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INVESTIGATING OF NANOCARRIERS INVASOMES OF METRONIDAZOLE FOR SKIN DRUG DELIVERY FOR TREATMENT OF MICROBIAL DISEASE

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ABSTRACT

Metronidazole is a commonly used antibiotic. It is available in capsule form, tablet form, and topical form, and suppository preparations for the treatment of various infections. To increase its efficacy this study aims at formulation of invasomal gel of metronidazole. In total six formulations of invasomes were created the average vesicle size varied from 236.65±0.32 to 265.58±0.14 nm. The entrapment efficiency was observed to be varied from 66.45±0.32% to 98.85±0.32%. It was seen that for F5 formulation the average vesicle size and % entrapment efficiency as 210.32±0.14nm &74.65±0.26% respectively. Further three formulations of gel were created the viscosity of gel varied from 3165.37 to 3452.27. The pH of formulation ranged from 6.65 to 6.85. The spreadibility of formulation lies in between 11.32 to 13.32 g.cm/sec. The drug content was found to be maximum for IG-2 formulation which is 99.25% respectively. The extrudability varied from 169.98 to 180.32. In 12 hours, about 99.15% drug is released in case of IG-1. Further the study of *In-vitro* drug release data for optimized formulation IG-2 was seen to be 98.78%. The formulation IG-2 follows, 0.985 Higuchi model. With R^2 value 0.985 In case of optimized gel formulation IG-2 at 100 µg/ml the zone of inhibition against *Escherichia coli and Staphylococcus aureus* was found to be 16±0.47 mm and 14±0.94mm respectively. Here, in this case it can be deduced that invasomal gel also shows more potent antimicrobial activity against *E. coli* than *S. aureus*. The stability studies of invasomal gel revealed that formulation IG -2 can remain stable for three months when kept at 4.0 ±0.2°C with normal physical appearance and 99.05% drug content. Thus, the formulated invasomal gel of metronidazole can be effectively used for topical drug delivery.

Keywords: Invasome, Invasomal gel, Metronidazol, Topical drug delivery

INTRODUCTION

Microbes are the oldest form of life on earth. They are very small living things and are sometimes termed as micro-organisms. A few harmful microbes, for example less than 1% of bacteria, can invade our body the host and make us ill. Microbes cause infectious diseases such as flu and measles. Colonization of the body by various microbes results in the infectious disease. There are many similar disease states that can arise from different causes, i.e., pneumonia can be caused by viruses, many types of bacteria, protozoa, and even fungi. There is also strong evidence that microbes may contribute to many non–infectious chronic diseases such as some forms of cancer and coronary heart disease.

Different diseases are caused by different types of micro-organisms (Neish *et al*., 2009; Sun and Chang, 2014).

Transdermal drug delivery system (TDDS) has gained numerous interests over the last two decades as it deals with many rewards over conventional and oral dosage forms. For transdermal products the penalty area is to maximize the instability of drug over the skin into the systemic circulation and minimize its retention and absorption in the skin It brings forth many attractive advantages over other routes of administration, like sustained and controlled delivery over a prolonged period of time, reduction in side effects associated with systemic toxicity, direct access to target or diseased site, convenient and painless administration and so on. The major limitation for topical drug delivery is the low diffusion rate of drugs across the stratum corneum (SC), which acts as the barrier SC is the outermost layer of skin and its structure is often compared to a brick wall, with the keratinrich corneocytes as the bricks surrounded by the mortar of the intercellular lipid lamellae (Jeong *et al*., 2021; Tanwar H, Sachdeva, 2016; Gaikwad, 2013).

Invasome a potential approach for transdermal drug delivery Invasomes are innovative vesicular systems, play an important role to improve transdermal penetration of active drug molecules as compare to other conventional vesicles. These vesicles are composed of phospholipids, ethanol and terpene or mixture of terpenes in their structures. These components worked as suitable transdermal penetrator with good penetration properties (Lakshmi *et al*., 2013; Pandey *et al*., 2021).

Invasomes are the soft liposomal vesicles embodying trivial quantities of ethanol and terpene or terpene assortments, which deed as potential transporters with amplified skin penetratio. These unique lipid vesicles are poised of phospholipids (i.e. phosphatidylcholine, phosphatidylserine, soya phospholipid, egg lecithin, phosphatidylinositol, phosphatidic acid and phosphatidylglycerol), low concentration of ethanol (3% to 3.3% v/v), terpenes or a mixture of terpenes (i.e. citral, cineole, limonene, eugenol; 1-5 % v/v) and water. Terpenes have general formula (C5H8)n, increases the percutaneous absorption of hydrophilic and hydrophobic drugs. The terpenes, which are constituents of essential oils obtained from natural sources, and used broadly as penetration enhancers. However, terpenes have additional advantages of low skin irritancy at low concentrations. Furthermore, FDA classifies terpenes as generally safe (Amnuaikit *et al*., 2018; Shailaja and Afreen, 2022).

The delivery of therapeutic agents through the skin has numerous benefits as compared to other delivery methods. It plays an important role in the delivery of drug with improved skin penetration and absorption of drugs. The improvement and modification of new technologies is leading to a radical development of the resource of drugs that can help from this delivery strategy and is further intensifying its impact in medicine. Invasomes, as typically fluidic system have been explored and proved to be beneficial in targeting to treat skin disorders. The targeting efficacy has been confirmed comprehensively by preclinical studies on different actives for dermal applications.

The attention seeking areas include exploring invasomes for delivery of biological macromolecules. Scale-up, acute and chronic toxicity aspects and clinical intricacies, need to be addressed before these systems can be put to commercial use (Gama et al., 2023; Padhan *et al*., 2022).

Metronidazole is a commonly used antibiotic, belonging to the nitroimidazole class of antibiotics. It is frequently used to treat gastrointestinal infections as well as trichomoniasis and giardiasis, and amebiasis which are parasitic infections. Metronidazole has been used as an antibiotic for several decades, with added antiparasitic properties that set it apart from many other antibacterial drugs, allowing it to treat a wide variety of infections. It is available in capsule form, tablet form, and topical form, and suppository preparations for the treatment of various infections (Hager and Rapp, 1992; Ceruelos *et al*., 2019). This study aims at formulation of invasomal gel of metronidazole.

MATERIALS & METHODS

Formulation and optimization of Metronidazole loaded Invasomes

Metronidazole (100mg) was loaded in to invasomes by mechanical dispersion technique. Soya Phosphatidylcholine (0.5 to 1.5% w/v) was added to ethanol and vortexed for 5 minutes (El-Nabarawi *et al.,* 2018). Drug (100mg) and terpenes (0.25%) were added under constant vortexing, this mixture was sonicated for 5 minutes. Fine stream of Phosphate buffer saline was added with syringe under constant vortexing. It was vortexed for additional 5 minutes to obtain final invasomal preparation.

Table 1: Formulation optimization of Metronidazole loaded Invasomes

Ingredient (%)	F1	F2	F3	F4	F5	F6
Metronidazole (mg)	100	100	100	100	100	100
Phosphotidylcholine (%)	1.0	1.5	2.0	1.0	1.5	2.0
Terpenes (%)	0.25	0.25	0.25	0.25	0.25	0.25
Ethanol (ml)	10	10	10	10	10	10

Preparation of gel base

Carbopol 934 (1-3%w/v Invasome based gel formulation i.e. G-1 of 1%w/v, G-2 of 2%w/v, G-3 of 3%w/v) was accurately weighed and dispersed into double distilled water (80ml) in a beaker. This solution was stirred continuously at 800 rpm for 1 hour and then 10ml of propylene glycol was added to this solution (Lakshmi *et al.,* 2014). The obtained slightly acidic solution was neutralized by drop wise addition of 0.05 N sodium hydroxide solutions, and again mixing was continued until gel becomes transparent. Volume of gel was adjusted to 100 ml and then sonicated for 10 min on bath sonicator to remove air bubbles. Final pH of the gel base was adjusted to 6.5. The same procedure was used to formulate Invasomes containing gel in which previously prepared Invasomes suspension was added. Invasomes preparation corresponding to 1% w/w of drug was incorporated into the gel base to get the desired concentration of drug in gel base.

Table 2: Formulation optimization of gel base

Ingredient (%)	G-1	G-2	G-3
Drug (Invasomes equivalent to 0.1%)	1	1	1
Carbopol 934	1	2	3
Propylene glycol	0.2	0.2	0.2
Water (ml)	100	100	100

Evaluation of Invasomes

Entrapment efficiency

Entrapment efficiency of Metronidazole Invasomes formulation was determined using centrifugation method (Haag *et al.,* 2011). The entrapment efficiency of acyclovir in invasomes vesicle was determined by ultracentrifugation, 10mL of invasomes formulation were collect in test tube. The amount of drug not entrapped in the invasomes was determined by centrifuging at 3,000 rpm and collect the supernatant, the supernatant layer was separated, diluted with water suitably and drug concentration was determined at 300nm using UV spectrophotometer.

$$\%\ Entrapment\ Efficiency = \frac{Therotical\ drug\ content - Practical\ drug\ content}{\text{Therotical drug content}} \times 100$$

Vesicle Size

Microscopic analysis was performed to determine the average size of prepared invasomes (Ota *et al.,* 2003). Formulation was diluted with distilled water and one drop was taken on a glass slide and covered with cover slip. The prepared slide was examined under trinocular microscopic at 400 X. The diameters of more than 150 vesicles were randomly measured using calibrated ocular and stage micrometer. The average diameter was calculated using the following formula.

$$AverageDiameter = \frac{\Sigma n.d}{\Sigma\ n}$$

Where n = number of vesicles; d = diameter of the vesicles

Table 3: Optimized formulation Invasomes

Formulation code- F5	
Metronidazole (mg)	100
Phosphotidylcholine (%)	1.5
Terpenes (%)	0.25
Ethanol (ml)	10

Evaluation of Invasomes containing gel

Measurement of viscosity

Viscosity measurements of prepared topical Invasomes based gel were measured by Brookfield viscometer using spindle no. 63 with the optimum speed of 10rpm.

pH measurements

pH of selected optimized formulations was determined with the help of digital pH meter. Before each measurement of pH, pH meter should be calibrated with the help of buffer solution of pH 4, pH 7 and pH 9.2. After calibration, the electrode was dipped into the vesicles as long as covered by the vesicles. Then pH of selected formulation was measured and readings shown on display were noted (Vaddi *et al.,* 2002).

Drug content

Accurately weighed equivalent to 100 mg of topical Invasomes gel was taken in beaker and added 20 ml of methanol. This solution was mixed thoroughly and filtered using Whatman filter paper no.1. Then 1.0 mL of filtered solution was taken in 10 mL capacity of volumetric flask and volume was made upto 10 mL with methanol. This solution was analyzed using UV-Spectroscope at λ_{max}278 nm.

Extrudability study

Extrudability was based upon the quantity of the gel extruded from collapsible tube on application of certain load. More the quantity of gel extruded shows better extrudability. It was determine by applying the weight on gel filled collapsible tube and recorded the weight on which gel was extruded from tube.

Spreadibility

Spreadibility of formulation is necessary to provide sufficient dose available to absorb from skin to get good therapeutic response. It was determined by method reported by Multimer *et al.* An apparatus in which a slide fixed on wooded block and upper slide has movable and one end of movable slide tied with weight pan.To determine spreadibility, placing 2-5 g of gel between two slide and gradually weight was increased by adding it on the weight pan and time required by the top plate to cover a distance of 10 cm upon adding 80g of weight was noted. Good spreadibility show lesser time to spread.

$$Spreadability = \frac{Weight\ tide\ to\ Upper\ Slide\ x\ Length\ moved\ on\ the\ glass\ slide}{Time\ taken\ to\ slide}$$

***In-vitro* drug diffusion study**

The *in-vitro* diffusion study is carried by using franz diffusion cell. Egg membrane is taken as semi permeable membrane for diffusion. The franz diffusion cell has receptor compartment with an effective volume approximately 60 mL and effective surface area of permeation 3.14sq.cms. The egg membrane is mounted between the donor and the receptor compartment. A two cm^2 size patch taken and weighed then placed on one side of membrane facing donor compartment. The receptor medium is phosphate buffer pH 7.4. The receptor compartment is surrounded by water jacket so as to maintain the temperature at 32±0.5°C. Heat is provided using a thermostatic hot plate with a magnetic stirrer. The receptor fluid is stirred by Teflon coated magnetic bead which is placed in the diffusion cell. During each sampling interval, samples are withdrawn and replaced by equal volumes of fresh receptor fluid on each sampling. The samples withdrawn and analyzed spectrophotometrically at wavelength of 278nm.

***In-vitro* antimicrobial efficacy evaluation**

The antibacterial activity of invasomal gel formulation was measured using inhibitory zone measurements against Gram-negative and Gram-positive organisms, including *Escherichia coli* and *Staphylococcus aureus*. The agar well diffusion method was used to ensure the antimicrobial properties of invasomal gel for bacteria testing (Balouiri *et al.,* 2016). The wells (6 mm in diameter) were prepared by using a sterile cork borer, and an overnight bacterial inoculum was uniformly spread using a sterile cotton swab over a sterile nutrient agar plate. The invasomal gel formulation (IG-2) and the Ciprofloxacin working standard solution were transferred into the wells and incubated for 24 h at 37°C. After incubation, confluent bacterial growth was observed. Inhibition of the bacterial growth was measured in mm.

Stability Studies

Stability study was carried out for drug loaded invasomal gel at two different temperatures i.e. refrigeration temperature (4.0±0.2°C) and at room temperature (25-28±2°C) for 3 weeks. The formulation subjected for stability study was stored in borosilicate container to avoid

any interaction between the formulation and glass of container.

RESULTS AND DISCUSSION

In total six formulations of invasomes were created the average vesicle size varied from 236.65±0.32 to 265.58±0.14 nm. The entrapment efficiency was observed to be varied from 66.45±0.32% to 98.85±0.32%. It was seen that for F5 formulation the average vesicle size and % entrapment efficiency as 210.32±0.14nm &74.65±0.26% respectively.

The outcome implies that a higher terpene content improved the formed invasomes' entrapment efficiency, which is because the lipophilic medication became more soluble. During vesicle formation, the lipophilic terpene and phosphatidylcholine dissolve in the vesicular bilayer, where the phospholipid acyl chains provide an environment that is conducive to the lipophilic drug and lipophilic terpene. It is therefore confirmed that an increase in terpene concentration makes more chains accessible, increasing the lipophilic medication's solubility in bilayers and, consequently, the efficacy of trapping.

Further three formulations of gel were created the viscosity of gel varied from 3165.37 to 3452.27. The pH of formulation ranged from 6.65 to 6.85. The spreadibility of formulation lies in between 11.32 to 13.32 g.cm/sec.

The gel's low spreadability coefficient value indicated that it spread easily and showed little grittiness. The formulation was easily spreadable with a modest amount of shear, as indicated by the lower spreadability value, which also suggests less effort needed to spread the gel over the skin. The drug content was found to be maximum for IG-2 formulation which is 99.25% respectively.

The extrudability varied from 169.98 to 180.32. The *In vitro* drug release study of optimized gel was then carried out. During application, gel extrusion from the packed tube is crucial. Gels with low viscosity exhibit excellent fluidity, while those with high consistency have poor extrudability.

In 12 hours, about 99.15% drug is released in case of IG-1. In IG-2 & IG-3 formulation the drug release in 12 hours was observed to be 98.78 % & 90.12% respectively. Further the study of *In-vitro* drug release data for optimized formulation IG-2 was seen to be 98.78%. The R^2 value for zero order, first order, Higuchi and Korsmeyer Peppas was found to be 0.962, 0.805, 0.985 and 0.982 respectively. Thus it is clear that formulation IG-2 follows Higuchi model.

The Antimicrobial activity of standard drug against selected microbes was then performed. The zone of inhibition against *Escherichia coli* and *Staphylococcus aureus* with the use of ciprofloxacin at 100 µg/ml was observed to be 18±0.74 mm & 15±0.5mm respectively. Thus it can interpreted that ciprofloxacin is more effective against E.coli than *Staphylococcus aureus*. In case of of optimized gel formulation IG-2 at 100 µg/ml the zone of inhibition against *Escherichia coli and Staphylococcus aureus* was found to be 16±0.47 mm and 14±0.94mm respectively. Here, in this case it can be deduced that invasomal gel also shows more potent antimicrobial activity against *E. coli* than *S. aureus*. The stability studies of invasomal gel revealed that formulation IG-2 can remain stable for three months when kept at 4.0 ±0. 2°C with normal physical appearance and 99.05% drug content.

Table 4: Entrapment efficiency and average vesicle size

Formulation Code	% Entrapment efficiency	Average vesicle size (nm)
F1	69.98±0.25	274.65±0.22
F2	66.45±0.32	265.58±0.14
F3	71.12±0.15	236.65±0.32
F4	69.96±0.31	255.85±0.52
F5	74.65±0.26	210.32±0.14
F6	98.85±0.32	236.36±0.32

Table 5: Characterization of optimized formulation of invasomes

F. code	Average vesicle size (nm)	% Entrapment efficiency	Zeta Potential (mV)
F5	210.32±0.14	74.65±0.26	-37.45

Table 6: Characterization of Invasomes gel

Gel formulation	Viscosity (cps)	pH	Drug Content (%)	Extrudability (g)	Spreadibility (g.cm/sec)
IG-1	3452.27	6.85	98.45	169.98	13.32
IG-2	3245.66	6.74	99.25	175.52	12.45
IG-3	3165.37	6.65	97.85	180.32	11.32

Table 7: *In vitro* drug release study of optimized gel

S. No.	Time (hr)	% Cumulative Drug Release*		
		IG-1	IG-2	IG-3
1	0.5	24.45	20.23	17.74
2	1	31.15	34.45	32.25
3	2	46.65	44.65	46.65
4	4	60.23	56.65	59.98
5	6	85.65	68.85	69.98
6	8	96.65	73.32	79.95
7	10	98.85	88.98	86.65
8	12	99.15	98.78	90.12

Table 8: *In vitro* drug release study of optimized gel formulation IG-2

S. No.	Time (hr)	% Cumulative Drug Release*
1	0.5	20.23
2	1	34.45
3	2	44.65
4	4	56.65
5	6	68.85
6	8	73.32
7	10	88.98
8	12	98.78

Table 9: *In-vitro* drug release data for optimized formulation IG-2

Time (h)	Square Root of Time(h)$^{1/2}$	Log Time	Cumulative* % Drug Release	Log Cumulative % Drug Release	Cumulative % Drug Remaining	Log Cumulative % Drug Remaining
0.5	0.707	-0.301	20.23	1.306	79.77	1.902
1	1	0	34.45	1.537	65.55	1.817
2	1.414	0.301	44.65	1.650	55.35	1.743
4	2	0.602	56.65	1.753	43.35	1.637
6	2.449	0.778	68.85	1.838	31.15	1.493
8	2.828	0.903	73.32	1.865	26.68	1.426
10	3.162	1	88.98	1.949	11.02	1.042
12	3.464	1.079	98.78	1.995	1.22	0.086

Table 10: Regression analysis data of optimized gel formulation IG-2

Batch	Zero Order	First Order	Higuchi	Korsmeyer Peppas
	R^2	R^2	R^2	R^2
IG-2	0.962	0.805	0.985	0.982

Table 11: Antimicrobial activity of *standard drug* against selected microbes

S. No.	Name of drug	Microbes	Zone of Inhibition (100 µg/ml)
1.	Ciprofloxacin	*Escherichia coli*	18±0.74
		Staphylococcus aureus	15±0.5

Table 12: Antimicrobial activity of optimized gel formulation IG-2 against selected microbes

S. No.	Formulation	Microbes	Zone of Inhibition (100 µg/ml)
1.	IG-2	*Escherichia coli*	16±0.47
		Staphylococcus aureus	14±0.94

Table 13: Stability of optimized formulation of invasomes gel

Characteristic	Time (Month)					
	1 Month		2 Month		3 Month	
Temp.	4.0 ±0. 2°C	25-28±2°C	4.0 ±0. 2°C	25-28±2°C	4.0 ±0. 2°C	25-28±2°C
Viscosity (cps)	3215.45	3362.23	3200.14	3256.33	3210.45	3345.66
Drug Content (%)	99.45	98.45	99.11	97.65	99.05	96.65
Physical Appearance	Normal	Turbid	Normal	High turbid	Normal	High turbid

CONCLUSION

Invasomes filled with metronidazole provided a stable, affordable alternative for delivering metronidazole to patients. The goal of the current work was to create a invasomal gel that would enable metronidazole to be administered transdermally and have better systemic availability. As indicated by the data, a invasomal gel formulation (IG2) was selected for the gel formulation that had the highest drug entrapment percentage, the best release percentage, and the best stability outcomes. the invasomal gel loaded with metronidazole demonstrated a higher release and longer duration of effect. All things considered, these findings suggest that invasomal gel may work well. As a result of this investigation, transdermal formulations of metronidazole in invasomal gel were effectively created and may be utilized as a substitute delivery method.

DECLARATION OF INTEREST

The authors declare no conflicts of interests. The authors alone are responsible for the content and writing of this article.

REFERENCES

- Neish, A.S. (2009) Microbes in gastrointestinal health and disease. *Gastroenterology*, 136, 65–80.
- Sun, J. & Chang, E.B. (2014) Exploring gut microbes in human health and disease: Pushing the envelope. *Genes and Diseases*, 1, 132–139.
- Jeong, W.Y., Kwon, M., Choi, H.E. & Kim, K.S. (2021) Recent advances in transdermal drug delivery systems: A review. *Biomaterials Research*, 25, 24
- Tanwar, H. & Sachdeva, R. (2016) Transdermal drug delivery system: A review. *International Journal of Pharmaceutical Sciences and Research*, 7, 2274.
- Gaikwad, A.K. (2013) Transdermal drug delivery system: Formulation aspects and evaluation. *Compr. J PharmSci*, 1, 1–10.
- Lakshmi, P.K., Kalpana, B. & Prasanthi, D. (2013) Invasomes-novel vesicular carriers for enhanced skin permeation. *Systematic Reviews in Pharmacy*, 4.
- Pandey, S. & Srivastava, V. (2021) An overview on invasomes: Novel vesicular carrier for transdermal drug delivery. *Research Journal of Topical and Cosmetic Sciences*, 12, 107–112.
- Amnuaikit, T., Limsuwan, T., Khongkow, P. & Boonme, P. (2018) Vesicular carriers containing phenylethyl resorcinol for topical delivery system; liposomes, transfersomes and invasomes. *Asian Journal of Pharmaceutical Sciences*, 13, 472–484.
- Shailaja, A.K. & Afreen, U. (2022) Formulation and evaluation of naproxen sodium loaded invasomes for topical delivery. Current nanomedicine (formerly: recent patents on nanomedicine), 12, 32–43.
- Gamal, A., Aboelhadid, S.M., Abo El-Ela, F.I., Abdel-Baki, A.S., Ibrahium, S.M., El-Mallah, A.M., Al-Quraishy,

S., Hassan, A.O. & Gadelhaq, S.M. (2023) Synthesis of carvacrol-loaded invasomes nanoparticles improved acaricide efficacy, cuticle invasion and inhibition of Acetylcholinestrase against hard ticks. *Microorganisms*, 11, 733.

- Padhan, D. (2022) A curcumin-nanosized invasome topical formulation evaluated against Bacillus subtilis Salmonella bongori. *Journal of Pharmaceutical Negative Results*, 13, 1883–1887.

- Hager, W.D. & Rapp, R.P. (1992) Metronidazole. *Obstetrics and Gynecology Clinics of North America*, 19, 497–510.

- Hernández Ceruelos, A., Romero-Quezada, L.C., Ruvalcaba Ledezma, J.C. & López Contreras, L. (2019) Therapeutic uses of metronidazole and its side effects: An update. *European Review for Medical and Pharmacological Sciences*, 23, 397–401.