CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES
SJIF Impact Factor: 7.187

Available online at: http://www.iajps.com *Research Article*

DEVELOPMENT AND CHARACTERIZATION OF TRANSFEROSOMES OF DRUG KETOCONAZOLE

Sunita[1], Dibya Kumari[1], Umesh Kumar Jain[1]*

[1]*Bhopal Institute of Technology and Science – Pharmacy Bhopal M.P.

Abstract:

The topical and transdermal routes of drug administration are long known to the field of pharmaceutics. These routes have been explored for the delivery of a wide range of therapeutic agents over centuries. However, the anatomy of the skin and the physicochemical properties of molecules limit their transport via these routes. Transferosomes are nanovesicles with different contents that play a role in various biological and pathological processes. It offers significant advantages over other delivery systems such as liposomes and polymeric nanoparticles. Although exosomes are expected to be effective therapeutic agents, their optimal use remains a challenge. The development of methods for large-scale production, isolation, and drug loading is necessary to improve their efficiency and therapeutic potential. The current challenges and potential directions of this new area of drug delivery that liposomal delivery of drugs was reported to be limited to the upper layers of skin. This led to the development of self-regulating and self-adaptable vesicles known as transferosomes. Among all the prepared formulations, one optimized formulation was chosen by the point prediction method and evaluated for drug-polymer compatibility, particle size, and surface charge analysis, followed by skin permeation and pharmacodynamic studies. The optimized transferosomal gel of ketoconazole showed all responses which confirm with the values predicted by the design. Results of the current study suggest that the development of such combinational delivery system can result in a rational therapeutic regimen for effective treatment of concomitant disease conditions of fungal disease patients.

Keywords: *Transferosome, Ketoconazole, Fungal disease, Gel, Vesicular system*

Corresponding author:

Dr. Umesh Kumar Jain,

Bhopal Institute of Technology and Science -Pharmacy,
Bangrasia Bhojpur Road, Bhopal, M.P,462047

QR code

Please cite this article in press ***Umesh Kumar Jain*** *et al,* ***Development And Characterization Of Transferosomes Of Drug Ketoconazole****.,Indo Am. J. P. Sci, 2024; 11 (6).*

INTRODUCTION:

Transferosome is a highly adaptable and stress-responsive, complex aggregate. Its preferred form is an ultradeformable vesicle possessing an aqueous core surrounded by the complex lipid bilayer [1]. Transferosomes possess an infrastructure consisting of hydrophobic and hydrophilic moieties together and as a result can accommodate drug molecules with wide range of solubility. Transferosomes can deform and pass through narrow constriction (from 5 to 10 times less than their own diameter) without measurable loss [2]. Transferosomes are chemically unstable because of their predisposition to oxidative degradation. Purity of natural phospholipids is another criteria militating against adoption of Transferosomes as drug delivery vehicles. The carrier aggregate is composed of at least one amphiphatic (such as phosphatidylcholine), which in aqueous solvents self-assembles into lipid bilayer that closes into a simple lipid vesicle. By addition of at least one bilayer softening component (such as a biocompatible surfactant or an amphiphile drug) lipid bilayer flexibility and permeability are greatly increased. The resulting, flexibility and permeability optimized, Transferosome vesicle can therefore adapt its shape to ambient easily and rapidly, by adjusting local concentration of each bilayer component to the local stress experienced by the bilayer. The transferosome thus differs from such more conventional vesicle primarily by its "softer", more deformable, and better adjustable artificial membrane. Another beneficial consequence of strong bilayer deformability is the increased Transferosome affinity to bind and retain water [3]. The characterization of Transferosomes is generally similar to liposomes, niosomes and micelles [4]. Transferosomes have been widely used as a carrier for the transport of proteins and peptides. Proteins and peptide are large biogenic molecules which are very difficult to transport into the body, when given orally they are completely degraded in the GI tract. These are the reasons why these peptides and proteins still have to be introduced into the body through injections. Various approaches have been developed to improve these situations. Transferosomes can also positioned nearly exclusively and essentially quantitatively into the viable skin region [5]. Human skin consists of various layers penetrated by hair shafts and gland ducts. The major skin layers, from inside to outside, comprise the fatty subcutaneous layer (hypodermis), the dermis of connective tissue and the stratified avascular, cellular epidermis. The epidermis consists of five layers, which from inside to outside are the stratum germinativum (basal layer), stratum spinosum (spinous layer), stratum granulosum (granular layer), stratum lucidum and stratum corneum (SC). The transepidermal pathway can be defined as the pathway where compounds permeate across the intact, unbroken stratum corneum. This pathway contains two micropathways. First, the intercellular route, which is a continuous but tortuous way through the intercellular lipid domains and secondly, the transcellular pathway through the keratinocytes, then across the intercellular lipids the transcellular pathway requires not only partitioning into and diffusion through the keratin bricks but also into and across the intercellular lipids. Thus, the intercellular lipids play a major role in the barrier nature of the SC [6]. Topical/transdermal delivery of drugs via lipidic vesicular nanocarriers (liposome, niosome, SLN, nanoemulsions gel) is explained on the basis of following proposed hypothesis as the penetration enhancing process by liposome components and niosomal surfactants by lowering the permeability barrier of the skin through mobilizing the unbound and bound lipids **[7].** Ketoconazole is widely recommended orally for the treatment of various fungal infections. One of the major disadvantages of oral therapy in the treatment of skin infections like Seborrhoeic dermatitis and Psoriasis is rapid relapse on the cessation of therapy and the risk of hepatotoxicity. Topical application of 2% cream or shampoo has shown beneficial effect in Seborrhoeic dermatitis. However, topical application of ketoconazole causes irritation, dermatitis or a burning sensation**.** Delivery via the transdermal route is an interesting option in this respect because a transdermal route is convenient and safe. This offers several potential advantages over conventional routes like avoidance of first pass metabolism, predictable and extended duration of activity, minimizing undesirable side effects, utility of short half-life drugs, improving physiological and pharmacological response, avoiding the fluctuation in drug levels, inter-and intra-patient variations, and most importantly, it provides patients convenience.

MATERIAL AND METHODS:

Analytical Profile of ketoconazole: Analytical method based on UV Spectroscopy was developed at pH 6.8 phosphate buffer. Absorption Maxima (λmax) of ketoconazole was obtained at 233 nm. UV scan (at various wavelengths) for pH 6.8 phosphate buffer as media shows **λ**max at respective wavelengths. Accurately weighed 10 mg of drug was transferred to a 10 ml volumetric flask, sufficient amount of pH 6.8 phosphate buffer as dissolution media was added to dissolve it and volume was made up to 10 ml (stock A; 1000 µg/ml) for the preparation of standard curve. 0.2 ml of stock A was taken into 10 ml volumetric flask and further diluted up to 10 ml with methanol

(stock B; 20µg/ml). Aliquots of stock B were further diluted up to 10ml to get concentration of 2, 4, 6, 8, 10 up to 20µg/ml. In order to establish the linearity of analytical method, a series of dilutions ranging from 2-20 µg/ml was prepared in the same manner.

Preparation of transferosomes: Transferosomes of drug ketoconazole with different combinations of lipid material were prepared by the high-speed homogenization method. The various formulations were prepared using varying amount of gelling agent and penetration enhancers. The composition of the formulation was prepared with nutmeg oil as a carrier, surfactant Tween 20, span 20 and glycerin in purified water by high-speed homogenization. Accurately weighted quantities of surfactants and cholesterol were taken to give the desired ratio and were dissolve in 50 ml of ethanol in a round bottom flask. Then, accurately weighted amount of drug was added to the solvent (ethanol). The solvent was evaporated in a rotary flash evaporator at temperature of 60°C at 120 rpm.The film was hydrated with the heated buffer by hand shaking for half an hour in orbital shaker. The transferosome was observed under microscope The prepared mixture was sonicated upto 10 min with ultrasonicator. The transferosomes suspension was formed and kept at 2 to 8°c for 24 hrs [9].

Table 1: A various composition of different transferosomes containing gel formulations

F. Code	Lipid material (mg)			Tween 20(%)	Span 20 (%)
	Cholesterol	Phosphatidylcholine	Triglycerides (Tristearin)		
KTG1	150	0	150	10	0
KTG2	0	150	150	10	0
KTG3	150	50	100	10	0
KTG4	50	150	100	10	0
KTG5	150	100	50	0	10
KTG6	50	100	150	0	10
KTG7	100	150	50	0	10
KTG8	100	50	150	0	10

Preparation and characterization of transferosomal gel: The transferosomes KT1 – KT8 was incorporated in to a gel base to prepare transferosomal gel formulations KTG1 – KTG8 and evaluated as parameters discussed earlier. The quantities of gel ingredients were weighed methyl paraben (50 mg), glycerine (10 ml), polyethylene glycol (5ml) were dissolved in about 50 ml of water in beaker. The dissolved mucilaginous materials were stirred at high-speed using mechanical stirrer. Then Carbopol 940 (1 g) and PVP (100 mg) were added slowly to the beaker containing above mixture liquid dispersion while stirring. After 1 h, the prepared gel solution was neutralized by the addition of the alkali triethanolamine (1 ml) act as gelling agents was added slowly while stirring to attain translucent gel structure with maximum viscosity. The transferosomal gel was finally transferred in aluminium collapsible tube and labelled [10].

Evaluation of transferosomal gel formulation:

Physical appearance: The physical appearance of prepared transferosomal gel was visually checked as parameters i.e. colour, appearance and feel on application.

Determination of vesicle size and size distribution: The average vesicle size and size distribution was determined by using zeta sizer. The sample of dispersion was diluted to 1:9 with distilled deionized water.

Determination of Zeta potential: The zeta potential of particles is the overall charge that the particle acquires in a particular medium Zeta potential of transferosomes formulations were assessed by p-hoton correlation spectroscopy using Zetasizer Nanoseries using a flow-through cell.

pH determination: The pH of transferosomal gel were determined by using the digital pH meter. 1 gram of gel was dissolved in 100 ml distilled water and stored for two hours. pH electrodes were completely dipped into the formulations and pH was noted. The measurement of pH of each formulation was done in triplicate manner and average values were calculated.

Extrudability determination: The transferosomal gel was filled into collapsible metal tubes. The tubes were pressed with same pressure by using fingers and the extrudability of the formulations was checked. The extrudability of the formulation was determined

in terms of weight in grams required to extrude a 0.5 cm ribbon of gel in 10 seconds.

Viscosity determination: The viscosity of the prepared transferosomal gel was measured by Brook field viscometer. The sufficient quantity of gel base was filled in wide mouth jar separately and it should sufficiently allow dipping the spindle. The RPM of the spindle was adjusted to 2.5 RPM. The viscosities of the formulations were recorded.

Spreadability: Spreadability means the extent of area to which transferosomal gel readily spreads on application to skin or affected part of skin. Lesser the time taken for the separation of two slides, better the spreadability. It is calculated by using the formula

S = M * L / T

where, M = Weight tied to upper slide; L = Length of glass slide; T = Time taken to separate the slides

Homogeneity: The transferosomal gel has been set in the container; all prepared gels were tested for homogeneity by visual inspection.

Grittiness: The transferosomal gel was evaluated microscopically for the presence of any appreciable particulate matter under light microscope.

Determination of Entrapment Efficiency: The entrapment efficiency was determined after separation of the unentrapped drug by the use of minicolumn centrifugation method, the drug content was determined using UV-Vis spectroscopy at 233 nm.

The percentage efficiency was determined by following equation:

$$\text{Percentage drug entrapment efficiency} = \frac{\text{Amount of entrapped drug}}{\text{Total amount of drug efficiency}} \times 100$$

In-vitro permeation studies: In-vitro permeation studies of the developed transferosomal gels were carried out using Franz-diffusion cell. The drug release studies of the ketoconazole transferosomal gel was carried out in 10 ml of phosphate buffer pH 6.8 saline maintained at 37±2° with a magnetic stirrer with constant heating equipment. Amount of drug diffused through the membrane was measured by using U.V. spectrophotometer at the wavelength 233 nm against phosphate buffer (pH6.8) as the blank [11].

RESULT AND DISCUSSION:

Analytical method: The UV spectrum of Ketoconazole in phosphate buffer pH 6.8 was scanned and λmax was found to be 233 nm.

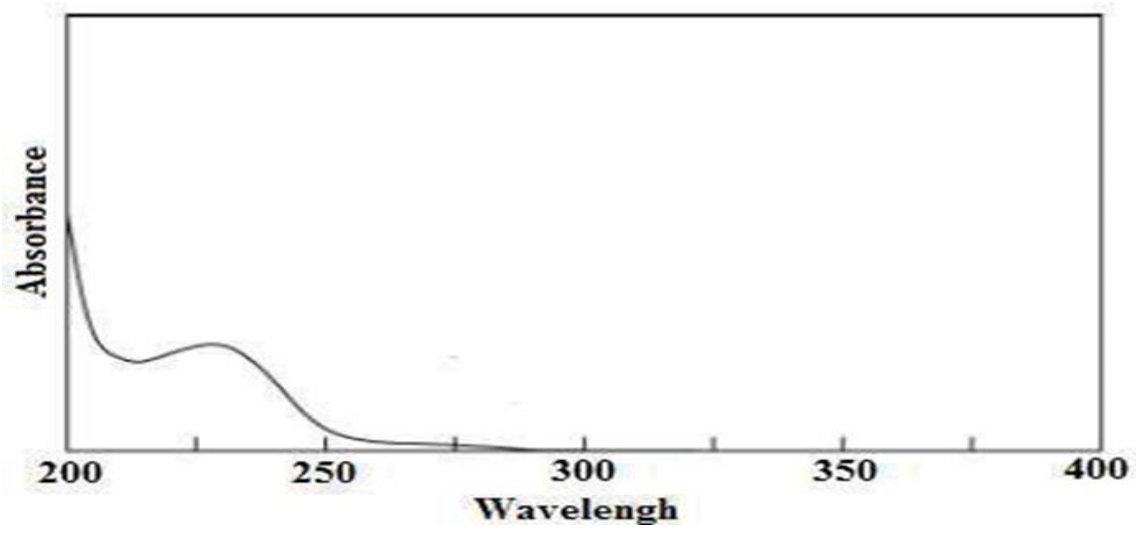

Figure 1: UV spectrum of ketoconazole showing absorption maxima at 233 nm

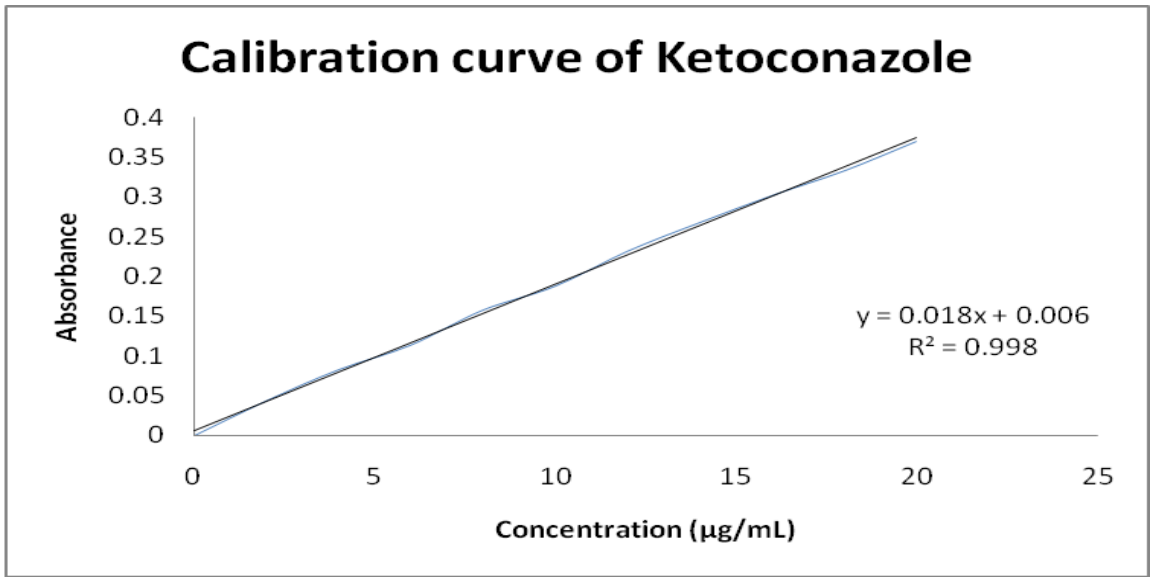

Figure 2: Calibration curve of Ketoconazole

Optimization of transferosomal gel: The physical evaluation as the colour of prepared transferosomal gels was light yellow colour to pale yellow colour in colour. The appearance of tarnsferosomal gel was transparent and translucent in nature and smooth on application at skin. The optimization transferosome gel was done on the result of Particle size, Zeta potential, Poly dispersity index of prepared various formulations transferosomes from KTG1 to KTG8. The result of all dependent variables were evaluated and among all the formulations KTG4 having phosphetidyl choline:cholesterol:triglyceride (0.5:1.5:1.0) was selected best formulation. The prepared transferosomes lipid layers ratio varying from 0.5 to 1.5 in with solid lipid triglycerides (Tristearin) provides a hydrophobic core. The result concluded that as the concentration of solid core varies and the amount of lipid content increase the particle size increase, thus increase the PDI and zetapotential action. The individual properties of prepared transferosomal gel was found to be smooth with pH ranges at 7.03 to 8.21 and good extrudibility due to having good viscous property. These transferosomal gels stored to accelerated stability testing with physically stable at all temperatures during storage period. The result concluded that KTG4 was best formulation under all observations.

Table 2: Characterization of ketoconazole containing transferosomal gel

Formulation Code	Particle size (nm)	Zeta potential (mV)	PDI
KTG1	123.02±1.02	-22.12±1.01	0.221±0.01
KTG2	124.01±1.01	-21.14±1.01	0.221±0.03
KTG3	131.01±0.02	-21.31±1.13	0.232±0.11
KTG4	125.11±1.01	-23.21±1.19	0.221±0.01
KTG5	129.11±1.01	-21.18±1.081	0.231±0.05
KTG6	128.11±1.12	-22.03±1.01	0.224±0.01
KTG7	130.21±1.01	-21.01±1.01	0.226±0.03
KTG8	129.11±1.01	-21.11±1.01	0.222±0.01

Table 3: Evaluation of transferosomal gel formulation

Parameters	Formulations
	KTG4
Colours	Pale yellow colour
Appearance	Translucent
Odour	Pleasant odour
Feel of application	Smooth
Spreadability (g.cm/sec)	8.4
Consistency	Poor
pH	6.18
Viscosity (cps)	0.94
Extrudability	Good
Drug content (%)	99.41

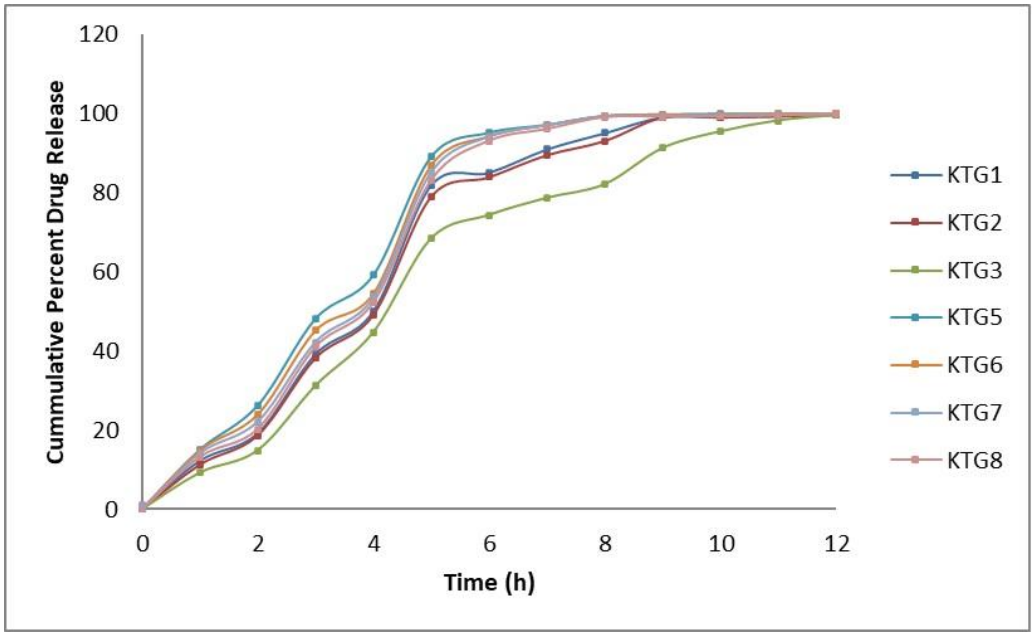

Figure 3: Zero-order in-vitro drug diffusion release kinetic study of transferosomal gel formulation (KTG1-KTG8)

SUMMARY AND CONCLUSION:

The formulation transferosomes was prepared with optimization of lipid ratio over the solid lipid content on the result of variables of prepared various formulations from KTG1 to KTG8. The content of solid lipid core triglycerides were coated with single layer of lipid content and double layer of combination of two layers of cholesterol and phosphetidyl choline. The various combinations of phosphetidyl choline:cholesterol:triglyceride optimize with constant concentration of surfactant as penetration enhancer of drug. The penetration enhancer affects the insertation of drug (Ketoconazole) amount inside the solid lipoid core (TG) through the single or double layer of lipid layers. The sonication time was creates unilaminar vesicles and the process also useful for overcome the problem of agglomeration of vesicles. The result concluded that as the concentration of solid core varies and the amount of lipid content increase the particle size increase, thus increase the PDI and zetapotential action. All the result of dependent variables concluded that the formulation KTG4 was selected for the optimization of effect of various surfactants in different concentration to identify the penetration rate or drug entrapment efficiency inside the solid lipid core. The gel base containing sodium CMC, Carbopol 934 was selected as the optimized concentration of gelling agent. The prepared

transferosomes was prepared to transferosomal gel and evaluated with various parameters.

REFERENCES:

1. Chaurasiya P., Ganju E., Upmanyu N., Ray S.K., Jain P. Transfersomes: A novel technique for transdermal drug delivery. J. Drug Deliv. Ther. 2019;9:279–285.
2. Modi C., Bharadia P. Transfersomes: New dominants for transdermal drug delivery. Am. J. PharmTech. Res. 2012;2:71–91.
3. Jain A.K., Kumar F. Transfersomes: Ultradeformable vesicles for transdermal drug delivery. Asian J. Biomater. Res. 2017;3:1–13.
4. Elsayed M.A., Abdallah O.Y., Naggar V.F., Khalafallah N.M. Lipid vesicles for skin delivery of drugs: Reviewing three decades of research. Int. J. Pharm. 2007;332:1–16.
5. Crommelin D.J.A., Fransen G.J., Salemink P.J.M. Stability of liposomes on storage. Target. Drugs Synth. Syst. 1986:277–287.
6. Xu X., Khan M.A., Burgess D.J. Predicting hydrophilic drug encapsulation inside unilamellar liposomes. Int. J. Pharm. 2012;423:410–418.
7. Szoka F., Papahadjopoulos D. Procedure for preparation of liposomes with large internal aqueous space and high capture by reverse-phase evaporation. Proc. Natl. Acad. Sci. USA. 1978;75:4194–4198.
8. Cevc G. Transfersomes, liposomes and other lipid suspensions on the skin: Permeation enhancement, vesicle penetration, and transdermal drug delivery. Crit. Rev. Ther. Drug Carr. Syst. 1996;13:257–388.
9. Mansat M, Picot M, Chicanne G, Nahoum V, Gaits-Iacovoni F, Payrastre B, Hnia K, Viaud J. Liposome-Based Methods to Study Protein-Phosphoinositide Interaction. Methods Mol Biol. 2021;2251:177-184.
10. Morbioli I, Casnati A, Esko JD, Tor Y, Sansone F. Calixarene-decorated liposomes for intracellular cargo delivery. Org Biomol Chem. 2021 Aug 5;19(30):6598-6602.
11. Rajan R., Jose S., Mukund V.P.B., Vasudevan D.T. Transferosomes—A vesicular transdermal delivery system for enhanced drug permeation. J. Adv. Pharm. Technol. Res. 2011;2:138–143.