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Formulation and Evaluation of Ornidazole Proniosomal Gel



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ABSTRACT

Proniosomes are converted into niosomes on hydration, the reason for adopting Proniosomal technology that they exists as a liquid crystalline state, this state of existence is much stable than normal niosomes. This research mainly emphasizes on formulating Proniosomal gels with span surfactants, cholesterol, soya lecithin and alcohol as aqueous phase. Ornidazole drug is chosen as an active ingredient in preparation of Proniosomal gels, these are prepared by coacervation phase separation method and the prepared formulations characterized for FTIR studies, Encapsulation efficiency, size distribution and In vitro release studies were carried. FTIR studies were carried and showed that there was no interaction between API and used excipient. The encapsulation efficiency of Proniosomal formulations are in the range of 38% to 78%. Morphological size and shape of the vesicles are characterized by using optical microscopy and scanning electron microscopy, particles are found to be spherical, size of the particles are in the range of 3.29 μ m to 30 μ m and permeation studies showed good control release for prolonged period of time. Span20 Nonlecithin formulation showed highest amount of drug release of 88% in 24 hours. *In vitro* rat skin permeation studies proved that good amount of drug is permeated than the marketed formulation. The results suggest that Ornidazole proniosome formulations can be used for a Topical drug delivery system for the treatment of skin infections.



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INTRODUCTION

Proniosomes are the vesicular drug delivery systems exist in liquid crystalline [1] state upon hydration forms vesicles that can bypass the skin membrane to reach the systemic circulation. Proniosomes are unique in nature for their stability and amphiphilicity. Proniosomes can entrap both hydrophilic and lipophilic drug molecules in their vesicles. Liposomes also exists in a bilayer lipid vesicle forms however stability of the formulations are less compared to it of niosomes[2] and proniosomal gels are additional stable than niosomes because the aggregation of vesicles are less usually seen during this gel state. Currently most of the cosmetic formulation permeation potentials and local action is created easily with this formulation are developed using this proniosomal gel formulation. [3, 4]

Proniosomes can act as drug reservoirs and the drug release rate can be controlled by modifying their composition. Proniosomes have shown advantages as drug carriers such as less cost, low toxicity [5] due to non ionic nature and chemical stability in comparison to liposomes but they are associated with problems related to physical stability, such as aggregation, sedimentation, leakage and storage.

Ornidazole is an Antibacterial and Antiprotozoal agent [6]. The antimicrobial activity of this Ornidazole is due to reduction of the nitro group to highly reactive amine group that attacks microbial DNA, terminates synthesis and leading to degradation of existing DNA. The objective of this research is to prepare an optimized ornidazole Proniosomal formulation [7, 8] that can act for both locally and systematically for the treatment of bacterial infections [9, 10]. Proniosomal formulation act as a reservoir system for the prolonged drug delivery through Transdermal route [11, 12] for better Bioavailability of the formulation. Proniosomes can bypass the skin mechanism by transcellular and paracellular way hence the plasma concentration of the drug for prolonged time can be maintained. Recent advancements in advanced drug delivery systems this ornidazole Proniosomal formulation can be used for the treatment of superficial skin infections and in Cosmeceuticals. [13]

This present research work mainly indicates preparation of Proniosomal gel by using different surfactants such as span 20, span 40, span 60 and cholesterol along with Lecithin acts as a membrane stabilizer. Ornidazole is chosen as an active ingredient for preparing these Proniosomal gels which was prepared by coacervation phase separation method. The prepared Proniosomal gels were finally characterized by optical microscopy, SEM,

Encapsulation Efficiency and evaluated by FTIR studies, In vitro permeation studies.

MATERIALS AND METHODS

Chemicals

Ornidazole was purchased from yarrow chemicals limited Mumbai, span 20, 40, 60 were purchased from Loba chemicals limited Mumbai, cholesterol was purchased from Finar chemicals Ahmadabad and soya lecithin was purchased from Sigma chemicals limited Hyderabad.

Preparation of Ornidazole Proniosomal Gel

Ornidazole Proniosomal gel was prepared by a coacervation-phase separation [14] methodology. Exactly weighed amounts of surfactant, lecithin, cholesterol and drug were taken in clean and dry wide mouthed glass ampoule of 5.0 milliliter unit capacity and alcohol (0.5 ml) was added to it. Once warming, all the ingredients were mixed well with a glass rod the open end of the glass bottle was covered with a lid to prevent the loss of solvent from it and warmed over water bath at 60-70°C for about 5 minutes till the chemical agent mixture was dissolved fully. Then the aqueous solution (0.1% glycerol solution) was added and warm on a water bath until a transparent solution was formed that was converted into Proniosomal gel on cooling. The gel therefore obtained was preserved within the same glass bottle in dark conditions for characterization.

Table 1. Composition of Proniosome gel formulations

Formulation	Surfactant	Cholesterol	lecithin	Drug	Alcohol
Span 20	900mg	100mg	100mg	100mg	0.5ml
Span 20 (NL)	900mg	100mg	-	100mg	0.5ml
Span 40	900mg	100mg	100mg	100mg	0.5ml
Span 60	900mg	100mg	100mg	100mg	0.5ml
Span20:Span40	450:450mg	100mg	100mg	100mg	0.5ml

Characterization of Proniosomal formulation Optical microscopy

The vesicle formation by the particular procedure was confirmed by optical microscopy in

100x resolution. Proniosomal gel before hydration clear liquid crystalline state was observed then upon hydration niosomal suspension made was placed over a glass slide and glued over by drying at space temperature, the dry skinny film of niosomal suspension determined for the formation of vesicles. The photomicrograph of the preparation was additionally obtained from the magnifier by employing a digital SLR camera. [15]

Scanning electron microscopy and Size Analysis (SEM)

Particle size of proniosomes is a vital factor. The surface morphology and size distribution of proniosomes were observed by SEM. A double-sided tape was affixed on aluminum stubs and the proniosomal powder was spread on it. The aluminum stub was kept in a vacuum chamber of scanning electron microscope (XL 30 ESEM with EDAX, Philips, Netherlands). The morphological characterization of the samples was examined using a gaseous secondary electron detector (working pressure of 0.8 torr, acceleration voltage-30.00 KV) XL 30, (Philips, Netherlands).[16, 17]

FTIR –Spectroscopy

Fourier transform infrared spectroscopy (FT-IR) is a simple technique for the detection of changes within excipient – drug mixture. The disappearance of an absorption peak or reduction of the peak intensity combined with the appearance of new peaks give a clear evidence for interactions between the drug and excipient. FTIR spectra of drug and excipients were mixed in combinations and were obtained by the conventional KBr disc/pellet method. The sample was grounded gently with anhydrous KBr and compressed to form pellet. The scanning range was 400 and 4000 cm^{-1} .

Encapsulation efficiency Centrifugation method

To evaluate the loading capability of Proniosomal system, a weighed quantity of proniosomal gel was distributed in phosphate buffer pH 7.4 and warmed or homogenized to make niosomes. Then the dispersion was centrifuged [18] at 18000 rpm for 40 min at 5⁰C (Remi cooling centrifuge). The clear fraction was used for the determination of free drug by victimisation of the subsequent equation:

$$\% \text{Encapsulation efficiency} = \{1 - (\text{unencapsulated drug} / \text{Total drug})\} \times 100$$

In vitro release studies

In vitro, release studies on proniosomal gel were performed by using Franz diffusion cell. The capacity of the receptor compartment was 15 ml. The area of the donor compartment exposed to receptor compartment was 1.41cm². Cellophane membrane was soaked in phosphate buffer pH 7.4 for 1 hr before carrying the experiment. The dialysis cellophane membrane (MMCO14KDC)[19] was mounted between the donor and receptor compartments. A weighed quantity of proniosomal gel was kept on one side of the dialysis membrane [20, 21] and was placed in phosphate buffer pH 7.4 which acts as receptor medium. The receptor compartment was enclosed by a vessel to maintain the temperature at 37±1°C. Heat was provided by using a hot plate with a magnetic stirrer bearing a magnetic bead which helps in stirring of the receptor fluid. At every sampling interval, 3ml samples were withdrawn and were replaced by equal volumes of fresh receptor fluid on every occasion and analyzed spectrophotometrically at 318nm. [22, 23]

In vitro permeation studies

The male albino rat of 3 months age was used to study the permeation of formulation through the skin [24, 25]. The rats were sacrificed and their hair was trimmed off with scissors. Their skin was subjected to depilatory for about less than 10 min which was then wiped off with cotton. An incision was made on the flank of the animal and the skin was separated from the underlying connective tissue using scalpel. The fat remaining on the skin was removed away by using a razor blade. The residual hypodermal fat was wiped off by a piece of cotton impregnated in isopropyl alcohol. The skin so prepared was kept in an aluminium foil at 4 °C and used within two days. [26, 27]

On the day of experiment, the rat skin was transferred to saline solution, where it was kept for about 1 h. Then skin was kept between donor and receptor compartments of Franz diffusion cell for carrying permeation study [28, 29]. Phosphate buffer of pH 7.4, temperature 37.5±1 °C 600 rpm were maintained. Heat was provided using a thermostatic hot plate with a magnetic stirrer. The receptor fluid was stirred by a magnetic bead placed on a magnetic stirrer. At each sampling time, 3ml of samples were withdrawn and replaced by equal volumes of fresh receptor fluid. Samples withdrawn were analyzed by using UV spectrophotometrically.

RESULTS AND DISCUSSIONS

Proniosomal formulations created with different non ionic surfactants among all span 20 has shown smart encapsulation potency and highest quantity of drug permeation through the skin membrane.

Optical microscopy

Proniosomal gel formulation exists as a liquid crystalline state upon hydration converts to niosomes, found to be spherical in photo micrographia on 100 X magnification (as shown in Figure 1).

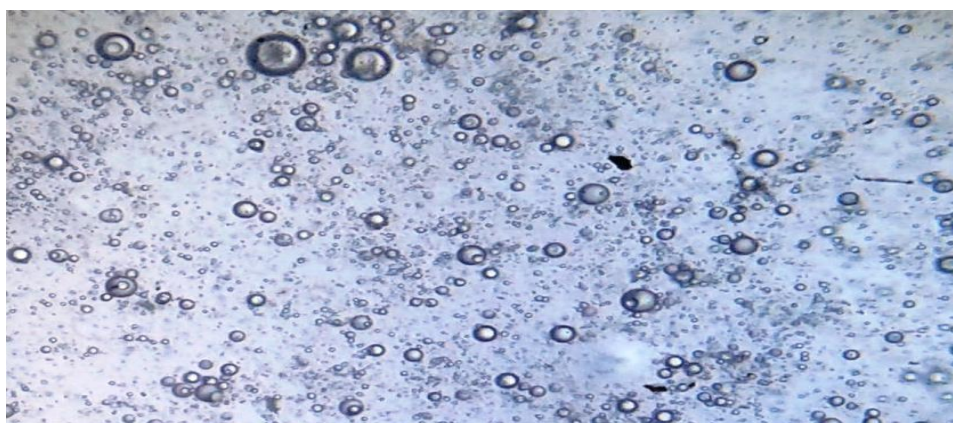


Figure 1. Photomicrographia of Niosomes on 100x magnification under optical microscope

Scanning electron microscopy

Scanning electron microscopy images of optimized span 20 formulations. Particle size of proniosomes may be a issue of prime importance. The surface morphology and size distribution of proniosomes were studied by SEM (as shown in Figure 3a and 3b).

Scanning electron microscopy images for the pure drug ornidazole are examined (shown in figure 2). They are found to be in crystal form. In the figure 3a and 3b Proniosomal ornidazole gel formulations are examined the images purely indicating spherical shaped particles with a size range of 3.29 to 30.9 μ m.

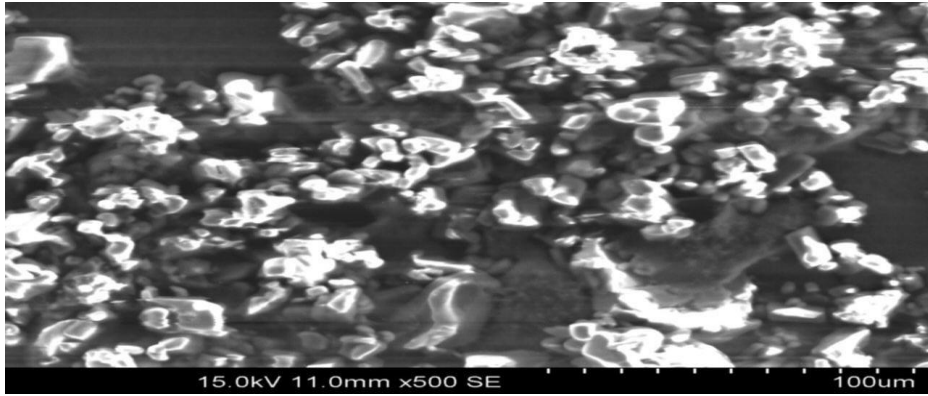


Figure 2. SEM image of Ornidazole pure drug

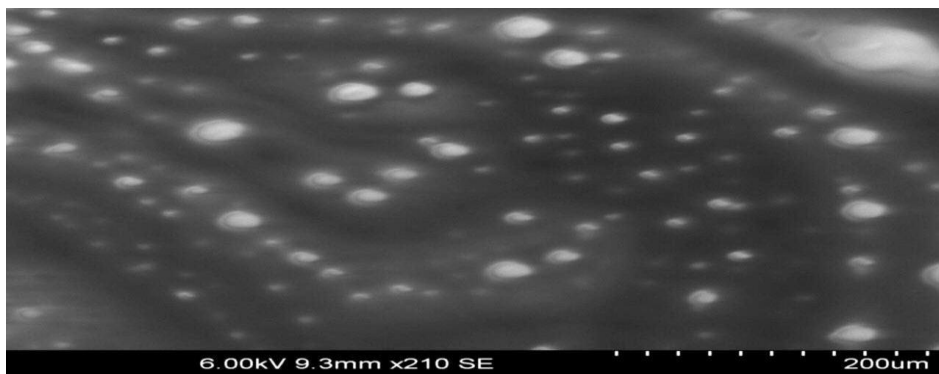


Figure 3a. SEM images of span 20 formulation

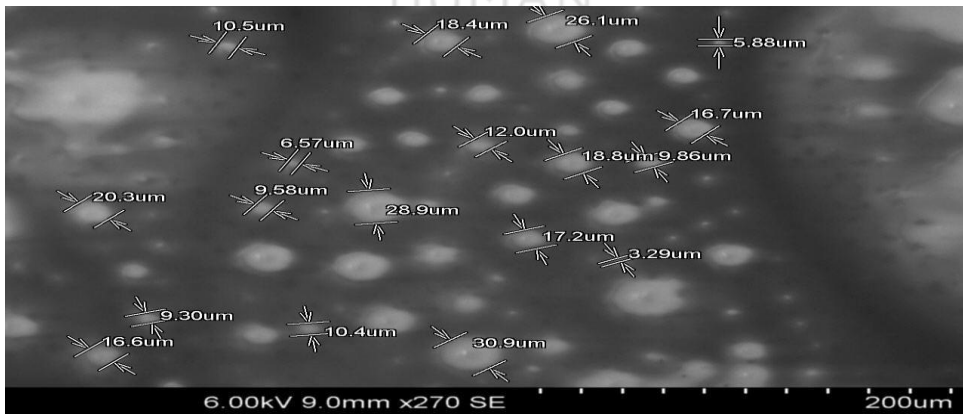


Figure 3b. SEM images of span 20 formulation

FTIR Studies

FTIR studies were done for API and span 20 optimized ornidazole formulations as shown in figures below (4 and 5). From these studies it was concluded that there is no noticeable change in the peak of the spectrums when the ingredients were analyzed individually as well

as in the form of a drug and excipients mixture. Individuality peaks of Ornidazole were clearly established without any interaction of excipients used in these formulation at $3387 \pm 4\text{cm}^{-1}$ (N-H stretch of amines), $2924 \pm 3\text{cm}^{-1}$ (O-H stretch of carboxylic acid), $1466 \pm 3\text{cm}^{-1}$ (C-H rock of alkanes), $1366 \pm 3\text{cm}^{-1}$ (N-O symmetric stretch nitro compounds) and 828cm^{-1} (C-Cl of alkylhalides). FTIR studies of drug and excipients results showed that there was no interaction between the ingredients.

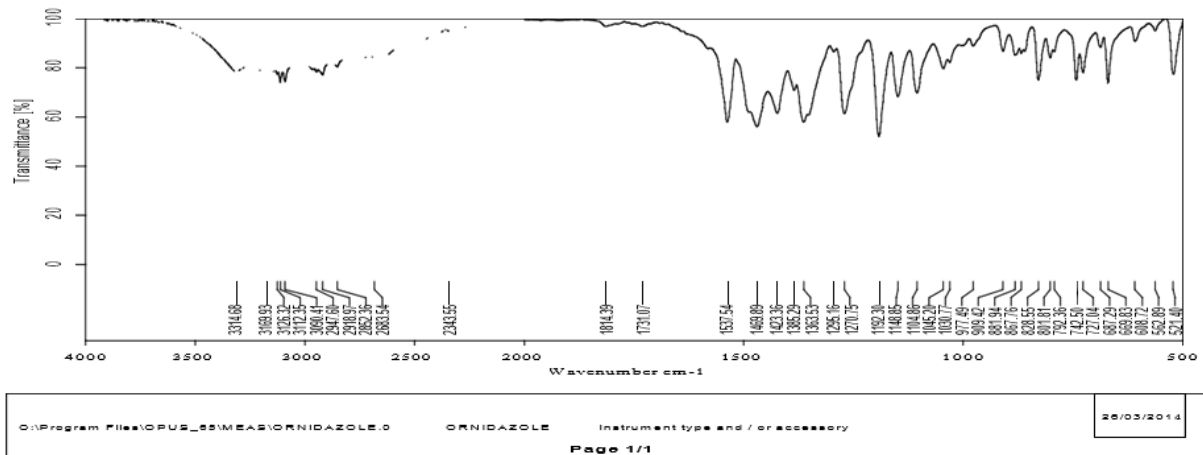


Figure 4. FTIR spectrum of API (Ornidazole)

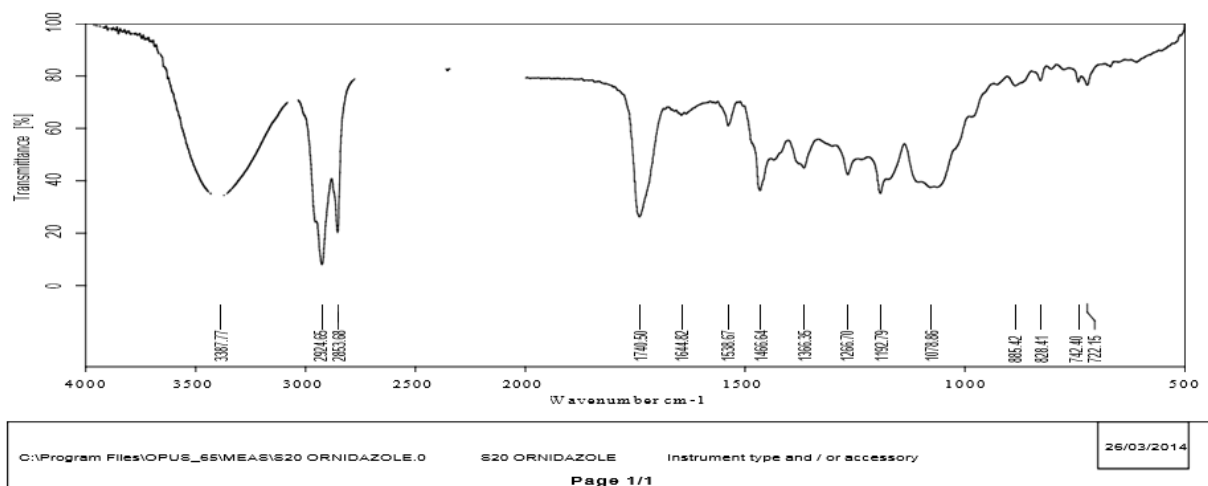


Figure 5. FTIR spectrum of Span20 Ornidazole formulation

Encapsulation efficiency

Span 20 among of all formulations showed highest encapsulation efficiency and mixed formulation of span20:span40 showed least encapsulation efficiency. The %Encapsulation efficiency of the formulations are listed in below Table 2.

Table 2. Percentage Encapsulation efficiency values of various formulations

Formulation	%Encapsulation Efficiency
Span 20	70±1.42
Span 20(NL)	55±1.03
Span40	66±1.18
Span 60	60±0.90
Span20:SP40	38±1.52

Represents mean ± S.D. (n=3)

In vitro release studies using dialysis membrane

The release studies (as shown in figure 6) for formulation prepared from span 20 without lecithin showed highest amount drug release of 88% and span 20 formulations showed 84% of drug release for 24 hours. In similar way span 40 and span 60 also showed around 70% of drug release, while marketed ornidazole aqueous base gel showed only 58% of drug release for 24 hours of study.

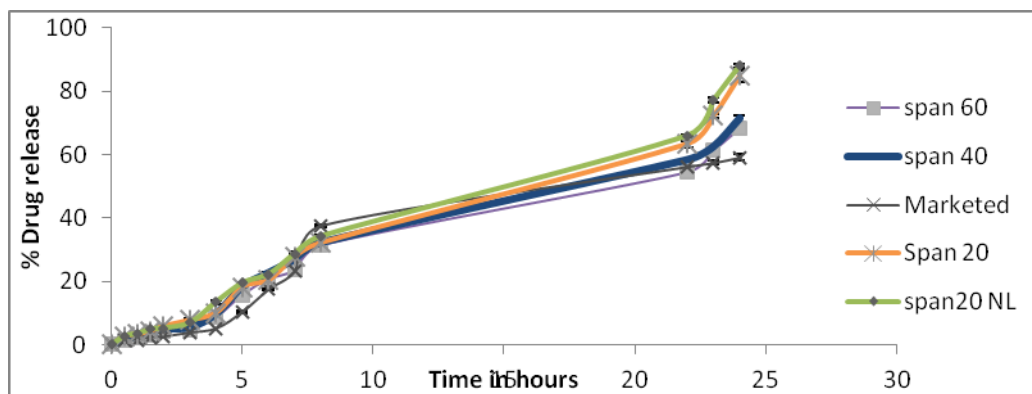


Figure 6. Comparative In vitro release profile of various proniosome gel formulations

In vitro rat skin permeation studies

In vitro permeation studies (as shown in Figure 7) were carried with rat skin for optimized

formulation of span 20 and compared with marketed aqueous based ornidazole gel for 24 hours. The amount of drug penetrating through skin for span 20 was 94% and marketed formulation showed only 64% of drug permeation for 24 hours of study.

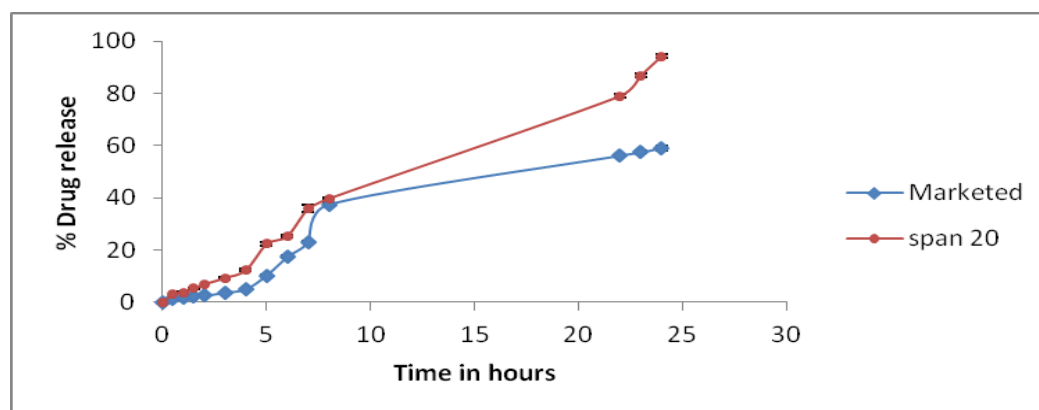


Figure 7. Comparative In vitro rat skin permeation studies of Span 20 and Marketed formulation

CONCLUSION

In conclusion, we are able to coat that proniosome ornidazole formulation acts as a reservoir system and shows continuous drug release out of all formulations made span 20 without lecithin exhibits highest quantity of encapsulation of drug compared thereupon of all alternative formulations. A mixed formulation of span 20 and span 40 reportable the least amount drug encapsulation, whereas in skin permeation studies span20 showed high quantity of drug permeation compared thereupon of the marketed ornidazole formulation. FTIR studies were carried and showed that there was no interaction between API and used excipients SEM analysis of optimized span 20 formulation indicated that the size of the particles area unit in vary from of 3 μ m to 30 μ m in size. The outcome of this research indicates that Proniosomal ornidazole gel formulation is used for treating bacterial infections locally and systematically for higher medical aid.

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