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Research Article

THERMOSENSITIVE IN SITU GEL OF TINIDAZOLE IN TREATMENT OF BACTERIAL VAGINOSIS: FORMULATION AND EVALUATION

Shital R. Nikhar[1], Deepali A. Bansode[2]*, Kakasaheb R. Mahadik[3]

[1]Research Associate, Department of QAT, Bharati Vidyapeeth University, Poona College of Pharmacy, Pune, Maharashtra, India.

*[2]Assosiate Professor, Department of Pharmaceutical Chemistry, Bharati Vidyapeeth University, Poona College of Pharmacy, Pune, Maharashtra, India.

[3]Principal, Bharati Vidyapeeth University, Poona College of Pharmacy, Pune, Maharashtra, India.

ABSTRACT

Bacterial Vaginosis (BV) is the leading cause of vaginal discharge. Because of its big surface area, wealthy blood supply, avoidance of the first-pass effect and high permeability to many drugs, the vagina offers a promising location for local impact as well as systemic drug delivery. *In situ* gels give several benefits, such as ease of administration in the respective body cavities, elevated spreadability at certain temperatures, reduced administration frequency, improved patient compliance and comfort compared to standard dosage forms. Tinidazole (TNZ) can give effective treatment over the BV. *In situ* gel of TNZ containing polaxomer 407and HPMC E100 or carbopol 941NF was optimized on the basis of various evaluation parameters. Gelation temperature (T_{gel}) and pH of all batches was found in range of 36.6 to38.0 ºC and 4.20 to 5.03, viscosity was found in range of 1100-2050 cps at 25ºC and 4800-6530 cps at 37ºC. The Spredability was found in range of 16-20 cm. From these evaluation parameters we selected best combination for the mucoadhesive property, antimicrobial study, *in vitro* drug release and for HET CAM irritation study. The optimized formulation gives satisfactory results. In this study we also compare the performance of two mucoadhesive polymer. Based on maximum desirability and cost effectiveness, *in situ* vaginal gel containing 20% polaxomer and 0.5% HPMC E100 could be considered as a highly promising treatment for bacterial vaginosis.

KEYWORDS: Bacterial Vaginosis, Gel, *In situ* gel, Thermosensitive gel, Tinidazole, Vaginal drug delivery.

INTRODUCTION

Vaginal infection is wide and over 80% of females experience such illnesses during their lifetime[1]. Vaginitis is an exceptionally basic gynecological issue in ladies of all age gatherings. There are three sorts of infectious vaginitis: candidiasis, trichomoniasis and bacterial vaginosis[2]. Vaginitis is generally achieved by single microorganism or multiple realizing mixed vaginal infections. Around 30% of all instances of vaginitis are brought about by concurrent contaminations with in any event at least two pathogens and if not treated well, there are odds of sexually transmitted disease[3].In females of childbearing age, bacterial vaginosis (BV) is the most common cause of vaginal discharge. A thin homogeneous white discharge, a vaginal pH higher than 4.5, an ideal amine test, and the existence of clue cells microscopically characterize it. There is an increase in the concentration and incidence of organisms including *Gardnerella vaginalis, Mycoplasma hominis* and anaerobic bacteria such as *Peptostreto cocci, Prevotella spp.* and *Mobiluncus spp*. in vaginal flora and significant change in the ordinary lactobacilli (LB) dominant vaginal flora with hydrogen peroxide manufacturing to flora with significantly reduced LB figures[4].

The vagina provides a promising site for local effect as well as systemic drug delivery because of its large surface area, rich blood supply, avoidance of the first-pass effect, relatively high permeability to many drugs and self-insertion[5]. However, due to the wide inter-individual variation influencing some physiological variables such as pH and the existence of limited vaginal secretions which depends on age and menstrual cycles, the vaginal route has not been widely utilized. Although different options are

currently being researched, only limited number of vaginal delivery systems are available in market[6] such as creams, foams, gels, irrigations, tablets, which have certain constraints such as leakage, messiness and comparatively poor residence time due to the vaginal tract's self-cleaning action and often require repeated daily doses to maintain the intended therapeutic effect[5]. Despite the fact that traditional system aids to control vaginal infections, effective treatment isn't carried out absolutely due to distinctly dynamic vaginal morphology and pharmacokinetics of drugs. There is also a need to establish a novel system to enhance local residence time of formulation and enhance therapeutic action against vaginitis. Before administration, *in situ* forming gel structures are liquid aqueous solutions but it gets convert into gel under physiological conditions[7].

In 1967 Tinidazole (TNZ), 1-[2-(ethylsulfonyl) ethyl]-2-methyl-5-nitroimidazole, was first implemented for the therapy of trichomonas vaginalis infections[8]. Oral metronidazole has long been founded in the treatment of BV as an efficient therapy. But it gives various GI side effects. Nowadays metronidazole and clindamycin intravaginal preparations are commonly used for the therapy of bacterial vaginosis. TNZ also proves effectiveness in BV therapy. Cure rates are analogous to metronidazole, but there has been no evidence of clinical superiority. TNZ, however, has a more favorable side effect profile, particularly with better gastrointestinal tolerability and less metallic taste, both of which are often involved in bad adherence to metronidazole treatment[4]. TNZ is effective against protozoa including *T. vaginalis, E histolytica* and *G lamblia.* It also has *in vitro* activity against several anaerobic bacteria including bacteriods, fusobacterium, and clostridium spp. Two comparative *in vitro* research showed minor improved *in vitro* activity against *G. vaginalis* with TNZ compared to metronidazole[9]. Khushbu S. Patel et al, formulate the ion as well as thermo sensitive *in situ* gel of TNZ for the treatment of periodontitis which are applied on the dental cavity[10].

The use of appropriate polymers to formulate *in situ* gel would make them more acceptable and convenient drug delivery system [11]. *In situ* gels give several benefits, such as ease of administration in the required body cavities, elevated spreadability at certain temperatures, reduced administration frequency, improved patient compliance and comfort compared to standard dosage forms[12]. There are numerous possible mechanisms leading to *in situ* gel formation such as ionic cross linkage, solvent exchange, UV irradiation, pH or temperature changes [13]. A synthetic triblock copolymer consisting of polyoxyethylene and polyoxypropylene that exhibits thermoresponsive behavior in aqueous solutions is Poloxamer (PLX) that was selected to prepare *in situ* gel formulation. They have great compatibility with extended release of the active ingredient. Formulation of *in situ* gel with thermosensitive polymer having low mucoadhesive property to increase the mucoadhesive property polymer like HPMC or carbopol was added to enhance the mucoadhesive and mechanical characteristics of *in situ* gel formulations and ensure long residence time [14].

In this article formulation and evaluation of thermosensitive and mucoadhesive *in situ* gel of TNZ is done to avoid hepatic first-pass metabolism, a reduction in the incidence and severity of gastrointestinal side-effects. This *in situ* gel is directly applied to the vaginal cavity for the treatment of BV. *In situ* gel has broad drug absorption peak and a longer drug residence time as compared to conventional dosage form. Therefore, in this proposed research work, *in situ* gel of TNZ was developed which was further characterized and evaluated for its antimicrobial efficacy.

MATERIAL AND METHODS

Material

TNZ was gifted by BDH Industry Ltd., Kandivali (east), Mumbai. HPMC was procured as a gift sample from Colorcon, Goa. Carbopol 941 NF was procured from BF Goodrich Co., Breckville Road, Cleveland, OH. Pluronic F127 NF Prill (Polaxomer 407) was obtained from BASF Corporation, Mount Olive, New Jersey. Preservative benzalkonium chloride (0.01% w/w) other chemicals and solvents were procured from licensed vendors of pharmaceutical grades.

Preparation of simulated vaginal fluid

The simulated vaginal fluid (SVF) was prepared by mixing various ingredients such as 3.51gL^{-1} of NaCl, 1.40 g$L^{-1}$of KOH, 0.222 g$L^{-1}$of $Ca(OH)_2$, 0.018 g$L^{-1}$of bovine serum albumin, 2 g$L^{-1}$of lactic acid, 1 g$L^{-1}$of acetic acid, 0.16 g$L^{-1}$of glycerol, 0.4 g$L^{-1}$of urea and 5 g$L^{-1}$of glucose. pH of the mixture was adjusted to 4.5 ± 0.02 by using 0.1 N Acetic acid[15].

Preparation of *in Situ* gel of the TNZ

In situ gel of TNZ was prepared by dissolving various polymers. Polaxomer 407 (pluronic F127) was dissolved in water by using "cold method" at 0-4^{0}C.Carbopol 941 NF/ HPMC E100 was dissolved separately in water. The two solutions were mixed by continuous stirring and were sonicated for 15 min. The accurate amount of drug (0.75%) was weighed and dispersed in the above polymeric solution. Lastly

preservative was added and sonicated for 15 min and was stored below 25^0C.PH resembles the formulation containing polaxomer 407 and HPMC E100 and PC resemble the formulation containing polaxomer 407 and carbopol 941NF. Various trials have been carried out for the formulation of the *in situ* gel of TNZ. The formulation compositions of some batches are depicted in (Table 1).

Table 1: Formulation composition

Batch No	Excipients Concentration			
	Polaxomer 407 (%w/w)	Carbopol 941NF (%w/w)	HPMC E100 (%w/w)	Preservativ (%w/w)
PH1	19	-	0.5	0.01
PH2	20	-	0.5	0.01
PH3	21	-	0.5	0.01
PC1	20	0.1	-	0.01
PC2	21	0.1	-	0.01
PC3	22	0.1	-	0.01

Characterization of *in situ* gel

Interaction studies (Drug excipients compatibility study)

Compatibility study of drug excipients was conducted to check compatibility between drug and polymers. Studies of compatibility with drug excipients were conducted using fourier transform infrared spectrophotometer (FT-IR) (JASCO FT / IR-4100). Liquid solutions and the physical mixture of HPMC E100, carbopol 941 NF and TNZ were prepared individually and in combination. To study interactions between distinct components, both spectra were compared for possible changes[10].

Appearance

For color, odor and presence of suspended particulate matter, if any, the formulations were closely noted. By observing them against a dark and white background, the clarity of the solutions was further evaluated. From the observations formulations have been classified as turbid (-), slightly turbid (+), clear and transparent (+ +)[16].

pH evaluation

The formulation pH was recorded using a pH meter (Mettler Instruments, Germany). Triplicate experiments were conducted[17].

Clarity and refractive index

The clarity of the formulations after and before gelling was determined alternately against white and black backgrounds by visual examination of the formulations under light. The Abbe's refractometer was used to determine the refractive indexes of the formulations. The Na Light monochromatic lamp was switched on and heated for 5 min. between illuminating and measuring prism, 1-2 drop of liquid was placed, the lower case of prism was closed. To align the X-Mark in the eye piece with the shadow boundary, the rotating knob was used to separate the dark and bright region seen in the field of view. The scale refractive index has been registered[17].

Drug content

About 1 mL of gel was dissolved in100 mL of phosphate buffer pH 4.0. After continuous stirring, it was then filtered and evaluated at 318 nm by UV spectrophotometer after appropriate dilution [18]. The experiment was repeated three times for each formulation.

Gelling temperature

2 mL of the formulation was placed in a test tube immersed in a water bath with a temperature control of 4°C. The bath temperature gradually increased with 1°C. At each temperature, the sample was permitted to equalize. The sample was examined with a 90° angle by tilting the test tube. The temperature at which the gel transition occurred was observed as the temperature of gelation stated when meniscus would no longer move after the test tube had been tilted. Repeat the experiment in triplicate and record an average of three readings [19].

Viscosity

A programmable Viscometer (Brookfield, RVDV pro II, USA) was used to determine the viscosity of the liquid formulations at 25°C and the preformed gels at 37°C. 5mL of formulation was transmitted to the sample cell, which was closely positioned within a tiny volume sample adaptor, to determine the solution viscosity. The guard leg was put around the adaptor by constantly stirring the sample. The sample viscosity at various rotations per min (RPM) ranging from 0.5 to 100 RPM at 25°C temperature was evaluated. The formulations were balanced at a temperature of 37°C and 25°C for 24 h to determine gel viscosity at 37°C and 25°C. The gels viscosity was determined using a spindle no. 7 of brookfield viscometer. The motion of the helipad was

regulated to prevent the spindle from touching any portion of the sample holder, particularly the bottom. Viscosity values were observed at each RPM. The test was repeated three times for the same gel sample, and the average reading was observed [20].

Spreadability

A sample of 0.5 g of each formulation was placed between two slides (separated into 5 mm squares) and left for about 5 min at room temperature (25-30^0 C)where no further spread was expected. Spread circle diameters were measured in cm and taken for spreadability as comparison values. The findings acquired are average of three determinations[21].

Mechanical Properties of Polymer Solutions (Textural analysis)

Textural assessment was conducted using Texture Pro CT V1.7 Build 29 software (Brookfield) fitted with 0.5 g load cell in Texture Profile Analysis (TPA) mode. Formulations were transferred at 37 °C into jacketed glass vials 20 mL. In this, an analytical probe (TA3/100) was compressed twice for each sample at a specified speed 2 mm/sec allowing a delay period 15sec from the end of the first compression to the start of the second. Mechanical parameters (hardness, compressibility, adhesiveness, cohesiveness and elasticity) were obtained from the resulting force-time curve and calculated[22]. At least three experiments were conducted.

Mucoadhesion studies

A tensile test was used to evaluate the mucoadhesive properties of the various formulations, where the measurement of maximum force, mucoadhesion as well as work of adhesion required to detach the formulations from a mucosal tissue was evaluated. Mucosal tissue was collected from freshly sacrificed animals in the slaughterhouse and separated from the underlying tissues, washed, cut into smaller parts and thoroughly rinsed. Using the Brookfield texture analyzer fitted with a 5 kg load cell, the mucoadhesive characteristics of formulations were assessed. Cyanoacrylate glue was used to attach sections (> 2 mm in thickness) taken from the inner part of the mucosal membrane surface to the reduced end of the texture analyser sample (10 mm in diameter). The gels have been packed into a holder and kept at 37°C. The probe holding the mucosa was lowered to the gel surface at a steady velocity of 0.1 mm/s until a contact force of 0.05N was applied for 2 min in contact with the gel surface. The probe was then shifted vertically up at a constant velocity of 0.1 mm/s and the resulting force distance graph determined the maximum detachment force (F) and the area under the curve (AUC, called the mucoadhesion). The work of mucoadhesion (Work, mJ cm-2) was calculated from the following equation:

$$\text{Work} = \frac{AUC}{\pi r^2}$$

where, πr^2 = the gel-contacted mucosal surface.

All analyzes have been repeated at least three times [23].

***In vitro* drug release and release kinetics.**

Studies of *in vitro* drug release were conducted using a dialysis bag in altered USP dissolution device I (Veego, DT50, India) in Simulated Vaginal Fluid (SVF) at pH 4.5. The dialysis bag (Himedia DM 50 membrane, molecular size cut off 12 to 14 KD, pore diameter, 2.4 nm which was earlier soaked overnight in simulated, a precise quantity 2 mL of *in situ* gelling formulation was placed. Both the dialysis bag ends were attached to the dissolution assembly basket rod. The bags were immersed at 37 ± 0.5 ° C with a stirring rate of 50 RPM in the 100 mL dissolution medium (Simulated Vaginal Fluid pH 4.5). The dissolution medium was removed at a specific interval and the release of the drug was evaluated at 318 nm by the UV spectrophotometer (JASCO UV spectrophotometer V630). In order to maintain the sink condition, the same amount of dissolution medium was substituted in the flask. The release of drugs was demonstrated as the average of three studies. The drug release kinetic models were implemented using disso software to determine the mechanism of drug release from *in situ* gelling formulations. To determine the values of the correlation coefficient, the drug release data were attached to zero order, first order, Higuchi matrix, and Korsmeyer-Peppas models[24].

Microbiological studies

The microbiological studies were carried out on the optimized formulation, Marketed formulation (Metrogyl Gel – Metronidazole gel) and 0.75% w/v drug solution for comparison against micro-organism. *Staphylococcus aureus* and *E. coli* were used as the test microorganism. The petriplate was permitted to solidify a layer of nutrient agar 30 mL seeded with the test micro-organism 0.2 mL. Using a 4 mm diameter sterile borer, cups were produced on the solidified agar layer. Then quantity of formulations comprising equal quantity of medication (optimized formulation and plain drug solution) was poured into the cups. The plates were incubated at 37°C for 24 h after maintaining petriplates at room temperature for 4 h. The zone of inhibition was recorded. An antibiotic zone finder will measure the diameter of zone of inhibition[25,26].

Irritation test (Hen's Egg Test-chorioallantoic membrane test)

Modified Hen's Egg Test-Chorioallantoic membrane (HET-CAM) test was performed [27].The HET-CAM has been shown to be a method of evaluating the probable irritation of specific chemicals. The prospective irritation of compounds identified after exposure to test chemicals by observing negative alterations that happen in the egg's chorionallantoic membrane. Briefly, eggs from the poultry farm were collected from fertilized hen. Three eggs have been chosen for each formula weighing between 50 and 55 g. These eggs were incubated for 3 days at a temperature of 37 ° C ± 0.5 ° C in a humidified incubator. On 3rd day, egg albumin 3 mL was removed from the pointing end of the egg using sterile techniques. The hole was sealed with the assistance of heated spatula by using 70 % alcohol sterilized parafilm (American Can Company, USA). The eggs were held away in the equatorial position for CAM growth in the shell. On the 5th day of incubation, the eggs were candled and every day, non-viable embryos were removed.

Formulations were directly instilled on the CAM surface on the 10th day and left in touch for 5 min. For vascular harm, the membrane was examined and the time taken to happen for injury was reported.

It was found practically non-irritating, a 0.9 % NaCl solution was used as a control. The results were recorded by the scoring scheme which is shown in (Table 2)[17].

Table 2: Scoring scheme for HET-CAM test

Observation	Score	Conclusion
No visible hemorrhage	0	Nonirritant
Just visible membrane discoloration	1	Mild irritant
Structures are converted partially due to membrane discoloration or hemorrhage	2	Moderately irritant
Structures are converted totally due to membrane discoloration or hemorrhage	3	Severe irritant

RESULTS AND DISCUSSION

Drug excipient compatibility study

Interaction studies were conducted to verify any interaction between components of the formulation. UV spectra were discovered to be identical before and after autoclaving. Identical graph of UV shows no interaction.

The FT-IR spectra of TNZ, physical mixture of all excipients and TNZ loaded *in situ* formulations are shown in (Fig. 1-5) respectively. FT-IR spectrum of TNZ showed characteristic peaks at 3000 cm^{-1}- 2913 cm^{-1} (C-H stretching), 1759 cm^{-1} (C=C imidazole ring), 1522.5cm^{-1} (C=N imidazole ring), 1456 and 1371 cm^{-1} (N=O stretching), 1289 cm^{-1} (S=O stretching), 1262cm^{-1} (C-O stretching)(Fig. 1). IR spectrum of poloxamer 407 was shown characteristic peaks at 3000cm^{-1} to 2900 cm^{-1}(C-H stretch aliphatic), 1345 cm^{-1} to 1355 cm^{-1} (O-H bending), and nearby 1100 cm^{-1} (C-O stretch). IR spectrum of Carbopol941NF has shown peaks at broad peak above 3000 cm^{-1}(O-H stretching), nearby 1700 cm^{-1} (carboxyl group)where, FT-IR spectrum of HPMC E100 showed characteristic peaks at 1729 cm^{-1} C=O (ester) stretching, which was the same in formulation loaded *in situ* of TNZ. Thus, there was no drug-excipient interaction.

Appearance, clarity, pH and drug content

All formulations were observed visually for transparency, they didn't show any precipitation. The refractive gel index ranges from 1.335 to 1.337, which proves gel transparency. The pH of formulations was reported to be between 4.20±0.05 and 5.03±0.05. This range of pH is appropriate to the vaginal cavity since the vaginal pH is within the range. The percent drug content of formulation was found in range of 95.9+0.34 to 104.1±0.25% respectively.

Gelling Temperature

T_{gel} is the temperature at which the liquid phase makes a transition to gel. An ideal *in situ* gel should be a free flowing liquid at room temperature so as to allow reproducible administration into the site of application where it undergoes *in situ* phase transition to form a strong gel. The human vaginal temperature is 37.2°C, So T_{gel} of vaginal thermo reversible gels were considered to be suitable if they were in the range of 25-37°C[17]. If T_{gel} is higher than 37°C, a liquid dosage form still exists at vaginal temperature, resulting in drainage of the formula from the vagina at an early stage. The gelling temperature of all batches was found in range of 37.0-38.0°C.

Viscosity

Shear thinning (reduction in viscosity with growing shear rate) in liquid as well as gel state owing to the pseudoplastic behavior of the gels. A Brookfield Viscometer was used to determine the viscosities of TNZ formulations at 25°C and at 37°C. (Fig. 6) showed the findings. Figure depicts that viscosity at 25°C is less compared to viscosity at 37°C because as temperature increases the formulation is convert into gel so the viscosity also increases.

Spredability

The spreadability plays a significant role in accordance with patients and helps to uniform application of gel to the skin. High spreadability of gel requires less time to spread and it will easily spread on mucosal membrane. The spreadability is the distance travelled by the formulations before the transition to gel. The rise in the concentration of poloxamer reduced the spreadability of formulations owing to a reduction in the temperature of gelation.

Summary of various parameters like appearance, pH, drug content, gelling temperature, viscosity and spreadability is depicted in (Table 3). From the various parameters which are mentioned in (Table 3) we selected two batches i.e. PH2 and PC2 for further evaluation as these batches gave satisfactory results. These formulations having favorable pH range as well as converts into the gel at 37 °C. Also, PH2 and PC2 having moderate spreadability therefore, it can easily spread on the surface.

Table 3: Evaluation parameter of formulations

Batch no	Appearance*	pH	Drug content (%)	Gelling Temp (°C)	Viscosity at 25°C (cps)	Viscosity at 37°C (cps)	Spredability (mm)
PH1	++	4.20±0.05	99.8±1.12	37.3	1100	4800	17
PH2	++	4.48±0.1	102.2±0.5	37.0	1200	5200	15
PH3	++	4.56±0.2	97.4±0.31	36.6	1250	5300	14
PC1	++	4.45±0.1	104.1±0.25	38.0	1890	6000	20
PC2	++	4.62±0.05	100.2±0.18	37.0	2000	6400	18
PC3	++	5.03±0.05	95.9±0.34	37.0	2050	6530	16

*turbid(-), slightly turbid (+), clear and transparent (+ +)

Mechanical Properties of Polymer Solutions

Texture profile analysis (TPA) is a mechanical test that defines the compressive stress and subsequent relaxation strength of pharmaceutical formulations. TPA offers data on the impacts of repeated shearing stress on formulations structural characteristics, a property called 'cohesiveness'. Optimized gel formulation containing HPMC and carbopol i.e. PH2 and PC2were tested for TPA.

Hardness is defined as the force required to achieve a deformation (Strength Unit, N). The compressibility determines the work required to deform the material during the first contraction of the probe (work unit, N mm). The adhesiveness of the formulation is the job needed to resolve the attractive forces between the surface of the sample and the surface of the probe. This parameters helped to understand performance of the formulation are viz. ease of application to the surface or from container and retention of gel on site of application. The textural properties of TNZ *in situ* gel formulations are shown in (Table 4).

Table 4: Mechanical properties of *in situ* gel of TNZ

Batch no	Hardness (N) ± SD	Compressibility (N.mm) ± SD	Adhesiveness (N.mm) ± SD	Elasticity ± SD	Cohesiveness ± SD
PH2 - 20^{0}C	0.012 ± 0.000	0.025 ± 0.001	0.038 ± 0.002	0.800 ± 0.036	0.574 ± 0.069
PC2 - 20^{0}C	0.009 ± 0.006	0.089 ± 0.058	0.035 ± 0.006	0.752 ± 0.009	0.155 ± 0.012
PH2 - 37^{0}C	0.374 ± 0.053	0.720 ± 0.000	0.765 ± 0.013	1.752 ± 0.029	1.00 ± 0.033
PC2 - 37^{0}C	0.204 ± 0.025	0.557 ± 0.003	0.40 ± 0.036	1.102 ± 0.025	0.682 ± 0.003

Mucoadhesion Studies

Quantification of mucoadhesion is essential in order to guarantee that the adhesion provided by formulations is adequate to assure prolonged retention at the application site, but not excessively, because may result in harm to the mucous membrane. Mucoadhesion study gives the thorough assessment of the detachment phenomenon of various formulations under examination, formulation PH2 (containing 20% poloxamer 407 and HPMC) is having highest mucoadhesion as compared to the formulation containing carbopol. The results are shown in the following graph (Fig. 7).

In vitro release kinetics

The formulations of *in situ* gelling revealed a biphasic pattern of release, an original burst release accompanied by the continued release of TNZ. TNZ's burst release at the beginning of dissolution signaled a delay in sol transition to gel. Continued release of drug further stated the slower diffusion of drug from the gel matrix. This will confirm the controlled release behavior of the formulation. The original burst release is useful as it helps to achieve the drug's therapeutic concentration in a minimum of moment followed by steady release to retain the sustained release of a drug.

Developed formulation PH2 showed 30.5% cumulative drug release after 1 h 53.60% after 4 h and 71.3% after 8 h and PC2 showed 39.08% cumulative drug release after 1h 62.44% after 4 h and 71.7% after 8 h (Fig 8). Burst effect might be due to initial migration of the drug toward the surface of the matrix. The release kinetics data is shown in (Fig 8) (Table 5). The formulation obeys the Higuchi matrix model i.e. it will indicate that initial drug concentration in the matrix is much higher than drug solubility and perfect sink conditions are always attained in the release environment.

Table 5: Release kinetics of *in situ* gel of TNZ

Batch no	Regression coefficient (R^2)				
	Zero order	First order	Higuchi matrix	Korsemeyer Peppas	Hixoncrowell
PH2	0.9035	0.9687	0.9931	0.9559	0.9515
PC2	0.7887	0.8769	0.9465	0.8882	0.8504

Microbiological studies

The antimicrobial activity of the formulation was tested against the strains *E coli* and *S aureous* by using the cup plate technique. Here in the study comparison of the formulation was done against the marketed formulation and plane drug solution. The zone of inhibition of PH2 and PC2 batches was given in the (Table 6) (Fig. 9 and Fig. 10) respectively. This indicated that the formulations showed greater activity than the marketed formulation.

Table 6: Zone of inhibition of optimized batches

		Zone of inhibition(mm)		
		Standard	Marketed Formulation	Formulation
E coli	PH2	30	28	31
	PC2	35	28	34
S. aureous	PH2	32	20	31
	PC2	34	25	34

Irritation test

Irritation test of the developed formulation was carried out by Hen's egg CAM test. It is a fast, delicate and cheap test. Incubated egg testing is a borderline event between *in vivo* and *in vitro* processes and does not conflict with ethical and legal requirements. Using this method, developed formulations were tested and the result contrasted with normal saline, which was used as a control that is intended to be non-irritating because 0 score was obtained for normal saline.

As PH2 batch have shown consistent better mucoadhesive as well as better *in vitro* release, hence it was selected for the study of Irritation test on hen's eggs. The optimized formulation PH2 was tested for irritation study and was found to be non-irritant up to 1 h (mean score 0), with a mean score of 0.66 up to 24 h as depicted in (Table 7).

Table 7: HET CAM ASSAY observations

Sample	HET- CAM Score								
	Time in min								
	0	5	15	30	60	120	240	480	1440
Control	0	0	0	0	0	0	0	0	0
Formulation									
Egg 1	0	0	0	0	0	0	0	0	0
Egg 2	0	0	0	0	0	0	0	0	1
Egg3	0	0	0	0	0	0	0	1	1
Mean	0	0	0	0	0	0	0	0.33	0.66

*0 Non irritant, 1 Mild irritant

CONCLUSION

This research outlined formulations of *in situ* gel of TNZ and assessed their rheological and textural characteristics. Polaxomer possesses low mucoadhesive characteristics, but its thermal sensitivity makes it simple to apply and cover the mucosa. Adding HPMC / Carbopol to the formula it decreased the temperature of the sol-gel transition and influenced the mucoadhesive and mechanical properties of formulation.

The results obtained from the texture characterization of 20% polaxomer and 0.5% HPMC E100 i.e. PH2 formulation showed the better mechanical properties as well as good mucoadhesive strength. This formulation also gives sustained drug release for 7 h. Although the PC2 Formulation having the good antimicrobial activity than the PH2 formulation but other factors are better in case of PH2 formulation. From the all observations and based on maximum desirability and cost effectiveness, *in situ* vaginal gel containing 20% polaxomer and 0.5% HPMC E100 could be considered as a highly promising treatment for bacterial vaginosis. Our findings have shown that the formulations created have been discovered worthy for further study.

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***Address for correspondence**
Dr. (Mrs). Deepali Bansode
Associate Professor,
Dept. of Pharmaceutical Chemistry and QAT
Bharati Vidyapeeth University,
Poona College of Pharmacy, Pune,
Maharashtra, India.
Mobile: 9970002387
Email: deepali_mhaske@rediffmail.com
deepali.bansode@bharatividyapeeth.edu

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Figures

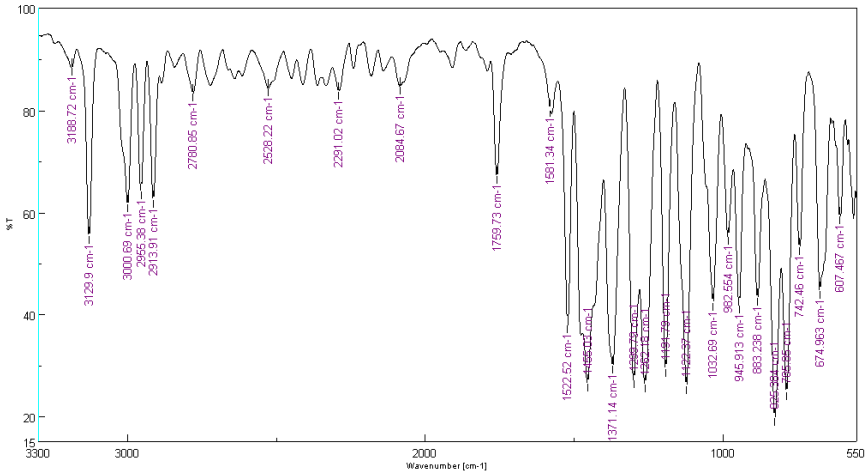

Figure 1: FTIR spectrum of TNZ (pure drug)

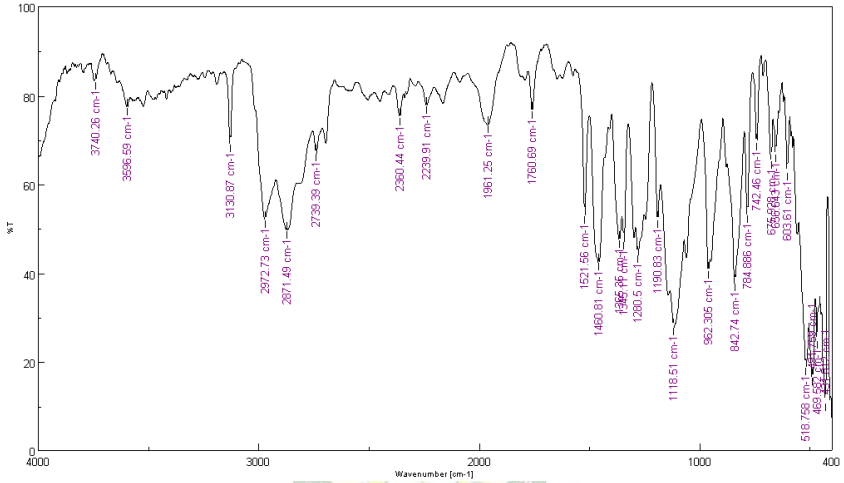

Figure 2: FTIR spectrum of physical mixture of polaxomer 407, HPMC E100 and TNZ

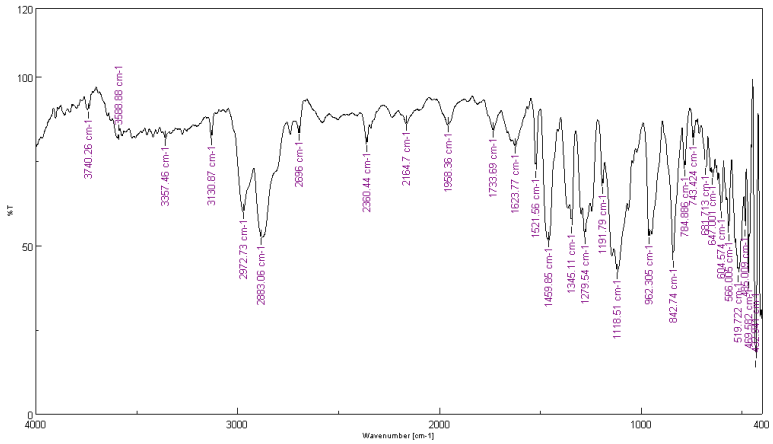

Figure 3: FTIR spectrum of physical mixture of polaxomer 407, carbopol 941NF and TNZ

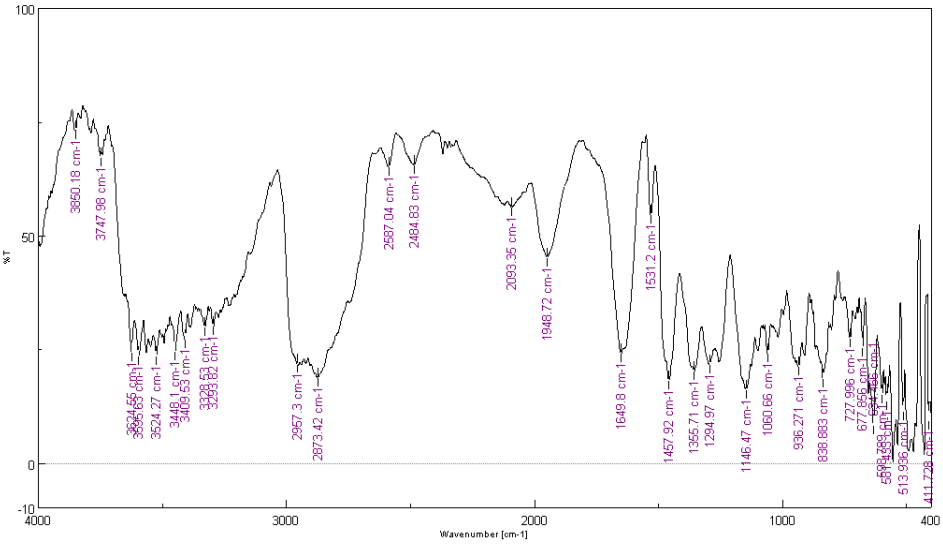

Figure 4: FTIR spectrum of *in situ* gel containing polaxomer 407 and HPMC E100

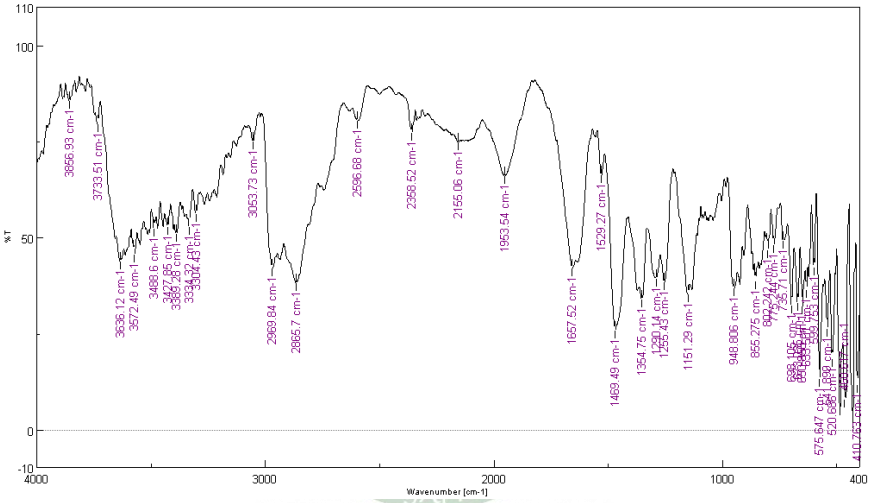

Figure 5: FTIR spectrum of *in situ* gel containing polaxomer 407 and 1657carbopol 941NF

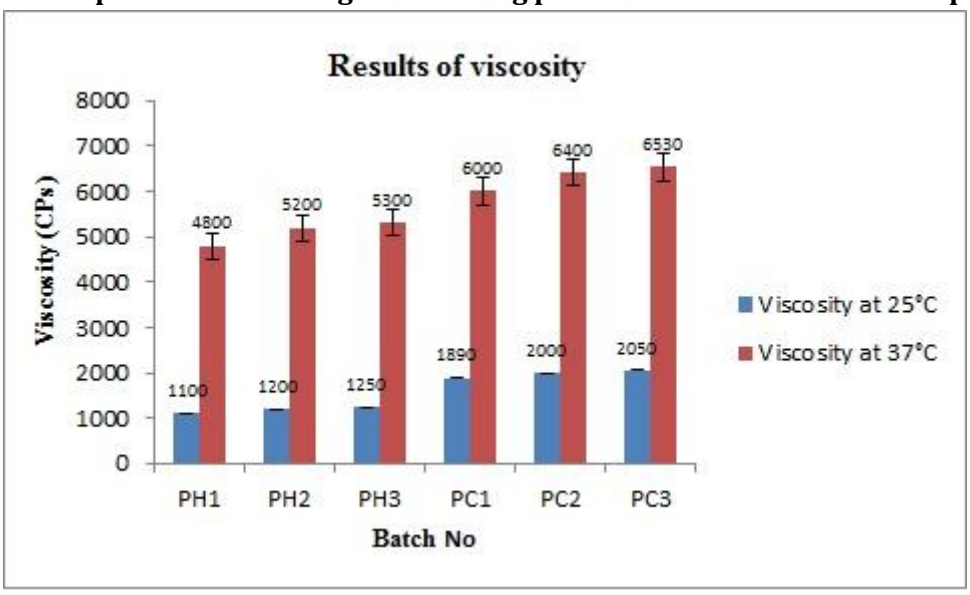

Figure 6: Results of viscosity

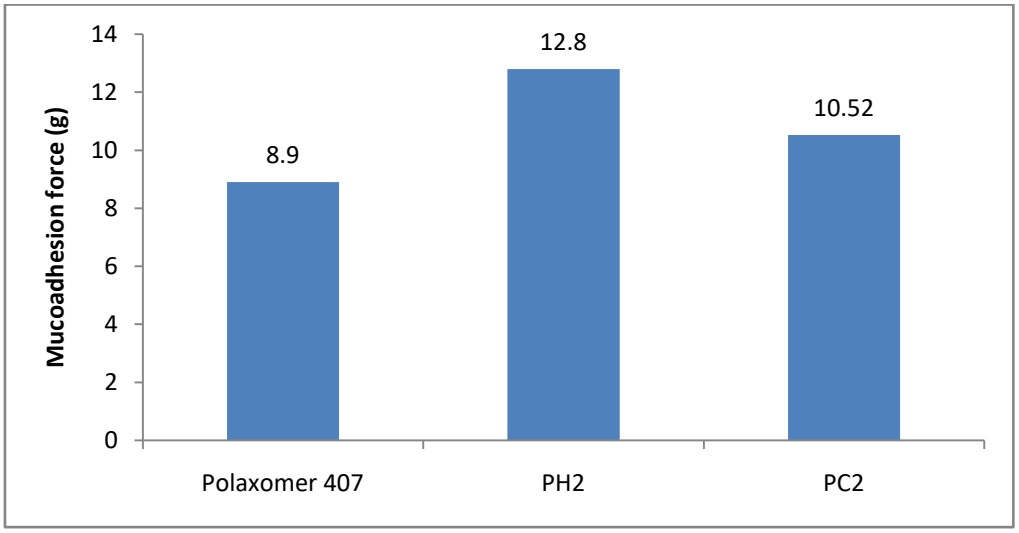

Figure 7: Mucoadhesive strength of formulation

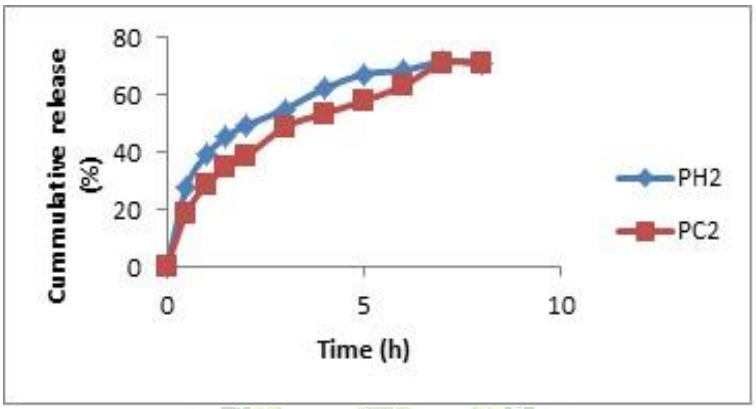

Figure 8: *In vitro* release of formulation

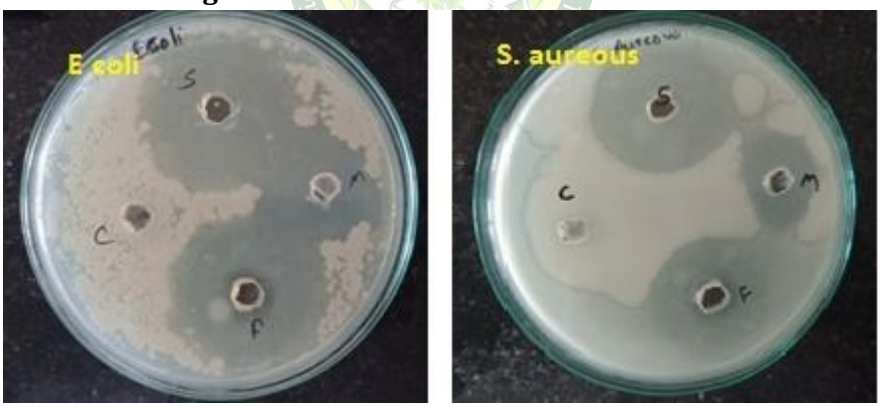

Figure 9: Zone of inhibition of optimized formulation PH2

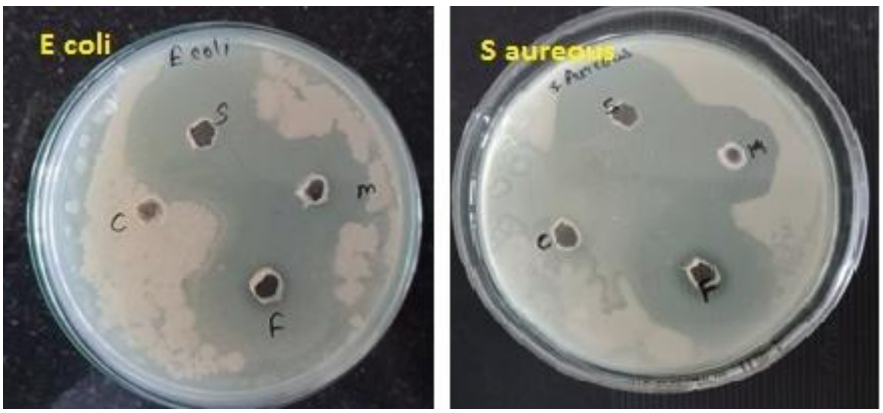

Figure 10: Zone of inhibition of optimized formulation PC2