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Formulation and Evaluation of ethosomal gel delivery system for metronidazole

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Abstract

The objective of the present work was to formulate ethosomal loaded with metronidazole and convert them to gel formulation. Ethosomes entrapping metronidazole were prepared using cold method and the effect of varying concentration of ethanol was considered for obtaining an optimized formulation. Lecithin (2%w/w) was used as the phospholipid to provide the structure to the vesicles and propylene glycol (10%) was used as the permeating agent. The vesicles were found to be of spherical to irregular shape ranged from 1.4 µm to 1.8 µm in size. The drug entrapment in the ethosomes was studied by analyzing the unentrapped drug using UV spectrophotometry at 340 nm and it was found that the maximum entrapment efficiency was found to be 92.07% for formulation ME3 and minimum 58.68% for formulation ME5, respectively. The *in vitro* permeation study suggested that the maximum permeation in the egg membrane occurred in ME3 (0.38 mg/cm^2) with 30% ethanol concentration while ME5 exhibited the minimum permeation (0.28 mg/cm^2). ME3 was incorporated into gel base to obtain gel formulations and the results revealed a good protection of the ethosomal gel when 2% carbopol was used as the gelling base.

Keywords: Metronidazole, ethosome, topical gel, entrapment, film hydration

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Introduction

Metronidazole is a commonly used antibiotic, belonging to the nitroimidazole class of antibiotics. It is indicated for the treatment of confirmed trichomoniasis caused by Trichomonas vaginalis (except for in the first trimester of pregnancy) and the patient's sexual partners, bacterial vaginosis, certain types of amebiasis, and various anaerobic infections. It is 20% bound to plasma proteins and has a half life of 6-10 h (drugbank, 2026). It is known to induce concentration-related skin irritation. Literature evidence suggests that the topical administration of drugs encapsulated in liposomes may be advantageous in reducing the irritation and itching and helps in improving the antibacterial efficacy and storage stability of the drug. It was further witnessed that ethosomes offered a higher advantage over the liposomes for transdermal delivery of drugs.

Ethosomes are lipid vesicles containing phospholipids, alcohol (ethanol and isopropyl alcohol) in relatively high concentration and water (Kumar et al., 2010; Bhalaria et al., 2009). Ethosomes have been studied was treating oral infections (Debata et al., 2022), topical bioavailability (El-Shenawy et al., 2020), sustained topical release (Fathalla et al., 2020), treat psoriasis (Chandra et al., 2019) and reduce skin irritation (Jain et al., 2018). The objective of this project is to develop, optimize and characterize metronidazole loaded ethosomes and formulate them as gel suitable for topical application.

Material and Methods

Metronidazole was obtained as generous gift from Medibios Laboratories Pvt Ltd, Tarapur. LR/AR/HPLC grade methanol, ethanol and propylene glycol were procured from Oxford Fine Chemicals, Mumbai. All the chemicals were used as received.

Preformulation Studies

The color, odor and taste of the obtained drug sample were observed with the help of the sensory organs. Melting point was observed by open capillary method. Calibration curve of methanol was prepared in phosphate buffer pH7.4 by measuring absorbance at 340 nm using UV-Visible spectrophotometer (Naveed & Qamar, 2014).

Preparation of ethosomes

Ethosomes were prepared by cold method. In brief the lecithin (3% w/v) was taken in a small round bottom flask and solubilized with ethanol (10-50% v/v) containing drug under mixing with a magnetic stirrer. The round bottom flask was covered to avoid ethanol evaporation. Distilled water was added slowly with continuous stirring to obtain the ethosomal colloidal suspensions. The final suspension of ethosomes was kept at room temperature for 30 min under continuous stirring. Formulations were stored in the refrigerator and evaluated for vesicle size, vesicular shape, surface morphology, entrapment efficiency, and in vitro drug permeation study (Mbah et al., 2014).

Table 1. Composition of ethosomal formulations

Formulation code	Lecithin concentration (%)	Ethanol concentration (%)	Polyethylene glycol concentration (%)
ME1	3	10	10
ME2	3	20	10
ME3	3	30	10
ME4	3	40	10
ME5	3	50	10

Evaluation of ethosomes

Shape and size

An optical microscope (Mkow) with a camera attachment was used to observe the shape of the prepared ethosomes formulation. Size and size distribution were determined by dynamic light scattering (DLS) using a computerized inspection system (Debata et al., 2022).

Entrapment efficiency

Aliquots of ethosomal dispersion were subjected to centrifugation using cooling ultracentrifuge (Remi) at 12000 rpm. The clear supernatant was siphoned off carefully to separate the unentrapped metronidazole and the supernatant was analyzed by UV spectophotometry. Sediment was treated with 1 ml of 0.1% Triton X 100 to lyse the vesicles and then diluted to 100 ml with methanol and metronidazole was analyzed by UV method. Amount of metronidazole in supernatant and sediment gave a total amount of metronidazole in 1 ml dispersion (Debata et al., 2022). The percent entrapment was calculated using the formula,

% entrapment= amount of metronidazole in sediment/amount of metronidazole added ×100

***In vitro* drug permeation study**

The *in vitro* permeation study was carried out by using modified Franz diffusion cell with egg membrane. The study was performed with phosphate buffer saline (pH 7.4). The formulation was placed (equivalent to 2.5 mg of drug) on the upper side of skin in donor compartment. The temperature of the assembly was maintained at 37±2º. Samples were withdrawn after every hour from the receptor media through the sampling tube and at the same time, same amount of fresh receptor media was added to make sink condition. Withdrawn samples were analyzed for metronidazole content using UV spectrophotometric method (Debata et al., 2022).

Formulation of ethosomal gel

Gel formulations were prepared by soaking varying concentration of Carbopol 934 in water for 24 h. The ethosomes equivalent to 2% w/w metronidazole were dissolved in ethanol and was added

to the gel with continuous stirring. The plasticizer and other ingredients were added and stirred to obtain the ethosome loaded gel formulation.

Table 2. Composition of gel formulation

Ingredients	MEG1	MEG2	MEG3	MEG4
Metronidazole ethosome (%)	2	2	2	2
Carbopol 934 (%w/w)	1	2	3	4
Propylene glycol (% w/w)	10	10	10	10
Ethanol (mL)	5	5	5	5
Triethanolamine (% w/w)	0.7	0.7	0.7	0.7
Water (g)	15	15	15	15

pH of gel

Accurately weighed quantity of 5 g of each gel formulation was mixed separately with 45 mL of distilled water and the pH of the solution was determined with the help of digital pH meter.

Viscosity of gel

The viscosity of each formulation was measured at 10 rpm by using Brookfield DV-1 viscometer employing a S94 spindle.

Spreadability of gel

Spreadability of the formulations was determined using indigenously developed apparatus. The apparatus consisted of a wooden block provided with a pulley at a one end. A rectangular ground glass was fixed on the block. An excess of cream (3-5 g) was placed on this plate sandwiched using another glass plate having the dimensions as that of fixed ground plate. A 1 kg weight was placed on the top of the plates for 5 minutes to expel air and to provide a uniform film of the cream between the plates. Excess of the ointment was scrapped off from the edges. Weight of 80 g was hung on the hook of the top plate with the help of string attached to the hook and the time (in seconds) required by top plate to cover a distance of 10 cm was noted. Spreadability of the formulation was determined by the following formula:

$S = M * L/T$; S – spreadability, L – distance travelled by the glass slide, T – time in seconds, M - weight in the pan

Results and Discussion

Preformulation studies

S. No	Parameter	Observation
1	Physical appearance	White
2	Odour	Odourless
3	Melting Point	157-159°C
4	Solubility	Water, ethanol, 0.1N HCl

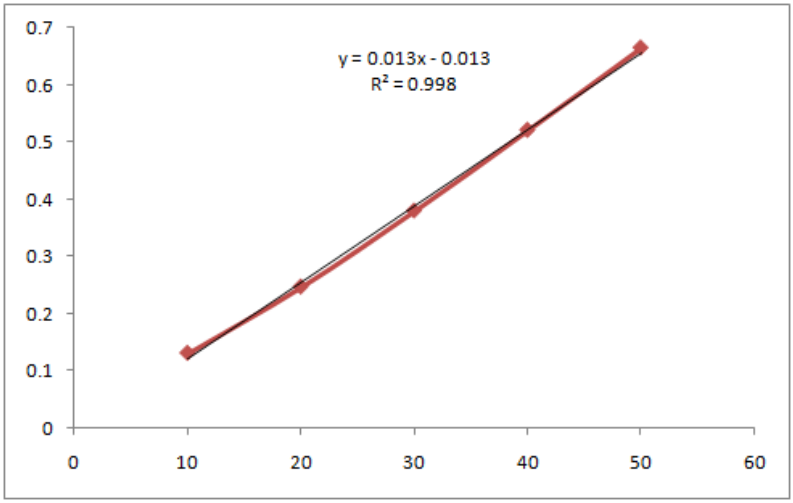

Figure 1. Standard curve of Metronidazole

Evaluation of ethosomes

The ethosomes were evaluated for shape and size, entrapment efficiency and in vitro permeation through egg membrane. The results of the study are presented in the following sections.

Vesicle shape and size

The vesicles were found to be of spherical to irregular shape ranged from 1.4 µm to 1.8 µm in size. The smallest particle size was found to be the formulation ME2 whereas the largest size was found to be of ME1 (Table 3).

Entrapment and drug permeation

The entrapment efficiency of ethosomes was determined for all formulations. Effect of ethanol concentration was observed on percent drug entrapment of ethosomes. The maximum entrapment efficiency was found to be 92.07% for formulation ME3 and minimum 58.68% for formulation ME4, respectively (Table 3). An increase in percent drug entrapment was observed with an increase in ethanol concentration, but when ethanol concentration exceeded 30%, a decrease in percent drug entrapment was observed. Improvement in aqueous solubility of

metronidazole was achieved with higher concentration of ethanol, which could be due to its co-solvent effect. Therefore, the more drug amount could be accommodated in the aqueous core of the vesicles however, as the concentration of ethanol increased above 30% resulting into leakage of drug from fluidized bilayer of vesicles.

Table 3. Evaluation parameters of ethosomes

Formulation Code	Vesicle Size (µm)	Shape	Drug Entrapment (%)	Cumulative amount of drug permeated (mg/cm^2)
ME1	1.73	Irregular	84.31	0.31
ME2	1.44	Spherical	82.18	0.34
ME3	1.64	Spherical	92.07	0.38
ME4	1.69	Irregular	70.11	0.34
ME5	1.56	Irregular	58.68	0.28

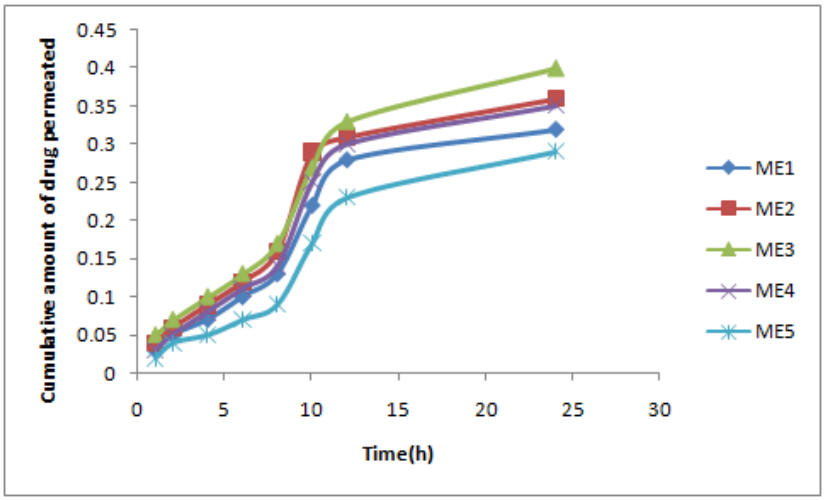

Figure 2. Drug permeation from metronidazole ethosomes

Evaluation of the ethosomal gel

The gel formulations were prepared using four concentrations of the gelling agent and were evaluated for physical appearance, pH, viscosity, drug content and in vitro diffusion of the drug. The gel formulations were found to be off-white in color, homogenous and sticky in feel. The pH of the all the formulations was between 6.4-6.7, rendering them suitable for topical application. The formulations were found to possess sufficient viscosity to make then suitable for application to the surface and extrusion from the collapsible tube in which they were packed. The drug content in all the formulations ranged from 96.7 to 98.39 % confirming the incorporation of the ethosomes into the gel base. The results of the evaluation parameters are presented in Table 4.

Table 4. Evaluation of the gel formulations

Formulation code	Color	Appearance	pH	Viscosity (cps)	Drug content (%)	Spreadability (g.cm/sec)	Extrudability (%)
MEG1	Off White	Sticky	6.44	7981	96.7	17.12	95
MEG2	Off White	Sticky	6.71	8186	98.11	16.08	84
MEG3	Off White	Sticky	6.41	8120	97.05	18.65	92
MEG4	Off White	Sticky	6.45	8512	98.39	15.26	87

Conclusion

The use of ethosomal flexible carriers has gained popularity as promising approach for transdermal drug delivery. Incorporation of metronidazole in the ethosomal carrier and formulating the same as gel formulation might help in reducing the dose of metronidazole as well as improving the anti acne efficacy (enhancing stability of metronidazole).

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