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FORMULATION AND EVALUATION OF TRANSFEROSOMAL GEL OF LULICONAZOLE AS AN ANTIFUNGAL AGENT

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Abstract

Transfersomes are particularly optimized, ultradeformable (ultraflexible) lipid supramolecular aggregates, which are able to penetrate the mammalian skin intact. Transfersome is a type of carrier system which is capable of transdermal delivery of low as well as high molecular weight drugs. Transfersomes penetrate through the pores of stratum corneum which are smaller than its size and get into the underlying viable skin in intact form. Luliconazole an optically active imidazole antifungal agent with broadspectrum activity, appears to inhibit ergosterol synthesis of fungi by inhibiting the enzyme lanosterol 14 α-demethylase. Inhibition of this enzyme's activity by lluliconazole results in decreased amounts of ergosterol, a constituent of fungal cell membranes. Especially, luliconazole inhibited growth of all filamentous fungi except zygomycetes at low concentration. In addition, luliconazole is the active R enantiomer of a chiral molecule. The drug entrapment in formulation – 3 is higher than the other formulations. The diffusion study of luliconazole transfersome gave extended release of the drug which suffices to decreased dose, lesser frequent dose of treatment and more patient compliance.

Keyword: Antifungal agents, Transferosomal gel,

Introduction

Transfersomes are particularly optimized, ultradeformable (ultraflexible) lipid supramolecular aggregates, which are able to penetrate the mammalian skin intact. Transfersome is a type of carrier system which is capable of transdermal delivery of low as well as high molecular weight drugs. Transfersomes penetrate through the pores of stratum corneum which are smaller than its size and get into the underlying viable skin in intact form.

The term gel was introduced in late 1800 to name sum semisolid material according to pharmacology. The USP defines gel as a semisolid system consisting of dispersion made up of either small inorganic particles or large organic molecules enclosing an interpenetrated by liquid. The inorganic particles form a three dimensional structure. Gels consist of two phase system in which inorganic particles are not dissolved but merely dispersed throughout the continuous phase and large organic particles are dissolved into the continuous phase.

Classification of gels:

Gels can be classified depending upon colloidal phases and nature of solvent used, physical nature and rheological properties.

Based on colloidal

- Two phase system (Inorganic)

If the partial size of the dispersed phase is relatively large and forms the three dimensional structure throughout gel such a system consists of floccules of small particles rather than layer molecules and gel structure in this system is not always stable. They must be thixotropic-forming semisolids on standing and become liquid on agitation.

- **Single phase system (organic)**

These consist of large organic molecules existing on the twisted stands dissolved in a continuous phase. These organic molecules either natural or synthetic polymer are referred as gel forms.

Based on nature of solvent used

- **Hydro gel (water based)**

In hydro gels water acts as a continuous liquid phase. E.g. gelatin, cellulose derivatives, poloxamer gel

- **Organic gels (with a non aqueous solvent)**

They contain a non aqueous solvent on their continuous phase. E.g.Plastibaseolag gel and dispersion of metallic state in oils.

- **Xerogels**

These are solid gels with low solvent concentration. They are formed by the evaporation of solvent leaving the gel framework behind. On contact with fresh fluid they swell and can be reformed. E.g. tragacanth ribbons, dry cellulose and polystyrene

Based on rheological properties they are classified into three types

- **Plastic gel**

Flocculated suspensions of aluminium hydroxide exhibit a plastic flow and the plot of rheogram gives the yield value of gels above which the elastic gel distorts and begins to flow.

- **Pseudo plastic gel**

For example liquid dispersion of tragacanth, sodium alginate etc exhibit pseudo plastic flow. There is a decrease in the viscosity of this type of the gel with the increasing rate of shear, the rheogram results from the shearing action on the long chain molecules of the linear polymer. As the shearing stress increased the disarranged molecules begin to align their long axis in the direction of flow with release of solvent from gel matrix.

- **Thixotropic gel-**

In this type of gel the bonds between the particles are very weak and can be broken down by shaking. The resultant solution will revert back to gel due to the particles colliding and linking together again, e.g. bentonite and agar.

Based on physical nature

- **Elastic gel**

Due to elastic behaviour of agar, pectin, guar gum the fibrous molecules being linked at the point of junction by relatively weak bond such as hydrogen bonds and dipole attraction. Example: alginate and carbopol.

- **Rigid gels**

In this type of gel macromolecules in which the framework linked by primary valance bond. Example: Silica gel

Preparation of gels

Gels are normally in the industrial scale prepared under room temperature. However few of polymers need special treatment before processing. Gels can be prepared by following methods.

Thermal changes: Solvated polymers (lipophilic colloids) when subjected to thermal changes causes gelatin. Many hydrogen formers are moresoluble in hot than cold water. If the temperature is reducing, the degree of hydration is reduced and gelation occurs (Cooling of a concentrated hot solution will produce a gel), e.g. Gelatin, agar sodium oleate, guargum and cellulose derivatives etc. In contrast,some materials such as cellulose ether owe their solubility in water to hydrogen bonding with water. Increasing the temperature of these solutions will break the hydrogen bonds and reduce the solubility, causing gelation. Therefore, this method cannot be used for the preparation of gels as a general method.

Skin

The skin is one of the most accessible organs for the human body of subject matter as well the main route of the drug delivery system. Fungal infection of the skin is one of common skin problems. In between wide selection of topics treatment from solid dose to semisolid forms and fluid volume the formation of clear gels is widely accepted in both cosmetics as well medicines.

A wide variety of vehicles ranging from solid to semisolids and liquid preparations are available for topical treatment of dermatological disease as well as skin care. Topical drug administration is a localized drug delivery system anywhere in the body through ophthalmic, rectal, vaginal and skin as topical route.

There are various medicated products that are applied to the skin. Such products are referred as topical or dermatological products. There are various Hydrophilic polymers such as carbopol 940,hydroxy propyl methyl cellulose (HPMC), Sodium alginate that are used in topical gel delivery system. Based on molecular fraction these polymers are used concentration between 1-5 % in topical formulation.

Antifungal Agents

Luliconazole: Luliconazole an optically active imidazole antifungal agent with broadspectrum activity, appears to inhibit ergosterol synthesis of fungi by inhibiting the enzyme lanosterol 14 α-demethylase. Inhibition of this enzyme's activity by lluliconazole results in decreased amounts of ergosterol, a constituent of fungal cell membranes. Especially, luliconazole inhibited growth of all filamentous fungi except zygomycetes at low concentration. In addition, luliconazole is the active R enantiomer of a chiral molecule.

METHODS AND PREPARATIONS:

Preparation of Transfersomes

All methods of preparation of transfersomes are comprised of two steps.

First, a thin film is prepared, sonicated vesicles are homogenized by extrution through a membrane filter. The mixture of vesicles forming ingredients, that is phospholipids and surfactant were dissolved in volatile organic solvent (chloroform-methanol), organic solvent evaporated above the lipid transition temperature using arotary evaporator. Final traces of solvent were removed under vaccum for overnight. The deposited lipid films were hydrated with buffer (6.4) by rotation at 60 rpm / min for 1 hr at the corresponding temperature. The resulting vesicles were swollen for 2 hr at room temperature. To prepare small vesicles; resulting LMVs were probe sonicated for 30 min at room temperature. The sonicated vesicles were homogenized by manual extrution through membrane filter.

Preparation of Topical Transfersome Gel Formulation:

Prepared transfersomes were incorporated in to carbopol-934 (1%) gel base in a 1:1 ratio. The carbopol-934 (1%) gel base prepared by soaking 30 min followed by continuous stirring with water.1% carbopol-934 gel base has a good consistency (gelling characteristic).

CHARACTERIZAITON OF TRANSFERSOMES

Particle Size Analysis of Transfersomes

The transfersomes were subjected to microscopic examination (S.E.M) for characterizing size and shape of the transfersomes. Microscopic examination revealed, spherical small uni-lamellar vesicles of 270-650, 360-550, 290-550 size range,for transfersomes of 3:2:1, 3:3:1, 3:4:1 ratios respectively. The average mean particle size of formulation 1, 2 and 3 were 405nm, 455nm, 420nm respectively.

Determination of Particle Size

The particle size of Acarbose Transfersomes was viewed and photographedusing Scanning electron microscopy. Acarbose Transfersomes were coated with goldby using Hitachi Vaccum evaporator. Coated samples were viewed and photographed in Hitachis-3000H SEM.

Size Distribution of Transfersomes

Transfersomes were subjected in to laser particle counter (L.P.C) for characterizing size distribution of transfersomes. Its shows that the particle size range 200-700nm, 200-600 nm, and 200-700 nm range for Luliconazole transfersomes of 3:2:1, 3:3:1, 3:4:1 ratios respectively. The average mean particle size of formulation1, 2 and 3 were 420nm, 450 nm, 430 nm respectively.

Entrapment Efficiency

The entrapment efficiency was determined by using direct method. Detergents are used to break the transfersome membranes1 ml of 0.1% Triton X- 100(Triton X-100 dissolved in phosphate buffer) was added to 0.1 ml Transfersomes preparations and made up to 5 ml with phosphate buffer then it was incubated at 37°Cfor1.5 hrs to complete breakdown of the transfersome membrane and to release the entrapped material. The sample was filtered through a Millipore membrane filter (0.25) µm. and the filtrate was measured at 425 nm for Acarbose. The amount of luliconazole was derived from the calibration curve.

The entrapment efficiency is expressed as:

Determination of viscosity

Viscosities of the gels were determined by using Brookfield Viscometer (model- RVTP).Spindle type, RV-7 at 20 rpm. 100gm of the gel was taken in a beaker and the spindle was dipped in it and rotated for about 5 minutes and then reading was taken.

Extrudability

It is useful empirical test to measure the force required to extrude the material from the tube. The formulations were filled in a collapsible metal tubes with anasal tip of 5mm opening tube extrudability was then determined by measuring the amount of gel, extruded the tip when a pressure was applied on tube gel. The extrudability of the formulation was checked and the results were tabulated.

Stability Study

The stability studies of transfersomal formulation were carried out at refrigeration temperature (4°C), Room temperature and 37±5°C. Physical evaluation of prepared transfersomes gel shown in the Table no. 12.Leakage of the drug from the prepared transfersome were analyzed in terms of percent drug retained storage under refrigerated condition showed promising results of 98.2%, 94.5% and 95.5% for formulation 1,2,3 respectively after 3 month. At room temperature the Percentage retained after 3 month was 94.28%, 87.98% and 88.88% for formulation 1, 2, 3 respectively and at 37°C the percentage of drug retained was 80.82%,78.72%, and 79.64 % for formulation 1, 2, 3 respectively. These showed that the formulation found to have more stable at refrigeration temperature, whereas good stability at room temperature and the drug degradation increased at 37°C.

***In-vitro* Drug Release**

Diffusion Study for luliconazole Transfersomes

The *in vitro* release of luliconazole from the transfersome formulations were studied by open ended cylinder method. This diffusion cell apparatus consist of a glass tube with inner diameter of 2.5cm, open at both ends. One end tied with artificial membrane, which serves as a donor compartment.

This study is performed for determining the permeation rate. The time needed to attain permeation flux at steady state and the information from *in-vitro* studies were used to optimize the formulations. Studies of drug release from transfersomes gel formulation were performed using the *in vitro* diffusion method at 37°C, 100 rpm, within a period of 24hr. A weighed amount of prepared transfersomes gel formulation was poured in to the glass cell and diffused against phosphate buffer pH6.4 as a diffusion medium. Aliquots were taken at regular intervals and analyzed spectro-photometrically at 425 nm using phosphate buffer pH 6.4 as blank.

***In-vitro* Release Studies**

In vitro release study is carried through open tubular method with artificial membrane at 37°C, 100 rpm, within a period of 24 hr. From this study we evaluated the percentage of drug diffused in the medium. The percentage of drug diffused from the luliconazole transfersomes were 70.62%, 73.35%, 84.70% from transfersomes formulation 1, 2and 3 respectively at the end of 24hrs.

Conclusion:

On the basis of these results, the luliconazole trnasfersomes (Formulation- 3) showed better characteristic behaviour.

The drug entrapment in formulation – 3 is higher than the other formulations. The diffusion study of luliconazole transfersome gave extended release of the drug which suffices to decreased dose, lesser frequent dose of treatment and more patient compliance.

This proved its targeting efficiency. So it was concluded that luliconazole transfersomes (Formulation–3) was a better and efficient formulation than existing formulations.

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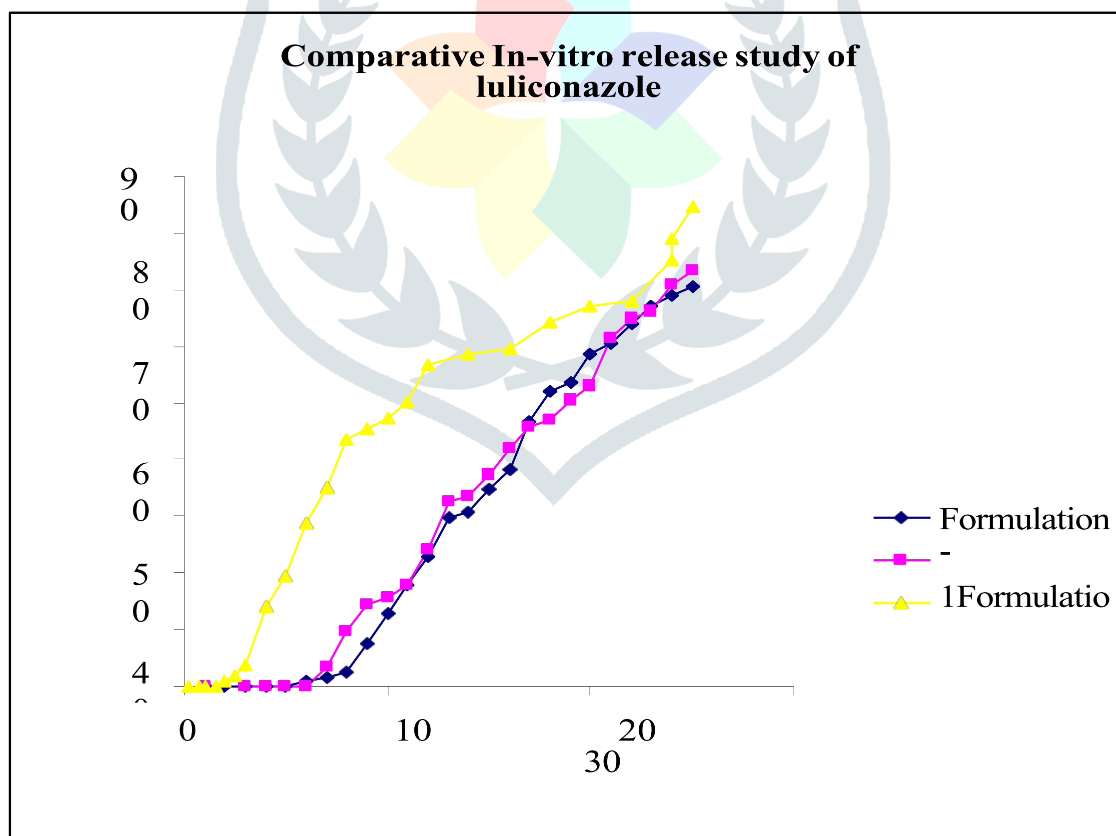

Fig No 01:Comparative In-vitro release study of luliconazole Transfersomes

S. No	Luliconazole Transfersome Formulations	% Drug diffused
1	Formulation-1	70.63
2	Formulation-2	73.36
3	Formulation-3	84.72

Table No 01:**Comparative *Invitro* release study of luliconazole Transfersome formulations**