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FORMULATION AND EVALUATION OF MUCOADHESIVE IN-SITU RECTAL GEL OF CLOTRIMAZOLE

[1]*Sohel Khan, [2]Shaikh Aaqueeb, [3]S. K. Shehzad, [4]Adnan Siddiqui, [5]Dr. Rehan Deshmukh and [6]Dr. G. J. Khan

[1]Department of Pharmaceutics, Ali-Allana College of Pharmacy, Akkalkuwa, Nandurbar, Maharashtra, India.

[2,3,4,5,6]Department of Pharmaceutics, Ali-Allana College of Pharmacy, Akkalkuwa, Nandurbar, Maharashtra, India.

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***Corresponding Author**
Sohel Khan
Department of Pharmaceutics, Ali-Allana College of Pharmacy, Akkalkuwa, Nandurbar, Maharashtra, India.

ABSTRACT

The purpose of this study was to develop a suitable mucoadhesive in situ gel formulation of clotrimazole (CLO) for the treatment of rectal candidiasis. For the formulation of Clotrimazole, In-situ Mucoadhesive gel poloxamer-407 was used as thermo reversible agent and carbopol-934 was used as mucoadhesive and pH sensitive agent. PEG 400 and 4000 was added to in situ gels in 0.5% ratio to improve the mucoadhesive and mechanical properties of formulations and to prolong the residence time in rectal cavity. After the preparation of mucoadhesive in situ gels; gelation temperature/time, viscosity, mechanical, mucoadhesive, syringeability, spreadibility and rheological properties and in vitro release determined. Based on the obtained results, it was found that gels prepared with PLX 407, Carbapol-934 and 0.5% PEG 400 and 4000 might be suitable for rectal administration of clotrimazole. In conclusion, the mucoadhesive in situ gels of clotrimazole would be alternative candidate for treatment of Rectal candidiasis since it has suitable gel properties with good rectal retention.

KEYWORDS: Clotrimazole; HPMC; mucoadhesive in situ gel; poloxamer; rectal candidiasis.

INTRODUCTION

The „in -situ gel" system has emerged as one of the best novel drug delivery systems, the in-situ gelling system helps for the sustained and controlled release of the drugs, improved

patient compliance and comfort by its special characteristicfeature of „Sol to Gel‟ transition. A formulation known as an in situ gelling system is one that, before entering the body, is in solution form but will transform into gel under certain physiological conditions. The sol to gel transition depends on various factors like temperature, change in pH, solvent exchange, UV radiation, andpresence of specific molecules or ions. The drug delivery systems having the above-mentioned properties "sol to gel transition" can be widely used for sustained delivery vehicle preparation of bioactive molecules. The "in situ gelling system" has many benefits, including simplified dosage administration, decreased administration frequency, and even protection of medicine against changes in ambient conditions. Various natural and synthetic polymers undergo in situ gel forming and potentially can be used for oral, ocular, transdermal, buccal, intraperitonial, parenteral, injectable, rectal and vaginal routes.[1-4]

The Rectal delivery was used for a long time as a route for drug delivery with the purpose to obtain a local and systemic pharmacological effect. The advantages of the rectal route are the avoid of first-pass metabolism, the reduction in the gastrointestinal side effects, the decrease in hepatic side effects of drugs and overcoming of pain, tissue damage, and infection observed in the case of parental routes. The conventional rectal dosage formulations (i.e. semi-solids, pessaries, liquid preparations, foams, and supposetories) are associated with limitations of poor retention, leakage and messiness causing inconvenience to users, leading to poor patient compliance and loss of therapeutic efficacy.[5-8]

MATERIAL AND METHODS

Material

Clotrimazole, Poloxamer-407, Carbopol-934, Sodium chloride, Potassium dihydrogen phosphate, Disodium hydrogen phosphate.

Method[9-16]

Preparation of clotrimazole In-situ Mucoadhesive Gel

Step 1: In-situ Mucoadhesive gel was prepared by dissolving poloxamer-407 as given in table no. 5 in 5 ml of cold water. Poloxamer-407 was added slowly in water with continues stirring with glass rod and water temperaturewas maintained at cool temperature using ice bath.

Step 2: Carbapol-934 was separately dissolved in small quantity of distilled water to make clear solution and slowly add with continues stirring into Poloxamer-407 solution prepared in step 1. Then add methyl paraben and Propyl paraben into it. Then add PEG 400 and PEG

4000 into it. The mixture was transferred to fridge at 4 °C for 24 hrs. to ensure complete wetting and removal of entrapped air bubbles.

Step 3: 100 mg of clotrimazole required for all formulation batches was dissolved in 10 ml of in situ gel formulation.

Table no. 1: Formulation batches of in-situ Mucoadhesive gel containing Clotrimazole.

Ingredient	Formulation Batch Code								
	F1	F2	F3	F4	F5	F6	F7	F8	F9
Clotrimazole	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Poloxamer 407	1.0	1.25	1.50	1.0	1.25	1.50	1.0	1.25	1.50
Carbapol 934	0.05	0.05	0.05	0.075	0.075	0.075	0.1	0.1	0.1
PEG 400	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
PEG 4000	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Methyl Paraben	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Propyl Paraben	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1

*All quantity is in mg.

Evaluation of In-situ Mucoadhesive gel for following parameters

- **Determination of pH**

The pH of the in situ gel was determined using a calibrated pH meter at 4 oC the readings were taken for an average of 3 samples.

- **Viscosity measurement**

Viscosity of gel was measured using Brookfield viscometer (model LVDVE, Engineering Laboratories, Middleboro, MA) spindle no 01 at 20 r.p.m. at temperature 4 ºC and 37 °C. The procedure was carried out in triplicate.

- **Syringeability study**

All prepared formulations were transferred into an identical 5 ml plastic syringe placed with 21-gauge needle to a constant volume (1ml). Passing solutions from the syringe was considered easy, while failing solutions required more effort.

- **Gelation Temprature**

The gelation temperature was determined using the test-tube inverting method. A volume of 2 ml of the sols was placed in a test tube, which was then immersed in a water bath at 15ºC. The water bath temperature was then gradually raised, samples were checked every two minutes, and the temperature at which the gel stops flowing upon test tube inversion at 90 degrees was noted. Three samples on average were used to take the readings.

- **Mucoadhesive strength**

Buccal mucosa of goat was freshly removed and kept in SGCF (simulated gingival crevicular fluid) freshly prepared isotonic phosphate buffer solution pH = 7.4 at 37°C. The buccal membranes of goat were then sticked on two inverted beakers. One beaker was tied with thread to another empty beaker through the pulley. The fixed amount of insitu gel (1gm) was placed in between two beakers with buccal membrane. Water was then added slowly in empty beaker, the weight at which two mucosal surfaces were detached was referred as mucosal strength. The detachment force in gm was then calculated by taking weight of pre tarred beaker which was having water and the weight was written as mucoadhesive strength (n=3).

- **Drug content**

A UV spectrophotometric approach was used to determine the amount of clotrimazole in various formulations. For this, 0.1 ml of each formulation was taken and diluted with distilled water up to 100 ml. The samples were analysed spectrophotometrically at λmax of clotrimazole is 264nm the concentration of drugs in samples was determinedusing UVVisible spectrophotometer (Shimadzu 1700, Japan) for Clotrimazole respectively.

- **In vitro drug release study**

A sample of 1 ml of in situ gel were placed into a dialysis membrane were thensuspended in 50 ml of (ethanol: water 1:1) preheated at 37 ± 0.5 oC in water bath at 37 oC and 100 rpm on magnetic stirrer. At predetermined time intervals of 24 hours, one milliliter sample was withdrawn and replaced with an equal volume offresh medium. The whole release media were changed and replaced with fresh media every day (2or 4 hours) during the release studies duration. Samples were diluted and analyzed using an UV spectrophotometer for clotrimazole concentration at λmax 264nm respectively. The cumulative amount of drug released was calculated. All experiments were done in triplicate.

- **Kinetic study**

To analyze the mechanism of drug release kinetics the obtained data were fitted to various kinetic equations including zero order, first order, and Higuichi and korsmeyer peppas models. The best fit model was selected on the basis of relatively high co-relation coefficient.

- **Stability studies**

To determine the physical and chemical stabilities of produced formulations, stability experiments were carried out. The optimized formulation was kept in air tight container covered with aluminium foil at refrigerated temperature, 4˚C for a period of 3 months. The formulation's visual appearance, gelation behaviour, viscosity, drug content, and in vitro drug release were all evaluated.

RESULT AND DISCUSSION

- **pH Measurement**

The pH of the formulations was found in the range of 6.4 to 7.2, which wasrequired pH for antifungal treatment.

Viscosity

Viscosities of the batches were found from 712 Cps to 947 Cps. The result data revealed thatas the concentration of Carbopol increases, the viscosity also increases. It means viscosity depends on the concentration of Carbopol.

- **Syringeability**

All the developed formulation was easily syringeable through 21-gauge needle at cold temperature. 9.3.1. D. Gelation temperature: Gelation temperatures of formulation batches were found from 32°C to 45°C. Here, batch no. F1, F4 and F5 showed high gelation temperature of and F8, F9 showed low gelation temperature below and F2, F3, F6 and F7 showed temperature. As the concentration of Poloxamer increases, gelation temperature decreases because of micelle formation, followed by micellar aggregation Therefore, the batch no. F2, F3, F6 and F7 were selected for further studies. And all other batches were rejected.

- **Mucoadhesive strength**

Mucoadhesive strengths of the batches were found to be from 17.75 dyn/cm to 22.95 dyn/cm. Because of increase in concentration of Carbopol mucoadhesive strength was also increased.

- **Syringeability**

All the developed formulation was easily syringeable through 21-gauge needle at cold temperature.

➢ **Drug content**

The data of drug content from all the prepared formulations showed that the values of Clotrimazole range between 96.33% and 99.22.

Table no. 2: Evaluation of In-situ Mucoadhesive gel.

Batch No Gelation	Ph	Viscosity	Mucoadhesion Strength (dyn/cm)	Temperature (0C)	Drug Connten
1	7.2	712	17.75	45	96.56
2	6.8	739	17.95	40	97.02
3	7.0	757	18.11	37	99.22
4	6.9	809	18.56	44	96.33
5	6.4	832	18.96	41	98.69
6	6.7	840	19.39	36	96.57
7	7.1	903	21.06	43	97.12
8	6.9	925	22.29	34	96.72
9	6.8	947	22.95	32	98.35

➢ **In vitro release study**

Cumulative percent drug released from the batches were shown in the Fig.no.1. All the batches showed drug release of clotrimazole 99.02'%,97.23%,97.27% and 97.8%,97.3%,95.68%,93.14%90.2 and 89.33% respectively Batch F3 showed 99.02% of clotrimazole respectively. Hence, Batch no F3 was selected as optimized. Batch no F3 have been selected for further studies like kinetic study and stability study.

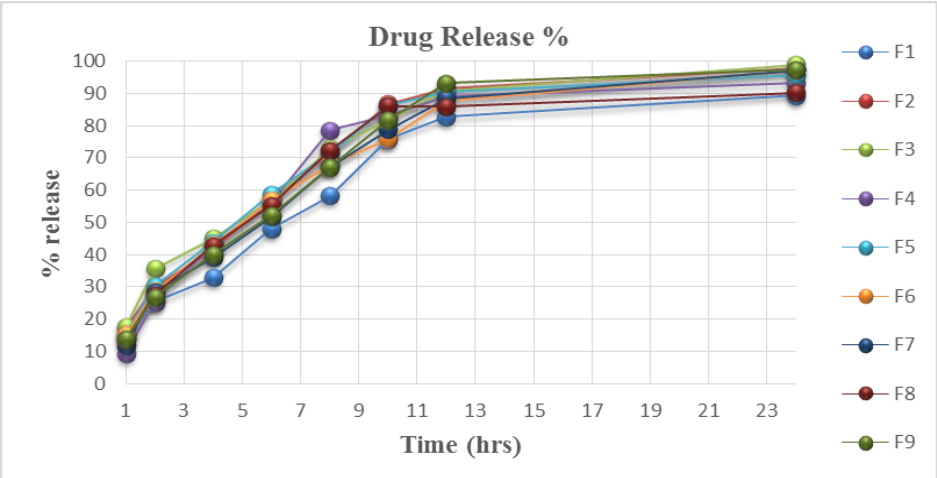

Fig. no. 1: Cumulative % Drug Release.

➢ **Kinetic studies**

Batch F3 was selected for kinetics studies. The slope n was computed to know whether the release was Fickian or Non-Fickian. For Non-Fickian release the n values falls between 0.5 and 1.0, while for Fickian diffusion n is less than or equal to 0.5. The slope values are tabulated in below chart. The values of n were more than 1 for all formulations.

Batch No	Zero order R^2 values	First order R^2 values	Higuchi R^2 values	Korsmeyer-Peppas R^2 values	n value
F3	0.8358	0.8987	0.9472	0.8093	1.596

➢ **Stability studies**

Formulation F3 was selected for stability studies. Results revealed that storage has no significant effect on the release pattern of the formulation. A slight increasein viscosity was observed with concomitant decrease in gelation temperature, Formulation F3 showed promising results regarding its physical prosperities, stability over storage period.

Evaluations	Initial	30 days	60 days	90 days
viscosity	757	751	745	739
Mucoadhesive strength	18.11	18.07	18.01	17.96
Gelation temperature	37	36.8	36.4	36.3
Drug release	99.29	99.26	99.1	98.78

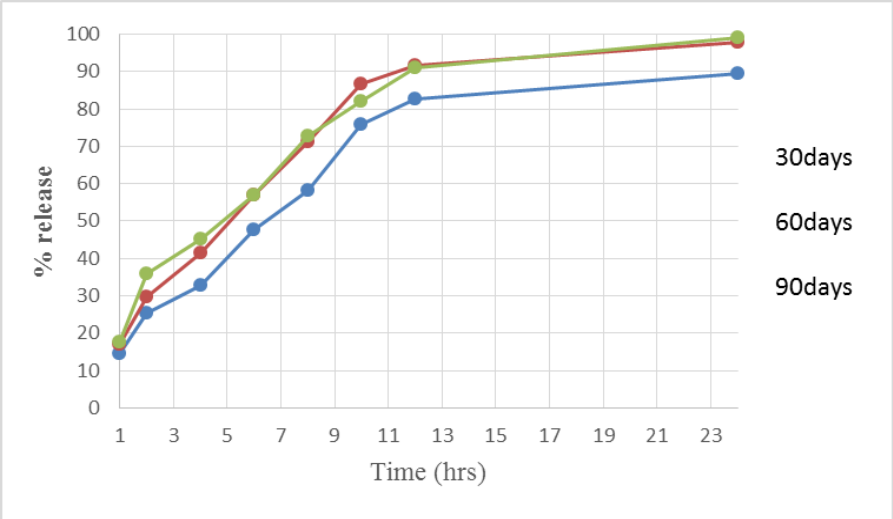

Fig. no. 2: Stability study of in-vitro diffusion for batch F3.

CONCLUSION

In situ gel as drug delivery systems offers an interesting Candidiasis to overcome crucial obstacle of delivery and retention of formulation in candidiasis. These are liquid preparations that can be easily injected into the rectal route and then (after temperature change) hardened

to form a gel with a custom geometry. The in situ gel remains in the form of a solution under non physiological conditions and forms a gel in physiological conditions under the control of stimuli such as pH, temperature present in the rectal route.

In situ mucoadhesive gel provides the drug release at a controlled rate for long period of time directly to the target site which reduces side effects, thus improving patient compliance. The main advantages of the implants of formation in situ are as follows: they can easily be injected into rectal route, harden to form a solid implant with customized geometry, the time-controlled release of drugs, and no need to remove the empty remnants.

For the formulation of Clotrimazole, In-situ Mucoadhesive gel poloxamer-407 was used as thermo reversible agent and carbopol-934 was used as mucoadhesive and pH sensitive agent.

Clotrimazole In-situ Mucoadhesive gel wereprepared and evaluated for viscosity, syringeability, gelation temperature, mucoadhesive strength, In-vitro release study.

Batch F3 showed good in vitro drug release and also shown good result for all parameters when compared with all other formulations. Hence Batch F3 was considered as the optimized formulations. Batch F3 was subjected for four different models viz. Zero order, First order, Higuchi matrix and Peppas model equations and the formulations best fit in to the Peppas model by giving the value of diffusion exponent (n) 1.596 for Clotrimazole that indicate the formulation had release the drug by case II transport.

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