



Research Article

FORMULATION AND EVALUATION OF TRANSFERSOMAL CREAM OF ACRIFLAVINE

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ABSTRACT

A burn can be major medical problems or life-threatening emergencies admitted to any hospital. Conventional topical burn formulations are required to be applied 3 to 4 times a day and previous application is needed to be removed prior to application of each new dose which is very painful to burn patients. In this research an attempt was made to increase the patient compliance by reducing the dosing frequency through sustained release vesicular drug delivery system like transfersomes. Transfersomes were formulated using by thin film hydration method and evaluated for entrapment efficiency, photomicroscopy, scanning electron microscopy, vesicle size, polydispersity and zeta potential. Optimized Transfersomal batch was incorporated in cream base and evaluated for pH, spreadability, viscosity, drug content and *in vitro* diffusion. Transfersomes prepared by using 1:3 ratio of sodium cholate with cholesterol and 200 mg drug showed highest entrapment (91.65%) and sustained release (65.18% in was 24 hrs) was obtained with transfersomal cream in comparison with conventional formulation (97.54% in 8 hrs).

Keywords: Transfersomes, burn, acriflavine

INTRODUCTION

Vesicular drug delivery can be defined as highly ordered assemblies consisting of one or more concentric bilayer formed as a result of self-assembling of amphiphilic building blocks in the presence of water. Vesicular drug delivery system is particularly important for targeted delivery of drugs because of their ability to localize activity of drug at the site or organ of action there by lowering its concentration at the other sites in body. Vesicular drug delivery system sustains drug action at a predetermined rate and simultaneously minimizes undesirable side effects. Origin of these vesicles was first reported in 1965 by Bingham and was given the name Bingham bodies. Vesicular drug delivery system has some of the advantages like; prolong the existence of the drug in systemic circulation, improves the bioavailability especially in the case of poorly soluble drugs, both hydrophilic and lipophilic drugs can be incorporated, delays elimination of rapidly metabolized drugs and thus function as sustained release systems¹.

Lipid vesicles are one type of many experimental models of bi-membranes which evolved successfully, as vehicles for controlled delivery. Transfersomes are the special type of liposomes consisting of phosphatidyl choline and an edge activator. The name means carrying body and is derived from the Latin word 'transfere' meaning 'to carry across' and Greek word 'soma' for a 'body'². These carriers are composed of at least one bilayer-forming lipid and one or more bilayer softening surfactant. The transfersomes are able to penetrate fine skin

pores smaller than its average size, driven by hydration gradient in the mammalian skin barrier, i.e. the epidermis, and more specifically spoken the stratum corneum. Transfersomes are a highly adaptable, stress responsive, complex aggregates³. Transfersomes offers an advantages like high entrapment efficiency in case of lipophilic drug near to 90%, high deformability gives better penetration of intact vesicles. They act as depot releasing their contents slowly and gradually, they can be used for both systemic as well as topical delivery of drug, they are biocompatible and biodegradable as they are made from natural phospholipids similar to liposomes, they protect the encapsulated drug from metabolic degradation, transfersomes possess an infrastructure consisting of hydrophobic and hydrophilic moieties together and as a result can accommodate drug molecules with wide range of solubility. They can act as a carrier for low as well as high molecular weight drugs e.g. analgesic, anesthetics, corticosteroids, sex hormones, anticancer, insulin, gap junction protein, and antibiotics.

Antibiotic encapsulated in transfersomes can be effectively used as sustained release system for the treatment of burn. Burns can be minor medical problems or life-threatening emergencies. Many people die each year from fire-related burn injuries. Electricity and chemicals also cause severe burns. Scalding liquids are the most common cause of burns in children. Treatment of burns depends on the location and severity of the injury. Sunburns and small scalds can usually be treated at home. Deep or widespread burns need immediate medical attention. Acriflavine is primarily indicated as a topical

antiseptic agent in conditions like burns, skin infections, wounds, and can also be given in adjunctive therapy as an alternative drug of choice in superficial infections. Acriflavine is derived from acridine dye^{4,5}. Marketed acriflavine cream formulations require two to three applications for treatment of burn; hence acriflavine is incorporated in transfersomal which may help in reducing the frequency of applications.

MATERIAL AND METHOD

Acriflavine was obtained from Sigma Aldrich, Mumbai India. Sodium cholate was obtained from, SD Fine Chemicals Limited, Mumbai India. Cholesterol, cetosteryl alcohol, white soft paraffin, glyceryl monostearate, propylene glycol, propyl paraben, methyl paraben and ethanol were obtained from Molychem, Mumbai India. Phospholipon G was kindly gifted by Lipoid KG, Germany.

Preparation of Transfersomes

Transfersome prepared (Table 1) by using film hydration method. In this method a thin film is prepared from mixture of vesicles forming ingredients that is phospholipon G, sodium cholate and cholesterol by dissolving in ethanol (10ml). Organic solvent is then evaporated at 60°C using rotary evaporator leaving a thin layer of solid mixture deposited on the wall of the flask which is then hydrated using 10 ml water at 60 rpm for 20 minutes at 60°C^{6,7}.

Table 1: Formulation and evaluation of transfersomes

Formula	F1	F2	F3	F4	F5
Drug (mg)	100	150	200	200	200
Phospholipon G (mg)	400	400	400	400	400
Sod. Cholate: Cholesterol	1:1	1:1	1:1	1:2	1:3
Entrapment Efficiency (%)	26.72	35.68	78.25	85.75	91.65

Preparation of Transfersomal Cream

Mixture of Cetosteryl alcohol (3.5%), glyceryl monostearate (1.5%), white soft paraffin (6%) propyl paraben (0.02%) were selected as oil phase and mixture of propylene glycol (4%), methyl paraben (0.18%) and water (q.s to 100%) was used as aqueous phase. Both the phases were heated separately to about 80°C and were cooled at room temperature with continuous stirring. Then the optimized transfersomal batch was added to above mixture to prepare the transfersomal cream containing 0.1% of acriflavine.

EVALUATION OF TRANSFERSOMES

Entrapment Efficiency (EE %)

Entrapment efficiency was carried out by centrifugation method. The aqueous dispersion containing transfersomes were separated from untrapped drug by centrifugation at 15000 rpm for 30 minutes at 4°C. The supernatant was discarded and the pellets were taken. These pellets were further dissolved in 10 ml methanol and were further diluted with phosphate buffer and the concentration of drug in the medium was determined by UV-spectrophotometer at 261.8nm. All the spectroscopic analysis was carried out in triplet and the values were averaged. Entrapment efficiency was obtained by using formula^{8,9,10}.

$$\text{Entrapment efficiency \%} = \frac{\text{Amount entrapped} \times 100}{\text{Total amount}}$$

Photo-microscopy

Optimized transfersomes (2 mg) were diluted with 0.5 ml of water in watch glass. Small amount of hydrated transfersomes were spread on glass slide and observed under compound

microscope at 40x magnification power and examined for vesicle structure¹⁰.

Scanning Electron Microscopy

The prepared optimized transfersomal formulation was also characterized for their morphology using scanning electron microscopy. The sample was sprinkled and fixed on a SEM holder with double sided adhesive tape and coated a layer of gold of 150⁰ A for 5 to 6 minutes using a sputter coater, working in a vacuum of (3x10⁻¹ atm) of argon gas. The sample was then examined using a scanning electron microscope at 10 Kv accelerating voltage^{10,11}.

Particle Size Analysis, Polydispersity Index, Zeta Potential

Mean diameter of optimized vesicles and polydispersity index (PDI) measurement was carried out by dynamic light scattering technique in triplicate using Horiba Nano Partica instrument (SZ-100, Japan). Small amount of optimized transfersomal sample was mixed with 10 ml of water and sonication for 5 minutes prior to PDI determination. Zeta potential was also measured in triplicate by laser Doppler electrophoresis technique at 25 °C using the same instrument^{11,12}.

EVALUATION OF TRANSFERSOMAL CREAM

Determination of pH

The pH measurement was carried out by using a calibrated digital type pH meter by dipping the glass electrode completely into the cream so as to cover the electrodes. Study was done in triplicate and the results were averaged¹³.

Determination of Spreadability

Spread ability of cream was determined by the apparatus which consists of a wooden block, which was provided by a pulley at one end. An excess of cream (about 2g) under study was placed on ground slide. The cream was then sandwiched between this slide and another glass slide having the dimension of fixed ground slide and provided with the hook. 50g weighted was placed on the top of the two slides for 5 minutes to expel air and to provide a uniform film of the cream between the slides. Excess of the cream was scrapped off from the edges. The top plate was then subjected to weight of 100g with the help of string attached to the hook and the time (in seconds) required by the top slide to cover a distance of 7.5 cm is noted. A shorter interval indicates better spread ability. Spread ability was calculated using the following formula:

$$S = M \times L / T$$

Where, S = Spread ability, M = Weight in the pan (tied to the upper slide), L = Length moved by the glass slide, T = Time (in sec.) taken to separate the slide completely each other^{14,15}.

Determination of Viscosity

Viscosity of cream was determined by Brookfield viscometer. The viscosity measurements were done using Brookfield viscometer using 6 number spindle. The developed formulation was poured into the adaptor of the viscometer and determined the viscosity of the test sample as per standard operating procedure of viscometer. The spindle was rotated at speeds of 100 rpm. The reading near to 100% torque was noted¹⁶.

Drug content

The formulation equivalent to 1 mg of drug was taken and dissolved in small quantity of methanol. Then the formulation is warmed on the water bath so that the drug present in the formulation was completely dissolved. Then the solution is filtered through whattman filter paper and diluted suitably with

phosphate buffer pH 7.4. Absorbance was measured by UV spectrophotometer at 261.8nm in triplicate.

In Vitro Diffusion Studies

The diffusion studies were performed using a Franz diffusion cell to compare the release pattern of acriflavine from transfersomes, transfersomal cream and conventional formulation. The cell had a 25 ml receptor compartment. The dialysis membrane was mounted between the donor and receptor compartments. The formulation equivalent to 0.1mg of drug was applied uniformly on the dialysis membrane and the compartments were clamped together. The receptor compartment was filled with the phosphate buffer (pH 7.4) and the hydrodynamics in the receptor compartment were maintained by stirring with a magnetic bead. The study was carried out for 24 hrs. 1ml of samples was withdrawn from the receptor compartment at pre-determined time intervals (0.5, 1, 2, 4, 6, 8, 10, 12 and 24 hrs) and an equal volume of buffer was replaced. The samples were analyzed after appropriate dilution for drug content spectrophotometrically at 261.8nm^{17,18,19}.

RESULT AND DISCUSSION

Evaluation of Transfersomes

Entrapment Efficiency

The Acriflavine transfersomes were prepared by thin film hydration method and optimized to obtain the high level of entrapment. Formula F1, F2 and F3 were prepared with 1:1 ratio of Sodium cholate and cholesterol with 100mg, 150mg and 200mg of drug. Formula F3 which was prepared with 200mg of drug and 1:1 molar ratio of Sodium cholate and cholesterol gave highest entrapment 78.25% in comparison with 26.72% and 35.68% with formula F1 and F2 respectively. It has been observed that entrapment efficiency of drug increases with the increase in drug quantity.

The Acriflavine loaded transfersomes were further prepared with different quantity of cholesterol in order to get highest entrapment. Formula F4 and F5 were prepared with 120mg and 180mg of cholesterol unlike 60mg cholesterol added in previous formula. It has been observed that with the increase in quantity of cholesterol, the entrapment efficiency of drug increases. Transfersomes prepared with 120 mg of cholesterol gave 85.75% entrapment, while Transfersomes prepared with 180 mg of cholesterol gave 91.65% entrapment²⁰.

Photomicroscopy

The photomicrograph of optimized acriflavine transfersomes (Figure 1) is shown in figure. Photomicroscopic observation of the optimized transfersomal formulation after hydration with buffer revealed the formation of vesicular structure.



Figure 1: Photomicrograph of acriflavine transfersomes

Scanning Electron Microscopy

SEM study was done on the optimized formula F5 to get more insight on vesicle formation and morphology of the system. It reveals the presence of well identified, unilamellar, nearly perfect spheres as shown in figure 2.

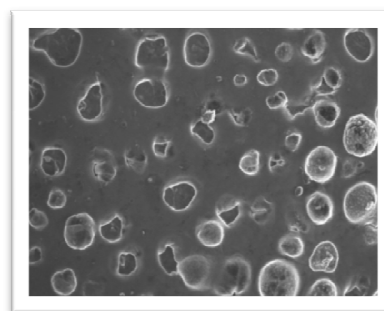


Figure 2: Scanning electron micrograph of acriflavine transfersomes

Particle Size Analysis, Polydispersity Index, Zeta Potential

Zeta potential study was also carried out on optimized transfersomes as it is an important indicator of particle surface charge, which can be used to predict and control the stability of colloidal or vesicular suspensions or emulsions (Woo et al., 2009). The zeta potential of formula F5 was found to be -37.3 mV, indicating prevention of aggregation and polydispersity index was found to be 0.387 which indicates broad particle size distribution²⁰. Vesicle size of optimized transfersomes was found out to be 195.2nm.

EVALUATION OF TRANSFERSOMAL CREAM

Table 2: Evaluation of Transfersomal Cream

Evaluation Parameter	Results
Ph	5.9
Spreadability	15gm/cm/sec
Viscosity	7200cps
Drug content	99.71%

Determination of pH

pH of topical formulation like cream should match with skin in order to avoid skin irritation. Transfersomal cream of acriflavine (Table 2) had shown the pH of 5.9 which was in acceptable range.

Determination of Spreadability

Good spreadability is important prerequisite for topical formulations in order to have ease in application. Spreadability of optimized transfersomal cream was observed to be 15gm/cm/sec

Determination of Viscosity

Viscosity of the topical formulation can affect the squeezing property from the collapsible tube container and also affect spreadability of formulations. Viscosity of optimized transfersomal cream was 7200cps.

Drug Content

Drug content of optimized transfersomal cream of acriflavine obtained was 99.71% indicating the minimum loss of drug during manufacturing.

In Vitro Diffusion Studies

This study was performed in order to compare release profiles of acriflavine transfersomes, transfersomal cream and marketed

formulation. The results are shown in Fig. 3. At the end of 8 h, 97.54% drug was released from marketed formulation of acriflavine whereas only 74.65% and 65.18% was released from transfersomes and transfersomal cream at the end of 24 respectively. We can infer that in comparison to marketed formulation, there was sustained release of drug from transfersomal cream which would result in improved patient compliance.

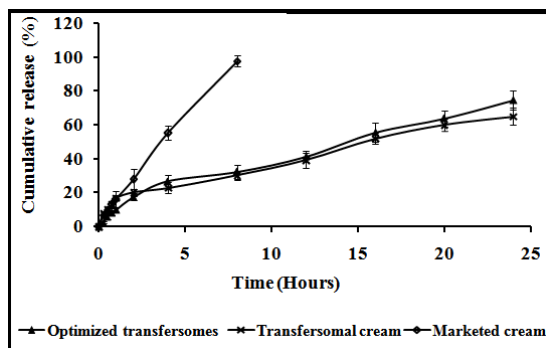


Figure 3: In Vitro Diffusion Study

CONCLUSION

Preparation of transfersomes as a vesicular drug delivery system of acriflavine was simple and easy with less number of excipients. The result from experimental work demonstrated the successful development of transfersomal cream of highly hydrophilic drug acriflavine with sustain release of drug which may help in reduction of frequency of application and thus increased the patient compliance.

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