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Formulation and Evaluation of Metronidazole Nanoparticle Vaginal Gel

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Abstract: Vaginal infection or vaginitis is a condition that cause infection or inflammation of the vagina, which may be caused due to bacterial infections, yeast infection, trichomoniasis, vaginal atrophy, irritants etc. Bacterial vaginosis is the inflammation caused by the overgrowth of bacteria in the vagina. Bacterial vaginosis is most common in women of reproductive age. The infection can cause thin, grayish-white discharge with unpleasant smell, itching and irritation. Several studies on metronidazole shown that metronidazole is very effective against vaginosis symptoms. The studies reveals that over 90% of the women had no symptoms after the treatment. Since Metronidazole is very effective antibiotic, it is being selected in this study. Metronidazole is a nitroimidazole class of antibiotics which can be used for pelvic inflammatory diseases namely bacterial vaginosis, trichomoniasis, endocarditis, amoebiasis, rosacea etc. Since nanoparticles can provide sustained release, cellular targeting and intrinsic antimicrobial properties and which can improve the efficacy of prophylactic and therapeutic modality, the nanoparticle- based drug delivery will have attention in the vaginal drug delivery, hence metronidazole is incorporated to nanoparticles. Gels have the worthy properties such as acceptability, feasibility, non-toxic, non-irritant nature towards vaginal mucosa, and also gels can avoid first pass effect, vaginal gels of metronidazole are efficacious for the treatment of vaginosis. The present study is to carry out the Formulation and Evaluation of Metronidazole nanoparticle vaginal gel. In this study nanoparticle was prepared by ionic gelation method. The optimized nanoparticle were characterized by different parameters such as FTIR, SEM, drug release study, entrapment efficiency study. The antimicrobial activity of 3 formulations showed good results. Then nanoparticle gel was prepared by using formulation 3 (F3) and evaluated for appearance, pH, viscosity, spreadability, extrudability, syringeability and in vitro release study. All these studies showed good results. The antimicrobial study of formulated gel and the marketed formulation was carried out and the formulated gel showed good zone of inhibition than the marketed formulation. The in-vitro release study showed a sustained release of metronidazole from the formulation.

Key words: Metronidazole, Carbopol 934, Nanoparticles, Ionic gelation method, Vaginal gel, Staphylococcus aureus, Zone of inhibition.

INTRODUCTION

The vaginal route has been studied as a highly effective pathway for the treatment of various female reproductive tract conditions, including sexually transmitted diseases, fungal and bacterial infections, and cancer. Traditionally, the vaginal cavity has been used for the delivery of locally acting drugs such as antibacterial, antifungal, antiviral, antiprotozoal, prostaglandins, steroids, labor-inducing agents and

spermicidal agents. However, due to the high permeability of the vaginal epithelium and expulsion of conventional soluble drug dosage forms, achieving sustained local drug concentrations in the vagina remains a challenge. Vaginal route of drug administration offers many advantages, such as avoidance of the harsh gastrointestinal (GI) environment and the hepatic first-pass effect, prolonged retention of formulations, reduced side effects. Additionally, for drugs that act locally in the female reproductive tract, topical vaginal application results in much higher drug concentrations and improved efficacy.

The major goals in designing nanoparticles as a delivery system are to control particle size, surface properties and release of pharmacologically active agents in order to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen. For instance, they help to increase the stability of drugs/proteins and possess useful controlled release properties (Mu L et al., 2003). As the nanoparticles can provide sustained release, cellular targeting, and even intrinsic antimicrobial properties that can improve the efficacy of prophylactic and therapeutic modalities, the nanoparticle-based drug delivery platforms have been receiving considerable attention for vaginal drug delivery (Laura M. Ensign et al., 2014). The drug release from nanoparticles can result in more controlled vaginal absorption compared to a drug depot like a vaginal gel, thereby requiring reduced amounts of drug. The nanoparticles can provide sustained release of microbicide drugs, which is necessary for maintaining protective drug concentrations between the time of dosing and the time of intercourse. Nanoparticles can be designed to contain multiple modalities, to target specific cells, and to have intrinsic antimicrobial activity. Appropriately designed nanoparticles can provide improved drug distribution to target cells and tissues, improving efficacy.

Vaginal formulations are cleared too fast due to the self-cleansing action of the vagina because the vaginal formulations failed to attain a proper distribution over the vaginal mucosa. Conventional vaginal preparations may cause leakage, discomfort, messiness, and frequent dosing. Gels are generally better tolerated than creams and ointments. Vaginal delivery of Metronidazole can provide high local tissue levels, provide rapid drug delivery, and reduce systemic exposure (J M Hanson et al., 2000). The present work was aimed to formulate Metronidazole nanoparticle gel by ionic gelation method and evaluate antimicrobial activity.

MATERIALS AND METHODS

Preformulation study

Delivery of any drugs needs a suitable dosage form to achieve optimum therapeutic efficacy. In this study, the preformulation studies conducted are solubility analysis, development of analytical range ($\lambda_{\max}$), physical characterization like colour, odour etc and FTIR studies.

Identification of metronidazole by FTIR

The FTIR analysis of Metronidazole was carried out to evaluate the functional groups that are involved in its formulation.

Solubility of Metronidazole in different solvent:

The solubility of Metronidazole was checked in different organic solvents such as organic solvent, inorganic solvent and water.

Determination of $\lambda_{\max}$ of metronidazole

100 mg of Metronidazole was accurately weighed and dissolved in 20 ml phosphate buffer pH 6.8 in a 100 ml volumetric flask and made up to 100 ml with phosphate buffer pH 6.8. 10 ml of this solution was taken and made up to 100 ml with phosphate buffer pH 6.8. Different concentrations of drug solutions were prepared and scanned (200-400 nm) against blank solution i.e. phosphate buffer pH 6.8.

Preparation of metronidazole loaded chitosan nanoparticles

Chitosan nanoparticle is prepared by ionic gelation method. Chitosan solution (1 mg/ml, 1.5 mg/ml and 2 mg/ml) were prepared by dissolving accurately weighed chitosan in 1% v/v glacial acetic acid and stirred with magnetic stirrer continuously at 600 rpm to get a clear solution. Drug solution was prepared by dissolving 1 mg/ml metronidazole in PEG 400 (solvent). Then 10 ml of chitosan solution is taken and add 10 ml of metronidazole solution and further stirred for 40 minutes. Sodium tripolyphosphate solution (0.5-2 mg/ml) was prepared in distilled water. Then add 8 ml of this solution drop wise into the metronidazole plus chitosan solution under constant stirring for 30 minutes in a sonicator. Then centrifuge at 16000 rpm

for 30 minutes at 25°C. Discard the supernatant and then add 10 ml distilled water and again centrifuged and the supernatant was discarded and re-suspended by adding 20 ml of distilled water. Finally lyophilise at -40°C or dried in microwave oven at 100°C for 3 hours to get powdered form (Rashi Rajput et al., 2016).

Each of the different concentrations are prepared by this method (Table: 1).

Table 1: Composition of Metronidazole NP

FORMULATIONS	METRONIDAZOLE	CHITOSAN (w/v)	STPP
F1	1 mg	1 mg	1 mg
F2	1 mg	1.5 mg	1.5 mg
F3	1 mg	2 mg	2 mg

EVALUATION OF METRONIDAZOLE NANOPARTICLES

Scanning electron microscopy

The SEM was carried out at Centre for Medicinal Plants and Research, Arya Vaidya Sala, Kottakkal. SEM was carried out by using Hitachi TM 3000 Tabletop Scanning electron microscope. SEM was carried out by placing sample on two-sided carbon conductive-adhesive tape pasted on the specific aluminium stub. Voltage and magnifications were set depending on the sample. SEM is done to characterize the surface morphology of nanoparticles.

Fourier transform infrared (FTIR)

The FTIR analysis was carried out at Centre for Medicinal Plants and Research, Arya Vaidya Sala, Kottakkal. The FTIR analysis was carried out to determine the functional groups that might be involved in the nanoparticle formulation. This is done by using Shimadzu-8400 FTIR system. The pellets were prepared in FTIR grade potassium bromide optics after background scan with potassium bromide and transmittance measured at the range of 400-4000 cm⁻¹.

Determination of drug entrapment

The drug entrapment efficiency of the prepared nanoparticles was determined by means of ultracentrifugation method. It was determined by adding 1ml of nanosuspension in to 4 ml of water. Then it was centrifuged at 16000 rpm for 30 minutes. The supernatant was analysed at 319 nm by UV- Visible spectrophotometer after further dilution with water.

The entrapment efficiency is calculated by

$$\% \text{ENTRAPMENT EFFICIENCY} = \frac{W (\text{Total drug}) - W (\text{Free drug})}{W (\text{Total amount of drug loaded})} \times 100$$

Invitro drug release study

The in vitro drug release of Metronidazole nanoparticle dispersion was determined using dialysis bag diffusion technique. Dialysis bag consists of 12000-14000 MW cut-off. Prior to the experiment, the membrane was washed with warm Milli-pore distilled water (70°C) for 1 hour and then rinsed thrice with Milli-pore water to remove the glycerine (Rai et al., 2013).

6 ml of suspension was placed inside the dialysis bag, tied at both ends and dipped in the dissolution media phosphate buffer of pH 6.8 at 37±0.5°C temperature. Two millilitres of aliquot were withdrawn at particular time intervals and replaced with an equal volume of fresh dissolution medium. After suitable dilution, the sample were examined spectrophotometrically by measuring the absorbance at 319 nm. The concentration of the test samples was calculated by using the regression equation of the calibration curve.

The percentage in-vitro drug release was calculated by using the formula:

$$\text{Percentage Drug release} = \frac{\text{Cumulative amount of drug release}}{\text{Amount of drug release}} \times 100$$

MATHEMATICAL MODELLING FOR DRUG RELEASE KINETICS:

Drug release kinetics was determined by the following kinetic equations such as zero order, first order, Higuchi model, Korsemeyer-Peppas model etc.

Zero- order model

Drug dissolution from dosage forms that do not disaggregate and release the drug slowly can be represented by the equation:

$$Q_0 - Q_t = K_0 t$$

Where Q_t – amount of drug dissolved in time t

Q_0 – initial amount of drug in the solution,

(most times $Q_0 = 0$ and K_0 is the zero order release constant expressed in units of concentration/time. To study the release kinetics, data obtained from in vitro drug release studies were plotted as cumulative amount of drug released versus time.

First order model

This model has also been used to describe absorption and/or elimination of some drugs, although it is difficult to conceptualize this mechanism on a theoretical basis. The release of the drug which followed first order kinetics can be expressed by the equation.

$$dc/dt = -Kc$$

Where, First order rate constant expressed in units of time⁻¹.

The data obtained are plotted as log cumulative percentage of drug remaining vs time which would yield a straight line with a slope of $-K/2.303$.

Higuchi model

Model expression is given by the equation:

$$F_t = Q = AD(2C - C_s) \sqrt{C_s t}$$

Where,

Q – Amount of drug released in time t per unit area A ,

C – Initial drug concentration,

C_s – Drug solubility in the matrix media,

D – Diffusivity of the drug molecules (Diffusion Coefficient) in the matrix substance.

In general way it is possible to simplify the Higuchi model as (generally known as the simplified Higuchi model).

$$F_t = Q = KH \times t^{1/2}$$

Where, KH – Higuchi dissolution constant.

The data obtained were plotted as cumulative percentage drug release versus square root of time.

Korsmeyer-Peppas model

To find out the mechanism of drug release, first 60% drug release data were fitted in Korsmeyer-Peppas model.

$$M_t/M_\infty = Kt^n$$

Where,

M_t/M_∞ - Fraction of drug released at time t .

K – Release rate constant.

n - Release exponent.

In this model, the value of n characterizes the release mechanism of drug. To study the release kinetics, data obtained from in vitro drug release studies were plotted as log cumulative percentage drug release versus log time. Diffusional release mechanism of polymeric films are given below (Beg S et al., 2016). (Table:2).

Table 2: Interpretation of diffusional release mechanism from polymeric film

RELEASE EXPONENT (n)	DRUG TRANSPORT MECHANISM
0.5	Fickian diffusion
$0.5 < n < 1.0$	Anomalous transport (Non-Fickian diffusion)
1.0	Case-2 transport
Higher than 1.0	Super Case 2 transport

EVALUATION OF ANTIMICROBIAL ACTIVITY OF METRONIDAZOLE AGAINST STAPHYLOCOCCUS AUREUS:

The organism used was *Staphylococcus aureus*. The Nutrient agar medium was autoclaved, cooled and poured into petridishes and left to solidify for 15 minutes. Sterile cotton swab was dipped in to prepared inoculums (broth) and selected all over the agar plates by rotating through an angle of 60° . Then wells were made in the inoculated plate with the help of sterile well puncture (5 mm diameter) and was labelled. 100 μ l of each prepared formulations (F1, F2, F3) were placed aseptically into the well with the help of micropipette. Then the nutrient agar plates were incubated at 37°C for 24 hours and observed for the zone of inhibition.

PREPARATION OF NANOPARTICLE GEL

Accurately weighed 1g of carbopol 934 was dissolved in 100ml of distilled water and stirred in mechanical stirrer for 2 hours. 1 mg of nanoparticle was added to the carbopol mixture and stirred at 1200rpm for 15 minutes in mechanical stirrer. Dissolve 0.05g propyl paraben and 0.1 g methyl paraben (preservatives) in water and then add 1ml of propylene glycol (penetration enhancer) and then add this to the metronidazole nanoparticle and carbopol mixture and then stirred continuously in magnetic stirrer. Triethanolamine (buffering agent) is added drop wise to adjust the pH and stirred until viscous homogenous gel is obtained.

EVALUATION OF NANOPARTICLE GEL

Physical Appearance:

The prepared formulation were evaluated for colour, odour, homogeneity (Swetha et al., 2013)

pH:

The pH of the prepared nanoparticle gel formulation was determined by using digital pH meter. 1g of gel was dissolved in 25 ml distilled water and then dip electrode and allow it to equilibrium for 1 minute and the reading was noted.

Syringeability:

Syringeability study was carried out using 22 gauge needle (Jones DS et al., 1997)

Viscosity study:

The viscosity of the nanoparticle gel was done by using Brookfield Viscometer using spindle no 64 at speed of 30rpm for 5 minute.

Spreadability:

Spreadability was developed to access the spreading property of the liquids, cosmetics etc. It was developed to study the amount of spread of sample.

1g of nanoparticle gel formulation was placed in between two slides of same dimension placed horizontally. A weight of 20g is tied in to the upper slide for 5 minutes. The spreadability can be

determined in terms of time in seconds taken by the slides to slip off from gel formulation. (Kaur et al, 2013)

$$S = M.L/T$$

Where S = Spreadability, M = Weight tied to the upper slide, T = Time taken to separate the slides, L= Length of glass slide slipped)

Extrudability:

10g of the nanoparticle gel formulation was taken in a closed collapsible tube and a clip is placed at the end to avoid any roll backs and 500 g weight is placed on the tube and remove the cap and the gel was extruded (Ciurba et al.,2015)

$$E = M/A$$

Where E = Tube extrudability' M = Weight applied on the tube, A = Area of the extrude gel

In-vitro diffusion study:

The diffusion study of formulated nanoparticle gel formulation was carried out using dialysis bag diffusion technique. Dialysis bag consists of 12000-14000 MW cut-off. Prior to the experiment, the membrane was washed with warm Milli-pore distilled water (70°C) for 1 hr and then rinsed thrice with Milli-pore water to remove the glycerine.

6 ml of suspension was placed inside the dialysis bag, tied at both ends and dipped in the dissolution media phosphate buffer of pH 6.8 at 37±0.5°C temperature. Two millilitres of aliquot were withdrawn at particular time intervals and replaced with an equal volume of fresh dissolution medium. After suitable dilution, the sample were examined spectrophotometrically by measuring the absorbance at 319 nm. The concentration of the test samples was calculated by using the regression equation of the calibration curve.

The percentage in-vitro drug release was calculated by using the formula:

$$\text{Percentage Drug release} = \frac{\text{Cumulative amount of drug release}}{\text{Amount of drug release}} \times 100$$

The percentage in-vitro drug release was calculated by using the formula

$$\% \text{ Drug release} = \text{Cumulative amount of drug release} / \text{Amount of drug release} \times 100$$

Comparison of antibacterial activity of prepared formulation with marketed formulation:

The comparison was done by well diffusion method. Bacteria *Staphylococcus aureus* was used.

Formulated gel containing drug loaded nanoparticles were aseptically transferred to the wells of inoculated agar plated and left to incubate for 2 days and same as for the marketed formulation. The zone of inhibition obtained was calculated.

RESULTS AND DISCUSSION

Preformulation studies

Nanoparticles prepared are subjected to preformulation studies. F₁, F₂ and F₃ are the formulations prepared by varying the concentration of drug and the polymer.

Formulations after visual inspection were found that the prepared nanoparticles is white in colour, odourless, and in powder state.

Solubility analysis

Table 3: Solubility of Metronidazole

CHEMICALS	SOLUBILITY
PEG 400	Slightly Soluble
DMSO	Highly Soluble

METHANOL	Highly Soluble
WATER	Slightly Soluble

FTIR spectroscopy of metronidazole

The result of FTIR analysis of metronidazole pure drug is depicted below. Spectra showed transmission peaks at 3452.34 cm^{-1} indicates the presence of alcoholic O-H stretching the peak at 2812.02 cm^{-1} indicates the presence of aldehyde C-H stretching, the peak at 1070.42 cm^{-1} indicates amine C-N stretching and the peak at 1514.02 cm^{-1} indicates nitro N-O stretching. (Figure 1)

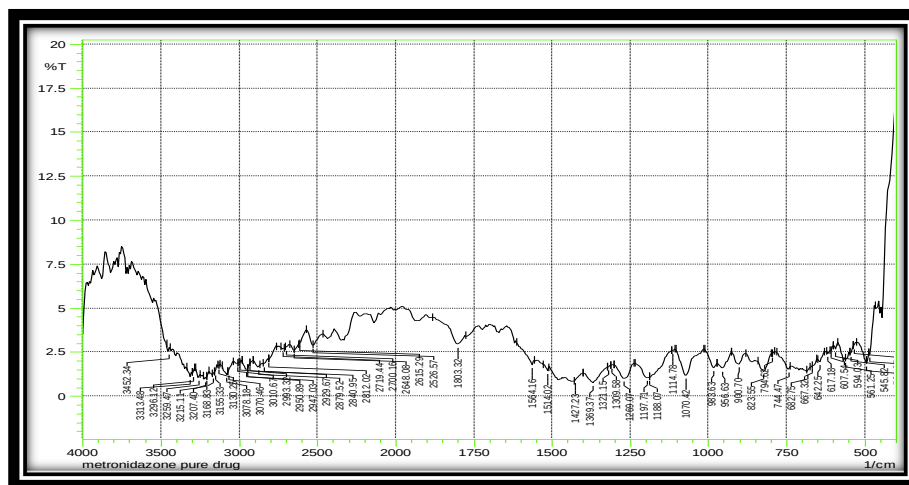


Fig 1: FT-IR spectrum of Metronidazole

FTIR spectroscopy of chitosan

The result of FTIR analysis of chitosan polymer is depicted above. Spectra showed transmission peaks at 2860.88 cm^{-1} at indicates presence of amine N-H stretching, peak at 1373.22 cm^{-1} indicates alcoholic O-H bending, peak at 1535.23 cm^{-1} indicates alkyl C-H bending, peak at 997.13 cm^{-1} indicates glucopyranose anti-symmetric stretching of C-O-C bridges. (Figure 2)

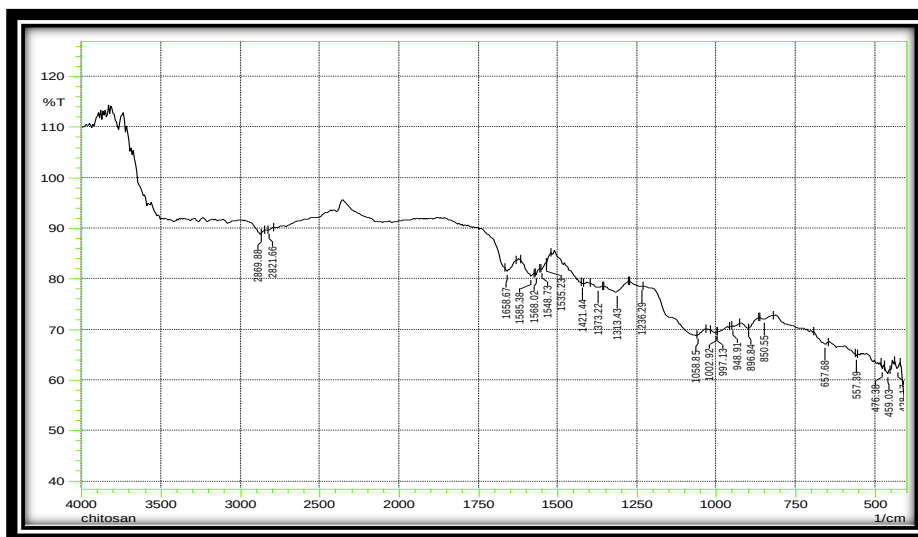


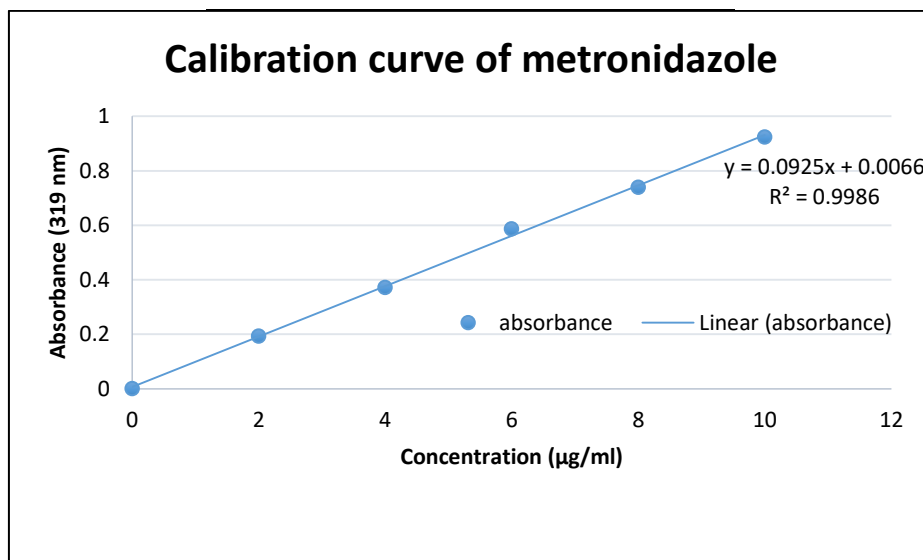
Fig 2: FT-IR spectrum of Chitosan

Determination of λ_{max} of metronidazole

The UV spectrum of metronidazole exhibits a wavelength of absorbance maximum of 319nm. This is close to the reported value. Calibration curve of metronidazole is plotted by taking concentration of the solution on X axis and the corresponding absorbance on Y axis. (Table 4) (Figure 3)

Table 4: Calibration values of Metronidazole

Concentration (µg/ml)	Absorbance
2	0.193
4	0.371
6	0.587
8	0.739
10	0.924

**Fig 3:** Calibration curve of metronidazole

EVALUATION OF NANOPARTICLES

Nanoparticle suspension were prepared by varying the concentration of polymer (Chitosan) in the ratio (1:1, 1:1.5, 1:2) by ionic gelation method. The nanoparticles were in the form of whitish suspension. The ratio 1:2 of nanoparticles showed more effective action compared to other ratios which was further confirmed by the evaluation methods.

Scanning electron microscopy

The Metronidazole nanoparticles were spherical in shape with varying particle size ranges. The larger nanoparticles may be due to the aggregation of the smaller ones. The SEM image of the metronidazole nanoparticles confirmed the development of nano stuctures. (Figure 4)

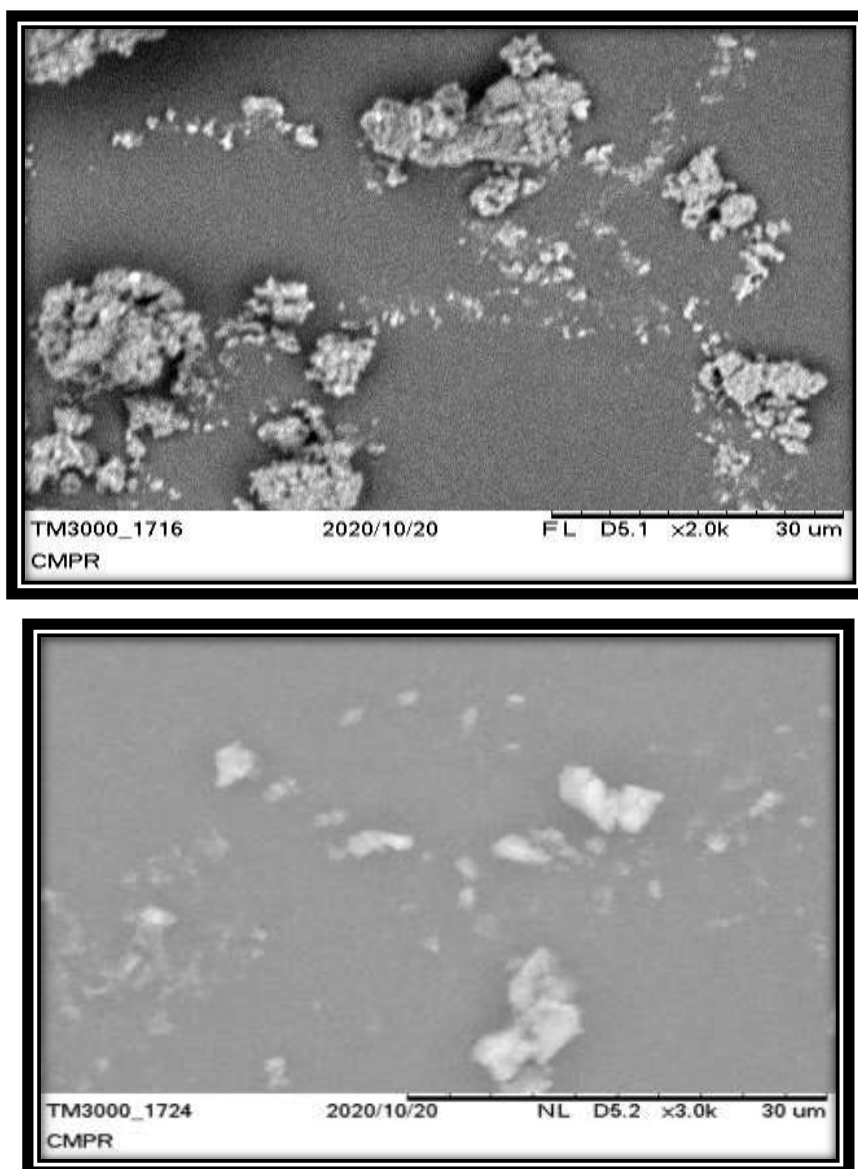


Fig 4: SEM images of NPs. Formulation 3

Fourier transform infrared spectroscopy

Spectra showed transmission peaks at 3541 cm indicates the presence of alcoholic O-H stretching, peak at 2727.6 cm indicates presence of aldehyde O-H stretching, peak at 1085.85 cm indicates presence of amine C-N stretching, peak at 1515.94 cm indicates presence of nitro N-O stretching. The result of FT-IR spectrum of drug and excipients did not differed with major peaks of Metronidazole i.e. there is no possible interaction between drug and excipients. (Figure 5)

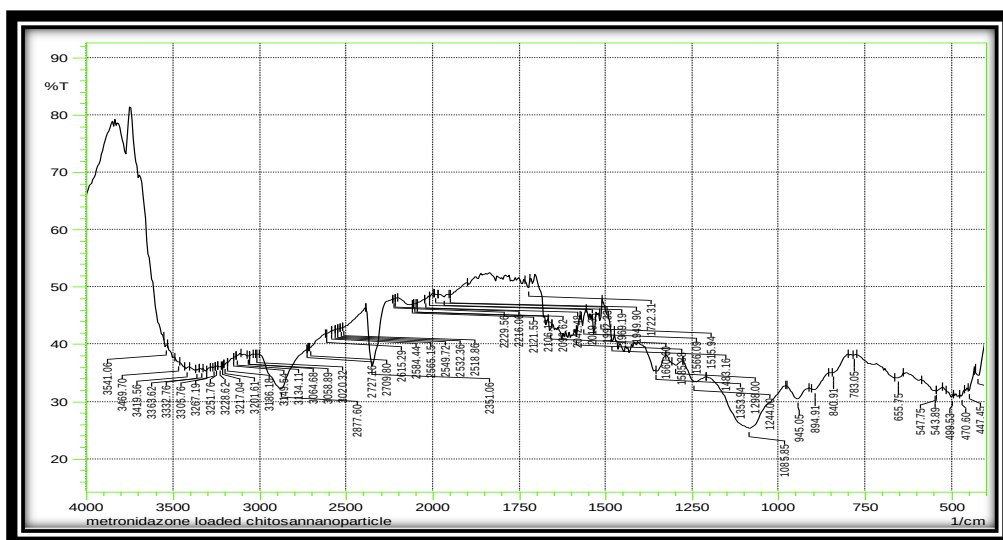


Fig 5: FT-IR spectrum of Metronidazole nanoparticles

Determination of drug entrapment efficiency

Surface adsorption is the principle behind drug loading. 1ml of nano suspension was centrifuged at 16000 rpm for 30 min. the nanoparticles free from untrapped drug were detected in the filtrate, which was then separated and its absorbance were examined by UV-Visible spectrophotometer at 319nm. The entrapment efficiency was calculated. (Table 6).

Table 6: % entrapment efficiency

Batches	Absorbance (319nm)	%EE
1	0.091	78.8
2	0.105	75.2
3	0.061	86

Three batches of samples was evaluated and the entrapment efficiency of samples was found to be 78.8%, 75.2%, 86%. Out of which, third batch shows optimum % EE compared to other two batches. Hence from these results the optimized %EE of the nanoparticle was found to be 86%.

In-vitro drug release of metronidazole nanoparticles

The in-vitro drug release study of the optimized formulation was carried out by Dialysis bag diffusion technique. (Table 7).

Table 7: Data of % drug release

Sl no.	Time (Hours)	Absorbance (319nm)	Concentration (µg/ml)	Concentration in 50 ml	Amount of drug release	Cumulative amount of drug release	% drug release
1	0	-	-	-	-	-	-
2	1	0.132	1.48	74	0.074	0.074	17.20
3	2	0.175	1.96	98	0.098	0.0994	23.13
4	3	0.221	2.43	121.5	0.1215	0.1234	28.69
5	4	0.315	3.41	170.5	0.1705	0.1729	40.20
6	5	0.359	3.98	199	0.199	0.2024	47.06
7	6	0.406	4.43	221.5	0.2215	0.2255	52.44
8	7	0.534	5.87	293.5	0.2935	0.2980	69.30
9	8	0.556	6.15	307.5	0.3075	0.3134	72.88

From the in-vitro drug release study of Metronidazole nanoparticles. % drug release was found to be 72.88 % at 8 hours.

4. Interpretation of Data

Determination of the order of release of drug from Metronidazole nanoparticles, using graphical method. The results obtained from in-vitro release studies were attempted to fit into various models as follows. Cumulative percentage drug released V/S Time (Zero order rate kinetics). Log cumulative percentage drug released V/S (First order kinetics). Cumulated percentage released V/S square root of Time (Higuchi's classical diffusion equation). Log of cumulative percentage drug released V/S Log Time (Korsmeyer-Peppas exponential equation).

Zero order kinetics:

The graph were plotted with % drug release on y axis and time x axis. The zero order rate constant (K₀) for Metronidazole nanoparticles was determined from the slope ($K_0 = \text{Slope} \times 2.303$), which was found to be 19.14. The regression coefficient were also determined to find the correlation between X axis and Y axis. The regression coefficient of Metronidazole nanoparticles was found to be 0.9835

First order kinetics:

The graph were plotted with log % drug remaining on X axis and time on Y axis. The first order rate constant (K₁) for Metronidazole nanoparticles was determined from the slope ($K_1 = \text{Slope} \times 2.303$), which was found to be 0.39. The Regression coefficient were also determined to find the correlation between X and Y values. The regression coefficient of Metronidazole nanoparticles was found to be 0.6608. These values were compared with R₂ values of the zero order kinetics plots. It was very difficult to predict exact order of kinetics of the formulations but the R₂ values showed a fair linearity, with the regression value 0.9835, that the Metronidazole nanoparticles was found to be close to be zero order kinetics, drug release was independent of concentration.

MECHANISM OF DRUG RELEASE:

Higuchi model:

In this model, the data obtained were plotted as cumulative percentage drug release versus square root of time. The R₂ value for metronidazole nanoparticles was found to be 0.8876 which indicated good linearity with n value. The n value for metronidazole nanoparticles was found to be 0.598.

Korsmeyer peppas model:

In this model, the graph were plotted between log% drug release and log time. The R₂ value for metronidazole nanoparticles was found to be 0.8876 which indicated fair linearity with n value. The n value for metronidazole nanoparticle was found to be 1.3543. (Table 8).

Table 8: R₂ value of kinetic models

Release kinetic model	R ₂ value
Zero order kinetics	0.9835
First order kinetics	0.6608
Higuchi plot	0.8876
Korsmeyer-Peppas plot	0.6679

In vitro drug release study data of the prepared formulation of metronidazole nanoparticles was subjected to goodness of fit test by linear regression analysis according to Zero order, first order and Korsmeyer-Peppas model to ascertain the mechanism of drug release. The model that best fitted the release data was evaluated by correlation coefficient R². The R² value of nanoparticles in various models are given above in the table. With the R² value of 0.9835, that metronidazole nanoparticles was found to be close to zero order kinetics, indicating that drug release was independent of concentration. The release profile of metronidazole nanoparticle could be best explained by Higuchi model, as the plot showed high linearity with R² value of 0.8876. The diffusion mechanism of the drug release was further confirmed by Korsmeyer-Peppas plot, that showed fair linearity (R² = 0.6679), with slope values more than 0.5, indicating that drug release mechanism from the prepared metronidazole nanoparticles show Non-Fickian diffusion. This findings in accordance with other reported works (Goodhart et al., 1974).

EVALUATION OF ANTIMICROBIAL ACTIVITY OF METRONIDAZOLE AGAINST STAPHYLOCOCCUS AUREUS:

From the study Formulation 3 (F3) showed more zone of inhibition compared to other formulations (F1 & F2). (Table 8).

Table 8: Antimicrobial activity of Metronidazole nanoparticle

SAMPLES	ZONE OF INHIBITION
F1	19 mm
F2	25 mm
F3	29 mm

EVALUATION OF NANOPARTICLE GEL

Physical Appearance/ Visual inspection:

The Nanoparticles gel was visually observed for the appearance and found to be colourless, clear, gelly and homogenous in nature.

pH:

The pH of the formulation was carried out by using digital pH meter and the pH was found to be 4.63 which is compatible with the vaginal pH. The normal pH of the vagina is in between 4 and 5.

Syringeability:

The nanoparticle gel formulation was found to be syringeable through 22 gauge needle.

Viscosity:

The viscosity of the prepared nanoparticle gel was determined by using Brookfield viscometer at 10 rpm in spindle no 64 and the viscosity was found to be 15266 cps

Spreadability:

The spreadability of the prepared nanoparticle gel was found to be 7.53 g.cm/sec.

Extrudability:

The extrudability of the prepared nanoparticle gel formulation from the tube was found to be 191.5708.

In-vitro diffusion study:

The in-vitro release study of prepared nanoparticle gel was carried out by using Dialysis bag diffusion technique. (Table 9).

Table 9: Data of % drug release

Sl no.	Time (Hours)	Absorbance (319 nm)	Concentration (µg/ml)	Concentration in 50 ml	Amount of drug release	Cumulative amount of drug release	% drug release
1	0	-	-	-	-	-	-
2	1	0.095	1.09	54.5	0.054	0.054	12.55
3	2	0.165	1.85	92.5	0.0925	0.0935	21.74
4	3	0.205	2.36	118	0.118	0.1198	27.86
5	4	0.281	3.11	155.5	0.1555	0.1575	36.69
6	5	0.299	3.34	167	0.167	0.1701	39.55
7	6	0.348	3.87	193.5	0.1935	0.1969	45.79
8	7	0.364	4.03	201.5	0.2015	0.2054	47.76
9	8	0.389	4.35	217.5	0.2175	0.2216	51.53

From the in-vitro drug release study of Metronidazole nanoparticle gel percentage drug release was found to be 51.53% at 8 hours.

Anti-microbial study:

Test – Prepared formulation.

Standard – Marketed formulation.

From the study the test formulation (F3) was found to have more zone of inhibition compared to the marketed formulation (F3). (Table 10)

Table 10: Zone of inhibition

SAMPLES	ZONE OF INHIBITION
Test	29 mm
Standard	27 mm

SUMMARY AND CONCLUSION

The present study deals with preparation of Metronidazole Nanoparticle by ionic gelation method and the best formulation has been incorporated into gel and compared with the marketed formulation. The goal of the present study deals with the preparation of nanoparticle gel for vaginal application for sustained release and for intrinsic antimicrobial activity and to ensure better therapeutic efficacy and patient compliance.

In this study, three formulations of Metronidazole nanoparticles were successfully prepared with varying concentration of polymer (Chitosan) (1:1, 1:1.5, 1:2). The entrapment efficiency and the antimicrobial activity of the 3 nanoparticle formulations were carried out. The optimized formulation was characterized by different parameters such as SEM, FTIR and drug release study.

The preformulation studies of the metronidazole nanoparticles were carried out and determined its organoleptic properties and analytical range. The SEM analysis confirmed the presence of nanostructures and the FT-IR studies confirmed the absence of incompatibility of metronidazole with the excipients. The entrapment efficiency of the metronidazole nanoparticles reveals that 86% of the drug was successfully absorbed into the NPs. The in-vitro drug release study was carried out and the cumulative drug release was found to be 72.88% at 8 hours and its release kinetics were also performed and found that the drug release kinetics follow zero order kinetics and the graph fitted into the Higuchi plot and Korsemeyer-Peppas plot. The antimicrobial activity of the nanoparticles were determined. It was performed in well diffusion method using staphylococcus aureus as bacteria and the zone of inhibition of three formulations were observed among which formulation 3 showed more zone of inhibition of 29 mm.

By analyzing evaluation parameters such as entrapment efficiency and antimicrobial test of metronidazole nanoparticles, it was observed that the formulation F3 obtained best results compared to other formulations so the optimized formulation was found to be formulation F3 and then the SEM, FT-IR, in-vitro drug release of the optimized formulation was carried out and then nanoparticle gel was prepared by using F3 formulation by dispersion method.

The appearance of nanoparticle gel was visually observed and was found to be colourless, clear and gelly in nature. The pH of the formulation was carried out by using digital pH meter and the pH was found to be 4.63 which is compatible with the vaginal pH. The normal pH of the vagina is in between 4 and 5. The nanoparticle gel formulation was found to be syringeable through 22 gauge needle. The viscosity of the prepared nanoparticle gel was determined by using Brookfield viscometer at 10 rpm in spindle no 64 and the viscosity was found to be 15266 cps. The extrudability of the prepared nanoparticle gel formulation from the tube was found to be 191.5708. From the in-vitro drug release study of Metronidazole nanoparticle gel percentage drug release was found to be 51.53% at 8 hours. The antifungal activity of the prepared nanoparticle gel was carried out by well diffusion method and compared with the marketed formulation. From this study the prepared nanoparticle gel showed more zone of inhibition compared with marketed gel with 29 mm.

Hence, the formulation F3 containing Metronidazole nanoparticle has met the objectives of the present study by designing the nanoparticle gel as drug delivery which has achieved sustained release and intrinsic antimicrobial activity. The project suggests that metronidazole loaded nanoparticle gel is more efficient than the marked formulation for vaginal infections. Thus this study may hold promise for future studies.

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