Research Article

DESIGN AND CHARACTERIZATION OF TRANSFEROSOMAL GEL OF REPAGLINIDE

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ABSTRACT

The main aim of current investigation is to formulate and evaluate transferosomal gel for effective transdermal delivery of repaglinide. Repaglinide is an oral anti hyperglycemic agent used for the treatment of non-insulin-dependent diabetes mellitus (NIDDM). The pre formulation study was carried out initially in terms of identification (physical appearance, melting point and IR spectra), solubility study and $\lambda_{\max}$ determination. The transfersomes were formulated by modified hand shaking method using surfactant such as Span 80 and Tween 80 in various concentrations and evaluated for their vesicle shape and type, entrapment efficiency, % drug content and *In vitro* drug permeation study. The shapes of most repaglinide containing transfersomes were found to be spherical from SEM analysis. The % entrapment efficiency of deformable vesicles formulations were found to be in the range of 82.51 % to 87.69 %. Entrapment efficiency of the RT6 formulation was high (maximum 87.69 %). *In-vitro* skin permeation study studies showed that, transfersome gels were found to increase the skin permeation and deposition showing a controlled effect. Stability studies were performed for RT-6 Transfersomes to study effect of different temperature conditions on percent entrapment and optimized gel formulation to study content uniformity and physical appearance for a period of 3 months respectively. Finally, in light of the current data, it can be concluded that transfersomes were a promising candidate for transdermal delivery, to prolong the release and to improve the site specificity of the drug repaglinide.

Keywords: Transdermal delivery system, transfersomes, repaglinide, vesicle shape, entrapment efficiency, *in vitro* drug permeation study.

INTRODUCTION

Oral and parenteral delivery is the most common therapy used in treatment of diabetes. Controlled drug delivery into a body is one of the novel methods in pharmaceutical research. The path way through the skin, for example is a predestined route for the drugs, which undergo degradation and metabolism in the gastrointestinal tract or the liver after oral application. Vesicular approaches, such as liposomes, niosomes, ethosome and transfersomes also have the potential of overcoming the skin barrier and have been reported to enhance permeability of drug through the stratum corneum barrier. A Transfersome is a highly adaptable and stress-responsive, complex aggregate.^{1,2} Its preferred form is an ultra-deformable vesicle possessing an aqueous core surrounded by the complex lipid bi layer. Interdependency of local composition and shape of the bi layer makes the vesicle both self-regulating and self-optimizing. This enables the Transfersome to cross various transport barriers efficiently and then act as a Drug carrier for non-invasive targeted drug delivery and sustained release of therapeutic agents. To date many chemical and physical approaches have been applied to increase the efficacy of the material transfer across the intact skin, by use of the penetration enhancers, iontophoresis, sonophoresis and the use of colloidal carriers such as lipid vesicles (liposomes and proliposomes) and nonionic surfactant vesicles (niosomes and proniosomes).

Vesicles have a unique structure which is capable of entrapping hydrophilic, lipophilic, amphiphilic and charged hydrophilic drugs. Vesicles are colloidal particles having a water filled core surrounded by a wall of lipids and surfactants (amphiphiles) arranged in bi layer. If the proportion of water is increased, these amphiphiles can form one or more concentric bi layers. Hydrophilic drugs find a place in the internal aqueous environment while amphiphilic, lipophilic drugs get entrapped in the bi layered wall with electrostatic and/or hydrophobic forces. The flexible or deformable vesicles are called elastic vesicles or Transfersomes. These are characteristic with transfersomes, because of the high vesicle deformability, which permits the entry due to the mechanical stress of surrounding, in a self-adapting manner. Flexibility of transfersomes membrane is governed by mixing suitable surface-active components in the proper ratios with phospholipids³. The resulting flexibility of transfersome membrane minimizes the risk of complete vesicle rupture in the skin and allows transfersomes to follow the natural water gradient across the epidermis, when applied under non-occlusive condition.

Composition of Transfersome

Transfersomes are composed of phospholipids like phosphatidyl choline which self assembles into lipid bi layer in aqueous environment and closes to form a vesicle. A bi layer softening component (such as a biocompatible surfactant or an amphiphile drug) is added to increase lipid bi layer flexibility and permeability. This second component is called as edge activator⁴⁻⁶. An edge activator consists usually of single chain surfactant that causes destabilization of the lipid bi layer thereby increasing its fluidity and elasticity. The resulting, flexibility and permeability optimized, Transfersome vesicle can therefore adapt its shape to ambient easily and rapidly, by adjusting local concentration of each bi layer component to the local stress experienced by the bi layer.

Figure 1: Deformability of Transfersomes

Mechanism of Transfersome

The mechanism for penetration is the generation of “osmotic gradient” due to evaporation of water while applying the lipid suspension (transfersomes) on the skin surface. The transport of these elastic vesicles is thus independent of concentration. The trans-epidermal hydration provides the driving force for the transport of the vesicles⁷. As the vesicles are elastic, they can squeeze through the pores in stratum corneum (though these pores are less than one-tenth of the diameter of vesicles)⁸. A Transfersome vesicle applied on an open biological surface, such as non-occluded skin, tends to penetrate its barrier and migrate into the water-rich deeper strata to secure its adequate hydration. During penetration through the stratum corneum, reversible deformation of the bi layer occurs. But it should be noted that while this deformation is occurring, vesicle integrity, gradient and barrier properties for the underlying hydration affinity should not be compromised. Since it is too large to diffuse through the skin, the Transfersome needs to find and enforce its own route through the organ. The Transfersome vesicles usage in drug delivery consequently relies on the carrier’s ability to widen and overcome the hydrophilic pores in the skin. Intracellular drug transportation may involve diffusion of vesicle lipid bi layer with the cell membrane like normal endocytosis. The mechanism is thus complex and involves advanced principles of elasto-mechanics combined with material transport and hydration/osmotic force.

MATERIALS AND METHODS

Materials

The drug repaglinide from mars therapeutics and chemicals pvt. Ltd, phospholipid soya lecithin from bright laboratories, edge activators span-80, tween-80 from merck specialist pvt. Ltd (Mumbai), India volatile solvents methanol and chloroform from S.D fine chemicals (Mumbai), India gelling agent carbopol-940 from research lab fine chem. Industry (Mumbai), India.

Methods

Preparation of Transfersomes Containing Repaglinide

Transfersome formulations were prepared by Hand shaking thin film hydration method using drug, Soya Lecithin and different surfactants (Tween-80, Span-80, Span-60 and Span-40). The amount of drug is kept constant in all the formulations. Different formulations were prepared by using different surfactants in different ratios. Lecithin, surfactants and the drug are dissolved in 5 ml of organic solvent (Methanol:Chloroform (3:1). The organic solvent is then removed by rotary evaporation (Rotary evaporator) under reduced pressure at 40°C. Final traces of solvent are removed under vacuum. The deposited lipid film is hydrated with the phosphate buffer (pH 6.8) by rotation at 60 rpm for 1 hour at room temperature. The resulting vesicles are swollen for 2 hours at room temperature. The multi lamellar lipid vesicles (MLV) are then sonicated using bath sonicator for 30 minutes at 4°C.

Preparation of Topical Transfersome Gel

As a vehicle for incorporation of transfersomes for topical delivery, carbopol gels were prepared. Transfersomes aqueous dispersion was utilized for the formulation of topical gel. Gel polymer such as carbopol 940 was utilized to prepare transfersome gel. 2 g of carbopol- 940 powder was dispersed into vigorously stirred (stirred

by magnetic stirrer Remi 5MLH) distilled water (taking care to avoid the formation of in dispersible lumps) and allowed to hydrate for 24 h. The dispersion was neutralized with tri ethanolamine to adjust the pH [6.8] by using pH meter (Lab India Sab 5000). (Table 1)

Characterization

Vesicle Shape

Transfersomes vesicles can be visualized by SEM and optical microscope. The Morphological characterization of transfersome vesicle such as shape and surface feature were projected by using optical microscope and SEM.

Determination of Entrapment Efficiency Percentage

The amount of Repaglinide entrapped in transfersome gel was estimated by centrifugation method. 1 g of Transfersome gel was taken and diluted with 100 ml phosphate buffer (pH 6.8). This suspension was sonicated using bath sonicator for 20 minutes. Later this solution was placed in centrifugation tube and centrifuged at 2000 rpm for 1 hour. Supernatant liquid was withdrawn and if necessary dilution is done. Check for absorbance measurement using UV spectrophotometer (UV-3200 Lab India) at 292 nm. This gives us the total amount of un-entrapped drug. Entrapment efficiency is expressed as the percent of drug trapped.

$$\% \text{ Entrapment} = \frac{\text{Total drug} - \text{Diffused drug}}{\text{Total drug}} \times 100$$

% Drug Content

1 g of transfersome gel formulation was taken and the vesicles were lysed with 10 ml of methanol by sonication [citizen, India] for 15 min. Later this solution was placed in centrifugation tube and centrifuged at 2000 rpm for 1 hour. Supernatant liquid was withdrawn and if necessary dilution is done. Check for absorbance measurement using UV spectrophotometer (UV-3200 Lab India) at 292 nm and drug content was calculated for Repaglinide.

$$\% \text{ drug content} = \frac{\text{Amount of drug obtained after centrifugation}}{\text{Amount of drug taken}} \times 100$$

In-vitro Drug Release Studies

Modified Franz diffusion cell with a receiver compartment volume of 30 ml and effective diffusion area of 2 cm² was used for this study. *In-vitro* drug study was performed by using egg membrane in phosphate buffer solution (pH 6.8). To perform *in-vitro* drug release study, egg membrane was mounted horizontally on the receptor compartment of Franz diffusion cell. The effective permeation area of donor compartment exposed to receptor compartment was 2 cm² and capacity of receptor compartment was 30 ml. The receptor compartment was filled with 18 ml of phosphate buffer (pH 6.8) maintained at 37 ± 0.5°C and stirred by a magnetic bar at 100 rpm. Transfersome gel formulation equivalent to 5 mg drug was placed on the skin and the top of the diffusion cell was covered. At appropriate time intervals 5 ml aliquots of the receptor medium were withdrawn and immediately replaced by an equal volume of fresh phosphate buffer (pH 6.8) to maintain sink conditions. The samples were analyzed spectrophotometrically at λ_{max} 292 nm.

Table 1: Quantity of Substances taken for Preparation of Transfersome

Formulation	Repaglinide (mg)	Lecithin (mg)	Tween 80 (mg)	Span 80 (mg)
RT1	8	95	5	--
RT2	8	90	10	--
RT3	8	85	15	--
RT4	8	95	--	5
RT5	8	90	--	10
RT6	8	85	--	15

In each of the formulation, 5 ml of chloroform and methanol ratio were added separately

Table 2: % Drug Entrapped and % Drug Content in Transfersomes

Formulation	% Entrapment Efficiency	% Drug content
RT1	82.51 $\pm$ 0.01	87.14 $\pm$ 0.005
RT2	85.92 $\pm$ 0.005	92.06 $\pm$ 0.006
RT3	83.11 $\pm$ 0.005	89.81 $\pm$ 0.006
RT4	85.45 $\pm$ 0.005	89.50 $\pm$ 0.03
RT5	86.90 $\pm$ 0.04	92.10 $\pm$ 0.03
RT6	87.69 $\pm$ 0.005	92.45 $\pm$ 0.005

Table 3: *In-vitro* Drug Release of Transfersome Gel (RT 1to RT 6)

Time (h)	% Drug release					
	RT1	RT2	RT3	RT4	RT5	RT6
0	0	0	0	0	0	0
1	19.51	21.5	23.1	22.1	26.3	20.6
2	23.28	32.04	27.81	31.5	30.1	26.4
3	27.41	39.41	30.44	46.3	35.7	28.4
4	33.51	43.82	34.91	50.1	44.9	35.9
5	40.28	49.61	42.84	56.2	52.4	44.6
6	46.17	54.81	44.90	68.4	65.5	56.1
8	57.78	58.31	52.28	74.4	70.4	77.4
12	60.53	67.80	63.94	86.4	87.7	94.2

Table 4: % Entrapment Efficiency and % Drug Content after Stability Studies

Number of Days	% Entrapment Efficiency at temperatures			% Drug Content at temperatures		
	4 $\pm$ 2°C	25 $\pm$ 2°C	37 $\pm$ 2°C	4 $\pm$ 2°C	25 $\pm$ 2°C	37 $\pm$ 2°C
15	87.5 $\pm$ 0.005	87.18 $\pm$ 0.005	88.19 $\pm$ 0.005	92.4 $\pm$ 0.005	92.1 $\pm$ 0.005	92.4 $\pm$ 0.005
30	86.3 $\pm$ 0.03	86.07 $\pm$ 0.03	87.41 $\pm$ 0.03	92.1 $\pm$ 0.03	91.5 $\pm$ 0.03	91.6 $\pm$ 0.03
90	86.0 $\pm$ 0.02	85.54 $\pm$ 0.02	87.06 $\pm$ 0.02	91.6 $\pm$ 0.02	91.7 $\pm$ 0.02	90.2 $\pm$ 0.02

Figure 2: Photomicrograph of Repaglinide Loaded Transfersome (RT 6)

Figure 3: SEM analysis of optimized formulation (RT6)

Figure 4: *In-vitro* Drug Release Study for Transfersome Gel Formulation RT 1 – RT 6

RESULTS AND DISCUSSION

Characterization Of Transfersomes

Vesicle Shape

The surface morphology was studied by Optical Microscopy. The shapes of most of the containing Repaglinide transfersomes were found to be spherical from SEM analysis, as shown in figures 2 & 3.

Entrapment Efficiency

The % entrapment efficiency of deformable vesicles formulations were found to be in the range of 82.51 to 87.69 % (Table 2). Entrapment efficiency of the RT6 formulation was high (maximum 87.69 %); because of the increase in the ratio of lipid volume in the vesicles as compared to the encapsulated aqueous volume. The effect of phospholipids and edge activator ratio in the lipid components of vesicles on the entrapment efficiency of lipophilic drug, the efficiency increased with increasing surfactant concentration and thus increased with increasing lipid concentration. (Table 2)

% Drug Content

The results obtained shows 87.14 - 92.45 % drug content in all the formulations, which shows that there is no degradation of the drug in the process.

In-vitro Drug Release Study

Comparison of results obtained from diffusion studies for all six formulations have been done. It was found that formulation RT6 shows higher drug release rate than other formulations. This result of dissolution profile showed slight initial burst release. This is probably caused by the release of drug absorbed on the transfersome surface or precipitated from the superficial lipid layer. Prolonged release in the later stage can be attributed to the slow diffusion of the drug from the lipid vesicle. (Table 3, Figure 4)

Stability Studies

It is clear from the results obtained that the transfersomes have shown the minimum drug lost at refrigerated condition and fairly high retention of drug inside the vesicles was observed. At this low temperature condition % remaining drug entrapped and drug content was good over a period of months. While, storage at higher temperatures $25 \pm 2^\circ\text{C}$ and $37 \pm 2^\circ\text{C}$ leads to less % remaining drug entrapped and drug content over a period of 3 months respectively. The higher amount of drug leakage at elevated temperature may be due to the degradation of lipids constituting bi layers resulting in

defects in membrane packing and loss of overall rigidity that makes them leaky. With the increase in temperature, there is also increase in the fluidity of lipid bi layers, due to phase transition phenomenon. So it can be inferred from the above discussion that the transfersomes formulation should be stored at lower temperature to minimize the drug loss and increase the stability of drug. (Table 4)

CONCLUSION

In light of the current data, it can be concluded that the optimized formulation RT6 which contains Lecithin: Span-80 in ratio 85:15 (% w/w) which is incorporated in 2 % carbapol gel has higher entrapment efficiency and maximum drug release; because of the increase in the ratio of lipid volume in the vesicles as compared to the encapsulated aqueous volume. The effect of phospholipids and edge activator ratio in the lipid components of vesicles on the entrapment efficiency of lipophilic drug, the efficiency increased with increasing surfactant concentration and thus increased with increasing lipid concentration. Finally, it was confirmed that Transfersomes gel improve the transdermal delivery, prolong the release and improve the site specificity of the drug Repaglinide. Transfersomes creates a new opportunity for the well-controlled transdermal delivery of a number of drugs that have a problem of administration by other routes.

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