Available online on 15.04.2019 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

Formulation and evaluation of topical Calcineurin inhibitor loaded transfersomal drug delivery for Vitiligo

Patel Dipti*, Sherasiya Zabir, Patel Kajal

Department of Pharmaceutics, Parul Institute of Pharmacy & Research, Parul University, Vadodara, Gujarat, India-391760

ABSTRACT

The aim of the present investigation was to formulate and evaluate topical calcineurin inhibitor loaded transfersomal drug delivery for vitiligo using rota evaporator followed by thin film method. Tacrolimus can restore the melanocytes if given in transfersomal gel. Fourier transform infrared spectroscopy (FTIR) had employed to study drug-excipients incompatibility. Analytical method was performed using UV spectrophotometer. Topical calcineurin inhibitor loaded transfersomal gel was evaluated for particle size, zeta potential, percent drug entrapment, surface morphology, in-vitro drug release study, in vitro permeability study and stability study. Optimization of process parameter was done by 3^2 full factorial Design Expert software. Topical calcineurin inhibitor was successfully prepared with drug:lipid(1:10), lipid:surfactant(9:1), water as hydration medium, chloroform:methanol(9:1) as solvent, HPMCK100 as mucoadhesive agent and extract of catechu powder to provide colour on skin. Optimization study of process parameter shows that batch prepared with hydration time 55 min,evaporation time 15 min, hydration temperature is 50°C and temperature to form thin film is 60°C as optimum condition for rota evaporator. Particle size, zeta potential, percent drug entrapment were found to be 155.5 nm, -49 mV, 80% respectively for optimized batch.

Keywords: Vitiligo, Tacrolimus, Transfersomal gel, Thin film hydration method, Vesicle size, PDE, Zeta-potential, Skin irritation study, Skin sustain study, Stability study.

Article Info: Received 17 Feb 2019; Review Completed 22 March 2019; Accepted 24 March 2019; Available online 15 April 2019

Cite this article as:

Patel D, Sherasiya Z, Patel K, Formulation and evaluation of topical Calcineurin inhibitor loaded transfersomal drug delivery for Vitiligo, Journal of Drug Delivery and Therapeutics. 2019; 9(2-s):88-91
http://dx.doi.org/10.22270/jddt.v9i2-s.2467

***Address for Correspondence:**

Patel Dipti, Department of Pharmaceutics, Parul Institute of Pharmacy & Research, Parul University, Vadodara, Gujarat, India-391760

INTRODUCTION

Vitiligo is an autoimmune disorder observing with progressive loss of skin pigmentation. Vitiligo is a cutaneous disease in which the melanocytes are damaged in discrete patches, resulting in lightened areas of variable size and location distributed throughout the skin of the body. Melanocytes are cells located in the stratum basale (bottom layer) of the skin's epidermis. The lightened lesions of the skin are immune-compromised and generally have greater susceptibility to the injurious effects of the sun, premature aging and possible cancer of the skin. The disease strikes about 1% of the world population, generally during adolescent years. The progressive loss of melanocytes from depigmenting vitiligo skin is accompanied by cellular infiltrates containing T lymphocytes. Infiltrating cytotoxic T cells with elevated similarity T cell receptors have likely escaped clonal removal in the thymus, allowing such T cells to enter the flow It is thought that through the appearance of cutaneous lymphocyte antigen, these T cells home to the skin where they express type 1cytokine profiles and mediate melanocyte apoptosis via the perforin pathway. As this state affects the skin and is readily visible to the public eye, there are many psychological and social trouble that can result[1,2].

Tacrolimus is first new topical drug for the treatment of moderate to severe vitiligo since the advent of topical corticosteroids. The mechanism of action of tacrolimus in vitiligo is not known. It has been demonstrated that tacrolimus inhibit T-limphocytes activation by first binding to an intracellular protein, FKBP-12. Tacrolimus is found to be effective in skin treating vitiligo with main side effect burning and irritant on skin.

Transfersomes are ultra-deformable vesicles, elastic in nature, which can squeeze itself through a pore which is many times smaller than its size due to its elasticity. Transfersome have been reported to enhance the transdermal delivery of drugs, when applied onto the skin non- occlusively biocompatible, biodegradable, posses high entrapment efficiency, act as depot so releasing their contents slowly and gradually, ability to deform and pass through narrow constriction, can be used for systemic as well as topical drug delivery. Apart from their benefits, transfersomes are associated with some drawbacks such as: chemical instability, expensive to formulate and purity of natural phospholipids.

MATERIALS AND METHODS

Tacrolimus was obtained as a gift sample from bills biotech. vadodara, phospholipids Soya phosphatidyl choline obtain from chemdyes corporation, vadodara, surfactant sodium cholate obtain from chemdyes corporation, vadodara, Muco-adhesive HPMCK100M obtained from chemdyes corporation, vadodara.

METHOD[3, 4, 5]

Method of Preparation Transfersomes:

Thin film hydration (TFH) method: The general elements of procedure involve preparation of lipid for hydration, hydration of lipid film, sizing of lipid suspension.

1. Preparation of Lipid for Hydration:

In this method, lipid mixture are dissolve in mixture of organic solvent in rotary evaporator flask and dry thin film of lipid is prepare using rotary evaporator.

2. Hydration of Lipid Film:

Flask is flushed with nitrogen and hydration of lipid is done by adding 10 ml of distilled water to be encapsulated and again use of rotary evaporator for making homogeneous milky white suspension. It is allowed to stand for 50 min at RT/above Tg (glass transition temperature) for complete swelling process. This process will finally produce the multi lamellar vesicles. Method of preparation of transferosomes by thin film method show in figure 1.

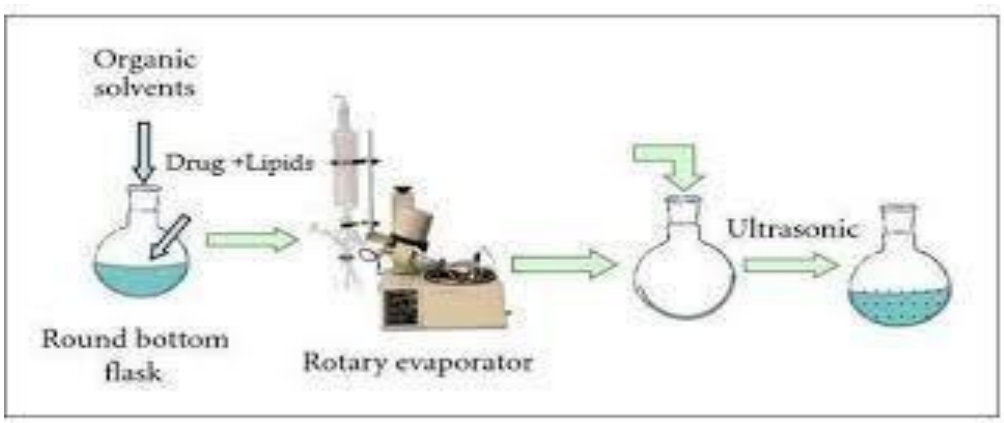

Figure 1: Method of Preparation of Transfersomes by thin film method

3. Sizing of Lipid Suspension:

Disruption of MLV suspension using sonic energy (probe sonication) typically produces small, unilamellar vesicle with diameter in the range of 15-50 nm. The most common instrumentation for preparation of sonicated particle is bath and probe tip sonicators. Probe tip sornicator deliver high energy input to the lipid suspension for these reasons, probe tip sonicator are the widely used instrumentation for preparation of SUV.

Transfersomal gel:

Transfersomal gel formulation to be prepared by incorporation of transfersomal into a structure vehicle such as HPMCK100 or carbopol polymer. Even though transfersomes offers several advantages for topical delivery, it is difficult to stabilize the system because of low viscosity. This problem can be overcome by formulating transfersomal based hydrogel using polymer such as various grade of hydroxypropyl methylcellulose (HPMC).

Thin film hydration method:[6]

Thin film hydration technique was basically used in preparation of Transfersomes. A thin film was prepared from the mixture of vesicles forming ingredients that is phospholipids and surfactant by dissolving in volatile organic solvent Chloroform: Methanol (8:2) which was transferred to RBF for 15 min at 80 rpm under 350mm Hg and at 65°C .The film was hydrated using 20 ml distilled water for period of 55 min at 50°C and 65°C temperature. The resultant transfersomal dispersion was sonicated for 3 min. Resultant transfersomal dispersion centrifuge at 3000 rpm for 15 min to remove free drug. Prepared transfersomes were evaluated for percentage drug entrapment, vesicle size and zeta potential measurement. Preliminary trial batches were prepared using different surfactant such as sodium cholate, tween 80 and span 60.Trial batches for preparation of transfersomes are shown in table 1. Result of preliminary trial batches for selection of drug: lipid and lipid: surfactant molar ratio are shown in table 2

Table 1: Preliminary trial batches for selection of drug:lipid and lipid:surfactant molar ratio

S.N.	Batch no	Drug: lipid molar ratio	Lipid: Surfactant molar ratio	SPC(mg)	Surfactant (mg)
1	Sodium choline	1:10	7:3	67.47	15.50
2	Sodium choline	1:20	9:1	41.85	2.58
3	Sodium choline	1:10	9:1	83.70	5.16
1	TWEEN-80	1:10	7:3	67.47	21.77
2	TWEEN-80	1:20	9:1	83.70	7.25
3	TWEEN-80	1:10	9:1	83.70	7.25
1	SPAN-60	1:10	7:3	65.10	15.42
2	SPAN-60	1:20	9:1	83.70	5.14
3	SPAN-60	1:10	9:1	83.70	5.14

Table 2: Preliminary trial batches for selection of surfactants

Sr no	Batch no	Drug: lipid molar ratio	Lipid: Surfactant molar ratio	PDE	Vesicle size (nm)
1	SC	1:10	7:3	71.15	358
2	SC	1:20	9:1	78.55	320
3	**SC**	**1:10**	**9:1**	**82.45**	**270**
4	TWEEN-80	1:10	7:3	35	882
5	TWEEN-80	1:20	9:1	31	375
6	TWEEN-80	1:10	9:1	78.42	364
7	SPAN-60	1:10	7:3	38	347.5
8	SPAN-60	1:20	9:1	44	216
9	SPAN-60	1:10	9:1	68.26	187

(SC-Sodium cholate)

RESULT & DISCUSSION

The objective of the present study was to formulate and evaluate calcineurin inhibitor loaded transfersomal drug delivery for vitiligo by thin film hydration method.

Solubility Study:

Based on solubility study in different solvent, it was found that methanol and chloroform maximum solubility for drug.show in table 3

Viscosity:

Measurement viscosity of gel is important because higher viscosity show difficulty on spreading while lower viscosity shows difficulty in retention of gel on skin. So optimum viscosity of gel was required for higher bioadhesion of gel on skin. The viscosity of transfersomal gel was found to be 54±0.0149 Pas. The results are shown in table 4

Spreadibility:

The potential usefulness as a topical dosage form with desired semisolid consistency was demonstrated by spreadibility values indicating the ease of application on the skin.

The spreadibility of transfersomal gel was found to be 4.69±0.521(g cm/sec) which shown in table 4

pH:

The pH of the developed formulation was in accordance with that of human skin pH rendering them acceptable. The develop formulation had pH near to that of skin.

Drug content uniformity:

Drug content for transfersomal gel and marketed tacrolimus cream was found to be 82.6±0.75 & 72.4±0.59 respectively shown in table 5

***In-Vitro* Drug Diffusion Study:**

Results obtained from in vitro diffusion studies evaluated with transfersomal gel and marketed ointment. The amount of Tacrolimus released in 24 hrs was found to be 82.25% and 72.34% from Marketed tacrolimus ointment and tacrolimus transfersomal gel respectively shown in table 6

Drug release:

The correlation coefficient (r2) of the higuchi model were found to be near to 1(0.972 & 0.968 for transfersomal gel and marketed gel respectively)compare to the first order, Hixson-crowell's cube root model, higuchi model. Hence the release of drug from the preparation followed predominantly zero order model shown in table 7

***Ex-Vivo* Permeation Study:**

Result obtained from *Ex-vivo* drug permeation studies were shown in table 8. Significant augmentation in the permeation of tacrolimus has been observed with transfersomal gel formulation.

Skin irritation study:

Skin irritation study was carried out by using transfersomal gel and marketed ointment which are shown in figure 2

Table 3: Stability data of tacrolimus containg transfersomal gel

Sr no	Parameter	Storage periods(Days) at 25°C±2°C Temperature and 65±5% Relative Humidity		
		Before Storage	After 15 days	After 30 days
1	Viscosity	54±0.0149	53±0.0524	52.5±0.0296
2	pH	5.2	5.2	5.1
3	%CDR	84.72±0.04	84.02±0.09	83.96±0.01

Table 4: Spreadibility and Viscosity

Parameter	Result
Viscosity	54±0.0149(Pa s)
Spreadibility	4.69±0.527(g cm/sec)

Table 5: Drug content uniformity of formulation

Formulation	Drug content
Tacrolimus Transfersomal gel	82.6±0.75
Tacrolimus ointment	72.4±0.59

Table 6: In vitro drug diffusion study of tacrolimus from transfersomal gel and marketed ointment

Time (hr)	Mean cumulative percentage drug release ±SD	
	Transfersomal gel	Marketed ointment
0.5	7.79±0.01	6.3±0.06
1	16.88±0.03	9.8±0.14
2	25.14±0.07	12.4±0.1
4	36.19±0.16	16.56±0.15
6	43.5±0.1	24.73±0.07
8	51.46±0.05	25.26±0.06
10	65.70±0.05	25.76±0.07
12	72.54±0.05	-
24	84.72±0.04	-

Kinetic modeling and mechanism of drug release

Table 7: Result of kinetic model fitted on transfersomal gel

Diffusion model	R^2 Value	
	Transfersomal gel (batch-7)	Marketed ointment
Zero-order plot	0.972	0.968
First –order plot	0.685	0.682
Higuchi plot	0.952	0.949
Hixon plot	0.798	0.789
Peppas plot	0.874	0.868

Table 8: Ex vivo permeability study of tacrolimus from transfersomal gel and marketed ointment

Time (hr)	Transfersomal gel	Marketed ointment
0.5	6.48±0.3	5.33±0.09
1	14.67±0.1	9.75±0.4
2	23.43±0.2	11.53±0.1
4	34.75±0.05	15.49±0.05
6	39.57±0.16	23.75±0.14
8	46.68±0.15	24.15±0.1
10	55.54±0.15	-
12	63.42±0.20	-
24	65.95±0.36	-

%Drug permeated per unit time ± S.D

A) Before B) After 24 hrs

Figure 2: Rat skin irritation study using transfersomal gel containing tacrolimus

ACKNOWLEDGEMENTS

The authors thank bills biotech, vadodara, for providing tacrolimus as gift samples for this work. They also thank Prof. Dr. D. H. Patel, Head of Department and Dr. T. Y. Pasha, Principal, Parul University, Vadodara (INDIA), for providing required facilities to carry out this research work.

CONCLUSION

Aim of research study was to prepare and evaluate topical calcineurin inhibitor loaded transfersomal drug delivery for vitiligo. Category of tacrolimus is an immunosuppressant and mainly used in vitiligo and acne. Skin irritation and burning sensation is main side effect of tacrolimus which can be overcome by incorporating into transfersomal carrier.

REFERENCES

2. Bhowmik D, Gopinath H, Kumar BP, Duraivel S, Kumar KS. Recent advances in novel topical drug delivery system. The Pharma Innovation. 2012; 1(9, Part A):12.
3. Hercogová J, Buggiani G, Prignano F, Lotti T. A rational approach to the treatment of vitiligo and other hypomelanoses. Dermatologic clinics. 2007; 25(3):383-92.
4. Kalyani V, Babu MK, Kishore M. Transferosomes: an emerging tool for effective transdermal drug delivery. J Chem Pharm Sci. 2014; 7:236-41.
5. Som I, Bhatia K, Yasir M. Status of surfactants as penetration enhancers in transdermal drug delivery. Journal of pharmacy & bioallied sciences. 2012; 4(1):2.
6. Lei W, Yu C, Lin H, Zhou X. Development of tacrolimus-loaded transfersomes for deeper skin penetration enhancement and therapeutic effect improvement in vivo. Asian journal of pharmaceutical sciences. 2013; 8(6):336-45.
7. Qushawy M, Nasr A, Abd-Alhaseeb M, Swidan S. Design, optimization and characterization of a transfersomal gel using miconazole nitrate for the treatment of candida skin infections. Pharmaceutics. 2018; 10(1):26.
8. Taglietti M, Hawkins CN, Rao J. Novel topical drug delivery systems and their potential use in acne vulgaris. Skin Therapy Lett. 2008; 13(5):6-8.
9. Garg BJ, Saraswat A, Bhatia A, Katare OP. Topical treatment in vitiligo and the potential uses of new drug delivery systems. Indian Journal of Dermatology, Venereology, and Leprology. 2010; 76(3):231.
10. Orecchia G, Sangalli ME, Gazzaniga A, Giordano F. Topical photochemotherapy of vitiligo with a new khellin formulation: preliminary clinical results. Journal of dermatological treatment. 1998; 9(2):65-9.
11. Varona S, Martín Á, Cocero MJ. Liposomal incorporation of lavandin essential oil by a thin- film hydration method and by particles from gas-saturated solutions. Industrial & Engineering Chemistry Research. 2011; 50(4):2088-97.