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# Formulation and Evaluation of Muco-adhesive *In-situ* Gel for Site-Specific Delivery of Clotrimazole.

**Jeevan Sen, Sujit Pillai, Gopkumar P, and Sridevi G***

GRY Institute of Pharmacy, Borawan, Khargone - 451228, India.

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***For Correspondence**

GRY Institute of Pharmacy, Borawan, Khargone - 451228, India.

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## ABSTRACT

Present research is about the *In-situ* gels for site specific delivery. In the present work, formulations with chitosan, bioadhesive and permeation enhancer and gellan gum ion activable gelling polymer and temperature sensitive polymeric systems was used for *In-situ* geliling. The developed formulations was characterized for various *in-vitro* parameters e.g. clarity, pH, isotonicity, viscosity, drug release profile, statistical release kinetics, bioadhesive force, retention time, microbial efficacy, irritation test and stability studies. Conventional topical application of clotrimazole to skin may cause localized irritation of the skin with a mild burning sensation, redness and itching. The entrapment of drug in polymer matrix was viewed to help in the localized delivery of the drug and an improved availability of the drug at the site and reduce the local side effects of drug. Therefore, it was envisaged to develop the mucoadhesive gels for topical delivery system which gives better patient compliance, controlled delivery of drug, reduce the local side effects of drug. The developed formulation was found to be non-irritant, bioadhesive with good retention properties. Developed formulation shows matrix model release kinetic. The developed formulation is thus a viable alternative to conventional vaginal dosage forms.

## INTRODUCTION

Present research is about the *In-situ* gels for site specific delivery. Difficulties associated with oral and parenteral delivery and poor oral availability promoted the impetus for exploring alternative routes for the delivery of such drugs. Consequently, other absorptive mucosa is considered as potential sites for drug administration [1]. Transmucosal routes of drug delivery (i.e., the mucosal linings of the nasal, rectal, vaginal, ocular, and oral cavities) offer distinct advantages over per oral administration for systemic effect. Significant progress has been reported in the area of vaginal microbicides [2].There are currently more than 50 potentially microbicidal products under development globally, of which 16 are in Phases I-III clinical trials. Recently, the vagina has been rediscovered as potential route for microbicide and contraceptive delivery. There are many pharmaceutical companies currently focusing on the development of novel vaginal drug delivery systems for contraception, treatment of vaginal infections, STDs and other gynaecological conditions. These innovative delivery systems may lead to extended product shelf life making the products competitive in the market place [3,4].

Conventional vaginal dosage forms frequently produce leakages and drip. There is a need for the development of innovative vaginal formulation technology that fulfills certain criteria such as desirable product dispersion throughout the vagina, retention for intended intervals, and adequate release of drug. These features can be achieved by the use of bioadhesive based novel delivery systems.*In-situ*gelation is a process of gel formation at the site of application after the composition or formulation has been applied to

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the site[5].Formulation and evaluation of one such bioadhesive based novel drug delivery system for an effective and patient friendly use of an antifungal drug clotrimazole in the form of *In-situ* gel was undertaken research work.

## MATERIALS AND METHODS

### Materials

Sodium citrate, Sodium alginate, CaCl2 and CaCO3 were obtained from Qualigens Fine chemicals, Mumbai. HPMC K4Mwas gift samples from Manbrove pharma, Mumbai, Gift sample of Clotrimazole was kindly provided by Plethico pharmaceuticals Pithampur.All the other ingredients used were of analytical grade and used as received.

### Method of preparation of in-situ gelling solutions[6,7,8,9]

Different concentrations of chitosan and gellan gum were prepared and evaluated for their gelling capacity in order to identify the compositions suitable for use as anin situ gelling systems. Chitosan, a pH sensitive polysaccharide was dissolved in 1% v/v acetic acid diluted further with phosphate buffer system, pH adjusted to 5.0. Gellan gum, an ion activated polymer was dissolved by adding the gum to deionised water containing 0.17% w/v sodium citrate and heated to 90° while stirring. After cooling to below 30°C appropriate amounts of calcium chloride (0.05% w/v) was added into the sol to form gel. The thermo sensitive polymers (Poloxamer 127) was dissolved in citric acid phosphate buffer system, pH adjusted to 5.0. The gelling capacity was determined by placing a drop of the system in a vial containing 2 ml of freshly prepared simulated vaginal fluid and visually assessing the gel formation, observing the time taken for gelation and time taken by gel formed to dissolve both with respect to pH and temperature.

**Table No.1: Optimized polymer concentration for Clotrimazole *In-situ*Gels**

| Sr. No | Ingredients (%W/V) | VF 1 | VF4 | VF6 | VF7 |
|---|---|---|---|---|---|
| 1 | Clotrimazole | 2 | 2 | 2 | 2 |
| 2 | Chitosan | 1 | 1.2 | 1 | 1 |
| 3 | Gellan gum | 0.8 | 0.8 | 1 | 1.2 |
| 4 | Poloxamer 127 | 0.5 | 0.5 | 0.5 | 0.5 |
| 5 | HPMC | 0.1 | 0.1 | 0.1 | 0.1 |
| 6 | Nacl % | 0.9 | 0.9 | 0.9 | 0.9 |
| 7 | Benzalkonium chloride | 0.02 | 0.02 | 0.02 | 0.02 |
| 8 | Water q.s | 100 | 100 | 100 | 100 |

Gelation temperature was determined by taking 10 ml polymer solution in transparent vial & gradually heated by electric heater with magnetic bid immersed in it. The magnetic rotor was set at 100 rpm, as temperature rise at gelling point bid stop rotating, corresponding temperature was noted down as gelation temperature. Gelation temperature was determined with & without Clotrimazole 2% w/w (CTZ).

Various concentrations of individual polymers & combination of thermo sensitive polymers (Poloxamer 127and Poloxamer 188) with pH activated polymers (chitosan) and ion activated polymer (gellan gum) were studied for the pH, ion concentration and temperature as gelling parameters [10].

### UV Absorbance Maxima and Calibration Curve of Clotrimazolein pH 4.5 Buffer

Stock solution of Clotrimazole in methanol and buffer with proper dilution was initially scanned in UV visible spectrophotometer within wavelength of 200-700 nm. For calibration, 100 mg of Clotrimazole was dissolved in100 ml of pH 4.5 buffer. The solution was then diluted with buffer solution to obtain 50 to 400 µg/ml solution. It was then measured by UV visible spectrophotometer at 260.3nm.

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### Identification of Drug by FTIR

Fourier-transform infrared (FT-IR) spectra were obtained using an FT-IR spectrometer (Shimadzu 8400S, Japan). The pure carbamazapine were mixed thoroughly with potassium bromide, an infrared transparent matrix, at 1:5 (Sample: KBr) ratio, respectively. The KBr discs were prepared by compressing the powders at a pressure of 5 tons for 5 min in a hydraulic press. Forty scans were obtained at a resolution of 4 cm-1, from 4000 to 400 cm-1.

### Evaluationof Optimized Formulations of *In-situ* gel

Optimized formulations of *In-situ*Gels and their sol forms were evaluated for their clarity, pH, viscosity, penentration rate, swelling index, spread ability, drug content, in vitro diffusion studies by using standard procedure reported in the journals. All studies were carried out in triplicate and average values were reported.

### Measurement of pH of sols and gels

Prepared formulations were evaluated for their Clarity, Texture and pH of sol before gel formation and after gel formulations were evaluated. The Texture and Clarity were evaluated by visual results and pH was checked under digital pH meter. Based upon the pH values of sol solution of different formulation pH adjustment were made between 5-5.5. Results of all these studies are reported in the table.

### Measurement of Gelling capacity

The gelling capacity was determined by placing a drop of the system in a vial containing 2 ml of simulated salivary fluid (pH 6.8) freshly prepared and equilibrated at 37° and visually assessing the gel formation and noting the time for gelation and the time taken for the gel formed to dissolve. Different grades were allotted as per the gel integrity, weight and rate of formation of gel with respect to time.

### Measurement of Viscosity of Sols and Gels

Viscosity determinations of the prepared *in situ* gels as well as sols were carried out on a cone and plate geometry viscometer (Brookfield, Massachusetts, USA), using spindle No 4. Viscosity of *in situ* gelling solutions was measured at different angular velocities at a temperature of 37°. A typical run comprised changing of the angular velocity from 0.0 to 100 rpm. The averages of two readings were used to calculate the viscosity. Evaluations were conducted in triplicate.

Simulated vaginal fluid (SVF) of pH 4.5 was added in increments of 25 ml to 200 ml of the formulations, and the viscosity at which gelation occurred were recorded. This allows the determination of relationship between applied shear rate and shear stress experienced by the test material. All the measurements were conducted using SC4spindle using about 4 ml sample volume at 20rpm. The tests were performed in triplicate, with a coefficient of variation of less than 5% being found. Since, in vivo, vaginal formulations will experience the dilution with vaginal fluids and it has been reported that the rheological behavior of gels could be affected by various factors such as copolymer compositions and solutes.

### Measurement of Drug content

The drug content of the formed gel was carried out by dissolving (0.5gm) gelequivalent to 10mg of drug wasdissolvedin10mlof methanol phosphate buffer. The volume is made with methanol up to 100 ml and 5ml of the this solution is further diluted with Methanol. After suitable dilution absorbance of the solution was recorded by using Shimadzu UV/ visible spectrophotometer at 260.3nm.

### Measurement of Extrudability

The extrudability test was carried out by using Pfizer hardness tester. A 15gm of gel was filled in aluminum tube. The plunger was adjusted to hold the tube properly. The pressure of 1kg/cm$^2$ was applied for 30 sec. The quantity of gel extruded was weighed.The procedure was repeated at threee qui distance places of the tube. Test was carried out in triplicates.

e-ISSN:2347-7857
p-ISSN:2347-7849

### Measurement of Mucoadhesive force

The mucoadhesive force of *in situ* gels on cellophane membrane specimen. The assemblies developed for in vitro measurement of bioadhesive strength are the modification of the previously reported bioadhesion test assembly. The method is based on the measurement of tensile strength or shear stress required to break the adhesive bond between a model membrane and the test formulation. The test formulation is sandwiched between two model membranes fixed on flexible supports in the assemblies for a sufficient period of time. After the adhesive bond has formed, the force (weight) required to separate the bond was measured and calculated as bioadhesive strength. The bioadhesive force, expressed as the detachment stress in grams/$cm^2$, was determined from the minimal weights needed to detach the tissues from the surface of each formulation, and the area of tissue exposed. Effect of varying contact time (1, 2, 3, 5 10 and 15 min) was investigated for some of the gel preparations to optimize initial contact time. In brief, formulations were allowed to be in contact with mucosa for carrying contact time (1, 2, 3, 5, 10 and 15 min.), and the bioadhesive force was determined as discussed above. Contact time that resulted in maximum bioadhesive strength was selected as optimum contact time (10min) required for adequate adhesion. All the above mentioned experiments were carried out in triplicates.

### Measurement of Spreadability:

For the determination of spreadability, excess of sample was applied between the two glass slides and was compressed to uniform thickness by placing 1000mg weight for 5 min. Weight (50 g) was added to the pan. The time required to separate the two slides, i.e. the time in which the upper glass slide moves over the lower plate was taken as measure of spreadability (S).

$$S=M\times L/T$$

Where M = weight tide to upper slide, L = length moved on the glass slide, T = time taken.

### Determination of*In-Vitro* release studies

*In-Vitro* release studies were conducted using diffusion cell using sheep stomach mucous membrane. Gel formulatione quivalentto10mg clotrimazole was spread uniformely on the surface of membrane (soakedinSVF pH 4.5 for24 hours before use). The assembly was fixed in such way that the lower endoftube containing gel was filled with diffusion medium i .e.100 ml of phosphate buffer of pH 4.5 contain edinbeaker which was maintained at 37±2$^0$C.

The membrane sac acts as a barrier between gel phase and buffer (sinkphase) thecontents were stirred using magnetic stirrer at 50±5rpm. 5ml of phosphate buffer of pH 4.5was replaced for every 5ml withdrawal each time. After suitable dilution there leased drug was estimated by using spectrophotometer at 260.3nm.

**Table 2: Standard curve of Clotrimazole in phosphate buffer**

| S No. | Concentration of Clotrimazole (µg/mL) | Absorbance | Regressed absorbance |
|---|---|---|---|
| 1. | 0 | 0 | 0 |
| 2. | 50 | 0.1022 | 0.102 |
| 3. | 100 | 0.2041 | 0.204 |
| 4. | 150 | 0.302 | 0.302 |
| 5. | 200 | 0.391 | 0.391 |
| 6. | 250 | 0.462 | 0.462 |
| 7. | 300 | 0.551 | 0.55 |
| 8. | 350 | 0.6706 | 0.670 |
| 9. | 400 | 0.7578 | 0.757 |

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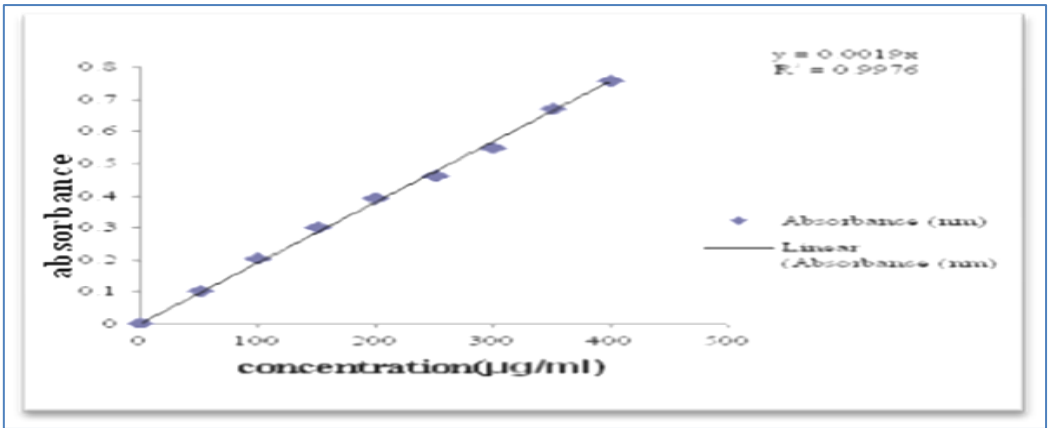


Figure 1: Standard curve of Clotrimazole in phosphate buffer pH

## Infrared spectroscopy

IR spectrum of the drug sample was found to be concordant with the reference spectrum. Figure showing the IR spectra of pure drug (CTZ-A), Gellan Gum (SA-B) and Drug and gum in combination. IR Spectra indicated No incompatibility between drug and excipient.

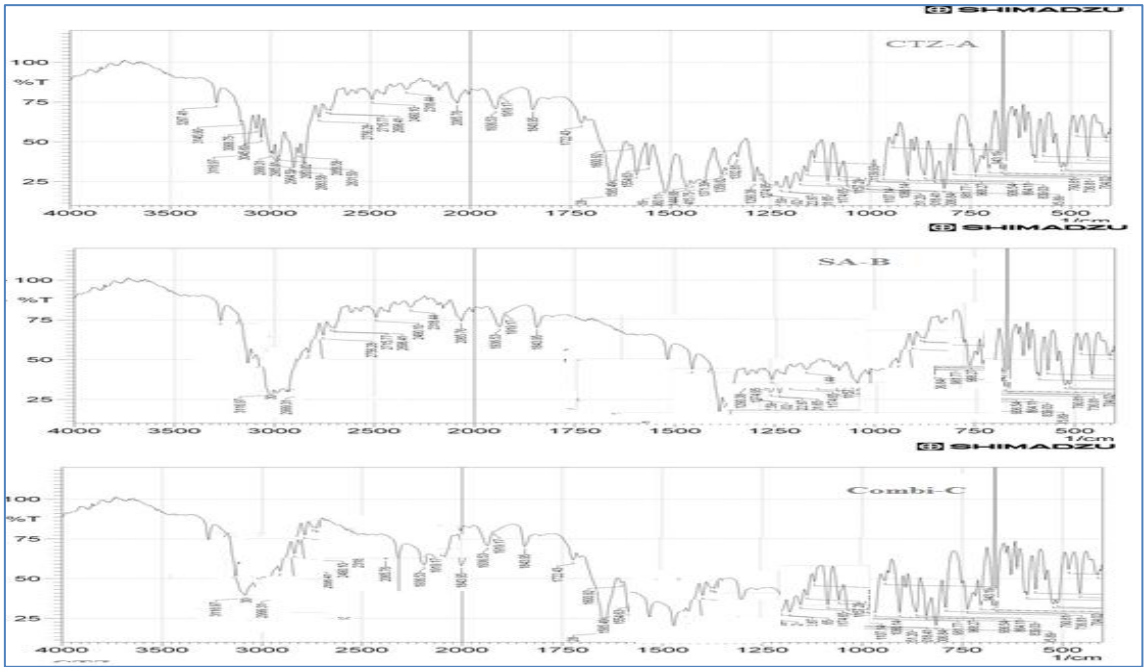


Figure 2: IR spectrum of Clotrimazole (A), Gellan Gum (B), and in Combination.

## Optimization of carrier systems

## Physical evaluation

The developed formulation was further characterized for various physical parameters, like clarity, viscosity and pH. The optimized chitosan/gellan/poloxomer gum vaginal gel is transparent in color. To avoid initial irritation due to difference in osmolarity of formulation and body fluid, the formulation was made iso-osmotic by addition of NaCl. The pH of the optimized formulations was found in the range of 5.0-5.5. Gellan gum converted into stiff gel in the presence of ions and results in sudden increase in the viscosity. And chitosan in acidic pH forms the clear gel with good consistency. Poloxomer help to get jelly consistency to the formulations at temperature around 35-37$^{o}$C. Formula consisting of combination of pH, ion and temperature sensitive polymers which in a said combination was able to yield in-situ gels with required behavior.

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Table 3: Measurement of pH of sols and gels, Clarity and Appearance

| Sr.No. | Formulation code. | pH of Sol | pH of Gel | Clarity of Sol and Gel | Appearance and Texture |
|---|---|---|---|---|---|
| 1 | VF1 | 5.3 | 4.3 | Clear | Fine, Acceptable |
| 4 | VF4 | 5.2 | 4.3 | Clear | Fine, Acceptable |
| 6 | VF6 | 5.5 | 4.2 | Clear | Fine, Acceptable |
| 7 | VF7 | 5.5 | 4.1 | Clear | Fine, Acceptable |

All the formulation prepared were able to form *In-situ*gel in SVF with 2 ml 2% ionic concentration of calcium. Formulation having more than 1.2% of chitosan or gellan gum were formed thick and stiffer gel and were still retaining gel consistency even after 12 hrs. Polymers (Chitosan and gellan gum) in the ratio 1.2:0.8, 1.2:1, 1:1, 0.8:1 and 0.8:0.8 formed the good *in-situ*gels and all these formulations were able to retain the gel for almost 12hrs more than 10hrs. But formulation VF2, VF3, VF8 and VF9 were able to retain gel up to 16 hrs. Formed *In-situ*gels were able produce similar viscosity as that of normal gel formulations.

Figure 4: Figure showing the consistency of *In-situ*gel in SVF after 12 hrs


Figure 4: Four optimized formulations selected based on the viscosity gelation time, consistency, drug release behavior

Table 4: Table of values showingMeasurement of Gelling strength GPC, Viscosity of sols and gels.

| For. code. | Gelling strength | Gel Persistent Capacity (GPC) | Viscosity of Sol ($25^0$ C) mCPS | Viscosity of Gel ($37^0$ C) mCPS |
|---|---|---|---|---|
| VF1 | firm gel, transparent, do not dissolve | > 8Hrs | $67\times10^3$ | $247\times10^3$ |
| VF4 | firm gel, transparent, do not dissolve | > 10 Hrs | $82\times10^3$ | $329\times10^3$ |
| VF6 | firm gel, transparent, do not dissolve | > 9Hrs | $71\times10^3$ | $268\times10^3$ |
| VF7 | firm gel, transparent, do not dissolve | > 10 Hrs | $79\times10^3$ | $317\times10^3$ |

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### Measurement of Drug content, Extrudability, Penetration rate and Mucoadhesive force

Formulation with lower concentration of added polymer showed better spread ability and free movement out of ointment tubes. 1g of gel formed was able to spread over 2-2.5 cm distance. Drug content studies showed that all the prepared batch of formulation contained the clotrimazole with in the official level between 90-110%

**Table 5: Table of values showing Drug content, Extrudability, spreadability and mucoadhesive force**

| For. code. | Drug content (%) | Extrudability (mg) | Spreadability (mm) | Mucoadhesive force (Grams/Cm$^2$) |
|---|---|---|---|---|
| VF1 | 93±0.053 | 89 | 22 | 21 |
| VF4 | 98±0.043 | 97 | 24 | 22 |
| VF6 | 98±0.023 | 92 | 28 | 22 |
| VF7 | 96±0.063 | 93 | 25 | 23 |

### *In-Vitro* Drug Release Studies

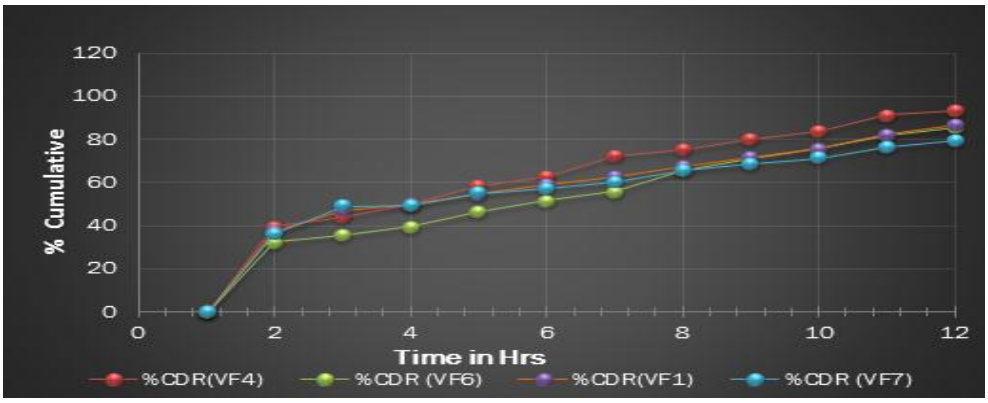


**Figure 5: *In-Vitro* Drug release studies from four selected optimized formulations.**

*In-Vitro* release studies conducted using SVF for selecting the formulations which shows better controlled release behavior for 12 hrs. Among the different formulations tested the formulations VF1, VF4, VF6 and VF7 are the formulations which showed better controlled release for 12 hrs duration. Formulation VF1 and VF7 were able to release more than 80 % of the drug in 12 hrs duration. And all the optimized formulations showed the initial burst drug release with almost 30 % in initial 2 hours which is required to maintain the initial loading dose.

Formulations VF1, VF4, VF6 and VF7 were able to retain their gelling consistency for more than 10 hrs duration. And kinetic of these drug release were further analysed using different release kinetics. The best fit regression coefficient of all these kinetics are tabulated in the table.

### Results of *In-Vitro* drug Release kinetics

**Table 5: Table showing the release kinetic model with regression coefficient values.**

| | Zero-order Model | | First-order Model | | Higuchi- Model | | Kors-peppas Model | | Best-Fit Model |
|---|---|---|---|---|---|---|---|---|---|
| Formulation Code | $K_0$ | $R^2$ | $K_1$ | $R^2$ | $K_H$ | $R^2$ | $K_{KP}$ | $R^2$ | |
| VF1 | 3.18 | 0.869 | 3.133 | 0.439 | 1.304 | 0.455 | -2.101 | 0.738 | Zero, peppas |
| VF4 | 11.9 | 0.939 | 4.94 | 0.539 | 1.02 | 0.539 | -1.91 | 0.785 | Zero, peppas |
| VF6 | 10.1 | 0.890 | 2.01 | 0.437 | 1.712 | 0.439 | -0.789 | 0.716 | Zero, peppas |
| VF7 | 12.5 | 0.835 | 0.67 | 0.431 | 1.29 | 0.475 | -0.919 | 0.701 | Zero, peppas |

*In-Vitro* release kinetics studies indicated that drug release from the formulated optimized batch of in-situ gels follows preferentially zero order and krosmeyer peppas model of drug release. Drug release kinetic study indicated that release of the drug from the gels is due to both diffusion and dissolution based.

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## DISCUSSIONS

Mucoadhesive *in-situ*gels of clotrimazole are formulated successfully, prepared gels were evaluated for various physicochemical properties. Optimized formulations of *In-situ*gels VF1, VF4, VF6 and VF7 batch of formulations were better compaired to other formulations in terms of softness of gel formed viscosity consistency, mucoadhesive property and GPC with good control over drug release for a period of about 12 hours. VF1 and VF4 batch of formulations were able to release more than 90% of drug in 12 hours duration in a well-controlled manner which is almost in a zero order fashion. Release kinetic analysis indicated *in-situ*gel formulations were following Mostly the zero and peppas kinetics of drug release.

*In-situ*gel formulations being better formulations of choice for body part applications as spray or as drops are more preferred comp aired to conventional gels or creams because of better patient compliance.

## CONCLUSION

Vaginal preparations, although generally perceived as safer most, still they are associated with a number of problems, including multiple days of dosing, dripping, leakage and untidiness, causing discomfort to users and expulsion due to the self-cleansing action of the vaginal tract. These limitations lead to poor patient compliance and failure of the desired therapeutic effects. For effective vaginal delivery of antimicrobial agents, the drug delivery system should reside at the site of infection for a prolonged period of time.

Conventional topical application of clotrimazole to skin may cause localized irritation of the skin with a mild burning sensation, redness and itching. The entrapment of drug in polymer matrix was viewed to help in the localized delivery of the drug and an improved availability of the drug at the site and reduce the local side effects of drug. Therefore, novel dosage form developed certainly gives better patient compliance, controlled delivery of drug, reduce the local side effects of drug.

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