



Formulation And Evaluation Of Metronidazole Benzoate Loaded Proniosomes For Topical Delivery

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Abstract: In the modern times, vesicular system is a well-known carrier for delivery of drugs. They can carry both lipophilic and hydrophilic drugs. Proniosomes are a vital member of novel drug delivery carriers. The present study was aimed to develop and prepare Metronidazole Benzoate loaded proniosomes with different ratios of cholesterol and non-ionic surfactants to prolong the drug release on topical administration. By slurry method, about fourteen formulations were prepared and evaluated for angle of repose, drug content, particle size, hydration rate, encapsulation efficiency, *in-vitro* release studies & *ex-vivo* drug permeation studies. The optimized formulation of proniosomes was developed into transdermal patch and was subjected to various evaluation studies. The *in-vitro* kinetic and diffusion study of the patch showed a release of 80.71% over 12hrs and fitted into zero order and non-fickian diffusion mechanism. It was summed up, that the formulation F12 containing surfactant: cholesterol as 1:1 was the best formulation. Incorporation into proniosomes can lead to reduced dose, improved bioavailability and prolonged release. Further formulating in form of transdermal patch allows controlled release of the drug. UV spectrophotometric method was developed for determining Metronidazole benzoate in 5% ethanol at 309nm. A regression coefficient value of 0.9977 was noticed. The SEM image as well as the FT-IR spectrum of the optimized formulation was taken to support the same. Hence, we conclude that the proniosomal formulation is a vital candidate for transdermal delivery of metronidazole benzoate in the treatment of various systemic as well as topical bacterial infections.

Keywords: Metronidazole benzoate, Proniosomes, Slurry method, *Ex vivo* permeation study, *In vitro* drug release, SEM

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I.INTRODUCTION

The skin is the largest organ of the body. Skin serves as a barrier for the drugs to pass through it. Percutaneous absorption involves the passage of the drug molecule from the skin surface into the stratum corneal under the influence of the concentration gradient and its subsequent diffusion through the stratum corneal and underlying epidermis, through the dermis and into the blood circulation. The resistance provided by the skin is the major rate limiting step in the percutaneous absorption.¹ Various technique have been employed to weaken the skin barrier and to deliver the drug into the body through the intact skin. Some of the techniques include iontophoresis, sonophoresis, electroporation, use of chemical enhancers and microneedles. These methods are not used now because of limited efficacy, skin irritation, complexity in usage and high cost. Due to this, many elastic lipid systems like niosomes, liposomes, transferases and proniosomes were developed in order to penetrate deeply and easily into skin. These vesicles squeeze themselves through the pores of the stratum corneal due to their highly flexible membrane². The absorption of drugs through the transdermal route improves bioavailability of drugs that might otherwise be metabolized by first-pass during their passage through the gastrointestinal tract. Drug absorption from the transdermal route is mainly via passive diffusion through the lipid membrane. Thus, transdermal route of drug delivery has attracted the attention worldwide for optimizing the drug delivery. Metronidazole is an antifungal agent and can be given through topical route. It has been used in the treatment of various topical as well as systemic diseases which includes: acne, pelvic inflammatory disease, endocarditis, bacterial vaginosis, amoebiasis, trichomonas. It has low aqueous solubility, which can affect its dissolution leading to poor Bioavailability³. Incorporation into proniosomes can lead to the reduced dose, improved bioavailability and prolonged release, hence the drug of choice. The aim of the study is to formulate the drug in different ratios and to achieve prolonged release of drug on topical administration. The proniosomes are promising drug carriers as they possess greater chemical stability and lack of many disadvantages associated with liposomes. Additional merit is its non-toxic nature due to the use of non-ionic surfactants in its preparation. They can be formulated as dry free flowing product which makes them more stable during sterilisation and storage and also ease of transfer, distribution and measuring make them a pronouncing versatile delivery system. Proniosomes are processed into transdermal dosage form as it is a non-invasive method of delivering drug to systemic circulation by bypassing first pass metabolism, which is a major drawback in oral drug delivery⁵. The plan of the study was to prepare proniosomes and evaluate them for angle of repose, drug content, particle size, hydration rate, encapsulation efficiency, *in-vitro* release studies and *ex-vivo* drug permeation studies and to incorporate proniosomes into transdermal patch. The problems of physical stability like fusion, aggregation, sedimentation, and leakage on storage can be reduced. Hydrolysis of encapsulated drug which limits the shelf life can be avoided. Since it's a dry formulation

provide convenience in storage and transportation. It also provides drug delivery with improved bioavailability and reduced side effects. Both hydrophilic and hydrophobic drugs can be entrapped. It shows controlled and sustained release of drugs due to depot formation. It is biodegradable, biocompatible & non-immunogenic to the body. These are the advantages of proniosomes.

2.1.MATERIALS AND METHODS

For Determination of λ_{max} of Metronidazole Benzoate, 10 μ g/ml of drug solution was prepared in ethanol. The solution was sonicated for 10 minutes. This drug solution was scanned in the UV region of 200-400nm & the overlay spectra was recorded. In the spectra, 309nm λ_{max} of 309 was observed.

2.1.1. Determination of Standard curve

Metronidazole Benzoate of 50mg was weighed accurately and added in 2.5ml of ethanol in a standard flask, and made up to 50ml with distilled water & 3mins in sonicated. Then the solution was serially diluted to get concentrations of 10, 20, 30, 40, 50 μ g/ml. The absorbance of the solutions was measured in UV spectrophotometer at 309nm. The calibration curve was plotted by taking concentration of the solution in μ g/ml on X-axis & absorbance on Y-axis & regression co-efficient R 2 was calculated.

2.1.2. Drug excipient compatibility studies

A physical mixture of the drug & the excipient (1:1) was prepared & mixed with suitable quantity of KBr. About 100mg of mixture was compressed to form a transparent pellet using a hydraulic press at 6 tons' pressure. It was scanned from 4000- 400cm⁻¹ in FTIR spectrometer. The IR spectrum of the pure drug was compared with the IR spectrum of the physical mixture & matching was done to detect any appearance or disappearance of peaks.

2.1.3. Surface morphology of proniosomes using SEM⁶

The surface morphology of the proniosomes was detected using Scanning Electron microscope of the complex was performed at STIC, Cochin University, Ernakulam.

2.1.4. Formulation of Proniosomes

The proniosomes were prepared using slurry method. The carrier was taken in RBF and to form slurry, the surfactant dissolved in the organic solvent was added in the entire volume. The flask was then attached to the rotary evaporator & vacuum was applied until a dry free flowing powder was obtained. It is kept under the vacuum for overnight after the flask was removed from the evaporator. The obtained proniosomal powder was stored at 4°C in a sealed container. In F3 cholesterol was replaced by lecithin to have a comparative study.

Table 3: Formulation of proniosomes

Formulation code	F ₁	F ₂	F ₃	F ₄	F ₅	F ₆	F ₇	F ₈	F ₉	F ₁₀	F ₁₁	F ₁₂	F ₁₃	F ₁₄
Drug(mg)	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Cholesterol(mg)	1000	1000	1000 lecithin	1000	1000	1000	1000	1000	250	500	1000	1000	1000	1000
Span40(mg)	1000	2000												
Span60(mg)			1000	2000					250	1000	1000	1000	500	750
Tween20(mg)					1000	2000								
Tween60(mg)							1000	2000						
Lactose(mg)									1000					
Mannitol(mg)	1000	1000	1000	1000	1000	1000	1000	1000		1000	1000	1000	1000	1000
Chloroform(ml)	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Ethanol(ml)	5	5	5	5	5	5	5	5	5	5	5	5	5	5

2.2. Evaluation of Proniosomes

2.2.1. Angle of repose⁷

The angle of repose of dry proniosomal power was measured by funnel method. The proniosomes powder was poured into a funnel which was fixed at a position, so that the orifice of the funnel is 2cm above the level surface. The powder flows from the funnel to form a cone on the surface & the angle of repose was then calculated by measuring the height of the cone & the diameter of the base. The angle of repose was then calculated from the following formula:

$$\text{Tan } \theta = h/r$$

Where,

θ = angle of repose, h = height of the cone ,

r = radius of the base

2.2.2. Optical microscopy⁸

It is done to confirm the vesicle formation. A proniosomal powder as a thin layer was placed on cavity slide. A drop of water was added through the sides of the cover slip into the cavity slide under microscope and observed.

2.2.3. Drug content analysis⁹

Proniosomes equivalent to 30mg of drug was taken in a standard volumetric flask. They were lysed with 10ml ethanol by shaking for 15min. Diluted to 100ml with distilled water and sonicated for 3min. Aliquots were withdrawn;

absorbance was measured spectrophotometrically at 309 nm and the drug content was calculated from the calibration curve.

2.2.4. Entrapment efficiency¹⁰

Proniosomes of 100mg was dispersed in little warm water to allow the formation of niosomes. Then 5% ethanol was added up to 10ml & sonicated for 10 min. Then the dispersion was centrifuged at 1800 rpm for 40 min at 5°C. The appropriately diluted clear fraction with distilled water & measured the absorbance at 309nm. The % encapsulation was calculated from the equation.

$$\% \text{ Encapsulation efficiency} = (\text{Total drug- Free drug} / \text{Total drug}) \times 100$$

In-vitro drug diffusion study through egg membrane

2.2.5. Drug diffusion through egg membrane¹¹

By using Franz Diffusion Cell, the in-vitro diffusion study was done. The capacity of the receptor compartment is 15ml. The egg membrane was mounted between the donor and the receptor compartments. Weighed amount of proniosomal powder (100mg) was placed in the donor compartment and the receptor compartment was filled with 5% ethanol. The receptor fluid was stirred continuously by a magnetic stirrer. At each sampling interval, samples were withdrawn for a period of 8hrs and were replaced by equal volumes of fresh receptor fluid to maintain sink condition. Withdrawn samples were analysed spectrophotometrically at 309nm.

2.2.6. Ex-vivo skin permeation study for optimised batch^{12,13}

The permeation Metronidazole Benzoate from proniosomal powder by using in-vitro Franz Diffusion Cell was investigated. The abdominal chicken skin was obtained from slaughter house & adhering subcutaneous fat was carefully cleaned. After removing the extraneous debris & leachable enzymes, the dermal side of the skin was kept in contact with physiological saline solution for 1hr before starting the permeation experiment. In the receptor compartment the skin was mounted with the stratum corneal facing towards the donor compartment. The receptor compartment was filled with 15ml of 5% ethanol maintained at 37°C & was constantly stirred using a magnetic stirrer. In the donor compartment, proniosomal powder Ig was placed on the skin. Samples were withdrawn for a period of 12hrs at each sampling interval; samples were replaced by equal volumes of fresh receptor fluid to maintain sink condition. Withdrawn samples were analysed spectrophotometrically at 309nm.

2.2.7. Rate of spontaneity¹⁴ (Hydration)

10 mg of proniosomal powder (optimized batch) was transferred to a test tube and spread uniformly. 1 ml of 0.9% saline solution was added along the sides of the walls and kept aside without agitation. After 15 min, a drop was withdrawn and placed on nebular chamber and the number of proniosomes eluted from proniosomes were counted.

2.2.8. Vesicle size analysis For optimized batch¹⁵

Hydrated proniosomal powder was observed under optical microscope at 100X magnification. The sizes of 100-200 vesicles were measured using calibrated stage and eye - piece micrometre fitted in optical microscopy.

2.2.9. Anti-fungal study¹⁶

Anti-fungal study was carried out to ascertain the biological activity of the optimized formulation and compared with the standard drug (pure Metronidazole) against Candida albicans. A layer of Savoured dextrose agar media (20ml) was seeded with 0.2ml of the test microorganism and allowed to solidify in the petri-plate. Cups were made with sterile borer at 4mm diameter on the solidified agar layer. 50 mg of the optimized formulation was taken and suspended in normal saline. The 12th hr release sample solution was poured into the cup for microbial assay. After keeping the Petri-plates at room temperature for 4hr, the plates were incubated at 37°C for 24 hrs. The diameter of zone of inhibition was measured.

2.3. Formulation of Transdermal Patches¹⁷

The proniosomes of optimized batch was loaded to the transdermal patch prepared by mixing 2% propylene glycol in solvent chloroform: ethanol (2:1) 200mg HPMC & poured into a Petri plate. The rate of evaporation of solvent was controlled by placing an inverted funnel over the Petri plate. After 24 hrs the dried film was take out.

2.4. Evaluation of Transdermal Patch

2.4.1 Physical appearance

All the transdermal films were visually inspected for clarity, flexibility colour, and smoothness.

2.4.2. Thickness uniformity¹⁸

The thickness of the formulated film was measured at 5 different points using a Vernier calliper and average thickness was calculated.

2.4.3. Folding endurance¹⁹

For the prepared films the folding endurance was measured manually. A strip of film 1cm² was cut and till it broke repeatedly folded at the same place. The more than times the film could be folded at the same place without cracking or breaking of folding endurance gives the value.

2.4.4. Percentage moisture absorption²⁰

Weighed accurately the films and placed in a 100 ml containing saturated solution in a desiccator of potassium chloride. The films were taken out after 3 days, and weighed again. By using this formula, the percentage moisture absorption was calculated:

$$\% \text{ Moisture absorption} = \frac{\text{Final Weight} - \text{Initial Weight}}{\text{Initial Weight}} \times 100$$

2.4.5. Percentage moisture loss²¹

The films were weighed accurately and placed in a desiccator containing anhydrous calcium chloride. The films were taken out after 3 days, and weighed again. By using this formula, the percentage moisture loss was calculated:

$$\% \text{ Moisture loss} = \frac{\text{Initial Weight} - \text{Final Weight}}{\text{Initial Weight}} \times 100$$

2.4.6. Drug content determination²²

A specified area of patch (1cm x 1cm) was dissolved in ethanol and made up the volume to 100ml with distilled water. Then ultrasonicated the whole solution for 15min. After filtration, the drug was estimated spectrophotometrically at wavelength of 309nm and determined the drug content.

2.4.7. Water vapour transmission rate²³

Equal diameter vial was used as transmission cells. They were thoroughly washed and dried in an oven. Then the film was prepared and fixed over the edge of the glass vial containing 3 gm of fused calcium chloride as a desiccant by using an adhesive. In the desiccator vial saturated solution of potassium chloride was placed. The vial was taken out often and weighed for a period of 72 h. The water vapour transmission rate was calculated using the formula:

$$\text{WVT} = \text{WL/S}$$

Where,

W: Water vapour transmitting in g

L: Thickness of patch in cm

S: Exposed surface area in cm

2.4.8. Percentage flatness test²⁴

This test is done to ensure that the patches do not constrict with time. Three longitudinal strips were cut out from each film: 1 from the centre, 1 from the left side and 1 from the right side. Measured the length of each strip and the variation in length because of non-uniformity in flatness was measured by determining percent constriction, with 0% constriction equivalent to 100% flatness. The percentage constriction can be calculated using the formula:

$$\% \text{ Constriction} = \frac{I_1 - I_2}{I_1} \times 100$$

Where,

I1: initial length of each strip

2.4.9. In-vitro diffusion study

The in-vitro diffusion study was done using Franz Diffusion Cell. The capacity of the receptor compartment is 15ml. The egg membrane was mounted between the donor and the receptor compartments. The formulated patch was cut into size of 2cm² and was placed above the egg membrane in the donor compartment. The receptor compartment was filled with 5% ethanol. Through the magnetic stirrer, the receptor fluid was stirred continuously. Samples were withdrawn for a period of 12hrs at each sampling interval, and were replaced by equal volumes of fresh receptor fluid to maintain sink condition. Withdrawn samples were analysed spectrophotometrically at 309nm.

2.4.10. Stability studies²⁵

The best formulation was tested for its stability. Stability studies were conducted according to the ICH guidelines, by storing the patch at accelerated temperature 40±2 °C and 75± 5% RH for 45 days. The samples were withdrawn

I2: Final length of each strip

initially, 30th & 45th day and analysed suitably for the physical characteristics, drug content and drug release.

3. RESULTS AND DISCUSSION

Determination of λ_{max} of Metronidazole benzoate by UV spectrophotometer

The standard stock solution of Metronidazole benzoate was prepared in ethanol and scanned by UV spectrophotometer between 200-400nm. The UV absorption spectrum of Metronidazole benzoate showed λ_{max} at 309nm & the same was used as analytical wavelength for further analysis.

3.1. Calibration curve of Metronidazole benzoate

The calibration curve for Metronidazole benzoate was developed in 5% ethanol at wavelength of 309nm using UV spectrophotometer. The linearity of the curve was found to be in the range (Fig 1) of 10-50µg/ml. The regression coefficient value was found to be 0.9977.

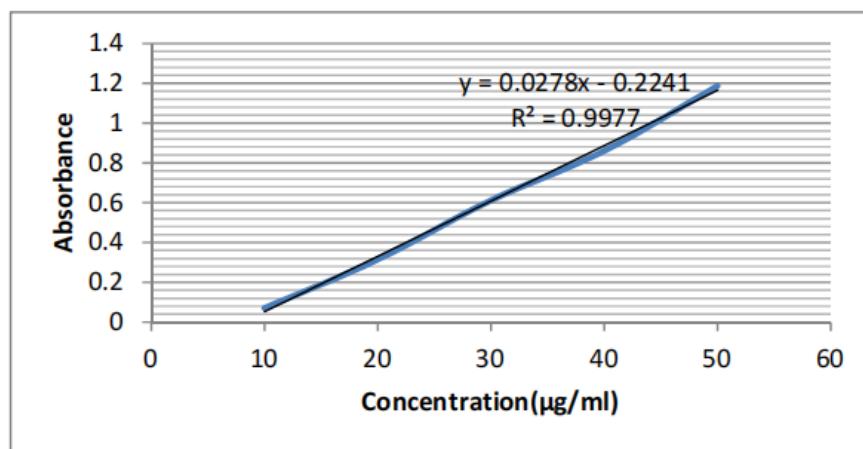


Fig1: Standard curve of metronidazole benzoate

3.2. Drug polymer compatibility studies

The drug with IR spectra and the drug with other excipients are interpreted as in table 2. The FT-IR study showed that there is no major change in the position of peak obtained in drug alone and in the mixture of drug with excipients (Fig 2-7). Hence it can be confirmed that there are no major interactions between the drug & the excipients.

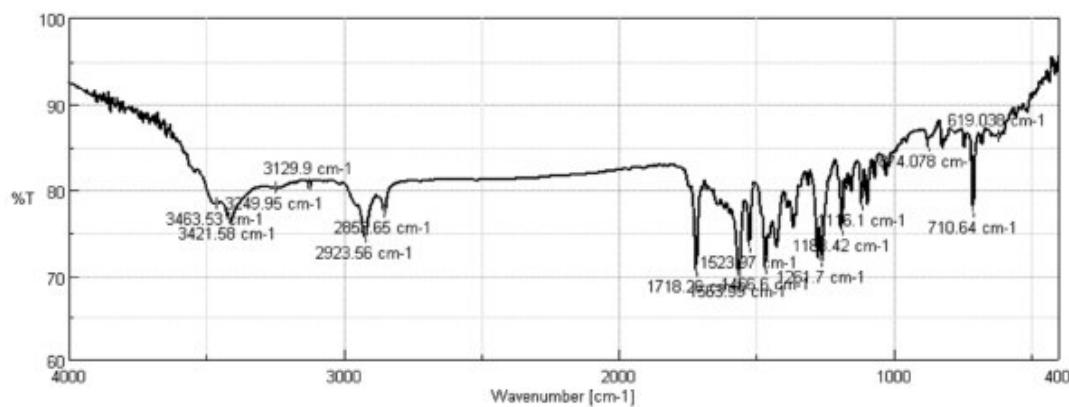


Fig 2 - FT-IR spectrum Metronidazole benzoate pure drug

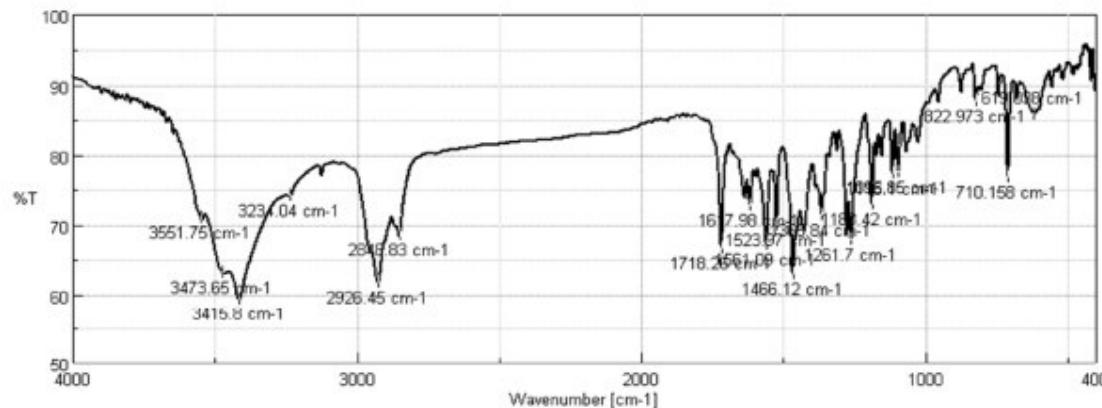


Fig 3 - FT-IR spectrum Metronidazole benzoate pure drug + cholesterol

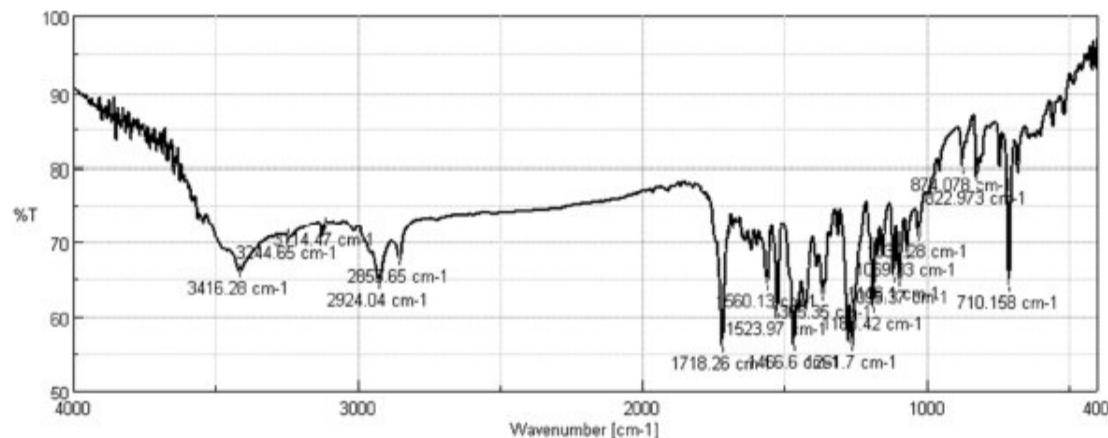


Fig 4 - FT-IR spectrum Metronidazole benzoate pure drug + lecithin

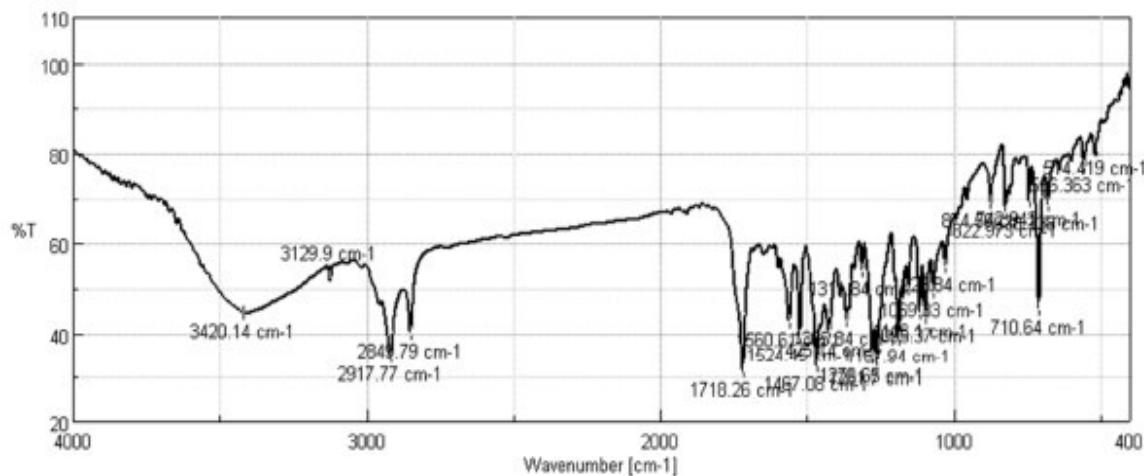


Fig 5 - FT-IR spectrum Metronidazole benzoate pure drug + span 40

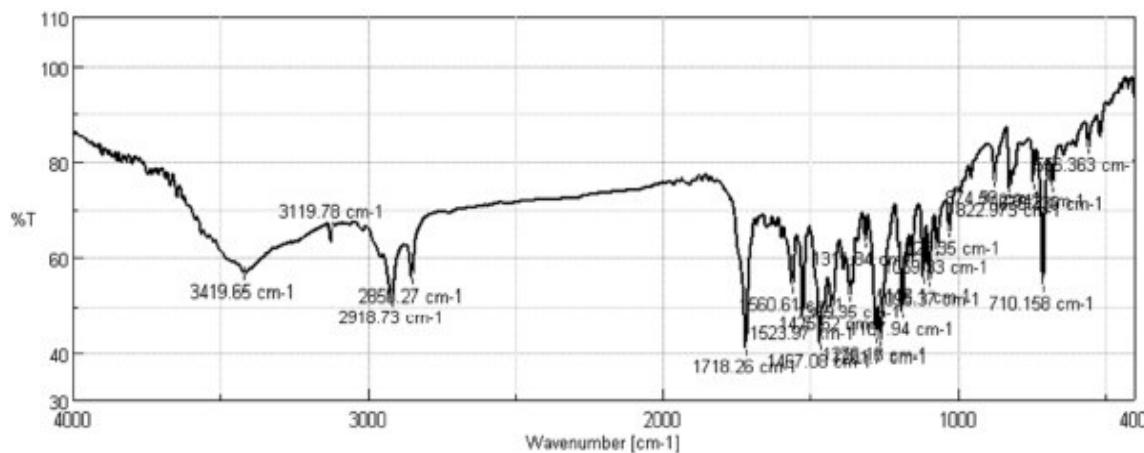


Fig 6 - FT-IR spectrum Metronidazole benzoate pure drug + span 60

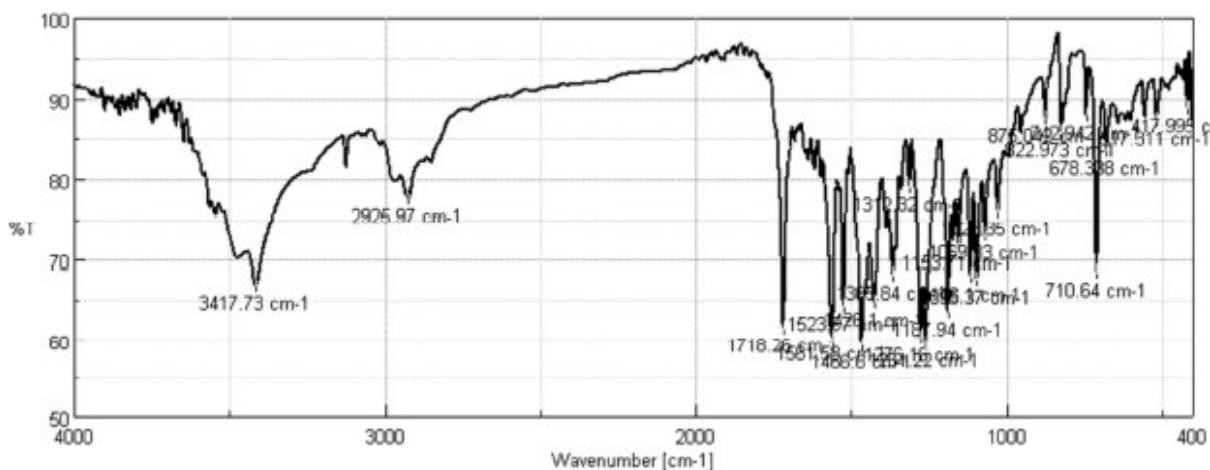


Fig 7 - FT-IR spectrum Metronidazole benzoate pure drug + HPMC

Table 2: Interpretation of FTIR spectra of drug and excipients

Drug & excipients	Functional group/wave number (cm ⁻¹)					
	Mono substituted benzene	C=O stretching	N-O stretching in aromatic ring	C=O stretching	Sp ² CH stretching	C-N stretching
Metronidazole benzoate	710.64	1718.26	1523.97	1261.7	3421.58	1466.6
MB+cholesterol	710.158	1718.26	1532.97	1261.7	3415.8	1466.12
MB+lecithin	710.158	1718.26	1523.97	1261.7	3416.28	1466.6
MB+Span40	710.64	1718.26	1524.45	1266.65	3420.14	1467.08
MB+Span60	710.158	1718.26	1523.97	1266.16	3419.65	1467.08
MB+HPMC	710.64	1718.26	1523.97	1261.22	3417.73	1466.6

3.3. EVALUATION OF PRONIOSOMES

3.3.1. Angle of repose

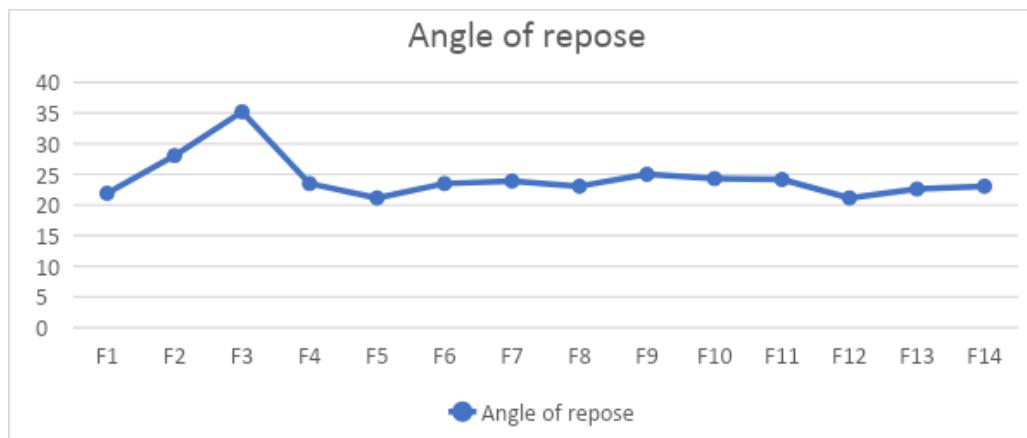


Fig 8: Angle of repose

From the above (Fig 8), it was observed that all the formulations had good to excellent flow character. F12 formulation showed the best flow property.

3.3.2. Optical microscopy

Optical microscopy was performed by viewing the formulations under microscope. It was observed that all the preparations showed vesicle formation. The vesicles formed uniform size and shape

3.3.3. Drug content analysis

From the drug content results, it was observed that formulations showed drug content in the range of 19.8-75.4%w/w. It was also observed that, as the concentration of the sorbitol surfactants were increased, it showed a decrease in the drug content. Conversely an increase in the concentration of the tween, gave increased drug content. The cholesterol content also influenced the amount of the drug present. Increasing the cholesterol content gave a rise in the drug concentration. This may be due to the lipophilic

nature of the drug. In F3 cholesterol was replaced by lecithin, but it showed less drug content. The formulation F12 showed maximum drug content²⁶.

3.3.4. Entrapment efficiency

The entrapment efficiency was calculated from the absorbance obtained from the supernatant solution. The formulations showed entrapment efficiency in the range of

45.85 - 89.85%. Formulations made from span showed more encapsulation of the drug than the one made from tween. In F10 & F11, the entrapment efficiency of F11 was more due to increased cholesterol content as low cholesterol content gives rise to smaller vesicles hence reducing the E.E. From all the preparations, the formulation containing span 60: cholesterol ratio 1:1 (F12) showed highest entrapment of the drug¹⁰.

3.3.5. In-vitro drug diffusion study

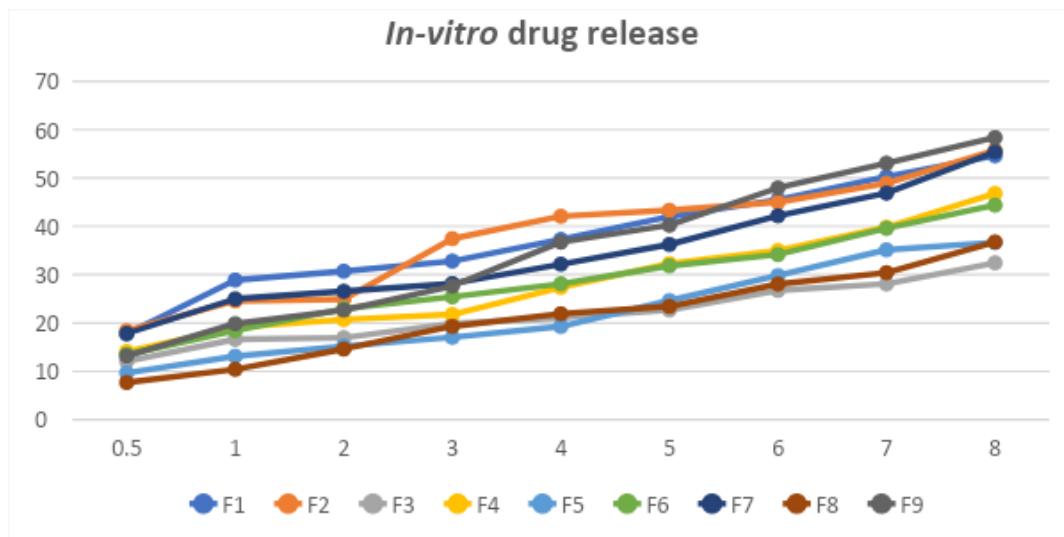


Fig 9: Results of drug release studies

The *in-vitro* diffusion studies of all formulations were carried out using Franz diffusion cell with egg membrane as the semi-permeable membrane. The data obtained is given in (Table 7) and plots were drawn between % cumulative release and time. It was observed that the formulations showed percentage cumulative release in the range of 32.44-82.05% over a period of 8 hrs. From span 60, the formulations made showed more release than the ones made from span40, tween 20 and 60. An increase in the cholesterol content

showed an increase in drug diffusion. This may be attributed to the increased lipophilic nature of the proniosomal complex. Span 60: F12 containing cholesterol in the ratio 1:1 was taken as the best formulation, since it showed highest and optimum drug release over a period of 8 hrs. Replacement of the membrane stabilizer cholesterol by lecithin gave least drug diffusion. Hence it cannot be used as an effective membrane stabilizer¹¹.

3.4. Evaluation of optimised formulation

3.4.1. Ex-vivo skin permeation study(optimized batch)

Table 3: Results of ex-vivo skin permeation study

Time in hours	Cumulative % permeation
0.5	5.8±0.58
1	11.75±0.42
2	18.14±0.02
3	21.90±0.7
4	26.96±0.12
5	35.69±0.33
6	46.52±0.17
7	56.43±0.5
8	63.99±0.68
9	66.45±0.1
10	71.92±0.38
11	75.82±0.49
12	84.71±0.55

± SD, p<0.01

3.4.2. Rate of spontaneity¹⁴

The proniosomes formulations having the maximum benefit can be speculated when abundant numbers of vesicles are formed after hydration. The number of vesicles formed per mm³ was found to be 4.

3.4.3. Vesicle size analysis

It was done using optical microscope at 100X magnification

The vesicular size was found to be $13.65 \pm 0.24 \mu\text{m}$ which is in correlation with the results obtained from SEM.

3.4.4. Vesicular size by SEM

The surface morphology of the formulated proniosomal derived niosomal vesicles (formulation F12) were confirmed by scanning electron microscopy shown in (figure 10). The vesicles are spherical in shape and smooth in nature.

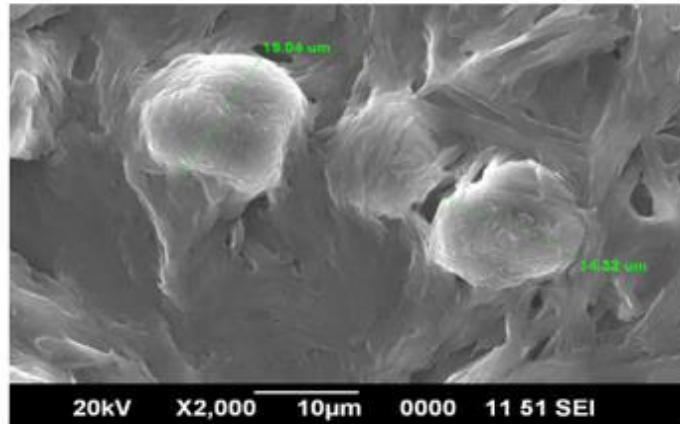


Fig 10: Scanning electron microscopic image of Metronidazole benzoate loaded proniosomal derived niosomes (optimised formulation)

3.4.5. Antifungal study

The microbiological assay of Metronidazole benzoate pure drug and 12th hour release sample solution of F12 proniosomal formulation was carried out. The diameter of the zone of inhibition was taken as a measure of the drug's

antifungal activity. The results in the zone of the pure drug & sample were found to be 26mm and 23mm respectively. Hence the results revealed that the developed proniosomal formulation is as effective as the pure drug in antifungal activity shown in (figure 11)²⁷.



Fig 11: Zone of inhibition obtained for Metronidazole benzoate proniosome (F12) and pure drug using *Candida albicans*

3.5. EVALUATION OF TRANSDERMAL PATCH

3.5.1. Physical appearance

All the transdermal films were visually inspected for colour, clarity, flexibility and smoothness. About 5 patches were prepared and the thickness was measured using Vernier calliper. All the prepared patches showed similar thickness. The average thickness was found to be 0.226 mm.

3.5.2. Folding endurance

The folding endurance of transdermal patches were measured manually. This is an important test check for the ability of sample to withstand folding. Evaluation of folding endurance involves determining the folding capacity of the film subjected to frequent conditions of folding. This also gives an indication of brittle nature; less folding endurance indicates more brittleness. The average folding endurance was calculated as 43 .

3.5.3. Percentage moisture loss

The average percentage moisture loss of the prepared patches was found to be 0.734%. The percentage moisture loss may be less due to the hydrophilic nature of the polymer.

3.5.4. Percentage moisture absorption¹⁸

The prepared patches were found to be 4.69% and this is the average percentage moisture absorption. The percentage moisture absorption may be more due to the hydrophilic nature of the polymer.

3.5.7. Percentage flatness test

Table 4: Results of percentage flatness test

Initial length (cm)			Final length (cm)			% constriction		% flatness
Trial 1	Trial 2	Trial 3	Avg	Trial 1	Trial 2	Trial 3	Avg	
3	5	3	3.66	3	5	3	3.66	0

The flatness study showed that the patch had the same strip length before and after their cuts, indicating 100% flatness (Table 4). Thus, no amount of constriction was observed. This reveals that the patch had a smooth and flat surface and the smooth surface could be maintained when the patch was applied to the skin.

3.5.8. In-vitro diffusion study of transdermal patch

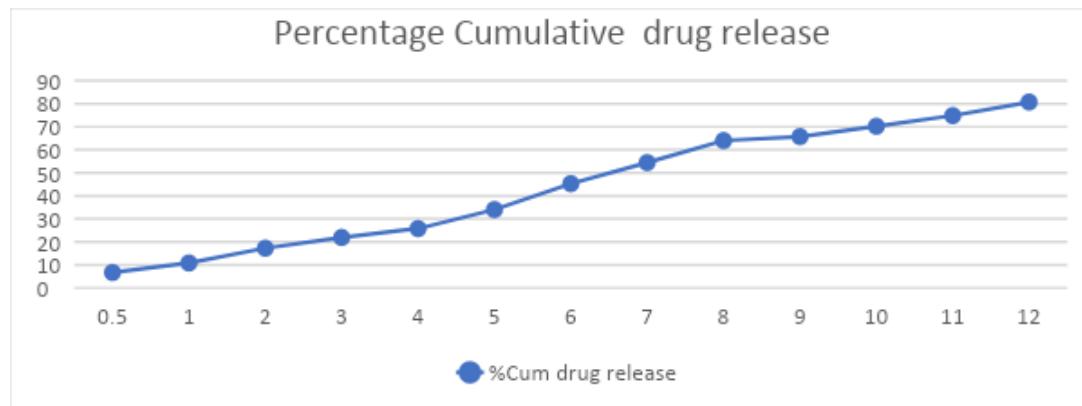


Fig 12: Results of in-vitro release study of transdermal patch

The *in-vitro* diffusion study of the transdermal patch incorporated with F12 was carried out using Franz diffusion cell with egg membrane as the semi-permeable membrane. The data obtained was drawn between % cumulative drug release and time as shown in the above figure 12. The percentage cumulative release over a period of 12 hrs was found to be 80.71%

3.5.9. Stability study

Stability study for the most satisfactory formulation F12 after incorporating into transdermal patch was carried out at accelerated temperature $40\pm2^\circ\text{C}$ and RH $75\pm5\%$ for 45 days. The patch was evaluated after 45 days for physical appearance, drug content and *in-vitro* release study. From the results obtained, it was observed that there was no major change in various parameters at accelerated temperature. Thus, it can be concluded that the formulation is stable at the specified temperature for a period of 45 days²⁸.

3.5.5. Drug content determination

Drug content in a small circular patch was analysed spectrophotometrically. Drug content of the patch was carried out to ascertain that the drug is uniformly distributed into the formulation. The prepared patch showed optimum drug content of 89.24%. Hence it can be concluded that the drug is uniformly distributed in the formulation.

3.5.6. Water vapour transmission rate

Water vapour transmission determines the permeability characteristics of the patch. The water vapour transmission rate of the prepared patch was found to be 1.55g. Hence the result reveals that the formulation is permeable to water vapour.

4.CONCLUSION

Proniosomes have been a promising carrier in vesicular research aimed at transdermal drug delivery. They are highly effective in terms to topical delivery of various agents. The prepared proniosomal transdermal patch of metronidazole benzoate showed a better diffusion as well as stability profile, hence providing an attractive carrier for prolonged and controlled topical delivery. Hence, we conclude that the proniosomal formulation is a vital candidate for transdermal delivery of metronidazole benzoate in the treatment of various systemic as well as topical bacterial infections. In future, further studies using animal models will throw more light on the effectiveness of the formulation.

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6. AUTHOR CONTRIBUTION

Mr. Muthukumar S gathered the data regard to this work and guided. Mr.Sankar C analysed these data and included necessary inputs to this manuscript. Ms.Swetha K discussed the methodology. Ms.Pradeepa R discussed the results. Mr.Muneeswaran B designed the manuscript. Ms.Noori irfna

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parvin M concluded this manuscript. Ms.Sangeetha J discussed the references.

7.CONFLICT OF INTEREST

Conflict of interest declared none.