NANOSIZED LIPID VESICULAR TOPICAL GEL OF BETAMETHASONE VALERATE FOR THE PSORIASIS TREATMENT

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Abstract

The safe and effective option for the therapeutic management of psoriasis was attempted by preparing transferosomes of Betamethasone valerate (BV) and incorporating into formulation of gel to get more skin permeation, more effectiveness in drug entrapment and sustained release. Batches of experimental design of transferosome containing 100 mg of BV were produced by using Lipova E 80 and Span 20 as surfactant in varying quantities and incorporated in 1.5% HPMC gel base. Formulation was optimized to attain maximum drug entrapment, % drug release, ex-vivo permeation, pH, viscosity, spreadability and extrudability. The size and stability was ascertained by using Zeta sizer and and TEM was utilized to assess the surface morphology. The optimized formulation entrapped 59.87 % of the drug, pH 6.57, the average size of the particle was found to be 108.93 nm and viscosity were 436.19 centipoise.The residual analyses by gas chomatography confirms the elimination of formulation's organic solvent and stability studies were conducted in accordance of the ICH guidelines. The developed formulation showed diffusion of BV through the rat's skin at sustained rate for 10 hrs while marketed preparation sustained for 7 hrs only. It promises of localized action at the affected psoriatic sites for prolonged period with better release characteristics as compared to marketed formulation. The optimized formulation was found to be stable during the period of study.

Key words: Psoriasis, Transferosomes, Betamethsone valerate, Topical gel.

INTRODUCTION

Psoriasis is a chronic, inflammatory condition marked with hyperproliferation of epidermal cells along with disorder of cell differentiation in granular layer resulting in overproduction of skin cells also referred to as hyperkeratosis.[1] Multiple physiologic and immune entities have been discovered to be involved in the pathway that mediates the development of psoriasis: Polymorphonuclear leukocytes, neuropeptides, T-lymphocytes, inflammatory cytokines, interleukins, TNF-alpha cells, etc.[2,3] Psoriatic plaques, or scaly patches caused by psoriasis are areas of inflammation and excessive skin production.[4]

Skin is an important target site for the application of drugs. When treating local diseases, topical drug delivery increases the therapeutic effect on the affected area and reduces systemic side effects. The utmost barrier -stratum corneum (SC) ought to be surpassed in order to reach therapeutic concentration of drug in underlying skin layers.[5] This process is influenced by many reasons, e.g., the drug's physiochemical properties and vehicle utilized for application, condition of the epidermal barrier etc. The various ways to improve the penetration of the drug though the skin include the use of chemical penetration enhancers, novel vehicle systems (e.g. liposomal based delivery systems and supersaturated formulations), Complex physical enhancement strategies (e.g., iontophoresis, sonophoresis and electroporation) etc.[6]

Topical steroids are the utmost commonly prescribed medications in the therapeutic management of the skin disorder.[7] Topical steroids work through three main mechanism-reduce inflammation, decrease mitosis, and by constricting the tiny blood vessels.[8]

Transferosome is very flexible and extremely adaptable, complex aggregate and receptive to stress. Its desired system is an

ultra-deformable vehicle that has an aqueous core and is enclosed by the complex lipid bilayer. Interaction of local configuration and shape of the bilayer makes the vesicle both self-regulating and self-optimizing, which crosses various transport barriers efficiently. It possesses hydrophobic and hydrophilic structural moieties together and hence it can accommodate molecules of drug with a wide range of solubility. It transports the medicament though the skin. Transferosomes squeeze along the stratum corneum's intracellular sealing lipids to reduce the penetration effort through the skin. [9] The carrier aggregate is actually made of phosphatidylcholine that is in aqueous solvents self-assembles into a lipid bilayer and closes into a simple vesicle.[10]

Another mechanism is "osmotic taxis" i.e. Whenever a vesicle is applied to an accessible biological surface, like non-occluded skin, it tends to break through its barrier and penetrate into the deeper water rich layer strata to ensure its adequate hydration.[11]

Hydrocortisone and Dexamethasone were loaded in extremely deformable carriers for vasoconstriction test and the study revealed higher degree of retention in the skin and lower treatment dose per area as compared to commercial preparations.[12] Transferosome of tacrolimus was formulated and assessed for deeper skin penetration and increased the efficacy for atopic dermatitis. The formulation exhibited good entrapment effectiveness, drug release and fast onset of action.[13] This study was then followed by formulation and evaluation of transferosome containing terbinafine, paramomycin sulphate, fluconazole, amphotericin B.

For efficient drug delivery to the skin an archaeosome of BV were formulated and comparison between archaeosome and conventional phospholipid was done.[14] Nanolipid carrier gel loaded with BV exhibited average particle size and entrapment efficacy with 74.55% in vitro drug release.[15] Betamethasone sodium phosphate loaded nanoparticles was formulated and evaluated for Ophthalmic drug delivery and proven that mucoadhesive chitosan-sodium alginate nanoparticles may be employed as a vehicle for the prolonged topical ophthalmic delivery of betamethasone sodium phosphate.[16]

MATERIAL AND METHODS

Betamethsone valerate was received as a gift from Wellona Pharma, based in Surat (Gujarat) India. Isopropyl Alcohol and oleic acid were procured from S.D. Fine chemicals (New Delhi). Propylene glycol mono caprylic ester (Sefsol 218®) received as a gift from Nikko Chemicals Co., Ltd, Japan. PEG 400, Tween 80, Tween 20, and ethanol got from Merck (Merck, India). Caprylo caproyl macrogol-6 glycerides (Labrasol), Plurol Oleique and diethyleneglycol monoethyl ether (Transcutol P) were received as a gift from Gattefosse (Mumbai, India). Phospoholipid LIPOVA E80, Surfactant Span 20, Cholesterol, Chloroform, methanol and all other chemicals were used of analytical grade.

Transferosome preparation:

Transferosomes were formulated by rotary vacuum evaporation method. Drug, Phospoholipid LIPOVA E80, Surfactant Span 20 and Cholesterol were mixed in organic solvent, Chloroform: Methanol (9:1). Organic solvent was evaporated by rotating in round bottom flask at 70-90 rpm for 1 h at the temperature and vacuum pressure of 50ºC and 620 psi respectively. The thin film was deposited which was then hydrated with 10 ml of suitable medium containing drug i.e. PEG: Water. The resulted suspension was then introduced to probe sonicator at amplitude of 60% for 2min to obtain desired size of vesicles. [17, 18, 19]

Evaluation of transferosome:

Vesicle size: Vesicle size was assessed by trinocular microscope at 40x magnification and Zeta sizer.

% Entrapment efficiency: Entrapment of drug was analyzed by centrifugation followed by analysis of unentrapped and entrapped drug by UV spectrophotometry and mass balance was evaluated from the data obtained for % entrapment efficiency.

Study of Surface morphology of Transferosomes: Transferosomes surface characteristic morphology was evaluated by using TEM.

Preparation of transferosomal gel:

Transferosomes (100 mg) of selected batch, were amalgamated in gel base (1.5 % HPMC and then kept for 7 days at room temperature to interpret the stability of gel base. It was observed that HPMC gel is good for incorporating transferosome in terms of pH, viscosity, spreadability, and stability [20].

Evaluation of Transferosome incorporated Gel:

Determination of pH: The pH of gel was checked by digital pH meter at room temperature.

Viscosity studies: Brookfield (LVDV 2) was used to determine the viscosity of gel.

Spreadability: The spreadability of gel was calculated by following formula: S = M.L / T Where, S = Spreadability, M = weight attached to upper slide (g) L = length of spread (cm) T = time taken in (s)

Drug content: 500 mg of the gel was triturated and diluted in 50 ml volumetric flask with methanol and drug content was measured spectrophotometrically at 240 nm using methanol as a blank solution.

In vitro and Ex vivo Drug release studies: In vitro diffusion studies was performed by using Franz diffusion cell and dialysis membrane (himedia) having pore size of 0.45µm. Samples (dorsal side of 5-6 weeks old rat) were mounted on donor

compartment after removing hair from the skin. Then it was clinched between the donor and the receptor chamber of modified diffusion cells with the stratum cornium facing the donor chamber. Then, 1 g of gel having 0.1% (w/w) BV was applied onto the donor chamber. 20 ml of PEG was filled in the receptor chamber: Water (5:4) was used as diffusion media in receptor compartment and the receptor medium was maintained at 37 ± 0.5°C and stirred at 600 rpm thoughout the experiment. Sample aliquots of 5 ml were taken from the receptor at time interval of 1 h. and then the same volume of pure medium was immediately added into the receptor chamber. All samples were analyzed by UV method. The cumulative amount of drug released across the membrane was determined as a function of time.

Residual solvent levels in the optimized formulation of Transferosomes: Residual solvents, chloroform and methanol, in transferosomal suspension were determined by gas chomatography using thermo scientific, DSQ 2 at SICART.

Stability Study According to ICH-Q1A (R2) guidelines, Stability studies of new drug substances and products were done at temperature (40°C ± 2°C) and relative humidity (75% RH ± 5% RH). [21, 22]

RESULTS AND DISCUSSION

Experimental Design-Central composite Design:

The experimental study batches were created with the help of central composite design (CCD) with 2 factors, quantity of Lipova E 80 and Span 20 at 2 levels and batches were assessed for Entrapment efficiency, drug release and vesicle size.

The highest % Drug entrapment of 59.87% was seen in the A2 batch (Table 1).

Table: 1 Results of experimental design batches (A1 to A13)

Batches	% Entrapment efficiency	Drug release after 8 h (%)	Average vesicle size (nm)
A1	47.12	69.79	145.19
A2	59.87	86.43	108.93
A3	58.23	65.74	116.33
A4	56.35	66.69	125.57
A5	49.67	81.57	110.26
A6	53.30	79.66	216.07
A7	42.22	74.20	156.14
A8	43.53	77.61	131.37
A9	47.73	56.75	176.12
A10	45.42	69.67	164.87
A11	46.15	66.53	124.36
A12	48.81	69.15	116.15
A13	56.72	84.97	204.32

Study of Transferosome's particle size and its distribution:

Evaluation of particle size were conducted by Malvern Zeta sizer was 108.9 nm (Figure 1) and Poly dispersity index (PDI) was 0.257 respectively (Figure1).

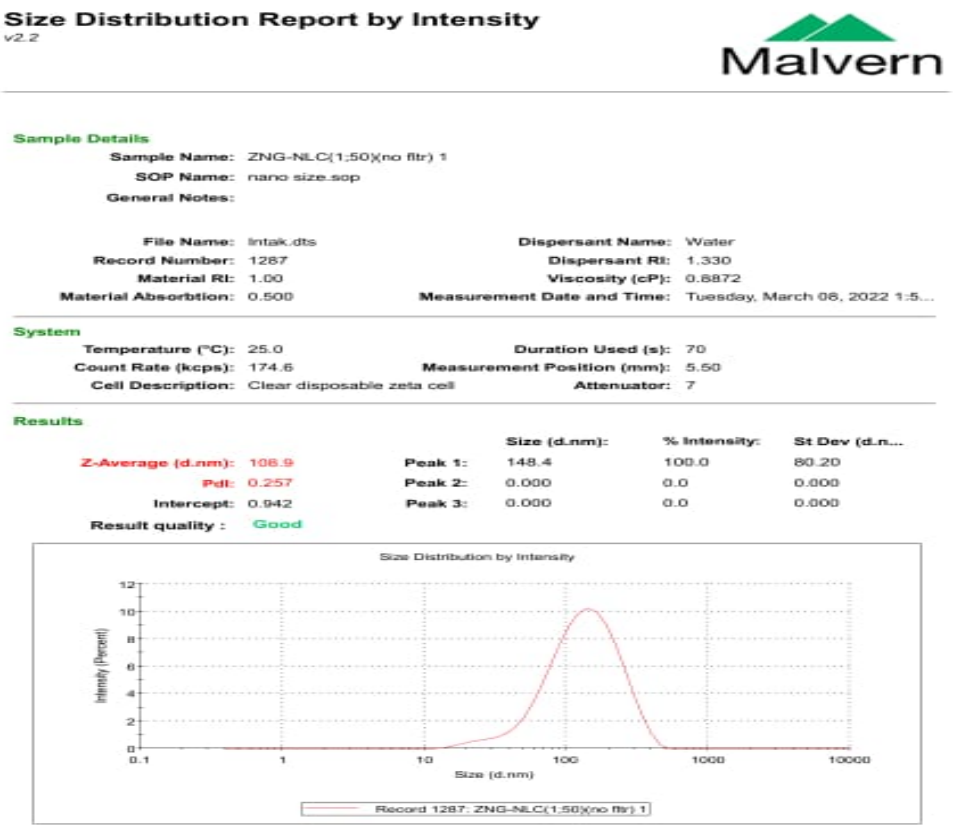

Figure1: Determination of Particle size and PDI by Zeta sizer of optimized formulation A2

Study of Surface morphology of Transferosome: Surface morphology of prepared Transferosome was determined by TEM. Transmission electron microscope images of transferosomal suspension of optimized batch were found to have spherical structure. The size of vesicles were in between 50 to 250 nm (figure 1)

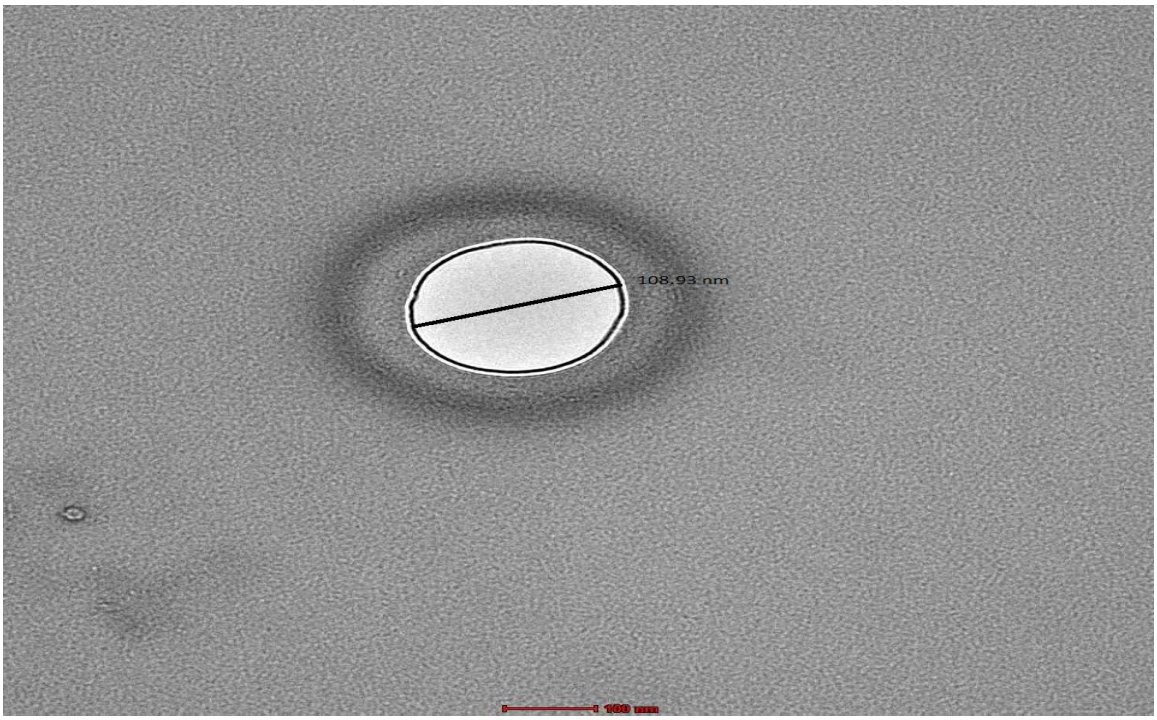

Figure 2: Determination of surface morphology by transmission electron microscopy (TEM) of optimized formula A2.

Incorporation of Transferosome in gel:-

Optimized batch of Transferosome was incorporated in 1.5 % HPMC gel and was evaluated for in-vitro release, pH, viscosity, extrudability and spreadibility. Results indicate that gel is compatible with skin and has ease of application .The drug content

was also found to be 96.99% (Table 3)

Table2: Evaluation of transferosomal gel

pH	Spreadability (gm.cm/sec)	Viscosity (cps)	Extrudability (gm)			%Drug Content
			Press 1	Press 2	Press 3	
6.57	0.892	436.19	3.142	2.968	1.979	96.99

Ex-vivo Drug Diffusion Studies:

The outcome of ex vivo studies of optimized batch showed that, 81.23 % of drug was released in 10 hours and a sustained release character was observed (Table 3).The marketed cream showed 75.34% of total drug released in 7 hours. Thus optimized batch had better release characteristics than the marketed formulation (Table 3).

Table 3: Drug Release of ex vivo studies in µg

Formula	**1 h**	**2h**	**3h**	**4h**	**5h**	**6h**	**7h**	**8h**	**9h**	**10h**
Optimized Formulation A2	10.9	25.21	33.48	50.32	52.77	60.81	65.31	73.29	80.91	81.23
Marketed preparation	24.32	39.51	5154.37	64.45	74.28	75.99	75.34	-	-	-

Residual solvent level analysis of optimized formulation of Transferosome: Prepared Transferosome are found to be safe to use for topical application after formulating as 2.30 ppm of chloroform was detected in the suspension while 60 ppm is permissible limit for chloroform as per ICH guidelines.

Stability study: The optimized formulation batches A2 were proven to be stable at 40ºC and at 60º C as there was no considerable change seen in its characteristics (Table 4).

Table 4: Result table of stability studies of optimized formulation A2

Test	**Before stability testing**	**A2 at 40°C**	**A2 at 60°C**
% Entrapment efficiency	59.87	59.89	59.33
Vesicle size (nm)	108.93	109.80	100.11
pH	6.570	6.730	6.840
Viscosity (CPS)	436.19	432.31	4302.33
%Drug content	96.99	96.87	96.98

CONCLUSION

The developed BV transferosome gel was found to exhibit sustained drug release characteristics better than marketed formulation with high % drug entrapment and more skin permeation. The formulation was also found to be easy to apply; we can conclude that BV transferosome incorporated gel can be a better option for treatment of psoriasis and a viable alternative to conventional treatment.

CONFLICT OF INTEREST

None

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