothness.

Uniformity of weight: About five patches of individual batch were weighed and calculation of the average weight was done. (El Maghraby et al 2001).

Moisture content: The weighed was patch and placed in desiccators having calcium chloride and dried for at least 24 h. The moisture content was the difference of the initial weight taken and constant weight was reported in terms of percentage (by weight) moisture content (Ali MF et al, 2015).

Thickness: Thickness of patch was measured using Vernier calipers. Thickness was determined by taking three different positions and average was calculated. The uniformity of the patch reflects the accuracy of the dose in each patch.

Folding endurance: Measurement of folding endurance was done manually. A part of patch (2 × 2 cm2) was cut and folded at the one place till it breaks. The brittleness of the patch was determined by the number of times the patch folded at the same place. (El Maghraby G.M et al 2000).

F. In vitro dissolution studies:

A vertical type of the Franz Diffusion cell was used for permeation study. The receptor compartment having diffusion area of 2.303 cm^2 and 22.5 ml phosphate buffer at pH 6.8 as the receptor fluid stirred at 100 rpm, and was maintained at 37 ± 0.5°C throughout the experiments (Guo F et al 2015). A semi permeable membrane was used for the study (**Stat-M®)** .The amount of the drug permeated was estimated by plotting cumulative amount permeated against time.

G. Stability studies

The formulation was subjected to accelerated stability studies as per ICH guidelines (40ºC ± 2ºC/75% RH ± 5% for 6 months). Parameters like morphology, drug leakage, and drug entrapment were evaluated (Honeywell-Nguyen P.L et al, 2003).

III. RESULTS AND DISCUSSION

Characterization of the drug: Lisinopril was identified and characterized as per official compendia. The drug purity was determined by IR spectra and melting point was found to be 146ºC. The partition coefficient of lisinopril in n-octanol: pH 7.4 phosphate buffer was found to be 1.62±0.16 (Jain S et al, 2003).

Different formulations of trasferosomes were prepared by using tween 80 and span 80 with soy a lecithin. The prepared nano vesicles were formulated and evaluated (Marwah H et al 2016).

Table 1: *Formulation of trasferosomes*

Formulation	Soya lecithin: Tween 80	Soya lecithin: Span 80	Solvent Chloroform : methanol
F1	3:1	-	2:1
F2	1:2	-	2:1
F3	2:1	-	2:1
F4	3:1	-	2:1
F6	-	3:1	2:1
F7	-	1:2	2:1
F8	-	2:1	2:1
F9	-	1:1	2:1

Table 2: *Evaluation of transferosomal formulations*

Formulation code	PDI	Particle size(nm)	% Drug loaded
F1	0.62	200	54.2
F2	0.43	389	62.4
F3	0.54	400	63.1
F4	0.61	142	72.2
F5	0.32	142	53.4
F6	0.71	186	49.3
F7	0.48	380	55.8
F8	0.46	233	69.1
F9	0.52	450	69.8

From the table 2 it is evident that all the formulations prepared are in nano rage and based on the drug loading the optimum formulation having 200mg of soyalecithin and 80mg of tween80 was found to have better properties compared to the other formulations. (Zeb A et al, 2016)

Fig. 1: *Particle size analysis of trasferosomes*

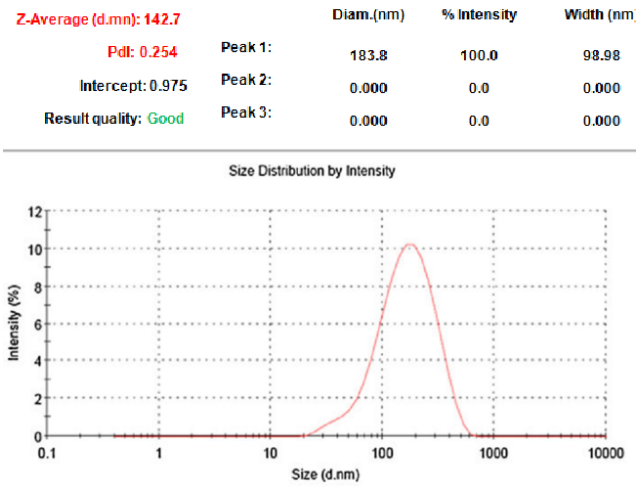


Fig. 2: *Zeta potential of trasferosomes*

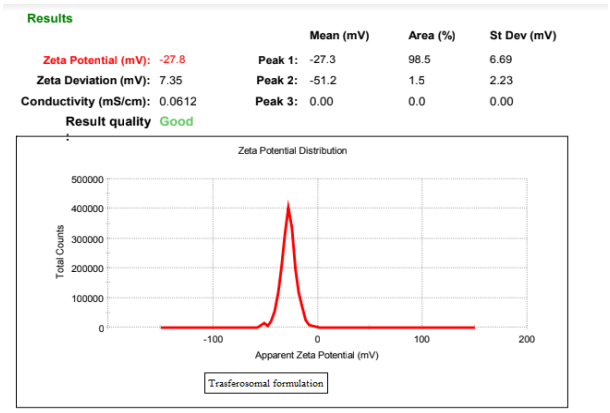
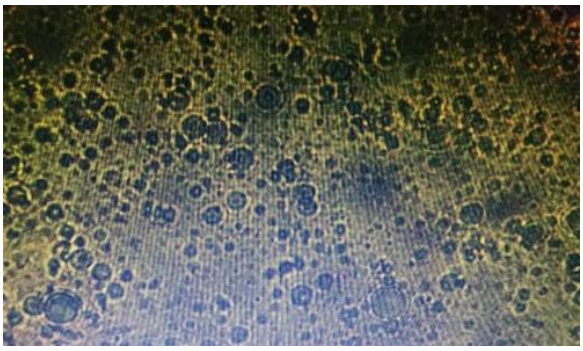


Fig. 3: *Fluorescence microscopic analysis of trasferosomes*



From Fig 3 it is inferred that the tranferosomes thus formed are spherical and bilayered

A. Formulation of transferosomal transdermal patch:

The patches were prepared using HPMC E15and polyvinylpyrrolidone (PVP), solvent and Propylene glycol (PEG) or glycerine (Ahad A et al, 2012). The transferosomal suspension (5ml) with optimum drug loading capacity was chosen and mixed with the above mixture. The prepared patches were subjected to evaluation and drug release.

Table 3: *Formulation of transdermal patches*

Formulation code	Polymer ratio HPMC E15:PVP	Plasticizer (30%W/W)	Solvent
P1	1: 2	Glycerin	water
P2	1:3	Glycerin	Water
P3	1:1	PEG	Water
P4	2:1	PEG	Water
P5	3:1	PEG	water

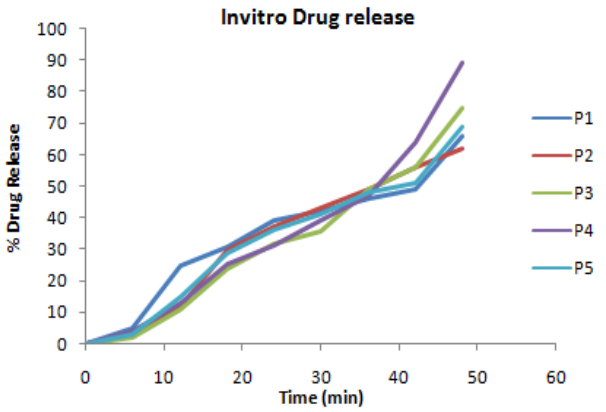
The prepared transdermal patches were evaluated for optimization

Table 4: *Evaluation of transdermal patch*

Formulation Code	Weight uniformity (mg)	Folding Endurance	Thickness (µm)	Moisture loss (%)
P 1	20.029	> 150	170.4	0.531
P 2	24.311	> 150	176.3	0.544
P 3	24.601	> 150	181.6	0.571
P 4	19.913	> 150	138	0.681
P 5	20.259	> 150	148.9	0.696

Sample size, n=3

Fig 4: *In vitro drug release studies*



From the evaluation tests it is found that all the formulation prepared have better patch characters and P4 formulation (HPMC E15 : PVP – 2:1) with PEG400 has good drug release properties when compared with other formulations. The prepared formulations were subjected to model dependent kinetics (Shaji J et al, 2014).

B. In vitro permeation studies:

The data of drug release from various formulations were evaluated using Equations.

Table 5: *Model dependent kinetics*

Model	Equation	Plot of graph	Parameters
Zero order	$Q_t = Q_0 + K_0 t$	% drug release versus time	K_0– release rate constant
First order	$\ln Q_t = \ln Q_0 + K_1 t$	Log % drug release versus time	K_1 – release rate constant
Higuchi release	$Q_t = K_H t^{1/2}$	% drug release versus square root of time	K_H– Higuchi constant
Korsmeyer-peppas	$Q_t Q_\infty = K_K t^n$	Log % drug release versus log time	n– release exponent

Table 6: *Permeation parameters of the prepared trasndermal patches*

Formulation Code	Q_{24}(µg/cm²)	Flux(µg/cm²/hr)	Permeation coefficient (cm/hr)	Enhancement ratio	Lag time (hrs)
Control	108.3± 1.5	4.96±0.04	0.069±0.2	1± 0.6	0.7± 0.9
P1	425±0.4	19.6±0.6	1.75±0.4	1.6 ±0.6	0.5± 0.9
P2	422±1.2	23.4±0.8	2.3±0.3	2.2 ± 1.6	0.6± 0.7
P3	540±0.8	22.24±0.5	2.5±0.2	1.1± 1.2	0.6± 0.6
P4	551±0.9	24.72±0.3	2.3±0.6	2.3 ± 1.01	0.5± 0.9
P5	456±0.3	20.40±0.5	2.6±0.1	2.3± 0.54	0.4± 0.6

[Note: Q_{24} - Cumulative amount permeated; all values are expressed as n=3]

Table 7: *Model dependent kinetics data for the transdermal patch*

Formulation codes	Zero order	First order	Higuchi Model	Korsmeyer-Peppas	Diffusional exponent (*n*)
P1	0.9232	0.6056	0.9340	0.9242	1.1855
P2	0.9941	0.7915	0.8524	0.9973	0.9937
P3	0.9941	0.8225	0.6798	0.9895	0.9827
P4	0.9974	0.7614	0.7863	0.9901	0.9353
P5	0.9982	0.7094	0.7224	0.9866	1.1723

From table no.6 it is inferred that all the prepared formulations follow zero order rate kinetics, and all the formulations follow Non-Fickian diffusion which was evident from the n value

C. Stability studies:

The studies were conducted for one month at 30^0C and ambient humidity. There was no degradation of the patch and drug content was uniform and there were no major changes in drug release. (Shaji J et al, 2014).

CONCLUSION

The transferosomal transdermal patch was prepared and evaluated. Based on the studies it was inferred that the P4 formulation having (HPMC E15 : PVP – 2:1) with PEG 400 which has transferosomal formulation having 200mg of soya lecithin and 80mg of tween80 was found to be better formulation in comparison with the other preparations. The drug release of the formulation was found to be 84% which is more better when compared to oral bioavailability of the formulation. Based on the permeation studies the flux was found to be 26.72±0.23 (µg/cm^2/hr) and cumulative amount of the dug permeated was found to be (Q_{24}) 751±0.94 µg/cm$^{2.}$. Further in vivo studies should be carried out to predict the pharmacokinetic parameters of the formulation.

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