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Development of Metronidazole Intravaginal gel for the treatment of bacterial vaginosis: Effect of Mucoadhesive Natural Polymers on the Release of Metronidazole

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Abstract: The purpose of this study was to achieve a better therapeutic efficacy and patient compliance in the treatment for bacterial vaginitis. Metronidazole (0.5 %) has been formulated in an intravaginal gel using the bioadhesive natural polymers such as Chitosan, Xanthan gum and Gelatine. To increase its aqueous solubility, Metronidazole was initially dissolved in a mixture of PVP K30 and water (5:3) and added to polymer dispersion. The intravaginal gel formulations were evaluated for pH, Spreadability, Syringeability, Viscosity, Bioadhesion test, Antimicrobial Susceptibility Test and In vitro drug release study. V7 and V2 mucoadhesive gel formulations were selected as optimum formulations based on evaluation studies. These formulations are showing control drug release with good mucoadhesion properties due to presence of chitosan alone and with combination of Xanthan gum. All the performed experiments confirm the applicability of intravaginal gel as a novel drug carrier system for the local treatment of bacterial vaginosis.
Key words: Intravaginal gel, Metronidazole, Polyvinyl pyrolidone K30, Prolonged release.

Introduction

Vaginitis is a common gynecological problem in women of all age groups. It may result from microbial infections, contact dermatitis, atrophic vaginitis, or allergic reactions[1]. The infectious vaginitis is of 3 types: candidiasis, trichomoniasis, and bacterial vaginosis. Vaginal infections are usually characterized by vaginal discharge, vaginal irritation or vulvar itching, and vaginal odor[2, 3]. The vaginal route has been traditionally used for the conventional delivery of several locally acting drugs like antimicrobial agents[4]. However conventional vaginal delivery systems such as creams, foams, pessaries and jellies reside at the targeted site for relatively shorter retentivity because of the self cleaning action of the vaginal tract which limits effective drug levels for a shorter period and fluctuation in drug dose level leads to increased dose frequency of the drug. This ultimately results into patient inconvenience and toxic conditions[5]. The use of prolong release bioadhesive vaginal gel was thought to offer numerous benefits including prolong residence time of the dosage form at the site of absorption due to bioadhesion to the vaginal mucosa, prolong drug release, improved bioavailability and decreased side effect of drug and ultimately improved patient compliance[6]. Metronidazole is classified

therapeutically as an antibacterial and antiprotozoal agent, indicated for the treatment of bacterial vaginosis (BV)[7]. For the treatment of vaginitis, local administration of metronidazole has been favored due to numerous side effects, toxicity, and teratogenic potential of the systemically applied drugs[8]. Metronidazole vaginal gel is the intravaginal dosage form of the synthetic antibacterial agent[9].

The objective of this study was to design a vaginal gel formulation with mucoadhesive properties to ensure longer residence at the infection site, using natural polymers such as Chitosan, Xanthan gum and Gelatine providing a favorable release profile for the antibacterial drug Metronidazole. The objectives of the study were to investigate the performance of natural polymers and effect on the release characteristics of the Metronidazole gels.

Materials and Methods

Materials:

Metronidazole was procured from Claris Lifesciences Ltd, Ahmedabad, India. Gelatine and Xanthan gum were procured from Thomas Baker, Mumbai and Loba Chemie Pvt. Ltd. Chitosan was obtained a gift sample from CIFT, Cochine. All other chemicals and reagents used were of analytical grade and used as received.

Preparation of Metronidazole mucoadhesive intravaginal gel [10, 11, 12]:

Metronidazole gel formulations were prepared using Chitosan, Xanthan gum and Gelatine as gelling agents. Gelling agent was dispersed in a small quantity of citrate phosphate buffer (0.1M, pH 4.0) and then stored overnight to ensure complete hydration. Metronidazole was initially dissolved in a mixture of PVP K30 and water (5:3) and added to polymer dispersion. Other excipients (methyl paraben and propyl paraben) were also added slowly with continuous stirring. The final weight of the gel was adjusted to 100 gm with citrate phosphate buffer (0.1M, pH 4.0). Entrapped air bubbles were removed by keeping the gels in vacuum desiccators. Table No. 1 shows the formulations of mucoadhesive gel.

Drug content analysis[13]:

About 6 gm of gel was weighed accurately and dissolved in citrate phosphate buffer pH 4.0 of sodium lauryl sulphate (1%w/v SLS). After appropriate dilutions, Metronidazole content was analyzed spectrophotometerically (Pharma Spec UV- 1700, Shimadzu, Japan) at 319 nm.

Viscosity Study[11]:

Viscosity of gels was studied on Brookfiel viscometer by using spindle number 3 at 60 revolutions per minute at constant temperature.

Syringeability study[14]:

Syringeability study was carried out by using a 22 gauze needle.

Spreadability of the mucoadhesive gels[15]:

Spreadability study was carried out by transferring the 6 gm of gel formulation to the center of a glass plate and compressed under several glass plates (100 ± 5 g each, every 1 minute) and the spread diameters recoded each time.

Bioadhesion study[16]:

In the present study, sheep stomach mucosa was used as a model mucosal surface for bioadhesion testing. The sheep stomach mucosa was procured from slaughter house, then excised and trimmed evenly from the sides. It was then washed in citrate phosphate buffer pH 4.0 and was preserved in the same or used immediately. The two sides of the balance were balanced with a 5 g weight on right hand side. The sheep stomach mucosa excised and washed was tied tightly with the mucosal side upwards using a thread over the protrusion in the rubber block which is covered with inert aluminum surface. The block was then lowered into the glass container, which was then filled with phosphate buffer (pH 4.0) kept at 37□C±1□C, such that the buffer just reaches the surface of mucosal membrane and keeps it moist. This was then kept below the left hand set up of the balance. The film was then glued at the border adhered to a aluminum surface hanging on the left hand side and the beam raised, with the 5 g weight removed on the right pan side. This lowered the aluminum surface along with the film over the mucosa, with a weight of 5 g. The balance was kept in this position for 8 min and then slowly water was added to the plastic container in the right pan by pipette. The addition of water was stopped as soon as the detachment of two surfaces was obtained. Weight of water was measured. The excess weight in the pan i.e. total weight minus 5 mg is the force required to separate the film from the mucosa. This gave the bioadhesive strength of the formulation in grams.

Dissolution rate study[17]:

The in vitro release of Metronidazole was determined from different vaginal gel formulations using a dialysis bag prepared by cellophane membrane placed in the release medium.

Treatment of cellophane membrane:

A cellophane membrane (cut to suitable size) boiled in distilled water for 1 hour, soaked

in absolute alcohol for half an hour and stored in citrate phosphate buffer pH 4.0 for 24 h before use.

The gel formulations (6 g) were packed into the cellophane membrane bags (50 mm) The release medium was 100 ml 0.1M citrate-phosphate buffer (pH 4.0) containing 1% Tween 80, providing sink conditions for Metronidazole. The medium was maintained at 37ºC and stirred at 100 rpm. At various time intervals, 5 ml of dissolution fluid was collected. Levels of Metronidazole in the samples were analyzed

with a Shimadzu UV-VIS 160A spectrophotometer at λ = 319 nm.

Antimicrobial Susceptibility Test[18]:

Formulations containing Metronidazole prepared and used in microbial assays. Drug equivalent to 30 mg formulations used for measurement of zone of inhibition. Under aseptic conditions the formulated gels placed on blood agar plates containing *Staphylococus aureus* and were incubated at 37□C for 40 hr. The zone of inhibition was measured at the end of 40 hours.

Results and Discussion:

V7 and V2 mucoadhesive gel formulations were selected as optimum formulations based on evaluation studies. These formulations are showing control drug release with good mucoadhesion properties due to presence of chitosan alone and with combination of Xanthan gum. The values of surface pH were within the range for all formulations. Spreadability of V7 mucoadhesive gel was schematically depicted in Figure 1. It indicated that spreadability area was increased with weight applied.

Drug content analysis

All the formulations exhibit fairly uniform drug content (Table No. 2). V1 formulation showed more drug content (99.4±0.02%).

Viscosity Study

The viscosity results showed in Table No. 2. The results indicating that formulations containing combinations of polymers are more viscous then the formulation containing only one polymers. Chitosan with combination of Xanthan gum containing formulations are showed more viscosity where as Gelatin containing formulations are showed less viscosity.

Syringeability study

The results of the syringeability study indicate that all the gel formulations were syringeable through 22 gauze needle.

Bioadhesion study

The bioadhesion was maximum with the V7 formulation and lowest for the V5 respectively (Figure 2), and all formulations showed satisfactory bioadhesion strength. The In-Vitro Bioadhesion study results are shown in Table No. 2. Results of bioadhesion indicating that formulations containing Chitosan alone or combination with other natural mucoadhesive polymers show more bioadhesion.

Dissolution rate study

The plot of cumulative drug release Vs time plotted for all formulations are depicted in Figure 3. The result revealed that release rate of the Metronidazole was controlled for a period of time (72 hr) with V7. Chitosan containing formulations showed better controlled release when compared to Xanthan gum and Gelatin. The drug release after 72 hr is in following order.

V7<V2<V1<V9<V4<V8<V3<V6<V5

Antimicrobial Susceptibility Test

The results of the antimicrobial studies of all the gel formulations against the *Staphylococcus aureus* indicates satisfactory zone of inhibition Table No 2. Bioadhesive polymers of the gel had prolonged drug release and provided better contact.

Conclusion:

Chitosan and Xanthan gum are the natural polymers of choice for bioadhesive vaginal gels. Bioadhesion studies justified the prolonged retention of the gels inside the vaginal tract. It can be concluded that the bioadhesive intravaginal gel formulations are more effective as well as having better patient compliance than existing conventional vaginal drug delivery systems.

Table No.1. Composition of Mucoadhesive Intravaginal gel formulations:

Ingredients	Formulation Code								
(gm)	V1	V2	V3	V4	V5	V6	V7	V8	V9
Metronidazole	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Chitosan	2.5	3.0	-	-	-	-	1.5	-	1.5
Xanthan gum	-	-	2.5	3.0	-	-	1.5	1.5	-
Gelatine	-	-	-	-	2.5	3.0	-	1.5	1.5
Methyl paraben	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Propyl paraben	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
Citrate phosphate buffer	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s

Table No.2: Drug content, Viscosity, Bioadhesion strength and Zone of Inhibition.

Formulation code	Drug content Mean ± SD (%)	Viscosity Mean ± SD (dyne/ cm2)	Bioadhesion strength (gm) Mean ± SD	Zone of Inhibition Mean ± SD (mm)
V1	99.4±0.02	1901.00±0.1	19±0.25	34±1
V2	98.9±0.1	1932.00±0.04	21±0.15	33±4
V3	98.5±0.04	1786.00±0.00	17±0.82	40±2.0
V4	99.01±0.01	1828.00±0.04	18±0.65	38±1
V5	99.1±0.12	1632.00±0.03	14±0.18	43±1.0
V6	98.8±0.14	1686.00±0.02	15±0.34	42±2.1
V7	99.4±0.07	1955.00±0.01	22±0.52	33±4
V8	99.5±0.01	1815.00±0.2	18±0.35	38±1
V9	99.03±0.02	1836.00±0.1	19±0.2	35±3

± S.D- Standard deviation for (n=3)

Figure 1. Spreadability of V7 formulation:

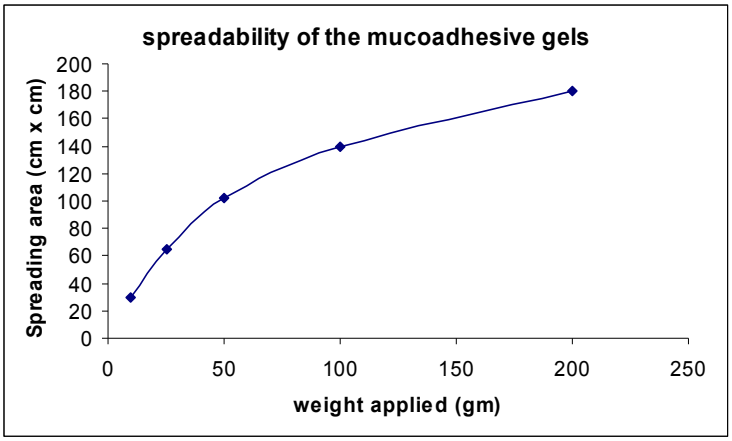


Figure 2. Bioadhesion strength of Mucoadhesive intravaginal gel formulations

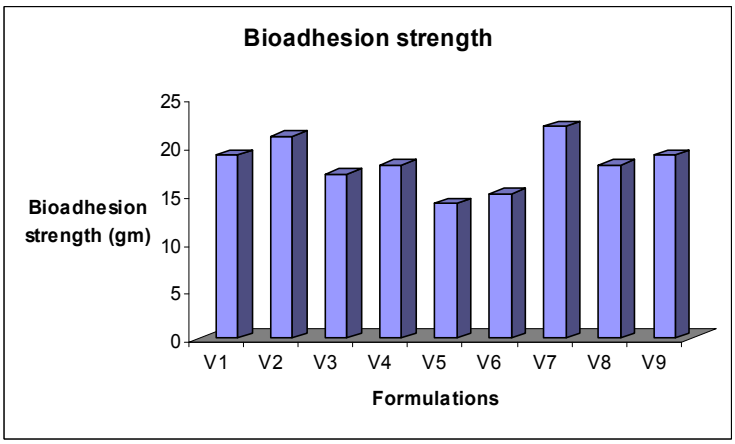
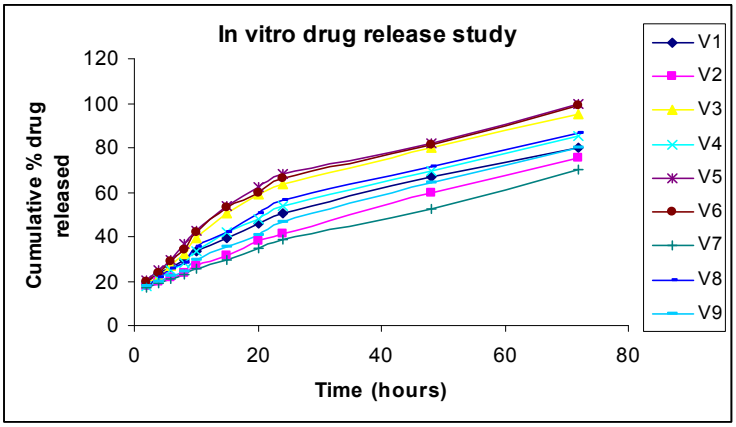


Figure 3. In vitro drug release study (cumulative % drug release)



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