



Formulation and evaluation of mucoadhesive vaginal gel containing novel combination of metronidazole and miconazole nitrate for the treatment of vaginitis

Dhanashree A. Mundhey*, Princy S. Morris, Govind K. Lohiya and Jasmine G. Avari

Department of Pharmaceutical Sciences, R. T. M. Nagpur University, Nagpur, India

Received: 18-03-2015 / Revised: 24-04-2015 / Accepted: 28-04-2015

ABSTRACT

Aim of present research work was to develop polycarophil based mucoadhesive vaginal gel comprising of novel combination of Miconazole nitrate (MIZ) & Metronidazole (MNZ) for the treatment of vaginitis. FT-IR studies revealed no interactions. Gel formulations were characterized for pH, spreadability, viscosity, rheological properties, mucoadhesive force, drug content, *in vitro* drug release study, drug release kinetics studies & antimicrobial efficacy studies. The pH & spreadability was found to be 4.19 & 5.5 to 8.1 cm, which is compatible with vaginal pH & indicates easy spreadability. F5 & F8 were selected as the best formulations with optimum gel viscosity of 22480 & 24800 cps respectively. The detachment stress of the optimized batch was found to be 81.06 & 88.11 respectively. Drug release was non-diffusion controlled. Microbiological studies revealed faster release of drugs than the commercial markets product of Metronidazole & Miconazole nitrate, expressed as inhibition zone. The stability study as per ICH guidelines revealed that the optimized batch holds promise for a high stability. It can be concluded that formulation batch F5 & F8 was considered optimized since it showed better release pattern of both drugs along with other parameters such as viscosity & mucoadhesive properties.

Keywords: Metronidazole, Miconazole nitrate, Vaginitis, Carbopol, Mucoadhesive vaginal gel.

INTRODUCTION

Vaginitis an inflammation of vagina associated with discharge, itching, pain & an infection of the vulva. The most common types of Vaginitis are Bacterial vaginosis which occurs due to overgrowth of an organism normally present in vagina i.e. *Gardnerella vaginalis*. Yeast infection, caused by naturally occurring fungus called *Candida albicans* & Trichomoniasis caused by a parasite *Trichomonas vaginalis*, commonly transmitted by sexual intercourse [1, 2, 9-13]. Ideally, vaginal drug delivery system is easy to use, painless to patient, cost effective, widely available & safe for continuous administration. Its advantages are [3]

- 1) Avoidance of hepatic first pass metabolism.
- 2) Reduction in the severity and intensity of gastro intestinal side effects.
- 3) Overcoming of pain, tissue damage and probable infection observed with parental routes.

Gels, three dimensional polymeric matrix comprising of a high degree of physical reticulation. They are formed of long disordered

chains that are connected at specific points & connections are reversible. Gels can present several advantages over other vaginal drug delivery system such as higher bioavailability, safety, versatility & are economical [12-15]. The desirable properties of vaginally administered gel against microbicides are acceptability & feasibility. They must be easy to use, non-toxic & non-irritating to the mucus membrane. In the treatment of vaginitis, Metronidazole & Clindamycin vaginal cream are found to be nearly as effective as orally administered drugs [4]. To evaluate the efficacy of an antibacterial vaginal cream in the treatment of bacterial vaginosis, Lamont et al. carried out a randomized, placebo controlled 3-day course study during the second trimester of pregnant women [5]. They found that the Metronidazole vaginal cream was well tolerated & more efficacious than placebo in the treatment. During the past few years, considerable work has been done on the development of hydrogel controlled release drug delivery systems. These hydrogels release drug in a controlled fashion by swelling in an aqueous environment and retains large volumes of water in

*Corresponding Author Address: Dhanashree A. Mundhey, Department of Pharmaceutical Sciences, R. T. M. Nagpur University, Mahatma Jyotiba Fuley Educational Campus, Amravati Road, Nagpur, 440033, Maharashtra, India.

their swollen structure. A swelling controlled release hydrogel delivery system for intravaginal administration of an antifungal drug, miconazole, has been reported [6, 16].

Bioadhesion defined as the state in which two surfaces, one of which is biological in nature, are held together for extended period of time by interfacial forces. If this attachment is due to mucous coating, the phenomenon is referred to as mucoadhesion. The main advantage of mucoadhesive systems is the possibility of increasing the time of residence in situ, thus reducing the number of applications. Ideally the formulation will be retained at the biological surface and the drug will be released closed to the absorptive membrane, with consequent enhancement of bioavailability [3].

Thus, aim of the present research work was to develop polycarbophil based mucoadhesive vaginal gel comprising of novel combination of miconazole nitrate (MIZ) & metronidazole (MNZ) to achieve better therapeutic efficacy & patient compliance in the treatment of bacterial vaginitis [17-20].

MATERIAL AND METHODS

Materials: Miconazole nitrate and Metronidazole were obtained as a gift sample from M/s Relief Lab. Kalmeshwar, Nagpur and M/s Unijules Lifesciences Ltd., MIDC, Nagpur respectively. Carbopol of different grades (CP-934, 971 & 974) were obtained as a gift sample from M/s Corel Pharma Chem, Cochin India. Propylene glycol, Polyethylene glycol 400, Triethanolamine, Disodium hydrogen orthophosphate, Potassium dihydrogen phosphate, Dipotassium hydrogen phosphate, L-Ascorbic acid & Glacial acetic acid were obtained from S.D. Fine Chem. Ltd. Mumbai.

Microbiological cultures of *C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. glabrata* and *Trichomonas vaginalis* were procured from Microbiology Dept. of Govt. Medical College & Hospital (GMCH), Nagpur. Sabouraud dextrose agar medium, anaerobic trypton soya agar & Nutrient agar were obtained from Hi media Laboratories Pvt. Ltd., Mumbai.

Methods

Selection of polymer: Various grades of Carbopol (CP-934, 971 & 974) were selected based on their mucoadhesive property & vaginal tolerance. Many dummy gels were prepared and the concentrations between 1% and 2% were selected for vaginal gel preparation.

Preparation of phosphate buffer pH 4.0 (PB pH 4.0): Accurately about 5.04 g disodium hydrogen phosphate & 3.01 g of potassium dihydrogen phosphate was dissolved in sufficient amount of DW to produce 1000 ml. Then pH was adjusted with glacial acetic acid.

Calibration curve of MNZ in PB pH 4.0: Accurately about 10.0 mg of drug was weighed & transferred to 10.0 ml of volumetric flask; volume was made with PB pH 4.0. 10.0 ml of this solution was pipetted, diluted to 100.0 ml with buffer. This solution of 100.0µg/ml was the working stock solution. Now 0.5, 1.0, 1.5, 2.0, 2.5 & 3.0 ml of stock solution were pipetted & volume made upto 10.0 ml in 10.0 ml of volumetric flask with PB pH 4.0 & absorbance was recorded at 318.8nm. Then calibration graph was plotted (Fig 1).

Calibration curve of MIZ in PB pH 4.0: Accurately about 100.0 mg of drug was weighed, transferred to 100.0 ml of volumetric flask & dissolved in 10.0 ml of methanol & volume made with PB pH 4.0. This solution 1000.0 µg/ml was working stock solution. Now 1.0, 2.0, 3.0, 4.0 & 5.0 ml of stock solution was pipetted & volume made upto 10.0 ml in 10.0 ml of volumetric flask with PB pH 4.0 & absorbance was recorded at 279 nm. Then calibration graph was plotted (Fig 2).

Infrared spectroscopy (IR): Fourier Transformed Infrared Spectrophotometer (Shimadzu-8101A, Japan) was used to scan the drug & polymer samples prepared as KBr pellets, over the range of 4000-400 cm⁻¹.

FT-IR Drug excipients compatibility study: All peaks observed in FT-IR spectrum of MIZ & MNZ also appeared unchanged in combination with the polymer (Carbopol 934,971 & 974). This clearly states that there is no interaction between the drug and polymer & hence, are compatible.

Preparation of gels: Propyl paraben and methyl paraben were taken as a preservative in a beaker containing propylene glycol. These were mixed properly by gentle stirring (100rpm) & heating (50° C) on a hot magnetic plate (Solution 1). A sufficient quantity of water was added in a beaker. Carbopol was dispersed slowly in water & allowed to soak for 24 h (Solution 2). Solution 1 was then thoroughly mixed with solution 2 (Solution 3). Required amount of drug i.e. MIZ (2%w/w) & MNZ (1%w/w) was dispersed in PEG 400 & then added to Solution 3 by vigorous stirring at 1200 rpm for 15 min with the help of mechanical stirrer. Triethanolamine (pH adjuster) was added dropwise to the final mixture & stirred thoroughly until viscous homogeneous gel was obtained. The pH of

the prepared gel was adjusted. The formulation code for gel preparation was shown in Table 1.

Organoleptic properties of vaginal gel: All formulations were evaluated for physical appearance. The evaluation parameters like appearance, colour & odour were studied.

pH of formulations: The pH of the gel was recorded using Digital pH meter (Elico Pvt. Ltd., India) by bringing it in contact with the gel & allowing it to equilibrate for one min. Prior to every measurement pH meter was calibrated by using pH 4.0 & 7.0 buffer solutions [12].

Spreadability: The spreadability of the vaginal gel was determined by measuring the spreading diameter of 1g of gel between 2 horizontal glass plates (20cm × 20cm) after one minute. The standard weight applied on the upper plate was 125.0 g.

Viscosity: The viscosity of the vaginal gel was determined by using Brookfield viscometer (Brookfield Engineering Labo.Inc. Stoughton, MA, USA) at 100 rpm using spindle no.6 & 7 at 25°C [12].

Rheological properties: The rheological properties of the vaginal gel were studied to determine flow behaviour of gel using Brookfield viscometer (Brookfield Engineering Labo.Inc. Stoughton, MA, USA). About 100 g of gel was taken in a beaker. The rate of shear was increased gradually from 5 to 100 rpm. The corresponding dial reading (shearing stress) was noted. Then the rpm was decreased gradually to the lowest value i.e. 5 and the dial reading was again recorded. Depending upon the viscosity of formulation spindle no. 6 was used for F1 and F4 whereas spindle no. 7 was used for remaining formulations. The rheograms were plotted by taking viscosity on Y-axis & angular velocity (rpm) on X-axis [3, 22].

Determination of mucoadhesive force: The mucoadhesive potential of each formulation was determined by measuring the force required to detach the gel from rat vaginal mucosal tissue by using a modified physical balance. A section of vaginal mucosa was cut from the rat vaginal cavity and instantly fixed on the mouth of the glass vial using a nylon thread with the mucosal side facing outwards. The vials were stored at 37 °C for 5 minutes. Then second vial with a section of mucosa was connected to the balance in inverted position, while first vial was fixed at the surface of the balance with the help of two way adhesive tape. A fixed amount of sample i.e; 0.5 g was placed onto the vaginal mucosa of first vial. Then the height of second vial was adjusted so that mucosal surfaces of both vials come in intimate contact for a period of five minutes. Then an increasing amount of weight was kept on the other side of weighing

balance until the vials get detached. The bioadhesive force as the detachment stress in dyne/cm² was determined from the minimal weight that detached the tissue from the surface of gel using the following equation [22, 23]:

$$\text{Detachment stress (dyne/cm}^2\text{)} = M \times g/A$$

Where,

M = weight required for detachment of two vials in grams.

g = Acceleration due to gravity [980cm/s²].

A = area of tissue exposed.

Drug Content: One gram of the gel was accurately weighed & placed in a tightly closed volumetric flask with 10.0 ml of methanol. The closed flasks were shaken for 10 minutes & diluted to 100.0 ml with PB pH 4.0 & was kept for 24 hr. The next day the supernatant was filtered & measured spectrophotometrically at 279.0 nm for MIZ. For MNZ aliquot of 1.0 mL was withdrawn diluted to 10.0 mL with PB pH 4.0 & measured spectrophotometrically at 318.8 nm for its drug content. Studies were performed in triplicate for all the formulations.

Determination of *in vitro* release of drugs from formulated gels: The *in vitro* release of MIZ & MNZ was studied by employing a Franz diffusion cell. A cellophane membrane was soaked overnight in PB pH 4.0. It was then fixed between the receptor and donor compartments of the Franz diffusion cell. Accurately weighed 1g of gel was evenly spread over the surface of cellophane membrane & the receptor compartment was filled with 20ml of PB pH 4.0. The receptor medium was agitated by magnetic stirrer at 37°C. Aliquots were withdrawn periodically for a period of 5 hr & equal volume of aliquot was replaced with drug free receptor medium to maintain the sink condition. The samples were analyzed by UV-Vis spectrophotometer at 279 nm & 318.8 nm for MIZ & MNZ respectively. The cumulative percentages of both the drugs were calculated from their calibration curve [21, 24-28].

Drug Release Kinetics Studies: Mathematical models such as Zero-order, First order, Higuchi, Hixon-crowell, Korsmeyer-Peppas were used to describe the kinetics of drug release from the vaginal gel batches. The criterion for selecting most appropriate model was based on the goodness-of fit test. All formulations were subjected to the drug release kinetics & the best fit kinetic model was determined for the optimized formulations using analysis software PCP Disso V2 [21, 24-28].

Antimicrobial Efficacy Studies: Antimicrobial efficacy studies were performed to ascertain the biological activity of the optimized vaginal gel batch F5 & F8 against various *Candida species* (*C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. Glabrata*) & against *Trichomonas vaginalis*. The antifungal activity of the optimized formulation F5 & F8 was compared with marketed formulation of MIZ cream whereas antibacterial activity was compared with marketed formulation of Metronidazole gel. This was determined by Agar Diffusion Test employing "Cup and Plate Technique". Marketed formulation of MIZ as a standard and optimized formulation were poured into cups bored into sterile Sabouraud dextrose agar medium previously seeded with *Candida* test organisms & same method was used for marketed formulation of MNZ where sterile TYM agar media was previously seeded with test organism *T. vaginalis*. The wells were filled with 0.16g of marketed as well as optimized formulation with the help of sterile syringe. The plates were kept at room temperature for 45 minutes to allow diffusion of gel in the inoculated medium. Thereafter, all the plates were kept at 27°C & 37°C to check for fungal & bacterial growth respectively in the incubator for 24 hrs. The zone of inhibition was measured around each cup with Hiantibiotic Zone Scale (Himedia Laboratories Limited) & was compared with that of standard [16].

Accelerated Stability Studies of vaginal gel: Stability studies were performed according to International Conference on Harmonization (ICH) guidelines. Optimized formulation batch F5 & F8 were previously placed in sterilized vials & then stored in desiccators containing a saturated solution of sodium chloride, which gives a, RH of 75±5%. The desiccators were placed in a hot air oven maintained at a temperature 40 ±2°C & at room temperature for a period of three months. The samples were withdrawn after 30, 60 & 90 days to determine the effect of temperature & aging. The change in the viscosity, pH & drug content was recorded.

RESULTS AND DISCUSSIONS

Evaluation of topical vaginal gel:

Organoleptic characteristics, pH, spreadability & viscosity of vaginal gel: The prepared vaginal gels were having creamy white colour with no odour. The pH of all the formulations was found to be 4.19 (Average value) which is compatible with vaginal pH & the spreadability was found in the range of 5.5 to 8.1 cm which indicates easy spreadability by small amount of shear & the formulation batches F5 and F8 showed optimum gel viscosity i.e. 22480 & 24800 cps respectively.

Rheological properties: The Rheological properties of all formulations are shown in Table 2. During vaginal application the shear rate on the preparation is large. If the viscosity is too high, this will result in irritation & difficulty in application & if it is too low, it will give rise to an increased drainage. So, the formulation should have an optimum viscosity for easy application to the vaginal mucosa. Generally viscosity values in an optimum range improve the contact time & mucoadhesive property. On the basis of rheological studies, formulation batch F5 and F8 showed optimum viscosity and good efficiency on vaginal application.

Determination of mucoadhesive force:

Mucoadhesive force is an important & crucial physicochemical parameter for vaginal gels since it prevents the formulation from rapid drainage & hence prolongs residence time. Optimum concentration of bioadhesive polymer produces maximum bioadhesion whereas in highly concentrated systems, adhesive strength drops significantly because the coiled molecules become separated from the medium hence chains available for interpenetration becomes limited. Study indicated that an increase in concentration of bioadhesive polymer increases bioadhesive strength upto a certain concentration i.e. 1.5% of Carbopol 934 & 974 (F5 & F8) whereas mucoadhesive strength decreases for 2% concentration of respective polymers. Thus, bioadhesive force for F5 & F8 was found to be 81.06 & 88.11 dynes/cm². The stronger the mucoadhesive force, more it can prevent the gel from leaching out of vaginal tract.

Drug Content: The percent drug content of MIZ for an optimized batch F5 & F8 was found to be 98.92 ± 0.26% & 98.26 ± 0.12% respectively & the percent drug content of MNZ for an optimized batch F5 & F8 was found to be 99.66 ± 0.31% & 98.94 ± 0.62 % respectively.

In-vitro Drug Release Studies: For formulations containing 1% concentration of different grades of Carbopol i.e. 971, 934 & 974, maximum cumulative drug release was obtained upto 4 hours after which there was no significant increase in the release rate of drugs & for 1.5% concentration release was obtained upto 5 hours & in the range of 70-72 % & 69-72% for MIZ & MNZ respectively. Whereas for 2% concentration of different grades of Carbopol maximum cumulative drug release was obtained upto 5 hours & was in the range of 55-60% & 57-66% for MIZ & MNZ respectively. Thus, with an increase in concentration 1% < 1.5% < 2% along with increasing grades of Carbopol

with respect to crosslinking 971 < 934 < 974 the % cumulative drug release for both the drugs decreases because of an increase in viscosity of formulation. The *in vitro* drug release study for vaginal gel formulation was considered optimum for upto 5 hours, considering the wash out period after certain time duration. From above results 1.5% was considered optimum & out of 1.5% concentration, formulation batch F5 & F8 was considered optimized batch since it showed better release pattern for both MIZ & MNZ.

Drug Release Kinetics Studies: Drug Release Kinetics Studies revealed that the optimized batch F5 & F8 containing both drugs MIZ & MNZ follows Korsmeyer-Peppas model with release diffusion coefficient (n) value of 0.5380 & 0.5255 for MIZ & 0.5400 & 0.5779 for MNZ, respectively, which indicates that the release pattern is Non-Fickian.

Antimicrobial Efficacy Studies: The antifungal & antibacterial activity of optimized batch was compared with marketed formulation of MIZ cream & also against marketed formulation of MNZ gel by using Cup and Plate technique. Table 3 & Figure 3 & 4 shows the result for antimicrobial efficacy studies that the zone of inhibition of optimized batch was found to be greater than that of marketed formulation.

Accelerated Stability Studies: Three months of stability studies revealed that there was no change in pH but slight change in viscosity was observed which was within acceptable limits (± 0.5) & studies of percent drug remaining revealed no significant change for drug degradation as shown in Fig 5 & 6. From the stability studies, it was confirmed that vaginal gel formulation batch F5 & F8 of MIZ & MNZ remained stable at ambient temperature & humidity.

CONCLUSION

From the results obtained in the present work it can be concluded that mucoadhesive vaginal gels can be a promising and innovative therapeutic system for the vaginal administration of MNZ & MIZ. Combination of MNZ & MIZ played a key role in treatment of Bacterial vaginosis which is not either a bacterial or fungal infection but it is a mixed infection. Vaginal therapy could be significantly improved if the vaginal residence time and bioavailability of drugs could be increased. The results revealed that formulation batch F5 & F8 was considered optimized since it showed better release pattern of both drugs along with other parameters such as viscosity & mucoadhesive properties. The pH of formulation was found in acidic range i.e. compatible with vaginal pH, thus allows re-establishment of normal physiology of vagina which itself is useful for treatment of the disease condition. A simple, reproducible and precise instrumental method was established for the evaluation of different parameters of the gel such as pH, viscosity, rheology, mucoadhesive force as well as spreadability. The stability study as per ICH guidelines revealed that the optimized formulation gel (F5 & F8) holds promise for a high stability.

ACKNOWLEDGEMENTS

The authors are thankful to Department of Pharmaceutical Sciences RTM Nagpur University, Nagpur for providing the facilities to carry out the research work. The authors are grateful to Dr. Pawar, Dean, Govt. Medical College & Hospital (GMCH), Nagpur for permitting to collect microbial samples of Bacterial vaginosis patients from the hospital. The authors are very grateful to M/s Relief Lab. Kalmeshwar, Nagpur & M/s Unijules Lifesciences Ltd., MIDC, Nagpur for providing drug samples & M/s Corel Pharma Cochin for providing polymer samples.

TABLE 1: FORMULATION CODE FOR GEL PREPARATION

Ingredients (%w/w)	F1	F2	F3	F4	F5	F6	F7	F8	F9
Miconazole Nitrate	2	2	2	2	2	2	2	2	2
Metronidazole	1	1	1	1	1	1	1	1	1
Carbopol 971	1	1.5	2	-	-	-	-	-	-
Carbopol 934	-	-	-	1	1.5	2	-	-	-
Carbopol 974	-	-	-	-	-	-	1	1.5	2
Polyethylene glycol 400	15	15	15	15	15	15	15	15	15
Propylene glycol	1	1	1	1	1	1	1	1	1
Methyl paraben	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Propyl paraben	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Triethanolamine	0.12	0.18	0.24	0.12	0.18	0.24	0.12	0.18	0.24
Water	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

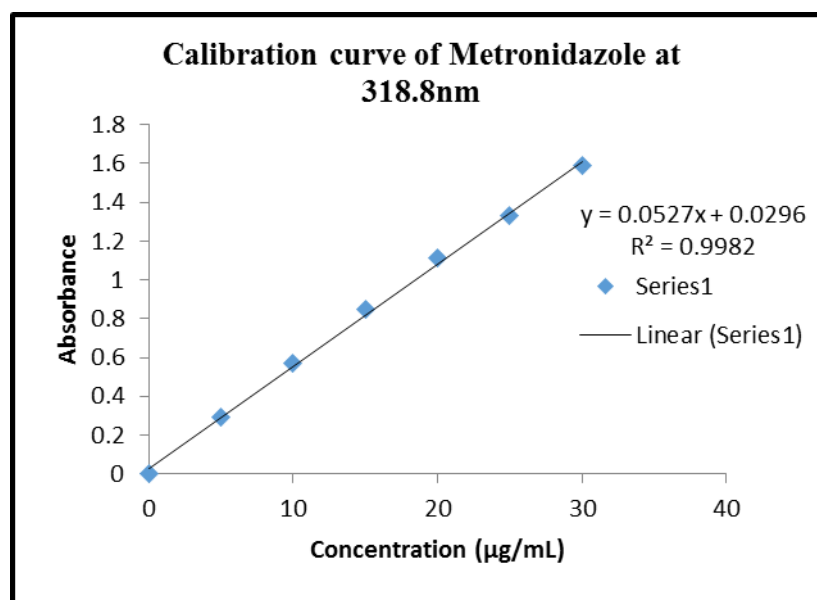
TABLE 2: RHEOLOGICAL BEHAVIOUR OF FORMULATED GELS

Rpm	Viscosity (in centipoises)								
	Carbopol 971			Carbopol 934			Carbopol 974		
	F1	F2	F3	F4	F5	F6	F7	F8	F9
5	71000	64000	128000	59000	92000	164000	124000	120000	232000
10	46000	42000	96000	41500	52000	120000	72000	84000	126000
20	28500	29000	66000	26250	32000	71000	41000	57000	74000
50	15000	16000	34400	14000	16000	38480	21600	30000	39600
100	9100	9600	11600	9800	22480	36880	14400	24800	38600

F* = Denotes number of formulation

TABLE 3: ANTIFUNGAL & ANTIBACTERIAL ACTIVITY OF OPTIMIZED VAGINAL GEL WITH MARKETED MICONAZOLE NITRATE CREAM FORMULATION & METRONIDAZOLE GEL FORMULATION

Fungal species	Zone of inhibition (mm)	
	Optimized formulation	Marketed MIZ cream formulation
<i>C. albicans</i> (CP)	38 mm	33 mm
<i>C. tropicalis</i> (CT)	31 mm	25 mm
<i>C. parapsilosis</i> (CP)	26 mm	21 mm
<i>C. glabrata</i> (CG)	35 mm	30 mm
Bacterial species	Zone of inhibition (mm)	
	Optimized formulation	Marketed MNZ gel formulation
<i>T.vaginalis</i>	21 mm	18 mm

Figure 1: Standard Calibration Curve of MNZ at λ_{max} 318.8 nm

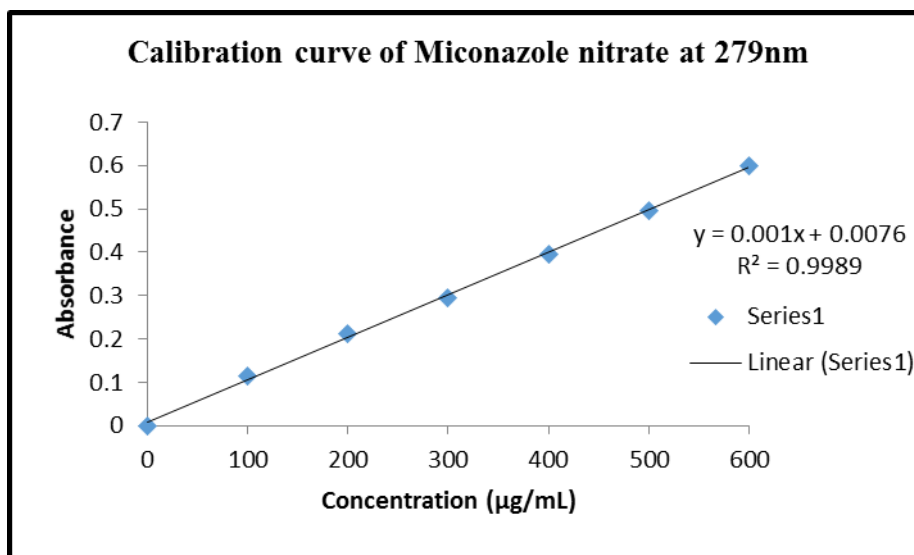


Figure 2: Standard Calibration Curve of MIZ at λ_{max} 279 nm

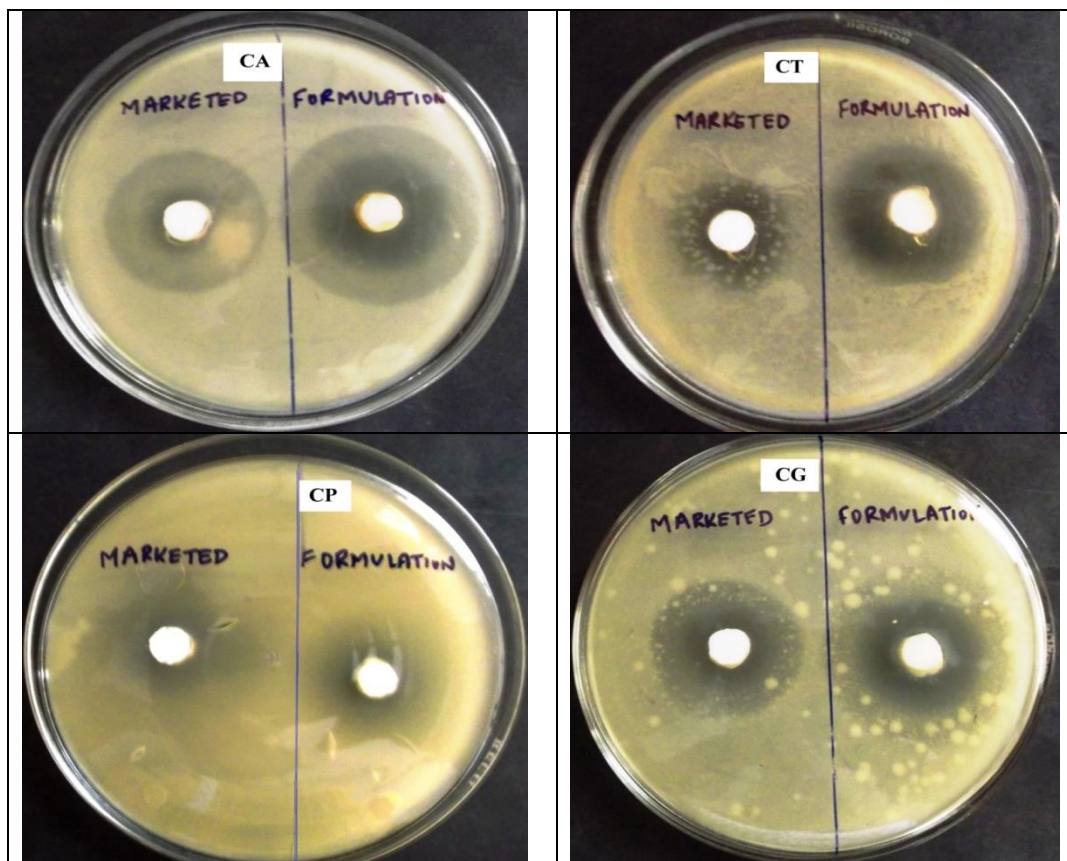


Figure 3: Comparison of antifungal activity of Optimized vaginal gel with marketed Miconazole nitrate cream formulation



Figure 4: Comparison of antibacterial activity of Optimized vaginal gel with marketed Metronidazole gel formulation

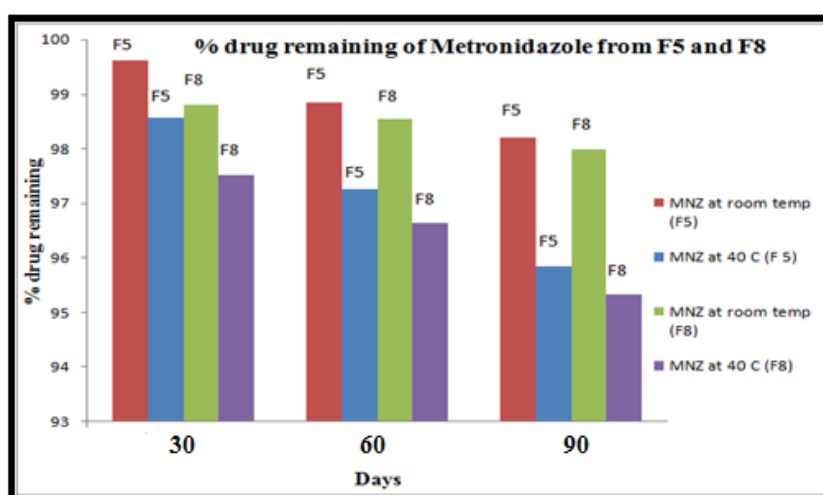


Figure 5: Results of stability studies for percent drug remaining (MNZ)

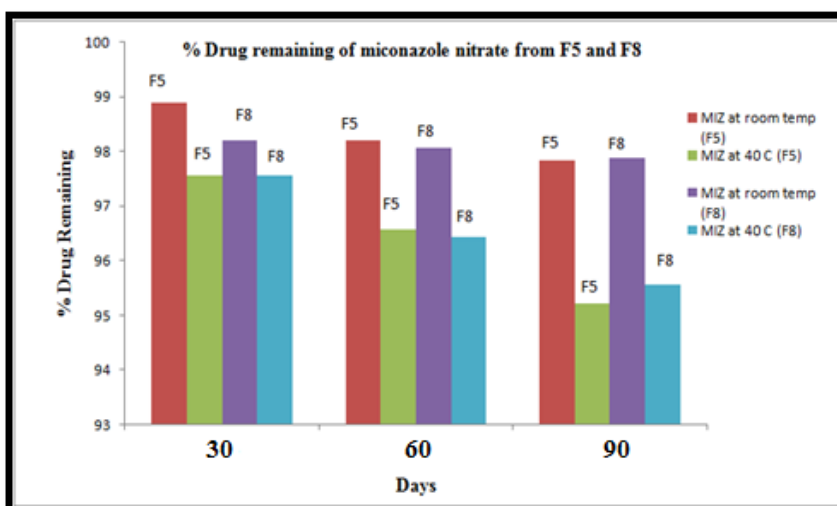


Figure 6: Results of stability studies for percent drug remaining (MIZ)

REFERENCES

1. Kukner S et al. Treatment of vaginitis. *Int J Gynecol & Obstet* 1996; 52: 43-47.
2. Thinkhamrop J et al. Vaginal fluid pH as a screening test for vaginitis. *Int J Gynecol & Obstet* 1999; 66: 143-48.
3. Jain NK. Controlled and novel drug delivery, 1st ed.; CBS publishers and distributors, 1997.
4. DuBouchet L et al. A pilot study of metronidazole vaginal gel versus oral metronidazole for the treatment of trichomonas vaginalis vaginitis. *Am Sexually Transmitted Dis Asso* 1998; 25: 176–179.
5. Lamont RF et al. The efficacy of vaginal Clindamycin for the treatment of abnormal genital tract flora in pregnancy. *Infect Dis Obstet & Gynecol* 2003; 11: 181-89.
6. D’Cruz OJ, Uckun FM. Clinical development of microbicides for the prevention of HIV infection. *Curr Pharml Des* 2004; 10: 315-36.
7. Mandal TK. Swelling-controlled release system for the vaginal delivery of miconazole. *Eur J Pharm & Biopharm* 2000; 50: 337–343.
8. Owen DH et al. Factors influencing nonoxynol-9 permeation and bioactivity in cervical mucus. *J Control Release* 1999; 60: 23-34.
9. Isham N, Ghannoum MA. Antifungal activity of miconazole against recent candida strains. *Mycoses* 2010; 53(5): 434-37.
10. Ralph ED et al. Susceptibility of *Trichomonas vaginalis* strains to metronidazole: response to treatment. *Am Sexually Transmitted Dis Asso* 1983; 10(3): 119-22.
11. Shwebke JR, Desmond R. A randomized trial of metronidazole in asymptomatic bacterial vaginosis to prevent the acquisition of sexually transmitted diseases. *Am J Obstet & Gynecol* 2007; 196: 517.e1-517.e6.
12. Shankar NB et al. Bioadhesive vaginal gel for prolong release of metronidazole: Formulation development, Characterization and comparative study with marketed formulation. *AAPS PharmSciTech* 2009; 1: 30-36.
13. Peixoto F et al. Efficacy and tolerance of metronidazole and miconazole nitrate in treatment of vaginitis. *Int J Gynecol & Obstet* 2008; 102: 287-92.
14. Neves JD, Bahia MF. Gels as vaginal drug delivery system. *Int J Pharm* 2006; 318: 1-14.
15. Hani U, Shivakumar HG. Development of miconazole nitrate thermosensitive bioadhesive vaginal gel for vaginal candidiasis. *Am J Adv drug delivery* 2003; 358-68.
16. Rai VK et al. Development of cellulosic polymer based gel of novel ternary mixture of miconazole nitrate for buccal delivery. *Carbohydrate polymers* 2014; 103: 126-33.
17. Drug Bank Open Data Drug and Drug Target Database. <http://www.drugbank.ca>.
18. British Pharmacopoeia. The Medicine & Healthcare products Regulatory Agency (MHRA) 2009.
19. United States Pharmacopoeia 30- NF 25; 2007.
20. Rowe RC et al. Handbook of Pharmaceutical excipients, 6th ed.; Pharmaceutical Press, 2009.
21. Umme H, Shivakumar HG. Development of Miconazole nitrate thermosensitive bioadhesive vaginal gel for vaginal candidiasis. *Am J of Adv Drug Delivery* 2013; 3: 358-68.
22. El-Sayed AI et al. Development and characterization of thermosensitive pluronic based metronidazole *in situ* gelling formulations for vaginal application. *Acta Pharm* 2012; 62: 59- 70.
23. Yadav VK et al. Mucoadhesive polymers: means of improving the mucoadhesive properties of drug delivery system. *J Chemical & Pharm Res* 2010; 2(5): 418-32.
24. Nayak BS et al. Development and characterization of bioadhesive gel of microencapsulated metronidazole for vaginal use. *Iran J Pharm Res* 2010; 9(3): 209-219.
25. David SJ et al. Mucoadhesive syringeable drug delivery system for controlled application of metronidazole to the periodontal pocket: In vitro release kinetics, syringeability, mechanical & mucoadhesive properties. *J Control Release* 1997; 49: 71-79.
26. Benoy BB et al. Formulation development and characterization of metronidazole microencapsulated bioadhesive vaginal gel. *Int J Pharm Pharm Sci* 2009; 1: 240-57.
27. Karthikeyan K et al. Formulation of bioadhesive carbomer gel incorporating drug loaded gelatin microspheres for periodontal therapy. *Trop J Pharm Res* 2012; 11 (3): 335-343.
28. Parashar B et al. Formulation and evaluation of gel containing miconazole nitrate an antifungal agent. *Int J Pharm Res & Rev* 2013, 2(6): 18-28.