Formulation, Development and Evaluation of Ibuprofen Loaded Nano-transferosomal Gel for the Treatment of Psoriasis

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Authors' contributions

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ABSTRACT

The present work was aimed to develop a transferosomal gel of ibuprofen (IBU) for the amelioration of psoriasis like inflammation. Three formulation of IBU loaded transferosomes (TFs1-TFs3) were prepared using different proportions of lipid (phospholipon 90H) and surfactant (tween 80) and further evaluated for vesicle size, zeta potential (*ZP*), entrapment efficiency and *in vitro* drug release. The IBU loaded transferosomes (TFs2) was optimized with vesicle size (217±8.4

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nm), *PDI* (0.102), *ZP* (-31.5±4.3 mV), entrapment efficiency (88.4±6.9%) and drug loading (44.2±2.9%). Further, the optimized IBU loaded transferosomes (TFs2) was incorporated into 1% carbopol 934 gel base and characterized for homogeneity, extrudability, viscosity and drug content. The *in vivo* pharmacodynamic study of gel exhibited reduction in psoriasis like inflammation in mice. The ibuprofen loaded transferosomal gel was successfully developed and has shown the potential to be a new therapy against psoriasis like inflammation.

1. INTRODUCTION

Psoriasis is a chronic autoimmune disease of skin that causes scaling on the skin surface. The quick grew up cells on the skin surface results in scaling. The scales around the psoriatic skin are thick red inflamed patches and are fairly common [1-3]. Psoriasis disease may be considered mild, moderate and severe, if it affects body less than 3%, 3-10% and more, respectively [4]. Topical treatment is the best option for the treatment of mild and moderate disease of psoriasis. Hence, topical treatment ensuring skin penetration would be recommended for psoriatic like inflammation [5-7].

Conventional topical dosage form for psoriasis treatment such as ointment, creams, gels have poor penetration power to the skin and patient discomfort due to stickiness and greasiness [8]. Lipid based nano-vesicles such as niosomes, ethosomes, transferosmes have been shown to provide a more useful drug carrier for antipsoriatic therapy with excellent penetration to skin, patient compliance and improved bioavailability [9-10]. Transferosomes are flexible nano-vesicles formulations composing of lipid and surfactant. The presence of surfactant in their structure, which help in solubilizing stratum corneum, bestows transferosomes with excellent skin permeation power. Furthermore, transferosomes have gained immense popularity in last few years due to sustained and efficient delivery of low and high molecular weight drugs. Transferosomal drug delivery have a better capacity to encapsulate hydrophilic and lipophilic drugs with no toxicity [11-14].

Ibuprofen (IBU) is a potent non-steroidal anti-inflammatory drug (NSAID) belongs to propionic acid class. It is administered orally and topically for the treatment of pain, inflammation and fever. Due to low bioavailability, first pass metabolism and gastric side effects, a need arises to develop a transdermal delivery system to provide consistent efficacy during therapy [15-17]. In this work, we have explored the potential of transferosomes to deliver ibuprofen into deeper skin. Further the anti-psoriasis efficacy of ibuprofen loaded transferosomes was assessed on mice.

2. MATERIALS AND METHODS

2.1 Materials

Ibuprofen and Phospholipn 90H was purchased from Sigma-Aldrich, St. Louis, USA". Tween 80 was purchased from Loba chemicals (Maharashtra, India). All other reagents were of analytical grade.

2.2 Preparation of Ibuprofen Loaded Transferosomes

Thin film hydration method were adopted for the preparation of ibuprofen loaded transferosomes [18]. Phospholipon 90H and tween 80 with different compositions were dissolved in 10 ml of organic solvent mixture (ethanol: chloroform: methanol) at molar ratio (2:2:1, v/v) (Table 1). A thin film was formed on rotary evaporator under reduced pressure at 50 rpm for 2 hours. A prepared thin film was hydrated with 10 ml of phosphate buffer (pH 6.8) containing 100 mg of ibuprofen by rotation at 60 rpm for 1 hour at room temperature. The formed vesicles were kept for swollen for 2 hours, and formed vesicles were sonicated on probe sonicator for 10 minutes at 40% power ("Ultrasonic processor, gx-130, Berlin, Germany"), and transferosome suspension was filtered through 0.22 μm membrane filter.

2.3 Characterization of IBU Loaded TFs

The mean vesicle size, zeta potential and polydispersity index (*PDI)* of all three developed IBU loaded TFs (TFs1-TFs3) were measured by using dynamic light scattering technique (Zetasizer Nano ZS instrument, Malvern Instruments, Ltd., Holtsville, NY, USA) at room temperature (25±2ºC). The light scattering angle of measurement was set at 90º [19]. The

samples were diluted with phosphate buffer saline (pH 6.8) appropriately and were filtered through 0.45 µm filter prior to measurement. Each samples were measured in triplicate. The zeta potential values of each TFs (TFs1-TFs3) were measured using Malvern Zetasizer (Malvern Instruments Ltd., Holtsville, NY, USA).

2.4 Percent Drug Entrapment and Loading Efficiency

An indirect method for the measurement of drug entrapment (%EE) and drug loading (%DL) of IBU loaded TFs (TFs1-TFs3) were followed. Briefly, freshly prepared suspension of TFs vesicles were centrifuged at 15000 rpm, for 10 minutes and supernatant was collected, filtered through 0.45 µm membrane filter and analyzed for the free drug content by using UV spectrophotometer (Jasco V-630, Tokyo, Japan) at λ_{max} of 220 nm [20]. The %EE and %DL was measured by using following equation:

$$\%EE = \frac{\text{Total amount of IBU used in TFs} - \text{Amount of IBU in supernatant}}{\text{Total amount of IBU used in TFs}} \times 100$$

$$\%DL = \frac{\text{Amount of IBU encapsulated in TFs}}{\text{Total weight of TFs}} \times 100$$

2.5 *In vitro* Release Studies

The release of developed TFs (TFs1-TFs3) was conducted using dialysis bag made of cellulose membrane (cut off mol wt. 12,000 dalton). The dialysis bag was soaked overnight in dissolution media before study. IBU loaded TFs were poured into dialysis bag which was then dipped in 50 ml of dissolution media (Phosphate buffer, pH 7.4). The beaker was kept in biological shaker and maintained a speed of 100 rpm, temperature 37±0.5°C during study. One ml aliquots were withdrawn at regular time interval (0, 0.5, 1, 2, 4, 6 and 12 hours) and an equal volume of buffer was compensated to maintain sink condition. The content of drug was analyzed by UV spectrophotometer at 220 nm [20]. Each measurement was performed in triplicate.

2.6 Scanning Electron Microscopy (SEM)

SEM is typically used to examination of surface morphology of optimized transferosomes (TFs2). One drop of transferosomal dispersion was mounted on a glass stubs, dried and pasted over grid by using double sided carbon adhesive tapes and coated with gold-palladium. A circular coverslip was gently placed over the stub to enable uniform conductivity. The transferosomes was viewed with LEO 435 VP (Carl Zeiss, Brighton, Germany) SEM operating at an accelerating voltage of 30kV, under high vacuum for shape, size, and other physical attributes. SEM software program (Carl Zeiss smartsem) was used for data analysis.

2.7 Preparation of IBU Loaded Transferosomal Gel

The optimized formulation of IBU loaded TFs (TFs2) was selected for the development of gel. The gel was prepared by dispersing carbopol (1%w/v) in a beaker with continuous stirring at 200 rpm for 2 hours, after complete dispersion, it was kept for swelling overnight then sonicated for 30 minutes. To the prepared blank gel, optimized IBU loaded TFs (TFs2) was incorporated with continuous stirring with glass rod until homogeneous mixture formed, and finally triethanolamine was added as neutralizing agent [21].

2.8 Characterization of IBU Loaded Transferosomal Gel

Homogeneity, Viscosity and pH: The homogeneity of the IBU loaded transferosomal gel was examined visually for appearance and presence of aggregates. The viscosity of IBU loaded transferosomal gel was measured using Brook-field viscometer with spindle number C-50-1 (Middleboro, USA).The pH of IBU loaded transferosomal gel was measured by pH meter (Adwa, Hungary).

Extrudability: The weight in grams to extrude a 0.5 cm ribbon like gel from a collapsible tube in 10 seconds is extrudability. The IBU loaded transferosomal gel was placed in a collapsible tube and pressed at the crimped end to extrude gel on removal of cap. The extrudability was calculated by using equation:

Extrudability = applied weight to extrude gel from tube (gm) / area (cm^2)

Measurement of drug content: Ibuprofen was extracted from IBU loaded transferosomal gel by adding ethanol and stirred for 1 hour, then the mixture was filtered through 0.45 µm membrane filter. The obtained filtrate was diluted

Table 1. Prepared IBU loaded TFs composition

Formulation code	Ibuprofen (mg)	Phospholipon 90H (mg)	Tween 80 (mg)
TFs1	100	70	30
TFs2	100	75	25
TFs3	100	90	10

appropriately and analyzed for the drug content by UV spectrophotometer at 220 nm [20].

2.9 Anti-psoriasis Activity

Animals: In the present study BALB/c male mice (approximately 10 weeks old and weighing 20-25 g), free from any pathogen, were used in this experiment. The animals were kept under standard laboratory conditions (12 hours light/dark cycle; 22 ± 2°C temperature and 55±5% relative humidity) during the whole study period and provided with free access to standard pellet diet and drinking water. All the procedure was carried out as per the standard guidelines and a protocol approved by animal ethics committee.

2.9.1 Induction of psoriasis-like skin inflammation in mice

2.9.1.1 IMQ-induced psoriasis-like skin inflammation in mice

Mice received a daily topical dose of 62.5 mg of commercially available IMQ cream (Aldara[TM] 5%; 3M Pharmaceuticals) on the shaved back for 3 consecutive days, as previously described [22]. This dose was empirically determined to cause most optimal and reproducible skin inflammation in mice (data not shown).

Experimental Design: Mice were divided into four groups in preventive treatment schedules. Group 1 served as control group and were treated only with petrolium jelly (VASELINE® PETROLEUM JELLY, Unilever, India;); group 2 served as IMQ group and applied a daily topical dose of commercially available IMQ cream (Aldara[TM] 5%; 3M Pharmaceuticals) for 3 days on their shaved back. Group 3 received drug-free transferosomal gel topically on the shaved back for 3 consecutive days. Group 4 served as treatment group and were applied with ibuprofen-loaded transferosomal gel topically 1 h before topical application of IMQ on the shaved back as described above for 3 days [22-23].

3. RESULTS AND DISCUSSION

3.1 Vesicle size, *PDI* and *ZP*

The mean vesicle size and *PDI* of IBU loaded TFs (TFs1-TFs3) are shown in Table.2. The size of the TFs (TFs1-TFs3) was measured in the range of 217–306 nm. The increase in vesicle size in TFs3 may be due increase in amount of lipid used in formulation that lead to increase in the viscosity. The *PDI* values of all vesicles was measured in the range of 0.092 – 0.249, which lesser than 0.3 that makes the dispersion homogenous. The *ZP* values for TFs1, TFs2 and TFs3 were found -25.9 mV, -31.5 mV and -21.5 mV, respectively (Table 2). Zeta potential plays an important role in stability of vesicles. Among three developed TFs, TFs2 was found optimum with vesicle size (217 nm), *PDI* (0.102) and *ZP* (31.5 mV).

3.2 Percent Drug Entrapment and Loading Efficiency

The %EE and %DL of developed IBU loaded TFs (TFs1-TFs3) was found as 47.8±4.8 -88.4±6.9% and 23.9±4.1 - 44.2±2.9%, respectively as shown in Table 2. It is clearly showed a significant effect of phospholipon 90H and tween 80 on encapsulation of drug. In the TFs3 formulae, the highest amount of lipid and lowest amount of surfactant was used. However, in TFs2 formulae lipid and surfactant amount was found optimum with maximum encapsulation efficiency. In spite of lipid, surfactant also play an important role for enhancement of encapsulation of drug. The maximum encapsulation and loading of IBU was found in TFs2.

3.3 *In vitro* Release Studies

The *in vitro* release study was conducted for the developed IBU loaded TFs (TFs1-TFs3). It was observed from the release profile that approximately 100% of drug was released from IBU solution in about 6 hours. However, IBU loaded transferosomes (TFs2) showed a release nearly complete release in about 12 hours (Fig. 1). The results of *in vitro* were fitted to different release models so that the mechanism of drug release from transferosomes can be correlated with best fitted model. The results showed that the release profile of TFs1 and TFs3 formulations followed Korsmeyer peppas model and TFs2 followed first order release pattern, respectively (Table 3). Based on particle characterization, encapsulation efficiency and drug release studies, TFs2 was found optimum

formulae and further subjected for animal studies.

3.4 SEM Studies

SEM studies of the optimized formulation (TFs2) showed slightly smooth, spherical structure (Fig. 2). The size shown by SEM studies are approximately in agreement with size measured by particle size analyzer.

3.5 Characterization of IBU Loaded Transferosomal Gel

IBU loaded transferosomal gel was evaluated for clarity, consistency, homogeneity, spreadability and pH. The gel was observed clear, homogeneous with excellent spreadability. The pH of gel was measured as 7.2±0.31, which was considered appropriate to skin application. The viscosity of the gel were measured as 4.61±0.52 Pa, which suggest that the gel have sufficient consistency to apply on skin. The prepared gel was extruded with a weight of 161±1.74 gm,

indicated that it would be taken out easily by application of thumb pressure. The drug content in gel was found 97.9±3.2%.

3.6 Anti-psoriasis Activity

Clinical signs of psoriasis, such as skin thickening, erythema, and scaling were consistently observed in Aldara®-treated BALB/c mice. Erythema developed following the second day treatment using Aldara, and soon thereafter, scaling appeared on the third day which continually increased in severity up to the end of the experiment in the Aldara® group (Fig. 3). No skin reactions were observed in animal group treated with drug-free transferosomal gel indicating the safety/tolerability of gel vehicle for IBU transferosomes. Of interest, pre-treatment with IBU-loaded transferosomal gel treatment group significantly suppressed skin reactions induced by Aldara® cream as manifested by the absence of any signs of erythema or scaling, observed with Aldara® cream-treated group (Fig. 3).

Table 2. Evaluation of developed transferosomes

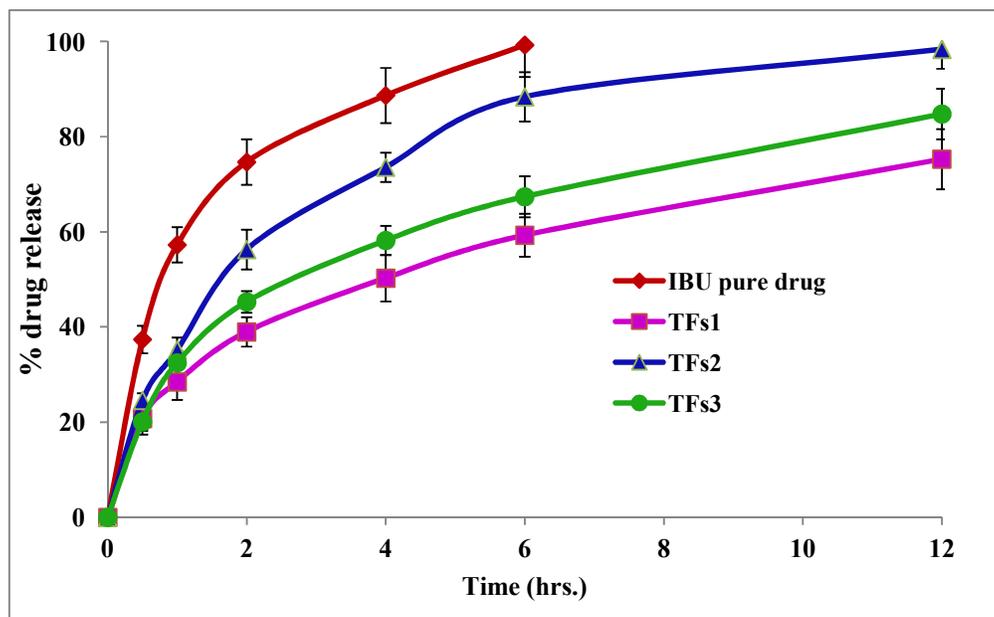| Formulation code	Size (nm)	*PDI*	*ZP* (mV)	%EE	%DL
TFs1	211±6	0.092	-25.9±3	47.8±5	23.9±4
TFs2	217±8	0.102	-31.5±4	88.4±7	44.2±3
TFs3	306±7	0.249	-21.5±4	67.7±8	33.8±4

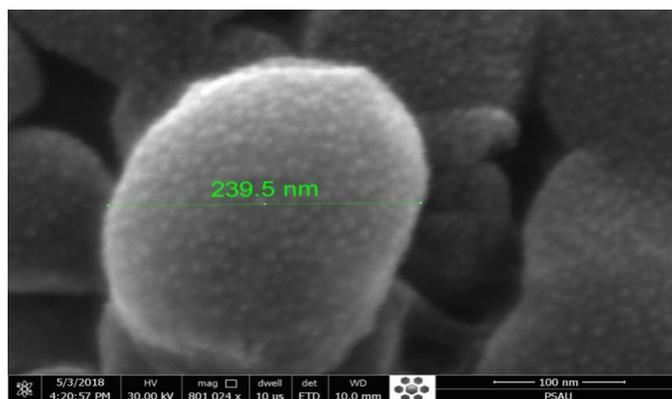**Fig. 1. *In vitro* drug release of IBU loaded transferosomes**

Fig. 2. SEM images of optimized transferosomes (TFs2)

Table 3. Drug release kinetics of developed transferosomes

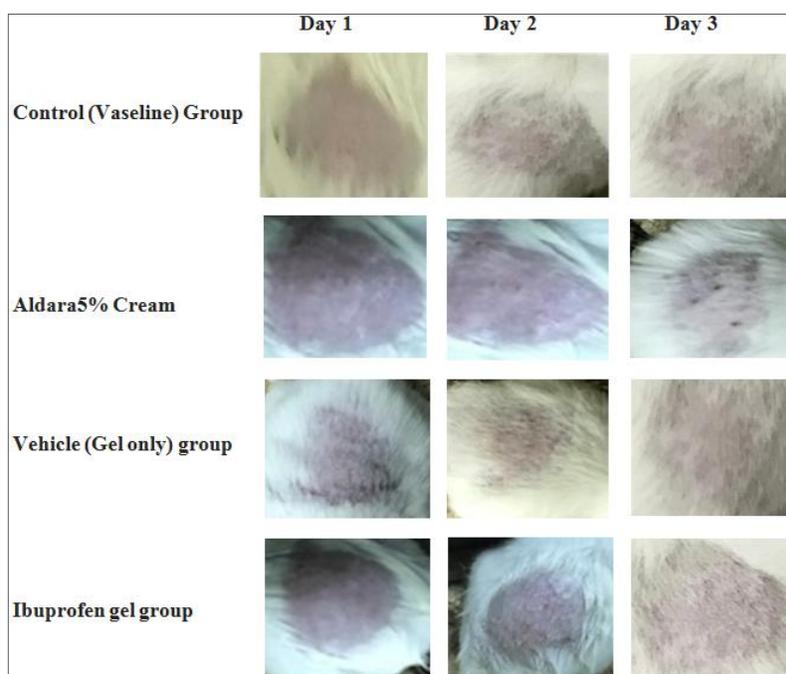| Formulations	Zero order	First order	Higuchi Model	Korsmeyer peppas model
TFs1	0.8191	0.9522	0.9823	0.9970
TFs2	0.7581	0.9980	0.9513	0.9661
TFs3	0.8070	0.9706	0.9766	0.9775

Fig. 3. Comparative psoriasis pattern of marketed cream and optimized transferosomes (TFs2)

4. CONCLUSION

In summary, the ibuprofen loaded transferosome had an optimum size and zeta potential for penetration through the skin. The optimized transferosome formulation was successfully incorporated into carbopol gel and evaluated for homogeneity, extrudability, viscosity, pH and drug content. The proposed drug loaded transferosomal gel was developed, evaluated for the anti-psoriasis activity and excellent results were obtained. Hence, ibuprofen loaded transferosomal gel can be a promising treatment approach for psoriasis.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All the procedure was carried out as per the standard guidelines and a protocol approved by animal ethics committee

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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