



FORMULATION AND EVALUATION OF MICRO BEADS LOADED BIO ADHESIVE VAGINAL GEL OF CLOTRIMAZOLE

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ABSTRACT

The objective of this study was to prepare and evaluate the micro beads loaded bio adhesive vaginal gel of clotrimazole. Micro beads provide the sustained release of drug and bio adhesive gel was act as a base for incorporation of micro beads to the vagina. Clotrimazole is an antifungal drug which is having a short half life (2hrs). To improve the bioavailability and stability of drug it was incorporated in micro beads from which it can be released slowly for prolonged period of time. Micro beads provide the sustained release of drug because of the presence of such sustaining and mucoadhesive polymers like sodium alginate, SCMC etc. Bio adhesive gel was prepared by using a bio adhesive polymer that is carbopol 934. Micro beads were prepared by ionotropic gelation method. Bio adhesive gel was prepared by accurately weighed carbopol 934 dispersed in distilled water containing glycerol and stirred the mixture with homogeniser until thickening occurred. Neutralization was done by added triethanolamine drop by drop and maintains the pH. Continue the mixing of solution until the transparent gel was obtained. Micro beads were analysed for drug loading, shape (scanning electron microscopy), flow properties, drug encapsulation efficiency, and the properties were found to be discrete, spherical with free flowing particles. The bio adhesive gel was evaluated for pH, spreadability, extrudability, viscosity, bio adhesion test. The final prepared formulation which is micro beads loaded in bio adhesive gel was evaluated for in vitro release study, drug release kinetics and antifungal studies. The range of swelling index was found to be 52-80% which shows moderate to good swelling property of micro beads. The percent drug loading was founded within the range of 66.23-92.59%. F5 shows the maximum drug loading that is 92.59 which show that F5 possess highest incorporated amount of drug within the micro beads. The percent encapsulation efficiency was founded within the range of 44.56-78.42% which indicates moderate to good efficiency. Spreadability measurement was found within the range of 5.54-9.59cm² in diameter. In vitro release study on all six formulations of clotrimazole micro beads loaded in bio adhesive gel were carried out in SVF 4.5 using cellophane membrane and modified apparatus. The cumulative percent release was founded within the range of 87.76-79.28% for all six formulations in 12 hrs. F1 shows the highest in vitro release of drug. From the results it was observed that as we increase the polymer concentration, release of drugs from micro beads loaded gel decreases.

KEYWORDS: Micro beads loaded bio adhesive gel, bio adhesion, antifungal activity.

INTRODUCTION

A modified dosage form or drugs, which provide the prolonged therapeutic action by releasing the drug in delayed form, known as sustained release dosage form. The release time of drug is decreased by producing delayed effect and for prolongs time in systemic circulation. Maintenance of uniform blood level of drug was achieved by sustained drug delivery system (SRDDS) so the patient compliance as well as efficacy of drug was also increased.

The dosing frequency of sustained release dosage form is lower as compare to conventional dosage form. Tablets which provide sustained release are generally taken once

or twice a day whereas the medication was needed to take three to four times in a day in case of conventional dosage form for producing the same therapeutic effect as like sustained release dosage form.^[1-4]

Vaginal route as a drug delivery system^[6-8]

Female reproductive tract consist of many parts, the vaginal cavity is coming under the important area of this tract. Now a day's vagina is preferred as a well established route for administration of drugs. Because of its advantageous features like avoidance of first pass effect, higher permeability, large surface area, rich blood supply and low enzymatic activity.

Vaginal cavity is a preferable site for uterine targeting. For the delivery of chemotherapeutic agents, vagina should be used as a novel route for treating uterine cancer. Vaginal drug delivery must have some ideal features which can not affect the dosage form as well as the targeting site. It includes.

1. It should be easy to use
2. Reversible application
3. Must improve patient compliance
4. Cost effective
5. Easily available
6. Must be safe for long and continuous application
7. Also provide self administration with minimal toxicity.

In recent years, a new type of controlled release form are developed which is known as bio adhesive preparation for the treatment of both topical and systemic diseases. The main application of such type of dosage form is retaining period which is extended for prolong period of time included days, hrs, and night that's why frequency is automatically reduced.^[6]

1.2.1. Advantage - Following advantages are included.^[7]

1. Easy and safe administration
2. Reduced side effects
3. Highly permeability to large molecular weight drugs
4. Rich blood supply
5. Large surface area
6. Relatively low enzymatic activity
7. Avoidance of first pass metabolism
8. Controlled form can provide prolonged therapeutic action with reduced frequency
9. Targeted site for most of the drugs
10. Quick onset of action

1.2.2. Disadvantages^[8]

1. Unawareness and gender specificity
2. Genital hygiene issues
3. Menstrual cycle associated vaginal changes
4. Coitus interference
5. Local side effects
6. Variable drug permeability

After bacterial vaginosis, the second most common cause of vulva and vaginal inflammation was the fungal infections. During the reproductive age of women, it was estimated that about three quarters of total population usually attacked by one episode of vulvovaginal candidiasis (VVC) and approx half population have two or more other episodes which is generally caused by the pathogen named as *Candida albicans*, founded in 85-95% of all cases. During one year of observation period it was founded that 70% of women was attacked by asymptomatic colonization (without any symptoms)

caused by *Candida* spp. It was also determined in 10-20% of women undergoing conisation for cervical intraepithelial neoplasia.^[12]

Clotrimazole is a broad spectrum antimycotic agent effective against pathogenic dermatophytes, yeast and several species of candida, trichopyton, micro-sporum, and malassezia. However clotrimazole is known to be very effective locally and causes no major side effects. The development of a controlled release delivery system for clotrimazole would provide long term therapeutic concentrations at the site of infection by a single dose leading to an eradication of the infection.^[13]

MATERIALS AND METHOD

Material

Clotrimazole was obtained as a gift sample from Ranbaxy laboratories Ltd, H.P. India, sodium alginate, calcium chloride, SCMC, carbopol 934 and almunium chloride were obtained in central drug house of department of pharmaceutical sciences Dehradun. All other reagents and Chemicals used in the experiments were of analytical grade.

Preparation of the Simulated Vaginal Fluid

Simulated vaginal fluid (SVF) was prepared from 3.51 g/l NaCl, 1.40 g/l KOH, and 0.222 g/l. Ca(OH)₂, 0.018 g/l bovine serum albumin, 2 g/l lactic acid, 1 g/l acetic acid, 0.16 g/l glycerol, 0.4 g/l urea, 5 g/l glucose. The pH of the mixture was adjusted to 4.5 using 0.1M HCl.

Preparation of micro beads by ionotropic gelation method

Sodium alginate is a major polymer used in case of ionotropic gelation technique. Sodium alginate and other co polymers were added in different proportion for the preparation of clotrimazole micro beads. After dispersing the sodium alginate and other copolymers in 100 ml of water the drug was added to it and mixed properly to form a thick solution which can be filled in a syringe having the needle size of 24. Then the filled solution was poured drop by drop from the syringe to the calcium chloride and or aluminium chloride solution which is previously prepared by mixing the calcium chloride and or aluminium chloride with sufficient water to produce the spherical micro beads. The droplets must retain for about 20 min in calcium chloride solution or aluminium chloride solution for stabilization and to complete the curing reaction for production of spherical rigid micro beads. After sometime micro beads were filtered through watman filter paper and collected in blotting paper to overcome the sticking problem and then air dried at room temperature for overnight and stored in a dry and cool place or dessicator.

Table no. 1: Different drug polymer ratio used for the preparation of micro beads

S.NO.	Formulation Code	Drug: Polymer ratio	Ratio
1	F1	Drug: sodium alginate: SCMC	1:2
2	F2	Drug: sodium alginate: SCMC	1:2

3	F3	Drug: sodium alginate: SMC	1:1.5
4	F4	Drug: sodium alginate: SMC	1:3
5	F5	Drug: sodium alginate: SMC	1:2
6	F6	Drug: sodium alginate: SMC	1:1

Determination of % drug loading, % yield and encapsulation efficiency

50 mg of micro beads was dissolve in 50ml of methanol until get dissolved completely, then filtered in a beaker and make suitable dilution to check the absorbance at 261nm. Drug loading, encapsulation efficiency and % yield was measured by using following formula.

% drug loading = (weight of drug/ weight of micro beads) X 100

Encapsulation efficiency = (actual drug content/ theoretical drug content) X100

%yield = (M/Mo) X 100

Where, M = weight of micro beads

Mo = total expected weight of drug and polymer

Morphological analysis by SEM

SEM analysis was done for determining the morphological characteristics and to confirm the spherical shape of micro beads. SEM analysis was done by using scanning electron microscope model Zeiss, Evo 40, India at suitable magnification at room temperature.

FTIR analysis

Accurately weight amount of drug and prepared micro beads were taken and mixed with KBr in a uniform way then this mixture was compressed with a pressure to form a semi transparent pellet and recorded the spectrum of the pellet from 450-4000cm⁻¹.

Preparation of bio adhesive gel (1%)

Bio adhesive gel was prepared by using carbopol 934 as a bio adhesive polymer. Accurately weighed carbopol 934 was dispersed in distilled water containing glycerol and stirred the mixture with homogeniser until thickening occurred. Benzyl alcohol was added as a preservative. When the thick mixture was formed it will sonicate for 20 min to reduce the insoluble particles to get solubilised. Neutralization was done by added triethanolamine drop by drop and maintains the pH. Continue the mixing of solution until the transparent gel was obtained.

Table no. 2: polymer concentration for preparing bio adhesive gel

S.NO.	Ingredients	Formulation Code (% v/v)					
		F1	F2	F3	F4	F5	F6
1.	Carbopol 934 (gm)	0.50	0.60	0.70	0.80	0.60	0.90
2.	Glycerol	10	10	10	10	10	10
3.	Benzyl alcohol	20	20	20	20	20	20
4.	Tri ethanolamine	50	50	52	50	50	52.0
5.	Water (% v/v)	100	100	100	100	100	100

Incorporation of Micros beads in bio adhesive Gel

Micro beads obtained from ionotropic gelation method were mixed properly into bio adhesive gel of carbopol 934 at 25 rpm for 2 min by an electrical mixture.

In vitro drug release

For the determination of in vitro drug release from clotrimazole micro beads loaded gel, an open diffusion cell was used. A semi permeable cellophane membrane was soaked overnight in SVF before the testing. Accurate amount of formulation was applied between the empty donor and receptor compartment containing 100ml of SVF. In every hour 5 ml sample was pipette out from the receptor compartment and replaced with equal amount of fresh medium solution till 12 hrs. Make the suitable dilutions and the spectrum data of the samples were obtained by UV spectrophotometer at 264nm.

In vitro Bio adhesion measurement

Bio adhesion test of prepared micro beads loaded gel was carried out by in vitro method by comparing it with

marketed clotrimazole gel. For testing the bio adhesion an agar plate was prepared in SVF (4.5) and 50 mg test sample was placed in the plate at center position. Then agar plate was attached to a USP disintegration test apparatus after 5 min and allows moving up and down in SVF (4.5) at 37±10C. The sample present at the agar plate was immersed into the solution to the lowest point and out of the solution to the highest point. The retaining time of test sample on agar plate was observed visually.^[12]

In vitro Antifungal activity

Antifungal activity was evaluated by using a cup plate method against candida albicans. Agar media was sterilised carefully and dispersed into three different petri dishes sterilised previously for solidification. 8mm bore containing pure drug and drug loaded micro beads in gel was made in each Petri dish by using borer at middle of the plates and incubated for 24 hrs at room temperature for allowing the growth of micro organisms. Zone of inhibition occurred by the samples was measured in the Petri dish and click the photographs for further use.^[14]

RESULT AND DISCUSSION

Table no. 3: Results of % drug loading, % yield and encapsulation efficiency $\pm$ SD (n= 3)

Formulation code	% yield	Encapsulation efficiency	% drug loading
F1	39.52 $\pm$ 0.01	50.8 $\pm$ 0.02	84.38 $\pm$ 0.01
F2	43.93 $\pm$ 0.02	66.03 $\pm$ 0.02	75.87 $\pm$ 0.01
F3	56.04 $\pm$ 0.01	56.62 $\pm$ 0.01	71.37 $\pm$ 0.01
F4	62.71 $\pm$ 0.02	69.12 $\pm$ 0.02	79.74 $\pm$ 0.05
F5	72.13 $\pm$ 0.02	78.42 $\pm$ 0.02	92.59 $\pm$ 0.01
F6	60.44 $\pm$ 0.02	44.56 $\pm$ 0.02	66.23 $\pm$ 0.01

SEM studies

The shape and surface characteristics of the micro beads were observed by Scanning electron microscopy (SEM) in **Figure 2**. SEM was performed to determine whether micro beads had been formed. Non-aggregated micro beads with spherical shape were obtained for all the formulations. Moreover, F5 showed a smooth surface indicating that CTZ was well dispersed inside the carrier.

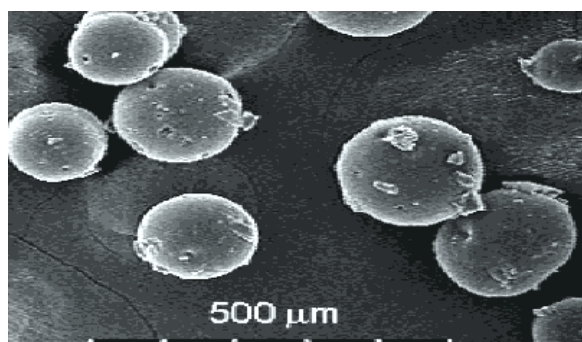
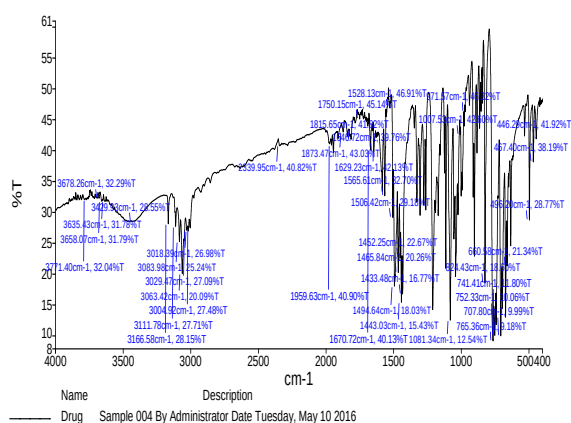


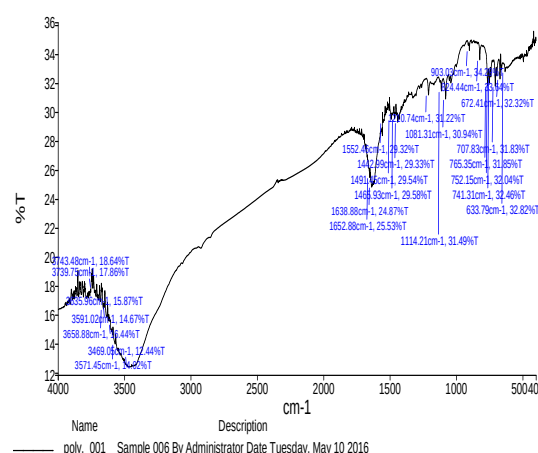
Figure no. 1: SEM analysis of F5

FTIR studies

The characteristic IR absorption peaks of CTZ at 3469 cm^{-1} (Aliphatic C-H stretch), 1552 cm^{-1} (Aromatic C=C stretch), 1442 cm^{-1} (Aromatic C=N stretch) were present in drug-loaded micro beads. The FT-IR spectra of the pure drug and formulation F4 indicated that characteristics peaks of CTZ were not altered without any change in their position after successful entrapment in the micro beads, indicating no chemical interactions between the drug and carriers used. These results indicate the method used to prepare micro beads do not affect the physicochemical properties of the systems.



FTIR spectra of DRUG



FTIR spectra of micro beads loaded drug

In vitro release studies

In vitro release study on all six formulations of clotrimazole micro beads loaded in bio adhesive gel were carried out in SVF 4.5 using cellophane membrane and modified apparatus. The cumulative percent release after 12 hrs was found to be 87.76-79.28. From results it was observed that as we increase the polymer concentration, release of drugs from micro beads loaded gel decreases. The release profile of micros beads clearly indicates that the concentration of polymers slows the release of CTZ from micros beads. The increase in the concentration of the polymers decreases the drug release from the matrices. F1 showed 87% drug release at 12h, F2 and F3 showed 86% and 83% drug release at the end of 12h, the slow release attributed to low permeability of the polymer sodium alginate. At the end of 12th h, in vitro drug release from formulation F4, F5, F6 was found to be 82, 81 and 79% respectively in the vaginal environment as shown in the **Figure 2**. Drug release from micro beads should theoretically be slower as the amount of polymer is increased because of an increase in the path length through which the drug has to diffuse. The total cumulative quantity of drug released at the end of the 12h dissolution test was below 100% for all dosage forms. This may in part be due to the relatively slow erosion of the matrix under these test conditions, with a resultant slow release of entrapped drug from the matrices undergoing testing. In comparison with other formulations F1 has shown 87% drug release in 12 hours with a good control release pattern. Hence F1 is selected as an optimized formulation and incorporated in the prepared gel.

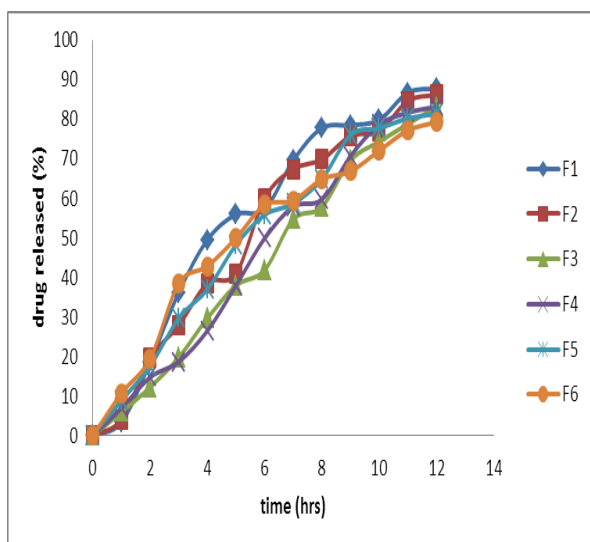


Fig no.2: In vitro release profile micro beads loaded gel in SVF.

In vitro Bio adhesion study

The bio adhesive potential of CTZ-MG was evaluated by in vitro method. The retention times showed by F6 was 22.5 ± 0.03 min, respectively ($n = 3$). The retention time shown by F6 was significantly higher as compared to other formulations. This clearly indicates that the CTZ-MG may have higher residence time in vagina as compared. The increased bioadhesivity of CTZ-MG can be attributed to the presence of Carbopol as its bio adhesive potential is highest at vaginal pH.

In vitro Antifungal activity

The results of antifungal activity of was done successfully. The zone of inhibition was found with the

CTZ-MG formulation containing loaded microspheres. Antifungal study with Sabourad Culture shows that the CTZ-MG was capable to control the growth of *C. albicans* for more than 12 h. CTZ-MG had prolonged drug release and provided better contact with the wells cut in the plate. Microbial activity and in vitro safety profile of CTZ were not negatively affected by formulating CTZ into CTZ-MG. F5 shows the greatest result which shows there was no significant change in antifungal activity of clotrimazole.

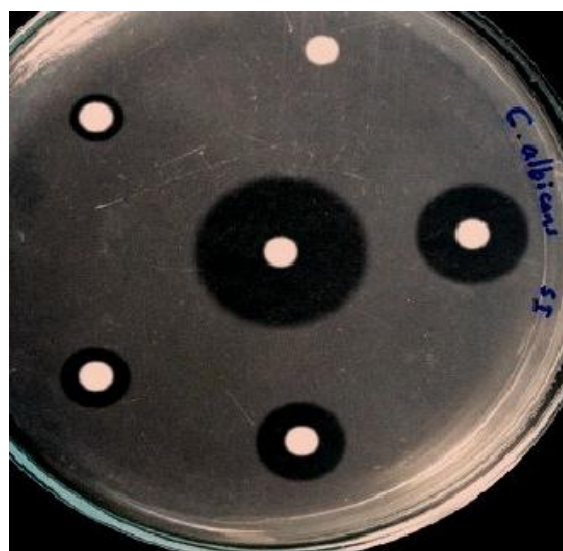


Figure no. 3: microbiological assay of clotrimazole micro beads loaded gel for F5

Table no.4: Zone of inhibition for F5

Formulation code	Zone of inhibition (mm) $\pm$ SD (n=3)
F5	19.3 ± 0.76

Table no. 5: Model fitting release profile of formulations F1-F6

Formulation code	Regression coefficient (r^2)			Best fit model	Slope (n) value		Mechanism of drug release
	Zero order	First order	Higuchi's		Korsmeyer peppas		
F1	0.927	0.985	0.951	First order	1.168		Super case II transport
F2	0.960	0.982	0.945	First order	1.131		Super case II transport
F3	0.992	0.964	0.921	Zero order	1.093		Super case II transport
F4	0.985	0.964	0.918	Zero order	1.053		Super case II transport
F5	0.964	0.986	0.959	First order	0.919		Super case II transport
F6	0.931	0.989	0.974	First order	0.787		Non fickian diffusion

CONCLUSION

Clotrimazole is an antifungal drug. Micro beads of clotrimazole loaded in bio adhesive gel was prepared by using a novel technique. Micro beads were prepared by ionotropic gelation method which is very common and gel was prepared by using a bio adhesive polymer carbopol 934. The bio adhesive gel was act as a base for incorporating the micro beads into vagina. It provides the stability to micro beads. Such micro beads loaded gel

could be used for topical, vaginal and rectal administration of drug materials. This method could be used to prepare spherical, free flowing micro beads and homogeneous gel which is finally matrix type drug polymer micro beads loaded in bio adhesive gel. From the reproducible results of the executed experiments, it can be concluded that:

- Micro beads were successfully prepared by ionic tropic gelation method and bio adhesive gel was prepared by using carbopol 934.
- Sodium alginate is a bio compatible polymer for the preparation of micro beads.
- In case of bio adhesive gel carbopol 934 is a bio compatible bio adhesive polymer for the preparation of gel.
- FTIR studies were carried out to find out possible interaction between the selected drug and polymer. The FTIR studies estimate that there was no interaction between the selected drug and polymer.
- FTIR studies were carried out to find out possible interaction between the selected drug and micro beads. The FTIR studies estimate that there was no interaction between the selected drug and micro beads loaded drug.
- SEM analysis revealed that the structure of micro beads is spherical and free flowing particles.
- Bio adhesion measurement estimates the strength of gel for imparting the retention time.
- Antifungal activity was done for the formulation which estimates the anti fungal activity of drug. Antifungal study with saboured culture shows that the clotrimazole micro beads loaded gel was capable to control the growth of *C. Albicans* for more than 12 hrs.
- The invitro dissolution studies revealed that as we increase the polymer concentration the release of drug from micro beads loaded gel was decreased.

The developed formulation overcome and alleviates the drawbacks and limitations of clotrimazole stability problems so that providing sustained drug delivery system in this era of patenting of novel and sustained drug delivery system.

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