FORMULATION DESIGN AND EVALUATION OF A NOVEL VAGINAL DELIVERY SYSTEM OF CLOTRIMAZOLE

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ABSTRACT: The present investigation was to prepare and evaluate bioadhesive vaginal gel containing clotrimazole loaded microspheres as a novel delivery system for vaginal use. Bioadhesive gel was prepared by incorporating drug loaded microspheres using bioadhesive polymer carbopol 934. Microspheres were prepared by spray drying technique using Eudragit RS-100 and RL-100 polymers with different drug/polymer ratios. Microspheres were characterized by SEM, DSC, FT-IR and particle size analysis and evaluated for morphology, drug loading and in vitro drug release in simulated vaginal fluid. The FT-IR and DSC spectra revealed that there was no chemical interaction between drug and polymers used. SEM revealed that microspheres were spherical with a smooth surface morphology indicating that CTZ was well dispersed inside the carrier with a mean particle size ranging from 17 -58 μm. The in vitro drug release from formulation M1 to M6 was found to be 83.10% to 98.88% at the end of 12th h. Among various formulations M4 was found to have good control release pattern as it has shown 99% drug release in 12 hours and hence to achieve bioadhesion to mucosal tissue formulation M4 was incorporated in the bioadhesive gel made of Carbopol 934P. Clotrimazole microspheres gel (CTZ-MG) was characterized by in vitro drug release and antifungal activity. The drug release was controlled up to 24 h. Inhibition effect on the C. albicans j1012 growth, suggested their effectiveness in the treatment of vaginal candidiasis. Result of this study suggests that CTZ-MG can be used as a novel controlled delivery system for local therapy of vaginal candidiasis.

INTRODUCTION: Microencapsulation is widely used in the pharmaceutical and other sciences to mask tastes or odors, prolong release, impart stability to drug molecules, improve bioavailability, and as multi-particulate dosage forms to produce controlled or targeted drug delivery. It is therefore a rapidly expanding technology for achieving sustained-release dosage forms. Up to now, only few studies reported the use of microspheres for the treatment of vaginal diseases. For instance bioadhesive microparticles, PLGA microspheres and polymer lipid based mucoadhesive microspheres were assessed as vaginal delivery systems. Moreover, chitosan based microspheres loaded with an antimicrobial drug were added to different mucoadhesive excipients (cellulose derivatives, sodium alginate or Carbopol® 974) to prepare mucoadhesive vaginal tablets.
Vaginal semisolids, particularly gels based on mucoadhesive polymers are currently receiving a great deal of interest as vaginal delivery systems.

The potential of mucoadhesive polymers was shown in ocular, nasal, vaginal and buccal drug delivery systems leading to a significantly prolonged residence time of sustained release delivery systems on these mucosal membranes. The vagina has been studied as a favorable site for the local and systemic delivery of drugs, specifically for female related conditions. The human vagina is colonized by microbes, and infections occur when the balance is disturbed. Under healthy conditions, vaginal flora is dominated by lactobacilli, which maintain acidic pH through production of organic acids at times other than menstruation. Disruptions of vaginal pH or lactobacilli may allow potentially pathogenic microorganisms to grow and dominate.

Vaginal candidiasis is a common condition and up to 75% of all women suffer at least one episode of this infection during their lifetime. Candida albicans j1012 is the most important cause of vaginal candidiasis, accounting for over 80% of the infection. Some of the antifungal agents used till date for vaginal candidiasis are clotrimazole, metronidazole, ketoconazole, fluconazole, itraconazole, secnidazole etc which are listed in review article of novel vaginal delivery systems.

Conventional vaginal formulations are associated with disadvantage of low retention to the vaginal epithelium, leakage and messiness causing inconvenience to the user. To circumvent this problems novel bioadhesive drug delivery systems are being propagated. Bioadhesive Gels can present several advantages over other vaginal drug delivery systems such as prolong residence time, safety, versatility, and economical savings.

Clotrimazole is a broad spectrum antimycotic agent effective against pathogenic dermatophytes, yeast and several species of candida, trichopyton, microспорum, 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.

The main goal of this research is to design and evaluate a vaginal delivery system for the local treatment of vaginal candidiasis. Clotrimazole microspheres were prepared using spray drying method and incorporated in the bioadhesive gel. The spray drying method of microencapsulation involves dispersion of a solution containing polymer and drug with an additional medium in which the drug and polymer cannot dissolve. It is a solvent evaporation process. The solvent in the droplets is removed very quickly due to heat energy provided in the spray drier. The technique is relatively simple and has been used to prepare microspheres of a variety of compounds using several different polymeric materials.

**MATERIALS AND METHODS:** Clotrimazole was a gift from Glenmark pharmaceuticals ltd. Eudragit RS 100 and Eudragit RL 100 were generous gifts from Vikram Thermo Ltd (Ahmedabad, India). Carbopol 934P, Bovine serum albumin and lactic acid were purchased from Loba (Ahmedabad, India). Carbopol 934P, Bovine serum albumin and lactic acid were purchased from Loba (Ahmedabad, India). Carbopol 934P, Bovine serum albumin and lactic acid were purchased from Loba (Ahmedabad, India).

**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.

**Spray drying method:** Clotrimazole microspheres were prepared by spray drying method using Eudragit RS-100/Eudragit RL-100 dissolved in dichloromethane as the system is Explosion proof for working with organic solvents. The drug was added to polymeric solution at room temperature to obtain the feed solution. CTZ-loaded micro-spheres were obtained by spraying the feed-solution with a spray-dryer (LSD-48 mini spray drier, JISL, Mumbai) using a standard 0.5mm nozzle.

The solution was fed to the nozzle with a peristaltic pump (29-35 ASP, corresponding to 50-60 mm –ve pressure), atomized by the force of compressed air and blown together with heated air to the chamber where the solvent in the droplets was evaporated. The dried microspheres were harvested from the apparatus collector.
Parameters for the preparation of microspheres are summarized in Table 1. Table 2 report the formulation chart of microspheres prepared.

### Table 1: Parameters for the Preparation of Microspheres

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Conditions</th>
</tr>
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<tbody>
<tr>
<td>Inlet temperature</td>
<td>80°C</td>
</tr>
<tr>
<td>Feed flow rate</td>
<td>5-6 ml/min</td>
</tr>
<tr>
<td>Compressed spray air flow</td>
<td>10 L/min</td>
</tr>
<tr>
<td>Air pressure</td>
<td>1.5 kg/cm²</td>
</tr>
</tbody>
</table>

**Determination of Drug loading, Encapsulation efficiency, and Microspheres yield:** The average drug content was determined by extraction of 100 mg of microspheres with methanol. Following filtration and appropriate dilution with SVF, the resultant concentration was determined using UV spectrophotometer (UV 1601 Shimadzu, Japan) at 264 nm and the percent drug loading was calculated using Eq. [1]

\[
\text{% loading} = \left( \frac{\text{weight of drug}}{\text{weight of microspheres}} \right) \times 100
\]

The encapsulation efficiency of the process was calculated using Eq. [2]

\[
\text{Encapsulation Efficiency} = \left( \frac{\text{actual drug content}}{\text{theoretical drug content}} \right) \times 100
\]

The percentage yield of the microspheres was determined for all formulations and was calculated using Eq. [3]

\[
\text{Yield} \% = \left( \frac{M}{Mo} \right) \times 100
\]

Where \(M\) is the weight of microspheres and \(Mo\) is the total expected weight of drug and polymer.

**Particle Size Analysis:** Particle size analysis was carried out by optical microscopy (Olympus BH2-UMA). About 200 microspheres were selected randomly and their size was determined using optical microscope fitted with a standard micrometer scale.

**Morphological Analysis by SEM:** SEM photographs were taken using scanning electron microscope Model Joel–LV-5600, USA, at suitable magnification at room temperature. The microspheres were sputter coated under an argon atmosphere with a thin layer of gold and then photographed.

The photographs were observed for morphological characteristics and to confirm spherical nature of the microspheres.

**FT-IR Analysis:** The FT-IR spectra of the samples were obtained using FT–Infrared Spectrophotometer (Shimadzu-8400 S, Japan) by KBr pellet method in the wave number range 600-4000 cm⁻¹. The samples were diluted with KBr and then compressed into a tablet, 10mm in diameter and 3 mm in thickness, using a manual tablet presser (Techno search) at 300 kg/cm for 1 min. The position of peak in FT-IR spectra of pure CTZ is compared with those in FT-IR spectra of CTZ plus excipients.

**Differential Scanning Calorimetry (DSC) studies:** Thermograms of CTZ and CTZ-loaded microspheres were obtained using a DuPont thermal analyzer 2010. Indium standard was used to calibrate the DSC temperature and enthalpy scale. The powder samples were hermetically sealed in perforated aluminum pans and heated at constant rate of 10 °C/min over a temperature range of 25 to 300 °C. The system was purged with nitrogen gas at the rate of 100 ml/min to maintain inert atmosphere.

**In vitro Release Studies of Microspheres:** Release studies for microspheres was carried out on all the formulation in triplicate, employing basket type dissolution tester-USP XXII, TDT-08L, using 650 ml of pH 4.5 SVF as dissolution medium at 25 rpm and 37 ± 0.5 °C. An aliquot of sample was withdrawn periodically at every 1h intervals and the volume was replaced with an equivalent volume of plain dissolution medium. Samples were analyzed spectrophotometrically at 264 nm.

**Preparation of 1% carbopol 934 gel:** As a vehicle for incorporation of microspheres for vaginal delivery, bioadhesive gel was made. Carbopol 934P (1 g) was dispersed in distilled water (88 g) in which glycerol (10g) was previously added. Mixture was stirred until thickening occurred and neutralized by drop wise addition of 50 % (w/w) triethanolamine. Mixing was continued until a transparent gel appeared.

**Incorporation of Microspheres in a Gel:** Optimized microsphere equivalent to 100mg of Clotrimazole (previously separated from the
unentrapped drug) was mixed into the 1g of 1% (w/w) carbopol 934P gel by an electrical mixer (25 rpm, 2 min) to get Clotrimazole microspheres gel.

**In vitro release Studies of Gel:** An open diffusion cell was used for drug release from the CTZ-MG and control gel. Commercial semi-permeable cellophane membrane was used as the permeation barrier. The membrane was soaked overnight in SVF before the study. 1g of gel was kept carefully between the donor and receptor compartment. The donor compartment as empty and open to the atmosphere but the receptor compartment contained 100 ml SVF. The contents of the receptor compartment were maintained at 37 ± 5 °C and stirred on a magnetic stirrer with a stirring speed of 25 rpm. Samples of 5 ml were withdrawn from receptor compartment every hour and replaced with equal volumes of fresh receptor medium. Samples were analyzed for CTZ by UV-Spectrophotometer at 264 nm.

**In vitro Bioadhesion study:** The bioadhesive potential of the CTZ-MG was evaluated in comparison with the marketed clotrimazole gel (Candid-VR gel) by an *in vitro* method reported by Bachhav et al. Briefly, an agar plate (1%, w/w) was prepared in pH 4.5 SVF. Test sample, 50mg was placed at the center of plate. After 5 min, the agar plate was attached to a USP disintegration test apparatus shown in Figure 1 and moved up and down in pH 4.5 SVF at 37±1 °C.

The sample on the plate was immersed into the solution at the lowest point and was out of the solution at the highest point. The residence time of the test samples on the plate was noted visually.

**Figure 1: Apparatus used for in vitro bioadhesion study**

**In vitro Antifungal activity:** Antifungal activity of Clotrimazole bulk powder, CTZ-MG and placebo gel was evaluated against *Candida albicans* j1012 by using a cup plate method. A volume of 20 ml of sterilized agar media was dispersed into three different sterilized petridishes and allowed to solidify. In each petridish, an 8mm bore was made using borer at the centre of petridish. Each bore was loaded with equal quantity of the Clotrimazole bulk powder, CTZ-MG and Placebo gel (gel without the drug). Petridishes were incubated at temperature of 37 °C for 24 h to allow the growth of microorganisms to take place. Zone of inhibition produced by the Clotrimazole bulk powder and CTZ-MG towards test organism was measured (mm) in the petridish and photographed.

**RESULTS AND DISCUSSION:**

**Spray drying:**

1. **Influence of inlet temperature and feed flow rate:** Spray drying is a solvent evaporation process. The solvent in the droplets is removed very quickly due to heat energy provided in the spray drier. The thermal efficiency of the spray drying is related to the heat energy input (controlled by inlet temperature) and the amount of heat used in the evaporation process. The optimum spray drying efficiency can be achieved from a balance of the amount of the energy input and the amount of the energy needed, which is related to the amount of the sample input. The inlet temperature used should be higher than the boiling point of the solvents used for the preparation of the solution to be dried.

The optimum inlet temperature for the preparation of CTZ microspheres was found to be 80°C. The feed flow rate was 5-6 ml/min. Increase in the feed flow rate results in the particle size reduction and decrease in the % yield of the microspheres. On the other hand, the decrease in feed flow rate less than 5-6 mL/min increases the particle size which is not suitable for pharmaceutical purpose. The solvent in the droplets could not be fully evaporated when the feed rate is more than 5-6ml/min. It was observed that some of the liquid dro
plets were attached inside the wall of the main chamber. Hence an increase of feed rate leads to higher moisture contents, especially at low inlet temperature. A higher inlet temperature promotes a decrease of residual moisture by enhancing water evaporation.

2. **Influence of compressed air flow and air pressure:** It was observed that compressed air flow and air pressure also influenced the shape as well as the size distribution of microspheres, possibly because of variable shear force experienced by the particulate system.

The average microspheres size increases with decrease in the spray flow. At the higher feed rate and pressure, the system tends to produce irregularly shaped microspheres which were not possible to distinguish individual microspheres.

3. **Effect of viscosity of liquid feed:** The viscosity of liquid feed also decides the shape and size of the droplet. The particle size depends on the size of the droplet formed during the spray drying. The low polymer concentration failed to produce the desired particle size as the droplet size was small. The liquid droplet consists of solvent which would evaporate, leaving small particles.

Better particles are formed by spraying the liquid feed which is having the viscosity 8 cps. The formulations containing lesser polymer ratios show lower viscosity i.e. less than 8 cps. As a result the particle size was decreased. On the other hand higher polymer ratios formed larger particles and they tend to form agglomerates.

4. **Encapsulation efficiency, Drug loading and %Yield:** Relatively high encapsulation efficiency was observed for all microsphere formulations. The encapsulation efficiency ranged between 55 and 88 %, and was found the encapsulation efficiency was increased with increasing amount of polymers in themicrospheres. Formulation M3 and M6 showed the relatively higher encapsulation efficiency as these formulations composed of high concentration of polymer.

Maximum drug loading was observed in formulation M4. During the process of microencapsulation, the mechanical variables cause loss of final product and hence process yield may not be 100%. Among all formulations, M3 and M6 showed maximum percentage yield. The obtained data is shown in Table 2.

**TABLE 2: POLYMER CONCENTRATIONS AND RESULTS FOR PARAMETERS EVALUATED.** Each value represents mean ± SD: * n = 3

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Drug: Eudragit RS100: RL100 (mg)</th>
<th>Theoretical Drug loading (%)</th>
<th>Actual Drug loading (%)*</th>
<th>Encapsulation Efficiency (%)*</th>
<th>% Yield*</th>
<th>Mean particle size (μm)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>1:1:0</td>
<td>50</td>
<td>27.91 ± 0.71</td>
<td>55.8 ± 0.76</td>
<td>54.81 ± 0.99</td>
<td>25±0.6</td>
</tr>
<tr>
<td>M2</td>
<td>1:2:0</td>
<td>33.33</td>
<td>22.29 ± 0.34</td>
<td>66.8 ± 0.59</td>
<td>67.55 ± 1.25</td>
<td>33±1.2</td>
</tr>
<tr>
<td>M3</td>
<td>1:3:0</td>
<td>25</td>
<td>20.30 ± 0.45</td>
<td>81.3 ± 0.43</td>
<td>83.06 ± 1.41</td>
<td>58±0.3</td>
</tr>
<tr>
<td>M4</td>
<td>1:0:1</td>
<td>50</td>
<td>28.63 ± 0.44</td>
<td>57.2 ± 0.56</td>
<td>60.54 ±2.04</td>
<td>17±1.4</td>
</tr>
<tr>
<td>M5</td>
<td>1:0:2</td>
<td>33.33</td>
<td>24.17 ± 0.87</td>
<td>72.8 ± 0.48</td>
<td>71.21 ± 1.01</td>
<td>36±1.3</td>
</tr>
<tr>
<td>M6</td>
<td>1:0:3</td>
<td>25</td>
<td>22.22 ± 0.61</td>
<td>88.4 ± 0.66</td>
<td>87.09 ± 1.22</td>
<td>53±0.4</td>
</tr>
</tbody>
</table>

**Scanning Electron Microscopy Studies:** The shape and surface characteristics of the microspheres were observed by Scanning electron microscopy (SEM) in Figure 2. SEM was performed to determine whether microspheres had been formed. Non-aggregated microspheres with spherical shape were obtained for all the formulations.

Moreover, M4 showed a smooth surface indicating that CTZ was well dispersed inside the carrier.
DSC and FT-IR studies: DSC and FT-IR were used to detect possible modifications of the physicochemical properties of the drug and/or of the carrier and possible interactions between the components of the formulations. DSC thermograms of CTZ and CTZ-loaded microspheres (M4) are depicted in Figure 3.

The DSC thermogram of pure CTZ showed a sharp melting endotherm at temperature 144.47 °C. This melting endotherm was also observed for CTZ-loaded microspheres (M4) at 144.23 °C, indicating absence of drug and polymer interactions. The IR spectra of CTZ and drug-loaded microspheres (M4) was found to be identical and presented in Figure 4.

The characteristic IR absorption peaks of CTZ at 3167 cm⁻¹ (Aliphatic C-H stretch), 1510 cm⁻¹ (Aromatic C=C stretch), 1450 cm⁻¹ (Aromatic C=N stretch) were present in drug-loaded microspheres. The FT-IR spectra of the pure drug and formulation M4 indicated that characteristics peaks of CTZ were not altered without any change in their position after successful entrapment in the microspheres, indicating no chemical interactions between the drug and carriers used.

These results indicate the method used to prepare microspheres do not affect the physicochemical properties of the systems.

Particle size determination: In general, the size of particles ranged from 17 to 58 µm. The particle size increased with increase in the concentration of polymer and particle size decreased with decrease in the polymer concentration. This can be explained to the fact that at higher concentration of polymer the viscosity of polymer solution increased, thereby producing bigger droplets during emulsification that were later hardened due to evaporation of dichloromethane.

In-vitro release studies of microspheres: The in vitro release studies were carried out for all microspheres in pH 4.5 SVF.
The quaternary ammonium groups in the RS and RL chemical structures play an important role in controlling drug release because they relate to water uptake followed by the swelling of the polymers. This is most likely because the number of quaternary ammonium groups of RS is lower than that of RL, which renders RS less permeable. The release profile of microspheres clearly indicates that the concentration of polymers slows the release of CTZ from microspheres. The increase in the concentration of the polymers decreases the drug release from the matrices. M1 showed 50% drug release at 6h, M2 and M3 showed 78% and 58% drug release at the end of 12h, the slow release attributed to low permeability of the polymer Eudragit RS 100. At the end of 12th h, in vitro drug release from formulation M4, M5, M6 was found to be 99, 90 and 88% respectively in the vaginal environment as shown in the Figure 5.

Drug release from microspheres 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 M4 has shown 99% drug release in 12 hours with a good control release pattern. Hence M4 is selected as an optimized formulation and incorporated in the prepared gel.

In vitro release studies of Gel: Formulation M4 which showed good controlled drug release 99 % at 12 h was incorporated in a 1% carbopol gel to get CTZ-MG and in vitro release study of CTZ-MG and control gel was carried out and compared. In vitro drug release was considerably retarded from the gel as compared to control gel. More than 70% of CTZ was released over the period of 12 h from CTZ-MG. Control tablet showed maximum 98% release of CTZ up to 12 h. CTZ-MG provided controlled release of CTZ up to 24h. From the control gel the drug release was faster in the initial period and about 65% of drug was released at 5th h and released 97.82% of the drug by 10th h. Where as in the CTZ-MG the drug release was controlled upto 24 h and there was about 72% drug release at 12h shown in Figure 6.

In vitro Bioadhesion study: The bioadhesive potential of CTZ-MG and commercial formulation (Candid-VR gel) was evaluated by in vitro method. The retention times showed by CTZ-MG and Candid-VR gel were 47±2.0 and 23±2.1min, respectively (n = 3). The retention time shown by CTZ-MG was significantly higher as compared to Candid- VR gel. This clearly indicates that the CTZ-MG may have higher residence time in vagina as compared to Candid-VR gel. The increased bioadhesivity of CTZ-MG can be attributed to the presence of Carbopol as its bioadhesive potential is highest at vaginal pH.

In vitro Antifungal activity: The results of antifungal activity of Clotrimazole bulk powder, CTZ-MG and placebo gel evaluated by cup-plate
method are shown in Table 3. The results were found encouraging. The placebo gel has not shown any zone of inhibition. 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. Like bulk powder, CTZ-MG effectively inhibited C. albicans growth. There was no significant change in antifungal activity of clotrimazole.

**TABLE 3: ZONE OF INHIBITION SHOWN BY THE CLOTRIMAZOLE BULK POWDER, CTZ-MG AND PLACEBO GEL FOR C. ALBICANS**

<table>
<thead>
<tr>
<th>Samples</th>
<th>Zone of inhibition, mm, mean ± SD (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clotrimazole bulk powder</td>
<td>19.4 ± 0.22</td>
</tr>
<tr>
<td>CTZ-MG</td>
<td>19.3 ± 0.76</td>
</tr>
<tr>
<td>Placebo gel</td>
<td>nil</td>
</tr>
</tbody>
</table>

**CONCLUSION:** The spray drying method using Eudragit polymers at optimum levels was effective for the formation of CTZ microspheres. Low feed rates of polymeric solution enabled to obtain best microspheres in term of morphology, the concentration of the polymer affected both morphology and dimensions of microspheres, an increase of air drying temperature induced a reduction of microsphere size and recovery and changes in flow nebulization did not affect microsphere characteristics (i.e. size, surface characteristics. Microspheres loaded with clotrimazole in a bioadhesive carbopol gel slowed down the release of the drug, confirming possible application of microspheres containing antimicrobial drugs as a novel delivery system for local treatment of vaginal candidiasis. The CTZ-MG prolonged drug release for about 24 h.

It may be concluded from present study that spray drying is one of the best method for microsphere preparation and clotrimazole microspheres gel can be used as a novel delivery system for the prolonged release of CTZ for treatment of vaginal candidiasis.

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**REFERENCES:**