

**FORMULATION OF NOVEL TRANSFERSOMAL LOADED DRUG
DELIVERY SYSTEM OF ACECLOFENAC FOR TOPICAL
APPLICATION****Rasvita Gawas*, Clyte Fernandes and Rajashree Gude**Department of Pharmaceutics, Goa College of Pharmacy, 18th June Road, Panaji, Goa-
403001.Article Received on
16 June 2017,
Revised on 06 July 2017,
Accepted on 27 July 2017
DOI: 10.20959/wjpr20178-9147***Corresponding Author****Rasvita Gawas**Department of
Pharmaceutics, Goa College
of Pharmacy, 18th June Road,
Panaji, Goa-403001.**ABSTRACT**

To avoid the gastro intestinal side effects of the drug on chronic oral administration, to provide faster action, transdermal formulation of Aceclofenac entrapped in transfersomes was formulated and evaluated. The aim of the present study involves formulation and evaluation of transfersomes of Aceclofenac by thin film hydration technique to improve the solubility and permeability of the drug. Transferomes were prepared using phosphatidylcholine and three different surfactants (Span 20, Span 60 and Span 80) as bilayer forming components. Formulations were evaluated for different parameters like; physicochemical properties (optical microscopy, particle size

distribution, zeta potential measurement, SEM), % entrapment efficiency, in vitro release and stability studies. The % entrapment efficiency of the formulations was found to be in the range of 52.96 % to 67.25 %. in vitro release studies constant release of drug for the period of 8 hrs and maximum release of drug was shown by formulation F2 (83.6%). The optimized transferomal dispersion was incorporated into gel base using Carbopol 934P and test results obtained were acceptable and transfersomes were found to be stable in gel base.

KEYWORDS: Transfersome, Phosphatidylcholine, Span, bilayer, vesicles**INTRODUCTION**

Recently advances have been made in transdermal delivery system of drugs by facilitating their delivery via vesicular delivery systems. Vesicles are highly ordered assemblies which consist of one or more concentric bilayers formed by self-assembling of amphiphilic building blocks in presence hydrating medium.^[1] Conventional liposomes do not deeply penetrate the

skin and are suitable for only topical drug delivery. Many strategies have been developed to improve this shortcoming of skin penetration ability of liposomes. One of the strategies includes the addition of a cationic or anionic surfactant in the lipid phase together with phosphatidylcholine, thus increasing the bilayer fluidity. These novel vesicular systems are referred to as 'Transfersomes'. Transfersomes are more deformable vesicles than the standard liposomes and are able to overcome permeation difficulty by squeezing themselves along the inter-cellular sealing lipid of the stratum corneum. After application on the skin due to flexibility of vesicle membrane the risk of complete vesicle rupture in the skin is minimised and it allows transfersomes to follow the natural water gradient across epidermis.^[2] Flexibility of the membrane can be adjusted by varying the composition of phospholipids and surfactant, this type of modification of vesicular composition or surface properties can adjust the drug release rate and deposition to the applied site.

BCS Class II drug NSAID Aceclofenac is used in management of pain and inflammation for the patients of Rheumatoid Arthritis, osteoarthritis and ankylosing spondylitis. Aceclofenac like other NSAIDs inhibits cyclooxygenase, a key enzyme involved in the inflammation cascade.^[3] However Aceclofenac on chronic oral administration produces side effects such as gastric irritation, ulceration, bleeding, diarrhoea, nausea and dyspepsia.^[4]

The aim of this study was to improve permeation of Aceclofenac through skin membrane and also to avoid gastric side effects caused by its oral administration in treatment therapy of Rheumatoid Arthritis, ankylosing spondylitis and osteoarthritis. Transdermal formulation of Aceclofenac was developed, with the drug encapsulated in the ultradeformable vesicles 'Transfersomes'. Transfersomes create drug depots in the skin which results in slow and gradual release of the drug encapsulated under the skin and/or systemic circulation without invasion.^[5] Transfersomal dispersion of Aceclofenac was prepared by thin film hydration technique and were evaluated for different parameters. Optimized batch was incorporated into a suitable gel base for ease of application.

MATERIALS AND METHODS

Materials

Aceclofenac was obtained as a gift sample from Geno Pharmaceuticals Ltd (Goa, India). Phospholipon 90 G was obtained as a gift sample from Lipoid GmbH (Germany). Span 20, Span 60 and Span 80 were obtained from Mohini Organics Pvt. Ltd (Mumbai). All excipients and solvents used in the study were of analytical reagent grade.

Preparation of Transfersomes

Aceclofenac transfersomes were formulated by using thin film hydration technique. Accurately weighed quantities of drug, Phospholipon 90G and surfactant were dissolved in chloroform:ethanol (1:1v/v) solvent system. The solvents were then evaporated by using rotary evaporator at 80 rpm in a thermostatically controlled water bath at $60^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and flask was rotated under reduced pressure for 1hr until uniform film was formed. Final traces of solvents from the dried film were removed under vacuum for overnight. The dried lipid film was then hydrated with phosphate buffer pH 7.4 by rotation at room temperature.^[6] Formulation codes of prepared transfersome batches are given in table 1

Table 1: Formulation code and variable used in preparation of transfersomes.

Sr. No.	Formulation code	Surfactant	Drug (mg)	PC:Surfactant
1	F1	Span20	100mg	80:20
2	F2	Span20	100mg	85:15
3	F3	Span20	100mg	90:10
4	F4	Span20	100mg	95:5
5	F5	Span 60	100mg	80:20
6	F6	Span60	100mg	85:15
7	F7	Span 60	100mg	90:10
8	F8	Span 60	100mg	95:5
9	F9	Span 80	100mg	80:20
10	F10	Span 80	100mg	85:15
11	F11	Span 80	100mg	90:10
12	F12	Span 80	100mg	95:5

Preparation of transfersomal gel

Gel base was prepared using simple gelation technique. Weighed quantity of carbopol was soaked in required quantity of distilled water for 3 hours. Carbopol was neutralized using triethanolamine followed by addition of mixture of other excipients in water. Transfersomal dispersion was added in the base with continuous stirring until uniform gel was formed.

Table 2: Composition of gel.

Sr No.	Ingredients	Quantity
1.	Optimised transfersomal dispersion	10 ml
2.	Carbopol 934G	1%
3.	Triethanolamine	q.s
4.	Methyl paraben	0.0125%
5.	Propyl paraben	0.00625
6.	Propylene Glycol	2.4 ml
7.	Glycerine	1.98 ml
8.	Distilled water	q.s

Evaluation of Aceclofenac Transferomes

Appearance

Transfersomal formulations were evaluated for color and appearance.

Optical microscopy

A drop of transfersomal dispersion was placed on a slide and spread. It was covered with a cover slip and examined under light microscope for vesicle shape.

pH determination

pH of the transfersomal dispersion was determined using PH meter at room temperature.

Vesicle size, distribution and Zeta potential determination

The average vesicle size and distribution were determined by dynamic light scattering using nano partica SZ-100 (HORIBA SZ-100 instruments). Zeta potential was measured using nano partica SZ-100 nanoparticle analyser.

Determination of Particle Shape and Morphology

Particle shape and morphology of transfersome vesicles was determined by Scanning Electron Microscopy.

Determination of entrapment efficiency

Indirect centrifuge technique was used to determine entrapment efficiency; transfersomal dispersion was centrifuged using mini centrifuge at 12000 rpm at 4°C for 30 minutes. The clear fraction i.e. the supernatant was collected and required dilutions were made with phosphate buffer pH 7.4. The resulting solution was analysed for determination of free drug by using UV-Visible spectrophotometer at 275nm.^[7] The % entrapment efficiency was calculated using the following formula.

$$\text{Entrapment Efficiency (\%)} = \frac{C_t - C_f}{C_t} \times 100$$

Where C_t is the concentration of total drug.

And C_f is the concentration of untrapped drug.

In vitro Drug Release Study

In vitro drug release study was performed using diffusion cell with phosphate buffer pH 7.4 as the diffusion medium. Semi permeable membrane was attached to one end of diffusion

tube and transfersomal dispersion was placed in the tube; this assembly was lowered in the diffusion medium such that the semi permeable membrane surface just touches the diffusion medium. 5ml of aliquot were withdrawn at hourly intervals for a period of 8 hours and filtered using whatman filter discarding first few drops and withdrawn sample was replaced with fresh medium to maintain sink condition throughout the experiment. Concentration of Aceclofenac in the filtered sample was analysed using UV spectrophotometer at 275nm. Data obtained from release study was subjected to kinetic analysis.

Stability Studies

Stability of prepared Aceclofenac transfersomes was carried out on optimized formulation at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and at room temperature for a period of 3 months. Leakage of drug from the vesicles was investigated over the storage period in the mentioned storage conditions. % entrapment efficiency test of transfersomal dispersion was done at monthly interval for a period of 3 months.

Evaluation of Transfersomal Gel

General Appearance

Transfersomal gel was evaluated for color, homogeneity and presence of any foreign particles.

pH Determination

Approximately 2.5g of gel was weighed and dispersed in 25ml of distilled water and the pH was measured by dipping the electrode of pH meter in the dispersion.

Spreadability Determination

A gel to be applied to skin should possess a good spreadability index so that it can be applied over a sufficient area without any difficulty and irritation to skin. The spreadability of gel was measured on the basis of 'Slip' and 'Drag' characteristics of gels. The apparatus consisted of a wooden plank with a glass slide fixed on it and had a pulley at one end. For measurement of spreadability an excess of gel formulation was placed on the fixed glass slide and a another glass slide having same dimensions was placed above. A weight of 100gm was placed on top of the two slides for 5 mins to expel the air and to provide a uniform film of gel between the slides. The top slide was subjected to weight of 80gm with the help of plastic cup attached to a string. Pulley was used to allow smooth motion of string with the weight. The time in

seconds required by the top slide to cover a distance of 7.5cm was noted.^[8] The following formula was used to calculate the spreadability

$$S = \frac{M}{T} \times L$$

Where S = Spreadability in g.cm/sec

W = Weight tied to upper slide in gms

L = Length of glass slide

T = Time in seconds required by the top slide to cover a specified distance.

Drug Content Test

The drug content of the formulated gel was carried out by dissolving accurately weighed quantity of gel equivalent to 5mg of drug in a 50ml volumetric flask and volume was made upto 50ml with phosphate buffer pH 7.4. The flask was shaken for 2 hours in shaker and the solution then filtered using whatman filter paper. The filtered solution was then analysed spectrophotometrically for drug content of Aceclofenac at 275nm.

Viscosity Studies

Viscosity of formulated gel was measured using a BrookField Viscometer at room temperature.

in vitro release studies

Same procedure performed for transfersomal dispersion was followed using a diffusion cell. Accurately weighed quantity of gel equivalent to 5 mg of drug was placed into diffusion tube onto the surface of semi permeable membrane. 5ml of aliquots were withdrawn at hourly intervals and were filtered. The concentration of Aceclofenac in the filtered sample was analysed using UV spectrophotometer at 275nm.

Skin Irritation Study

The skin irritation test was done according to the method described by Draize et al using albino wistar rats. The hair on the back was shaved exposing a bare patch of skin and the rats were divided into 3 groups.

Group I: Standard irritant (0.8% v/v Formalin)

Group II: Transfersomal Gel

Group III: Distilled water

After 24 hours each group was treated with respective solution and formulation on the shaved area of skin patch. The treated area was observed for any visible changes such as erythema/edema at 24, 48, 72 hrs post treatment. Animal ethics committee approval was taken to carry out the test.

Stability Studies

Stability study for a period of 3 months was done to ascertain the stability of gel formulation at different temperatures. The gel formulation was at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and at room temperature for period of 3 months. Effect of temperature on the gel formulation was studied on the basis of drug content and release studies. Drug release studies were conducted for a period of 8 hrs and samples were withdrawn at hourly intervals. % CDR was measured at the end of 8th hr.^[9]

RESULTS AND DISCUSSION

All batches of formulated transfersomal dispersion were white in color and there was no separation or settling or two phases observed. When observed under light microscope the shape of the vesicles was found to be spherical and some of them were found to be elongated in shape (Figure no 1). pH of the formulations was measured using digital pH meter and was found to be 6.5 ± 0.2 .

The mean particle size of the vesicles was found to be 549.1nm and size distribution curve confirmed normal distribution of vesicles. Polydispersity index was found to be 0.387, which indicated narrow size distribution between the vesicles of the formulation. Zeta potential was measured using nano partica SZ 100 nanoparticle analyser and was found to be -63.4 mV. The negative zeta potential is due to net charge of the lipid composition in the formulation since phosphatidylcholine is a zwitterionic compound with an isoelectric point 6 and 7. Particle size and morphology was observed by SEM; transfersomes vesicles were found to be almost spherical in shape (Figure No.2).



Figure 1: Photomicrograph of transfersomal formulation.

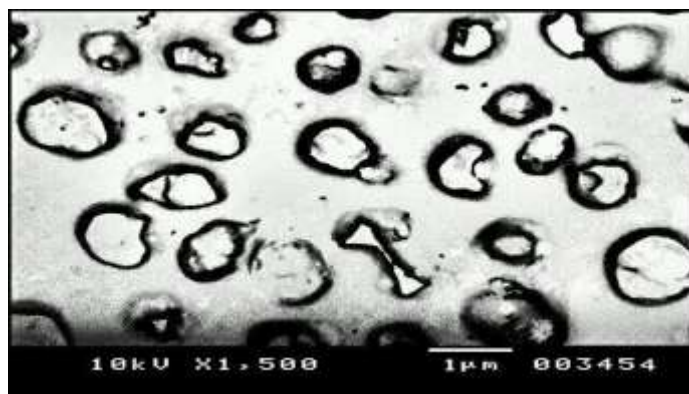


Figure 2: SEM images of transfersomal formulation.

Entrapment Efficiency

The % entrapment efficiency of the transfersomal formulations was found to be in the range of 52.96% to 67.25%. Percentage entrapment efficiency of all batches is given in table no. iii. Formulation F2 had highest entrapment efficiency (67.25%) and lowest was of formulation F12 i.e. 52.96% having Span 80 as edge activator. In all 3 batches of transfersomes formulated with 3 different surfactants as edge activators, batches with ratio of phosphatidylcholine:edge activator 85:15 showed highest entrapment when compared to other three ratios. Lipid layer have been found to incorporate only certain amount of drug after which the lipid bilayer starts sedimenting upon formation. Gradual increase in surfactant concentration helps in incorporation of drug in the bilayer, however at higher concentration value of edge activator the entrapment efficiency decreases. The surfactant at higher concentration may start forming micelles in bilayer which may result in pore formation of vesicles and conversion of vesicle membrane into mixed micelle.

Table 3: Results of % entrapment efficiency and in vitro drug release studies results.

Sr. No.	Formulation Codes	%Entrapment Efficiency	in vitro drug release(%CDR)
1	F1	66.46%	67.4%
2	F2	67.25%	83.6%
3	F3	65.12%	75.2%
4	F4	64.05%	37.4%
5	F5	56.64%	49.3%
6	F6	62.45%	71.72%
7	F7	60.96%	64.79%
8	F8	55.86%	48.2%
9	F9	55%	35.76%
10	F10	56%	58.31%
11	F11	54%	44%
12	F12	52.96%	40.27%

In vitro drug release

In vitro drug release study data shows that the drug release from formulation was increased gradually over a period of 8 hours of study. Results are shown in table no. 3. Maximum drug release i.e. 83.6 % is shown by formulation F2 transfersome dispersion in which surfactant Span 20 is used as an edge activator and lowest release was shown by Formulation F11(54%). The in vitro release results showed that the release was increased when compared to in vitro release of pure drug dispersion (11.36%) over a period of 8 hours. The release data was subjected to kinetic analysis and found to follow First order kinetics. On the basis of results obtained Formulation F2 was selected to be the optimised batch of Aceclofenac transfersomal dispersion.

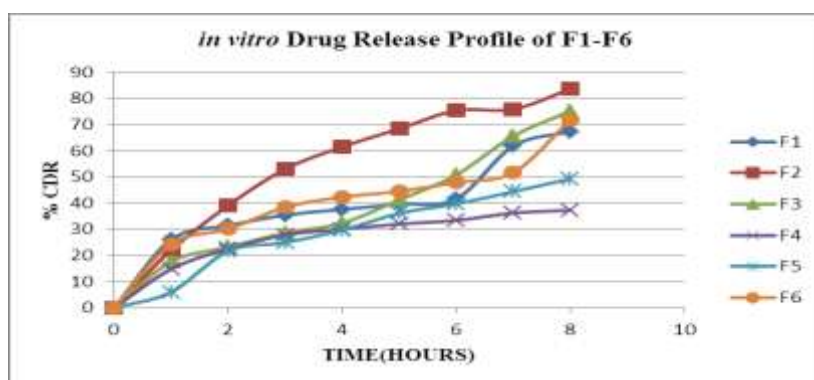


Figure 3: in vitro drug release plots of formulation F1-F6.

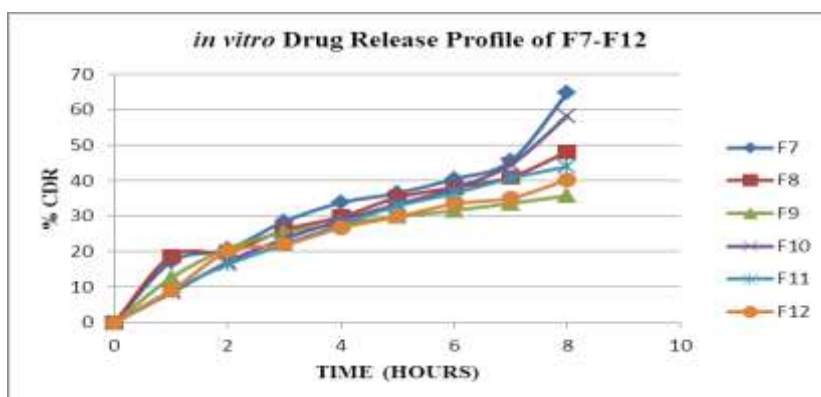


Figure 4: in vitro drug release plots of formulation F7-F12.

Stability Study

Stability study was conducted of optimised batch formulation F2 at $4^{\circ}\text{C} \pm 2^{\circ}\text{C}$ and at room temperature. Formulation was found to be stable at both temperature and was more stable at refrigerated temperature than at room temperature.

Results of Evaluation of Transfersomal Gel

Optimised batch F2 was incorporated in a gel base using Carbopol 934P. The formulated transfersomal gel was white in color, non-gritty and free from foreign particles. pH is an important parameter in a gel formulation as it has to be close to skin pH to avoid any irritation after gel application on the skin and pH of the formulation was found to be 6.4. Spreadability of the gel was determined on the basis of 'Slip' and 'Drag' characteristics of gels. Spreadability was found to be 38.96 gm. cm/sec which was satisfactory and time taken was less, therefore it was concluded that the formulated gel was easy to apply.

Drug content of Aceclofenac in the gel was found to be 98%. Viscosity was determined by using Brookfield viscometer and was found out to be 60000cps which was acceptable as viscosity is an important property for application of gel on skin.

In vitro drug release study was done using modified diffusion cell for a period of 8 hours. Drug release at the end of 8 hours was found to be 62.82%; which was low as obtained for the transfersomal dispersion (83.6%). This may due to slow diffusion of drug loaded transfersomal vesicles through the gel matrix for permeation across the diffusion membrane. Skin irritation test results indicated that prepared transfersomal formulation is non-irritating and safe for application for transdermal use. Rats used for skin irritation study did not show any signs of allergic reactions, erythema or redness upto 3 days after application.

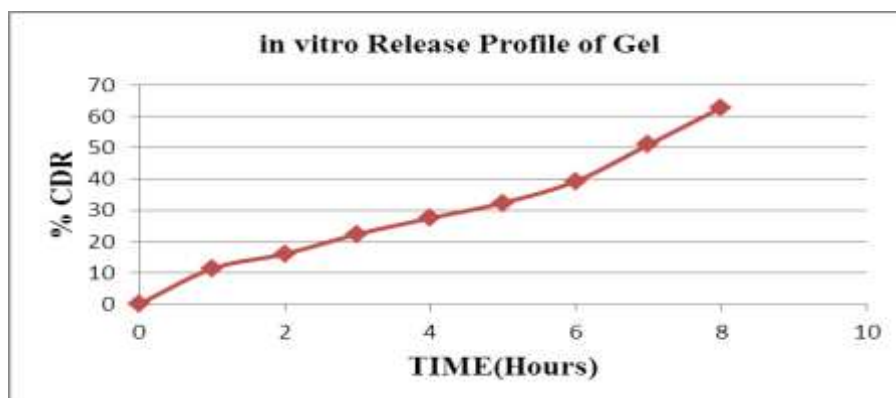


Figure 5: in vitro drug release plots of transfersomal gel.

Stability study of transfersomal gel was conducted for 3 months at two different temperatures i.e. room temperature and refrigerated temperature. Gel had acceptable appearance and the viscosity was also maintained at the end of 3 months of the study. Drug content and % cumulative drug release were found to be more decreased in samples stored at room temperature as compared to those stored at refrigerated temperature.

CONCLUSION

Novel vesicular drug delivery systems are investigated and used in today's research era since they have proved to be an effective system for delivery of drugs along with improved efficacy. In this research project an attempt was made to increase the permeability and also solubility of Aceclofenac drug across transdermal membrane for topical application. After encapsulation of Aceclofenac in transfersome vesicles the permeability and solubility of drug was increased and it will produce faster action along with avoidance of gastric side effects of drug due to its oral administration. Based on the evaluation tests results formulation F2 (85:15 ratio of phosphatidylcholine:Span 20) with highest drug release (83.6%) was considered to be optimized formulation. Transfersomal gel was formulated by incorporation of optimized formulation in gel base. *in vitro* release of drug from the transfersomal gel was found to be lower than that from dispersion but the release was satisfactory. Further studies can be done to improve the release of drug encapsulated in transfersomes from the gel matrix. Overall the permeation and solubility of drug was found to be enhanced as compared to that of pure drug.

ACKNOWLEDGEMENT

I express my sincere gratitude to my guide Mrs. Rajashree Gude, Associate Professor, Goa College of Pharmacy for her support and guidance. I thank Geno Pharmaceuticals, Goa for providing me with the gift sample of drug Aceclofenac. Also I am thankful to Lipoid GmbH (Germany) for generous help in providing gift sample of Phospholipon 90G.

REFERENCES

1. Jadhav S, Morey P, Karpe M, Kadam V. Biomarkers in Oncology. *Journal of Applied Pharmaceutical Sciences*, 2012; 3(4): 183-202.
2. Venkatesh D.N, Abid S. K, Kiran C, Kalyani K, Tulasi K. Transfersomes: A Novel Technique for Transdermal Drug Delivery, 2014; 3(4): 266-276.
3. Arslan S, Tirnaksiz F. A Nonsteroidal Anti-inflammatory Drug Aceclofenac. *J. Pharm. Sci*, 2010; 105-118.
4. MedIndia http://www.medindia.net/doctors/drug_information/aceclofenac.htm. accessed on, 15/04/2017.
5. Morsi N, Aboelwafa A, Dawood M. Improved Bioavailability of Timolol Maleate via Transdermal Transfersomal Gel: Statistical Optimization, Characterization and Pharmacokinetic Assessment. *Journal of Advanced Research*, 2016; 7: 891-701.

6. Tejaswini, Swapna S, A Madhu, Bakshi V. Formulation and Evaluation of Fluconazole Loaded Transfersomal Gel. International Journal of science and Research Methodology, 2016; 3(3): 7.
7. Malakar J, Nayak A, Sen K. Formulation, Optimization and Evaluation of Transfersomal Gel for Transdermal Insulin Delivery. Saudi Pharmaceutical Journal, 2012; 20: 355-368.
8. Shetkar M, Potinas V, Shete M. Development and Evaluation of Aceclofenac Gel for Topical Application. American Journal of Pharmacy and Health Research, 2014; 2(12).
9. Preeti, Kumar M. Development of Celecoxib Transfersomal Gel for Treatment of Rheumatoid Arthritis. Indian Journal of Pharmaceutical and Biological Research, 2014; 2(2): 7-13.