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RESEARCH ARTICLE

Formulation Development and Evaluation of Novel Bioadhesive Vaginal Gel

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ABSTRACT

The aim of this formulation was to achieve better patient compliance by increasing residence time and thus increase in bioavailability. Due to the wide significance such as large surface area rich blood supply, avoidance of first pass metabolism. Ciclopirox Olamine (CPO) is an antifungal, antibiotic, anti-inflammatory agent and is a class II drug. Various combinations of polymers and excipients were selected. CPO was dissolved in water followed by dispersing the polymers. Cellulose derivatives and natural polymer dispersions were prepared by overnight soaking to achieve complete hydration. The other water soluble ingredients were dissolved in water. These systems were combined and mixed properly to get homogeneous mixture. From the various combinations of polymer studied for this formulation, the HPMC K4M and carbopol 940 were showed good bioadhesive properties and thus longer residence time. So this combination was selected. The 99.06% drug release was obtained at the end of eight hour, and due to mucoadhesive polymers retention of the drug was increased. The mucoadhesive strength of the optimized formulation was found to be 0.52±0.01N which increased residence time of the formulation. Stable formulation was obtained containing lactobacilli without any growth. The spreadability and viscosity of the optimized batch were compared with the marketed formulation Candid-V gel and it was found that the spreadability of the optimized batch was better than that of the marketed formulation. The viscosity of the optimized batch was higher than that of the marketed one thus release was prolonged.

KEYWORDS

Ciclopirox Olamine, Antifungal gel, HPMC K4M, Carbopol 940

INTRODUCTION

Among of all the routes for the drug delivery, vaginal route is the most promising one for local and systemic drug delivery. Human vaginal tract is relatively unexplored route for the drug delivery despite of its several advantages over the other routes of administration. The larger surface area, rich blood supply, avoidance of first pass effect makes it favorable route of administration for many sensitive drugs.[1]

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It has been known that for several decades that various pharmacologically active agents, such as steroids, may be effectively absorbed through the vaginal mucosa. The feasibility of vaginal absorption was first demonstrated experimentally by the intra-vaginal administration of progesterone via drug-impregnated suppository formulation[2,3,4] This route of administration materialized in 1970 with the development of a medicated, resilient vaginal ring. In subsequent years a series of clinical trials with different designs and sizes of vaginal rings containing various doses steroids were undertaken in an

attempt to develop a contraceptive vaginal ring that would consistently inhibit ovulation.[5]

Bioadhesion (or mucoadhesion) is generally understood to define the ability of a biological or synthetic material to "stick" to a mucous membrane, resulting in adhesion of the material to the tissue for a protracted period of time. Mucus is a mixture of large glycoproteins i.e. mucin molecules, water, epithelial cells, electrolytes, bacterial enzymes etc. The term mucoadhesion is just the version of bioadhesion where the target is a biological tissue i.e. vaginal tissue. Ideally the polymers for vaginal drug delivery system should possess some important characteristics, they should be non-toxic, non-irritant, and should not get absorbed through the mucus membrane, they should preferably form mucoadhesive bond.[6,7,8]

Here in the present work bioadhesive gel formulations were prepared using different combinations of natural, synthetic, and semisynthetic polymers such as HPMC K4M, sodium alginate, guar gum, HPMC K15M, chitosan, polycarbophil, and carbopol 940. From the various combinations of polymer studied for this formulation, the HPMC K4M and carbopol 940 were showed good bioadhesive properties and thus longer residence time. So this combination was selected. Then trial batches of different combinations of HPMC K4M and carbopol 940 were prepared and the optimum concentration of these polymers were decided. A total number of nine batches were formulated, compositions of which are stated in the table 1.

MATERIAL AND METHODS

Materials

Ciclopirox Olamine was a gift sample from Glenmark Pharmaceuticals, Mumbai. HPMC K4M and Carbopol 940 were procured from Colorcon Labs and Thomas Baker Pvt. Ltd, Mumbai respectively. All other remaining chemicals were procured from Thomas Baker Pvt. Ltd. All other materials were of analytical reagent grade.

Methods

Preparation of Gel

Accurately weighed quantity of HPMC K4 M and Carbopol 940 was soaked in sufficient amount of water for 24 hr. Poloxamer 10% solution is prepared separately and kept in refrigerator for 24 hr. 0.05M sodium hydroxide (NaOH) is prepared as per the factor for sodium hydroxide. Measured volume of sodium hydroxide 0.05M and Poloxamer was mixed in a test tube. Accurately weighed quantity of CPO was added to the above solution. The two polymers which soaked previously were mixed into each other slowly with help of mechanical stirrer. The shaft of mechanical stirrer was maintained just above the bottom of beaker, so as to prevent the incorporation of bubbles. Other water soluble ingredients like Lactobacilli, EDTA, β-Cyclodextrin and methyl Paraben were added in water and the solution was added to polymeric mixture and stirred till the homogenous solution was obtained. Drug solution was then added to polymeric solution and mixed for 20 min. After the complete mixing the gel was subjected to sonication for 30 min so as to remove the bubbles.

Evaluation of Gel

Physical Appearance

The prepared gel formulations were inspected visually for their color, homogeneity, consistency.

pH

pH of all formulations were determined by using pH meter (Digital pH meter, Systronics, India). The pH meter was calibrated before each use with standard pH 4 and 7 buffer solutions. 2.5gm of formulation was stirred in distilled water till forms a uniform suspension. The volume is made up to 25 ml and pH of the dispersion was measured using pH meter.[14]

Rheological Study

The viscosity of different gel formulation was determined at 37^0C using a Brookfield viscometer (RVDV-II+ Pro, Brookfield Engineering Labs, US). Spindle no.95 was used to measure the viscosity at the speed of 0.5 rpm.

Table 1: Composition of Gel

Sr. No.	Ingredients	G1 %w/w	G2 %w/w	G3 %w/w	G4 %w/w	G5 %w/w	G6 %w/w	G7 %w/w	G8 %w/w	G9 %w/w
1.	CPO	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
2.	HPMC K4M	4.5	3	1.5	4.5	3	1.5	4.5	3	1.5
3.	Carbopol 940	1.2	1.2	1.2	0.8	0.8	0.8	0.4	0.4	0.4
4.	Lactobacilli	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
5.	Poloxamer 407 (10%)	5	5	5	5	5	5	5	5	5
6.	β-cyclodextrin	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7	0.7
7.	NaOH (0.05M)	15	15	15	15	15	15	15	15	15
8.	EDTA	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25	6.25
9.	Methyl Paraben	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
10.	Distilled water	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.

Procedure: Sample to be tested was placed in a beaker, the spindle 95 was attached to the hook of viscometer, and care was taken to maintain complete sink condition of spindle. The measurements were taken by changing the speed, and readings of viscosity were noted. Speed of 0.5 rpm was found to be favorable for further evaluation of all vaginal gel formulations.[10]

Spreadability

Spreadability was determined by the apparatus developed by Mutimer et al (1656), it consists of a wooden block, fixed at platform and with a pulley at one end. The drag or slip characteristics of gels were determined as a function of time.

A glass slide (20*20) was fixed on the wooden block on opposite side of pulley, to another glass slide of the same size a thread was attached at one surface and was tied to a weight pan. The thread was carried over the pulley; an excess quantity of gel sample was placed on the fixed glass slide. The other glass was placed on it exactly and a weight of 50gm was put over it for sufficient time, then the weights were kept into the pan to allow the sliding of glass slide. The time required to separate slides completely from each other was recorded and the spreadability was calculated by the formula,

$$S = M.L/T$$

Where, S= spreadability,

M=weight added to the pan,

L= length of the glass slides,

T= tome taken to separate the slides completely from each other

Drug Content Determination

Drug concentration in gel was measured by spectrophotometer. CPO content in gel was measured by dissolving known quantity of gel in solvent (methanol) by Sonication. Absorbance was measured after suitable dilution at 298 nm in UV visible spectrophotometer (Jasco V-630, Japan) and % drug content was calculated.[5]

In-Vitro Release Test

Franz diffusion cell was used for the determination of drug release, with the effective diffusion area of about 3.14cm^2 and 15.5ml cell volume. The gel (200mg) was kept on the surface of egg membrane evenly; the egg membrane was pre-socked in simulated vaginal fluid for about 24 hours. The egg membrane was clamped in between the donor and receptor compartment of the diffusion cell. The receptor compartment was filled with freshly prepared simulated vaginal fluid and was stirred with magnetic stirrer. The sample was collected after suited time interval and analysed for its drug content by UV visible spectrophotometer.[11]

Mucoadhesion Study

The mucoadhesion study was performed on lab scale, the apparatus for mucoadhesion study was developed at lab scale using one pan balance. The pan of the balance was removed. A vial stopper was taken. It was attached to the arm of a balance by the use of thread in inverted position. A vial containing the simulated vaginal fluid was kept in a beaker. The beaker was filled with water to maintain the temperature.

A piece of vaginal mucosa was kept for 24 hrs in simulated vaginal fluid (SVF) before the test and then removed just before the test from the SVF and tied at the opening of the vial. One gram of gel was placed on the fat surface of the stopper and it was placed over the mucosa. Mucosa and gel were kept in contact for several minutes in order to achieve intimate contact. After few minutes, the weights were on the balance in order to achieve the detachment of mucosa and gel formulation. Weight in grams required to detach the two surfaces was recorded for the gel formulation.[8,9]

In Vitro Antifungal Activity

The in vitro Antifungal studies were carried out by the ditch plate technique using *Candida Albicans* microbial species. Sterilized Sabouraud's agar medium was used for the study. The petri plate were washed, cleaned and dried and then sterilized. The sterile Sabouraud's agar medium was the poured into petri plates, and allowed to solidify. The wells were prepared into the solidified media. The suspension of *Candida Albicans* was uniformly spread over the media. Measured volume of control and the formulation was then added to the wells with help of syringe. Plates were covered with lids and incubated at 32^o C for seven days. The zone of inhibition was measured after seven days.[12,13]

Study of Effect of Temperature

Study of effect of temperature was studied by analyzing the optimized batch kept at room temperature and for three months. After storage the samples are tested for their physical appearance, pH, viscosity and drug content.

RESULTS AND DISCUSSION

Physical Appearance

All the formulation showed transparent appearance with absence of lumps due propylene glycol and Poloxamer407. The gel consistency of all the formulation was obtained due to the polymers Carbopol and HPMC K4M. All the batches G1-G9 showed the transparent gel like structure and consistency and were homogeneous, the pH values of all formulations were found in the range 6 to 7. Hence all the formulations are satisfactorily complying with pH values needed for vaginal infections.

The batches B1 showed the maximum in-vitro diffusion form the vaginal gel as compared to the other B2 and B3 batches, but the viscosity of this

formulation was not in ideal range. The batch B2 was the most ideal among the all three batches, as it showed good spreadability, pH and mucoadhesion strength and in-vitro diffusion of 98.55±0.04%. Therefore the formula for B2 batch was then selected as the optimized formula, for the preparation of bioadhesive vaginal gel. The viscosity and percentage release data of batch B1-B3 was shown in the table 2.

Rheological Study

Gel formulations from G1 to G9 contain varying concentration of polymers carbopol and HPMC K4M. From G1 to G3 the viscosity increases due the highest concentration of carbopol 940 and from G7 to G9 the viscosity decreases due lowest concentration of carbopol 940. The formulations from G4 to G6 show the optimum level of carbopol and hence viscosities are much in the range. The formulation G5 and G7 showed the ideal viscosity in the range of 90.00-120.00 poise. Hence viscosity of formulation increases with increased concentration of carbopol 940. Therefore, G5 formulation was found to be best suited as per the ideal range of viscosity for gel formulations.

Fourier Transform Infrared Analysis

The obtained IR spectrum of CPO Fig.1 showed the principle peaks 3207cm^{-1} for the O-H stretch, 3001 cm^{-1}, 2872 cm^{-1} peaks foe C-H stretch, 1587 cm^{-1} for C=C stretch, 1681 cm^{-1} for C=O stretch, 1485 cm^{-1} for C-C stretch and 893 cm^{-1} for N-H wag. From these principle peaks obtained it was clear that the drug was pure.

The IR spectrum of formulation mixture Fig.2 shows the drug principle peaks 3238 cm^{-1} for O-H stretch, 3097 cm^{-1} and 3111 cm^{-1} for C-H stretch,1556 for C=C cm^{-1} stretch, 1693 cm^{-1} for C=O stretch, 1450 cm^{-1} for C-C stretch and 893 cm^{-1} for N-H wag. So this indicates that there was no physicochemical interaction in between drug and the used excipients. Results of the preformulation study suggested that all the studied excipients were compatible with CPO.

Spreadability and Viscosity

From the above spreadability results we can conclude that, gel has capability to easily spread on the affected area. The formulation G5 and G3 had moderate concentration of the carbopol and HPMC K4M and had the moderate viscosity; hence it took the optimum time to spread over the area.

The spreadability and viscosity of the optimized batch were compared with the marketed formulation Candid-V gel and it was found that the spreadability of the optimized batch was better than that of the marketed formulation. The viscosity of the optimized batch was higher than that of the marketed one. Thus, the release was prolonged.

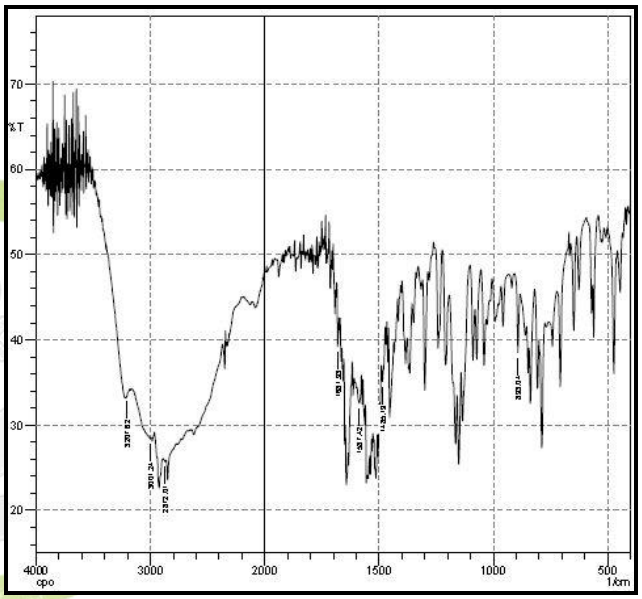

Figure 1: IR Spectrum of Ciclopirox Olamine

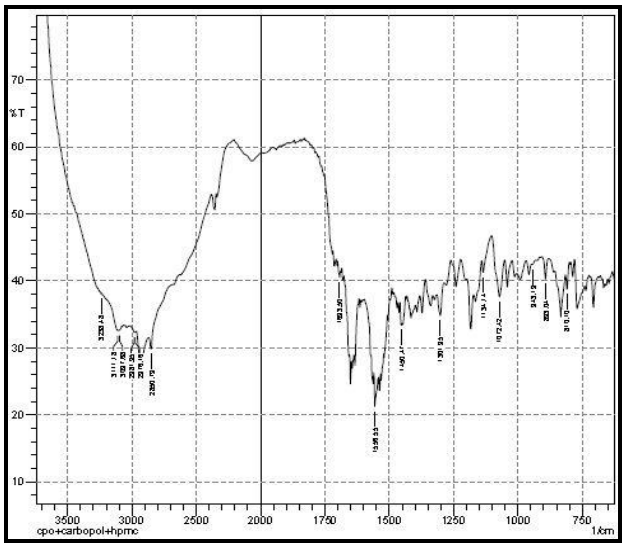

Figure 2: IR Spectrum of Ciclopirox Olamine + Carbopol 940 + HPMC K4 M Mixture

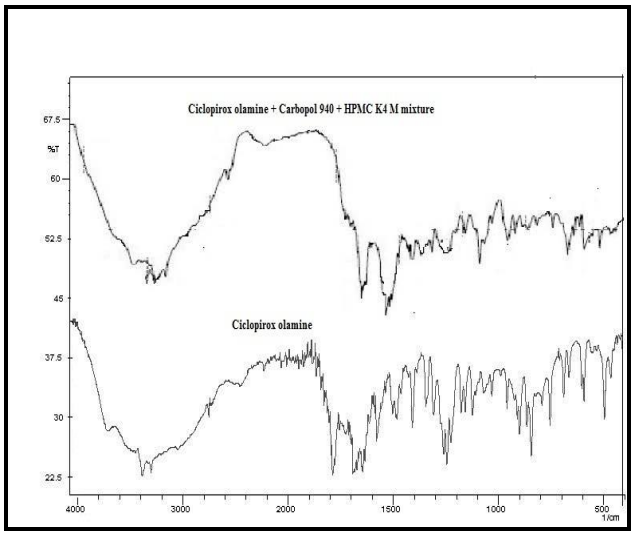

Figure 3: Combined IR Spectrum of Ciclopirox Olamine and Ciclopirox Olamine + Carbopol 940 + HPMC K4 M Mixture

Drug Content

The drug content of all formulations was found in the range. Hence uniformity of drug content was found satisfactory and was within acceptance limits i.e. 90-110%. The formulation G5 showed 97.26±0.87 and G8 showed 96.2±0.82. The drug content of optimized gel formulation was found to be 97.56±1.[15].

***In-Vitro* Diffusion Study**

The formulation G5 showed more diffusion i.e. 100.08 % after 8 hours as compared to the other batches.

Form the optimization step it was found that batch G5 and G9 showed the maximum diffusion of the drug from the gel formulation. But the viscosity of G9 formulation was not ideal for the vaginal gel, but that of G5 was found to be optimum. Therefor G5 formulation was selected for the further studies. The release kinetics of optimized batch was applied to various diffusion models such as Zero order, First order, Higuchi, Korsemayer-peppas and Hixon Crowell. The best fitted model gives the highest R^2 value 0.987 and least slope value -7.556. Thus, zero order kinetics model fits best for the dissolution data of the optimized batch as it showed the highest value for R^2.

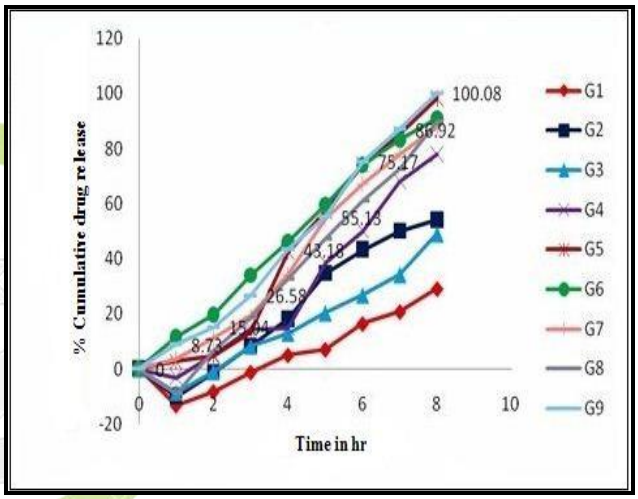

Figure 4: Graphical Presentation of Comparative Diffusion Profile of All Formulations

Table 2: The Selected Batches from the Solutions of Design Expert Software

Batches	Carbopol 940 (gm)	HPMC K4 M (gm)	Predicted % diffusion	Actual % diffusion	Predicted viscosity (Poise)	Actual viscosity (Poise)
B1	0.14	0.39	95.84	94.22± 0.01	56.00.42	49.23± 0.5
B2	0.16	0.62	98.89	98.55±0 .04	93.03.09	90.02± 0.2
B3	0.13	0.81	96.18	96.6±0.06	90.48.18	71.24± 0.4

Mucoadhesion Strength

The mucoadhesive strength of the optimized batch was found to be 0.524±0.01 which is higher than the marketed formulation 0.34±0.01.

Antifungal Study

The antifungal studies showed that the zone of inhibition obtained for standard drug was similar as that of obtained from optimized formulation.

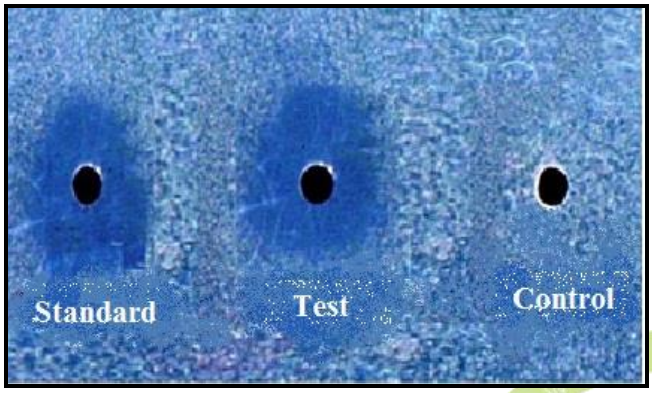

Figure 4: Zone of Inhibitions

Stability Study

The stability studies showed that there was no change in the formulation after 90 days. The antifungal studies showed that the zone of inhibition obtained for standard drug was similar as that of obtained from the formulation B2. Hence the antifungal activity of CPO was not changes after formulating it into a gel form.

CONCLUSION

The bioadhesive vaginal gel of CPO was formulated successfully with use of Carbopol 940 and HPMC K4M polymers. The 99.06 % drug release was obtained at the end of eight hour, and due to mucoadhesive polymers retention of drug increased. The mucoadhesion strength of the optimized formulation was found to be 0.52± 0.01N which increased residence time of formulation. The rheological behavior of the optimized batch was found to be pseudoplastic, which is ideally required for the vaginal gel formulation. The spreadability and viscosity of the optimized batch were compared with the marketed formulation Candid-V gel and it was found that the spreadability of the optimized batch was better than that of the marketed formulation. The viscosity of the optimized batch was higher than that of the marketed one thus release was prolonged. Stable formulation was obtained containing lactobacilli without any growth. From the antifungal study it showed that zone of inhibition of standard and test were similar hence one can conclude that drug is effective against *Candida Albicans*.

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