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Pharmaceutical development of metronidazole loaded transferosome gel for skin delivery

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Abstract--Objective: The focus of this research was out whether a transfersomal gel formulation for transdermal delivery of Metronidazole. Methods: Azole Antifungals include metronidazole, which is used to treat fungal and yeast infections. Transferosomes are supra-molecular aggregates that are ultra-flexible and have a high ability to penetrate mammalian skin intact. Drug encapsulation in various transfersomal formulations containing various ratios of different drug concentrations (0.05, 0.1, 0.2, 0.3, 0.4, 0.5 g) and Carbopol-934 (0.5, 1, 2 g) is being researched for use as a transferosomal gel. Results: Entrapment efficiency (EE %), drug content, in-vitro skin permeation tests, and stability investigations were performed on the produced formulations. The vesicles were spherical in shape, as confirmed by Transmission Electron Microscopy. Metronidazole was successfully pinned with a standardised drug content in all formulations, according to the results. The 0.1 g Metronidazole optimised transferosome formulation MG2 showed promising results, with maximum drug release (94.32%) and maximum drug release (94.32%). Conclusion: According to this report, transferosomes are a promising long-term delivery mechanism for metronidazole and have reasonably good stability. This study suggests that transferosomes containing Metronidazole may be used as a transdermal drug delivery tool for fungal skin infections.

Keywords---Transferosome, Edge Activator, Felixibility, Penetration, Metronidazole.

Introduction

A transferosome is a mechanism that can transfer drugs when it is applied to a specific site and penetrates the skin easily [1-3]. The interdependence between local composition and bilayer form is what gives transferosomes their self-regulating and self-optimizing properties. They can be used as an alternative drug carrier for sustained release of therapeutic agents and non-invasive targeted drug delivery because of these properties. [4, 5] The approaches resulted in two new vesicular carriers: Transferosomes and ethosomes, which are flexible elastic lipid-based vesicles. [6] . Each transferosome's aqueous phase is the innermost layer. The addition of an agent known as edge activator allows a lipid bilayer to surround and coat the inner aqueous layer with certain special modifications [7].

The antibiotic metronidazole is used to treat a wide range of infections. Bacterial infections of the vaginal canal are also treated with vaginal metronidazole gel. [8]

Materials and Methods

The pure metronidazole drug was obtained as a gift sample from Max-Med Laboratories in India. Delpha Drugs and Pharmaceuticals India given soya lecithin, methanol, and chloroform. S.D. Fine Chemicals Ltd., India, had given soya phosphatidyl choline, carbopol, isopropyl alcohol, and potassium dihydrogen orthophosphate. All of the chemicals used in the experiments were of analytical grade. Purified water that had been freshly prepared was used.

Pre-formulation studies

Compatibility studies through FT-IR

FTIR spectra obtained through the compatibility of the pure drug and excipients was observed using Bruker FTIR [9]. The spectra were reported at wave numbers ranging from 3500 to 500cm⁻¹. The results have been shown in the Figure 1,2,3,4

Metronidazole Standard Curve in Phosphate Buffer Saline pH 7.4

A UV visible spectrophotometer was used to conduct the calibration curve, which was measured at 227 nm. [10]

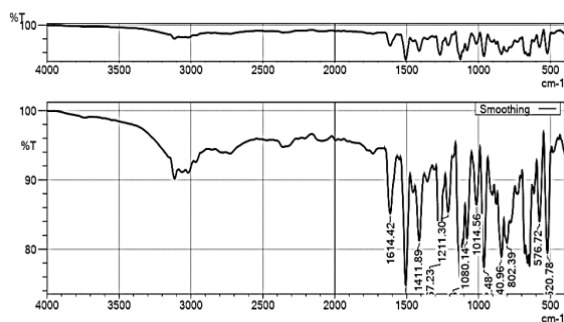
Formulation of Metronidazole loaded Transferosomes

Metronidazole, soya lecithin, and sodium deoxycholate were utilized to make transferosomes. Each formulation has a different amount of medication (Table 1). In a clean, dry bottom flask, dissolve sodium lecithin, sodium deoxycholate, and the medication in 10 mL of a (3:1) v/v mixture of two organic solvents (chloroform: methanol). The organic solvent was carefully evaporated using a magnetic stirrer to generate a lipid coating on the flask wall, and a phosphate buffer solution (pH 7.4) was hydrated by rotation at room temperature at 60 rpm for 1 hour and left at room temperature for 2 hours to allow swelling. A probe sonicator is used to sonicate the multilaminar lipid vesicles (MLV) for 10 minutes (Heldolph vcx750). [11].

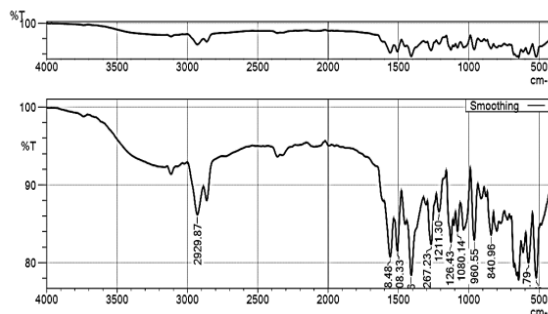
Table 1: Formulation table of Metronidazole Loaded Transfersome

Formulation code (mg)	Metronidazole Drug (mg)	Soya phosphatidyl choline (mg)	Sodium deoxycholate (mg)	Chloroform : methanol ratio
M1	50	50	30	3:1
M2	100	50	30	3:1
M3	200	50	30	3:1
M4	300	50	30	3:1
M5	400	50	30	3:1
M6	500	50	30	3:1

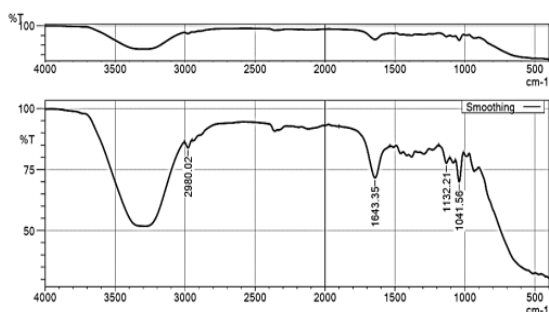
Evaluation of Transfersomes Preformulation studies



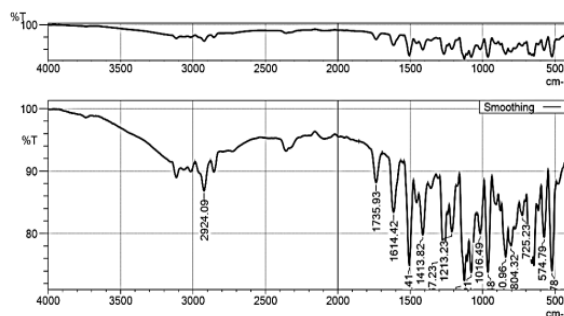
**Fig.1: FTIR Spectra of Metronidazole
Metronidazole and
Sodium Deoxycholate**



**Fig.2: FTIR Spectra of
Metronidazole and
Soya Lecithin**



**Fig.3: FTIR Spectra of Metronidazole
and Soya Lecithin**



**Fig.4: FTIR Spectra of Metronidazole
and Soya Lecithin**

Vesicular size determination:

The diameter of the vesicle can be determined using photon correlation spectroscopy. A sample is made with distilled water. The samples are diluted with filtered saline after passing through the 0.2 mm membrane filter. [12].

Zeta Potential Analysis

Using Zetasizer (DTS Version 5.03, Malvern) and the light scattering technique, commonly known as photon correlation spectroscopy, the zeta potential, size distribution, and vesicle size of the optimised formulation were measured (PCS). In this arrangement, the zeta sizer is set at 25°C and at a 90° angle. Water is utilised as a dispersion for estimating Zeta potential and determining size^[13]

Entrapment Efficiency:

Entrapment efficiency is the percentage of what is added expressed in the amount of drug present. To isolate the trapped drug, mini-column centrifugation is used. Vesicle disruption is conducted using 50 % of the total n-propanolol.

Formula:
$$\frac{\text{Amount entrapped}}{\text{Total amount added}} \times 100$$

Dilutions are carried out 5 times with 0.9 % NaCl solution for non-sonicated transferosome formulations. For further study optical microscope and Haemocytometer can be used. For composition and optimization of other process variables, this is an essential parameter.

Percentage Entrapment Efficiency:

Centrifugation method is used to determine the amount of drug Metronidazole being entrapped in the transferosome. 10ml of phosphate buffer (pH 7.4) is diluted to 1ml of transferosome formulation. For half an hour bath sonicator is used to sonicate this suspension. Centrifugation is carried out a speed of 14000 rpm with this solution for 30 minutes. 0.5ml of the supernatant was withdrawn and diluted 20 times before using UV spectrophotometer (UV-3200 Lab India) for the measurement of absorbance at 261nm. Unstrained drug is obtained from this procedure. The percent of drug trapped gives the entrapment efficiency of the drug^[14].

$$E.E\% = [(C_t - C_f) / C_t] \times 100$$

Percentage Drug Content

For the determination of percentage drug content transferosome formulation of about 1gm was taken. Sonication was carried out with ethanol to lyse the vesicles for 15 min. For half an hour, centrifugation at a speed of 14000 rpm was carried out by placing the solution in a centrifugation tube. Methanol of 100 ml was used to dilute the clear solution obtained. 100 ml phosphate buffer of pH 7.4 was made by diluting 10 ml of the prepared solution Aliquots were withdrawn after regular time intervals and by using UV spectrophotometer at 261nm the drug content was calculated for Metronidazole.

***In-Vitro* Drug Release Studies:**

The cellophane membrane (Molecular weight cut off 12000-14000, HI Media Ltd, Mumbai, India) is used to test different transfersomal formulation drug release trends. On a membrane placed between the donor and receptor chambers (Franz-diffusion cell apparatus) with an accessible diffusion region, an exact amount of formulation is spread out. The receptor compartment is filled with a continuously stirred phosphate buffer pH 7.4 with a small magnetic bar at a rate of 50 rpm held at a temperature of 37 ± 0.5 °C. The 5 ml aliquots were removed at different time intervals and replaced with the same amount of phosphate buffer solution. The samples were analysed in a spectrophotometer after which a graph is shown with the accumulated quantity of drug permeating through the membrane across time to build the invitro drug release ^[15].

Preparation of Carbopol Gel:

Three different quantities of carbopol resin 940—1.1000 mg, 2000 mg, and 3.3000 mg—were dissolved in distilled water (30ml). The mixture was mixed up until it became thick. PEG-400 (5 ml) was gradually included into the Carbopol-940 aqueous dispersion after complete dispersion. Then, additional chemicals including 1ml of triethanolamine (TEA), 5ml of propylene glycol (PG), and 5ml of isopropyl alcohol (IPA) were added. To ensure a uniform dispersion of the gel, distilled water (q.s.) was additionally added for every 100g of gel. To find the optimum batch of Carbopol gel, these three distinct gel formulations were tested using a variety of evaluation criteria. ^[16].

To the prepared carbopal gel transfersomes of metronidazole which is equivalent to 100 mg drug was incorporated as shown in the table 2

Table 2: Formulation Table For Metronidazole Loaded Transfersosomal Gel

S.NO	Transfersome (mg)	Carbopol (mg)	Triethanol Amine (ml)	Propylene Glycol (ml)	Iso-Propyl Alcohol (ml)	Water (ml)
MG1	100	1000	1	5	5	Q.S
MG2	100	2000	1	5	5	Q.S
MG3	100	3000	1	5	5	Q.S

Evaluation For The Formulated Transfersomal Gel:

Appearance:

Three different formulations of Carbopol gel were developed and tested for physical appearance through visual observation.

p H Value of Topical Transferosome Gel :

For determining the pH, each of the gel formulations was taken to measure pH using digital pH meter. The measurement of the pH of each system was replicated thrice [17].

Grittiness:

The light microscope was used to determine microscopically the presence of particles in all the formulations prepared. The gel formulation shows satisfying results of freedom requirement from grittiness and the particular matter as it is a desired characteristic for any topical formulation^[18].

Spreadability Test:

The gel formulation of 350 mg which was taken on one glass slide and another glass slide was containing about 5.8 ± 1 g of gel which was allowed to drop from a 5 cm distance. After 1 min, the spread gel was examined to determine the diameter of the circle [19].

Extrudability Test:

After applying the weight in gram necessary to extrude a gel ribbon of at least 0.5 cm in length in 10 seconds, the amount of gel (g/cm²) that was extruded from the lacquered aluminium collapsible tube was calculated. The formula provided can be used to calculate the extrudability. [20].

$$\text{Extrudability} = \frac{\text{Weight applied to extrude gel from the tube (g)}}{\text{Area in cm}^2}$$

Transmission Electron Microscopy Studies:

Transmission electron microscopy was used for determining the formulated gel^[21].

Scanning Electron Microscopic Studies:

Scanning electron microscopic studies are utilized to gain knowledge about the morphology of surface^[22].

Drug Content :

The vesicles in 1 g of a transferosome gel formulation were lysed in 25 ml of ethanol using a sonicator for 15 minutes. Later, a centrifuge tube containing this solution was filled, and it was centrifuged for 30 minutes at 14000 rpm. Methanol was used to dilute the clear solution to 100 ml. Then, using a phosphate buffer with a pH of 7.4, 10 ml of the solution was made into 100 ml. Metronidazole's drug content was determined after aliquots were taken out using a UV spectrophotometer set at 227 nm.^[23,24,25]

***In-vitro* Release Study :**

The invitro drug release study was carried out as mentioned in the transferosome evaluation^[26,27].

Stability Study:

For the stability study evaluation, the formulation was maintained at room temperature ($25 \pm 2^\circ\text{C}$) for two months. To evaluate the formulation, pH, spreadability and extrudability were checked after the 1st and 2nd month^[28,29,30].

Results and Discussion**Preformulation Studies:****Drug Excipient Compatibility Study by FT-IR:**

The identification of the drug excipient interaction has been made possible with the help of IR spectrophotometry. The figures below show the IR spectrum of the physical mixture of metronidazole, sodium deoxycholate, soy lecithin, and carbopal. The bands at 1506.41 cm^{-1} (N=C stretch), 3107.90 cm^{-1} (aromatic -C-H stretch), 2963.15 cm^{-1} (aliphatic -C-H stretch), 2979.21 cm^{-1} (-CH₂ bending), and 3203.12 cm^{-1} were identified in the FTIR spectrum of metronidazole (O-H). There were no significant differences between the primary peaks recorded for Metronidazole alone and those seen in the physical combinations and Metronidazole Transferosomes. There were no interactions between the medication and excipient, according to the IR spectra.

Evaluation of Metronidazole Loaded Transferosomes**Zeta Potential Analysis :**

The size of the vesicles and its distribution is confirmed by the obtained size distribution curve The normal.

Table 3 Z-Average Size, Zeta Potential And Pdi Of Optimized Formulation

PARTICLE SIZE & Z-POTENTIAL ANALYSIS		
Formulation	Z-Average size (d.nm)	PDI
M2	254.9 ± 60.2	0.783

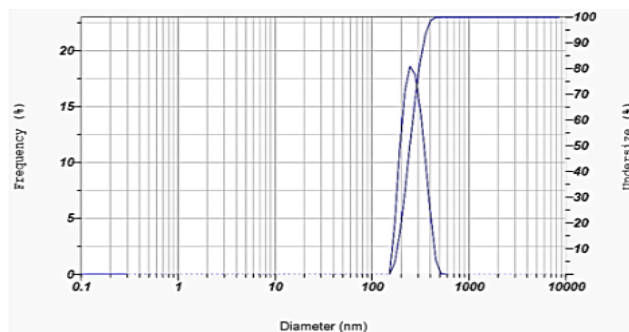


Fig. 5: Particle size of Optimized Formulation

Drug Content and Drug Entrapment Efficiency

The drug content and entrapment efficiency studies revealed M2 formulation seems to be above 90 % as shown in the table 4

Table 4: Percentage Entrapment Efficiency and Percentage Drug Content After Stability Studies

Formulation	% Drug Content	% Entrapment Efficiency
M1	81.32 ± 0.05	80.22 ± 0.01
M2	94.32 ± 0.21	92.34 ± 0.05
M3	84.21 ± 0.5	85.07 ± 0.08
M4	89.08 ± 0.34	89.97 ± 0.04
M5	83.15 ± 0.04	82.15 ± 0.03
M6	91.78 ± 0.18	90.53 ± 0.01

Invitro drug release of metronidazole loaded transferosomes

The invitro drug release of metronidazole loaded transferosomes shows optimum release for 12 hrs as shown in the figure 6

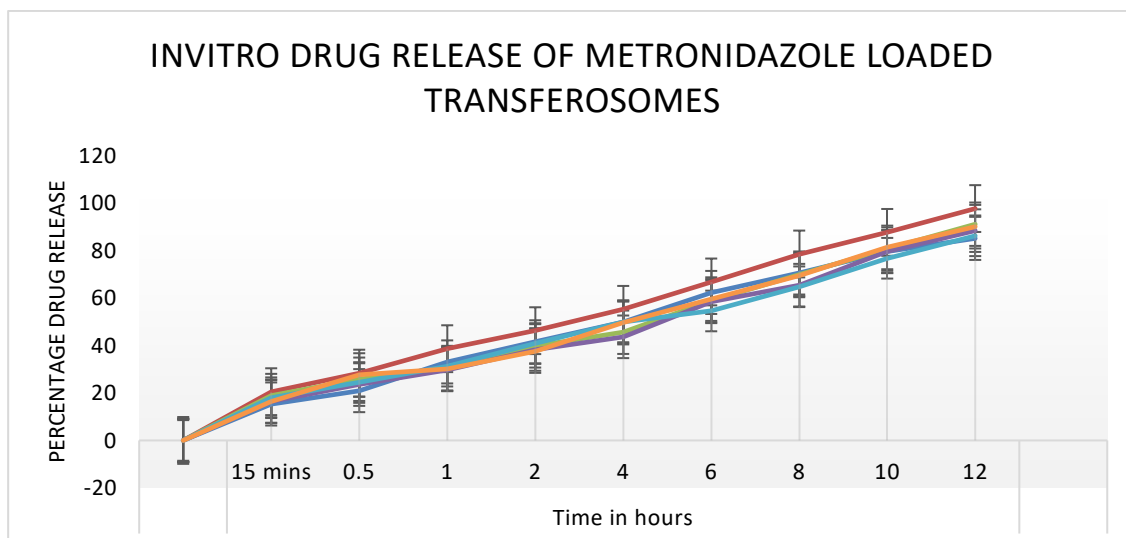


Fig. 6: *In vitro* Release Profile of all the Formulated Metronidazole

Transfersome Batch

Incorporation of Transfersomal Drug Formulation In Carbopol-940 Gel:

Carbopol gel contained a dispersion of transfersomes [16]. Finally, a mechanical stirrer (Remi Instruments, Mumbai, India) was used to mix a transfersomal dispersion (free of untrapped drug) into a plain vehicle (Carbopol-940 gel) for 5 minutes. Through drug content and invitro release experiments, the best batch of the three Carbopol gel formulations will be determined.

Evaluation Of Transfersomal Metronidazole Formulation In Carbopol-940 GEL:

Appearance:

The appearance of the five differently formulated nanogels was tabulated in table

Table 5: Appearance of Transfersomal Gel Batches

S. No	Formulation code	Appearance
1	GT1	White and opaque
2	GT2	Clear and soft
3	GT3	Highly viscous

Transmission Electron Microscopy:

Transmission Electron Microscopy (TEM) of formulation MG2 as shown in the figure 7.

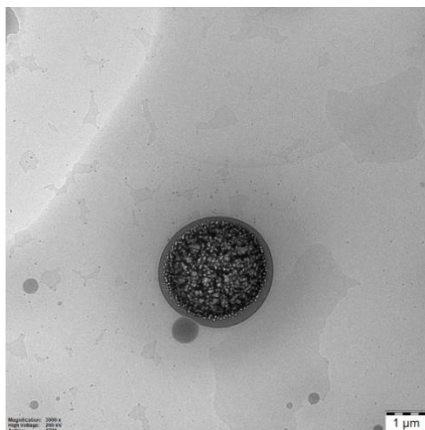


Fig.9: TEM Micrograph Of Transfersomal Gel Formulation MG2

Scanning Electron Microscopic Studies:

The scanning electron microscopy of MG2 is shown in the figure 10

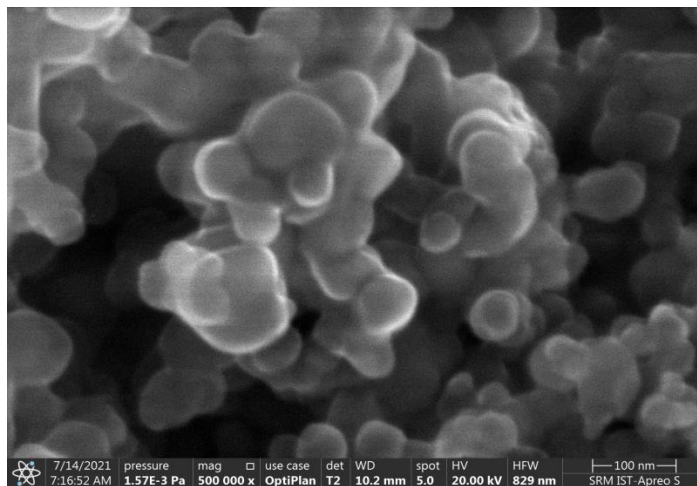


Fig.10:SEM of Transfersomal Formulation MG2

pH Value of Topical Transfersome Gel :

The value of pH of topical transfersome gels was measured by using a digital pH meter (LabindiaSab 5000 pH meter) at the room temperature. For skin delivery of a drug to understand its suitability, the pH of the formulation is given major importance. A pH range of 6.8-7 was determined for the three various formulations prepared, of which the optimised formulation MG2 had a pH value of 6.8 as shown in the table 7.

Table 6 :Entrapment Efficiency Of Transfersomal Gel

The entrapment efficiency and drug content is shown in the table 6

Number of days	% Entrapment Efficiency		% Drug content	
	Before (25 ± 2° C)	After (25 ± 2° C)	Before (25 ± 2° C)	After (25 ± 2° C)
30 days	92.34 ± 0.05	90.74±1.854	94.32 ± 0.21	92.32±0.987

Table 7: Ph, Grittiness,spreadability, extrudability and percentage drug content of transferosomal gel

S. No	Formulation Code	pH	Grittiness	Spreadability (Gm.Cm/Sec.)	Extrudability	% Drug Content
1.	MG1	7.0 ± 0.7	No	3.76 ± 0.5	5.5±0.25	87.38 ± 0.85
2.	MG2	6.8 ± 0.8	No	1.70 ± 1.9	6.2±0.20	93.12 ± 0.91
3.	MG3	6.9 ± 0.5	No	2.06 ± 0.1	7.8±0.20	84.06 ± 0.05

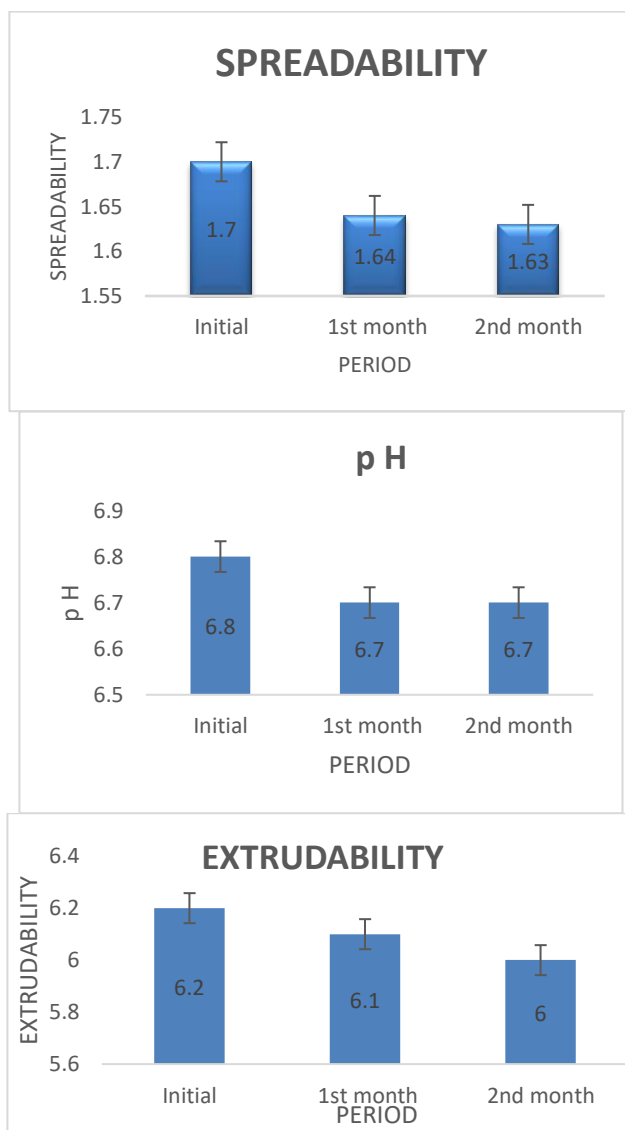


Fig.11: Spreadability, p^H, Extrudability of Metronidazole loaded transfersome gel

***In-vitro* Drug Release Study:**

The three different transfersomal gel formulations MG1, MG2, MG3 were examined for *in-vitro* diffusion study. For this study Modified Franz diffusion cell along with dialysis membrane was used. This study is carried out for 6 hours in phosphate buffer of pH 7.4. Figure 12 summarises the data obtained from the diffusion study of three formulations. The amount of drug release from the formulated gel containing Metronidazole was found to be in the order:

MG1 < MG3 < MG2

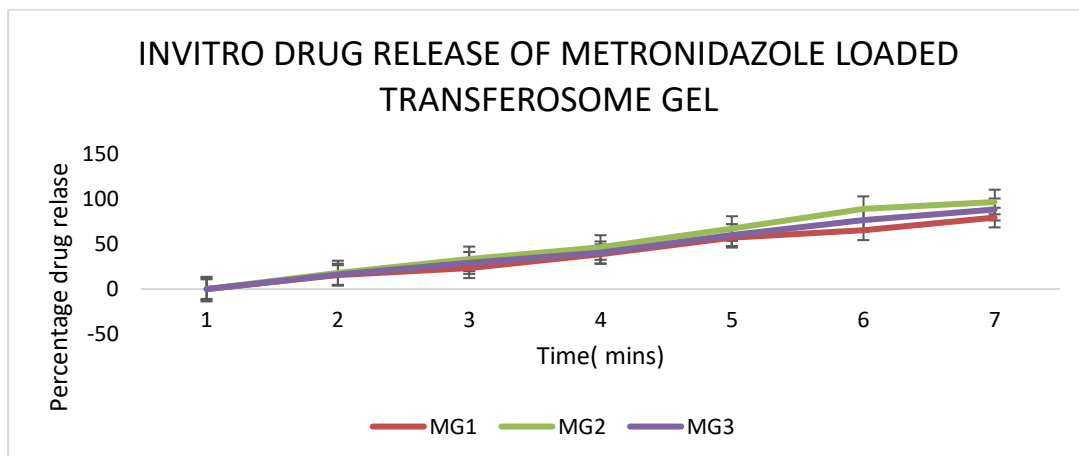


Fig.12: In-vitro Drug Release Study of all three Gel Formulations

Anti Fungal Activity: *In-vitro* anti-fungal activity was determined using the cup plate technique. The media was prepared by dissolving 10 gm. of peptone, 20 gm of agar, and 40 gm of dextrose powder in per liter of distilled water and was sterilized using autoclave at 121 °C for 20 min. The plates were sterilized in a hot air oven at 160 °C for 60 min. *Candida albicans* culture was introduced into the plate, and 20 ml of sterile sabouraud dextrose agar was poured into the plate. The plate was agitated carefully to allow for both an even distribution of the sabouraud dextrose agar and test organism in the plate. The plates were allowed to harden, and three cups, of each 6 mm in diameter, were bored in the medium. The marketed product (Daktarin® Gel 2% w/w) was used as a standard. The standard, pure metronidazole drug and optimized metronidazole loaded transfersomal gel (test) were taken into cups. The plate was incubated for 48 h at 25 °C. The zones of inhibition were measured in mm after 48 h for the test, standard and pure metronidazole drug.

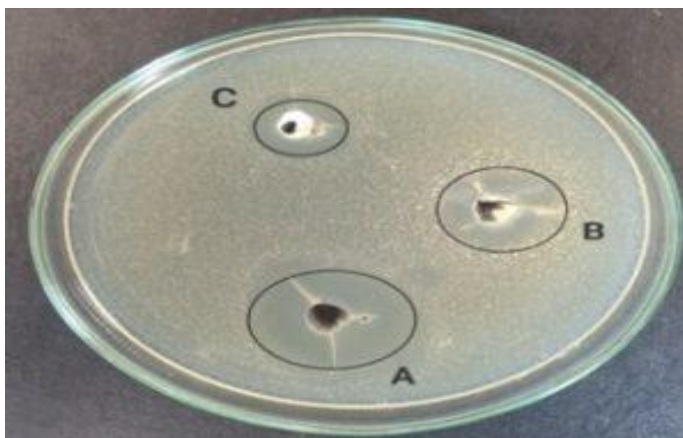


Fig.13: Antifungal Activity

Stability Study:

The stability study was carried out for a period of two months in room temperature for the batch MG2. The results showed that there was not much variation with the results within the period of two months. The results are shown in the table 8

Table 8: Stability Studies For Spreadability And Extrudability Of MG2 Batch

Evaluation	Initial	1st month	2nd month
pH	6.8 ± 0.8	6.7 ± 0.9	6.7 ± 0.7
Spreadability	1.70 ± 1.9	1.64 ± 0.19	1.63 ± 0.82
Extrudability	6.2 ± 0.20	6.1 ± 0.81	6.0 ± 0.12

Discussion

The drug Metronidazole demonstrated maximum absorption at wavelength 227 nm in methanol and all acquired values, according to the estimation procedure. Beer's law was observed by the standard curve at the specified concentration of 10 g/ml, and regression analysis revealed a linear relationship between concentration and absorbance with a regression coefficient value of 0.9989.

Transmission electron microscopy was used to examine the surface morphology. Most of the transferosomes identified to carry metronidazole had spherical forms. The spherical vesicles' contour and core could be seen in the transmission electron micrograph of the studied transferosome (M2), demonstrating its vesicular properties. The M2 transferosome under examination has a vesicular form and measures 200 nm and 100 nm.

The range of percentage entrapment efficiency for deformable vesicles formulations was determined to be 80.22 ± 0.01 to 92.34 ± 0.05, with the formulation M2 showing the highest percentage of entrapment (maximum 92.34 ± 0.05).

The results demonstrate that there is no drug degradation during the process because all formulations had a drug content ranging from 81.32 to 94.32 %. The outcomes show that the method used to make the Transferosomes was able to create formulations with predictable medication contents.

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The outcomes show that the method used to make the Transferosomes was able to create formulations with predictable medication contents.

Each Metronidazole transferosome formulation was subjected to *in vitro* drug release studies using a Franz diffusion cell. The cumulative amount of drug release was calculated for each formulation. Results revealed that the M2 (formulation with 100mg Metronidazole) had the highest cumulative amount of drug release (95.64%) up to 12 hrs as compared to other Metronidazole transferosome formulations.

The stability studies were carried out at $25 \pm 2^\circ$ C for a period of 30 days. At frequent intervals percentage entrapment efficiency and the drug content were evaluated. It is clear from the results obtained that the Transferosomes have shown the minimum drug loss at room temperature, and fairly high retention of the drug inside the vesicles was observed.

Scanning electron microscopy was used for determining the shape of formulated Transferosomes. The morphological structure found was spherical in shape as shown in Figure

Conclusion

It is concluded from the study that transfersomal gel formulated using sodium deoxycholate, soya lecithin, Carbopol, chloroform and methanol along with the pure drug Metronidazole can be used to improve the site specificity, increase the transdermal flux and prolong the release of the drug. Metronidazole could be entrapped into Transferosomes for penetration into skin pores much narrower than the vesicle diameter. The optimized transferosome formulation M2 containing 0.1 g Metronidazole showed promising results having maximum drug release (94.32 %) and higher entrapment efficiency (92.34 %) when compared to other formulations with concentrations of drug being the only variable factor. Similarly the transfersomal gel formulation (MG2) showed better results having maximum drug content (93.12 %) and cumulative percent drug release (96.78%). Transferosomes can alternatively be used as carriers for other transdermal drug delivery system as they possess simple scale up and can also act as a penetration enhancer by itself with easy production. Finally it is confirmed that transfersomal gel formulation of Metronidazole is therapeutically effective for the treatment of local skin infections and can be developed successfully as a commercial product to improve the antifungal activity of the drug.

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Authors Contributions

All the authors contributed equally.

Consent For Publication

Not applicable.

Conflict Of Interests

Declared none

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