Formulation And Evaluation of Cyclodextrin Based Clotrimazole Nanogel For Vaginal Delivery

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Abstract- The objective of this study was to improve the solubility and dissolution profile of clotrimazole in the vaginal fluid for the treatment of fungal infection, a gamma-cyclodextrin-based clotrimazole nanogel was created for vaginal administration. The emulsion solvent evaporation process was used to prepare gamma-cyclodextrin-based nanogel. Aqueous phase was prepared using γCD, NaOH, EGDE and HPMC (1 %,2% w/w). Organic phase was prepared using Span 80(0, 0.5, 1, 2% w/v) in organic solvent dichloromethane. Aqueous phase was added to organic phase and homogenization was done followed by lyophilization. Formed solution was treated in an ultrasonic bath. The water was evaporated using a rotavapour. Schrodinger molecular docking software revealed a glide score of - 2.85 kcal/mol for the gamma-cyclodextrin-clotrimazole complex, indicating the establishment of a hydrogen bond with a length of 2.4. The AL type of curve with a stability constant of 545 M⁻¹ was visible in the phase solubility diagram, indicating the production of 1:1 complexes. F8 was chosen as the optimal formulation batch. The mucoadhesion force (g) for batch F8 was 13.53g. When compared to pure Clotrimazole in the simulated vaginal fluid, the solubility of Clotrimazole in loaded nanogel increased by 15.45 fold. At the conclusion of six hours, there had been a 79.67% drug release with a drug flux of 1.3596 g/cm²/h. The Korsmeyer-Peppas model was used in the formulation of nanogel, with a r² value of 0.9984. The final formulation showed the highest zone of inhibition which is of standard zone of inhibition.

Keywords: Clotrimazole, Gamma-cyclodextrin, EGDE, Nanogel.

INTRODUCTION

Each year, more than 1.5 million people die of fungal disease and more than 1 billion people are treatments, to name a few. If an accurate diagnosis is made early, antifungal affected. Despite knowing that most deaths from fungal infections are preventable, public health officials continue to ignore this issue. Serious fungal infections can be caused by a variety of other health problems. People suffer from asthma, AIDS, cancer, organ transplants, and corticosteroid drugs can be started immediately. Unfortunately, this is often not done or available, causing death, serious chronic illness, or blindness.[1,2] Nanogels have gotten a lot of attention as nanoscopic drug carriers, especially for site-specific or time-controlled administration of bioactive mediators. The versatility of polymer systems and the ease with which their physicochemical properties can be changed has resulted in versatile nanogel formulations. Nanogels offer exceptional stability, drug loading capacity, biologic consistency, strong penetration ability, and the ability to respond to environmental stimuli.[3,4] Nanogels have shown great promise in a variety of sectors, including gene delivery, chemotherapeutic medication delivery, diagnostics, organ targeting, and many more. Thenano-particulates drug delivery system offers plenty of advantages over conventional dosage forms for example reduced toxicity, enhanced bio-distribution and improved patient compliance.[5] Sudden outbreak in the field of nanotechnology have introduced the need for developing nanogel systems which have proven their potential to deliver drugs in controlled, sustained and targeted manner. With the emerging field of polymer sciences it has now become inevitable to prepare smart nano-systems which can prove effective for treatment as well as clinical trials progress.[6,7] Enhanced drug targeting, solubility, and bioavailability due to large surface area and nanosize range. [8,9] The polymer of choice for building nanosystems and nanogels has been cyclodextrin, which has greater advantages over cyclodextrin inclusion complexes due to its flexibility in altering behaviours, targeting, and API loading. As a result, nanogel delivery is more efficient than traditional macroscopic delivery. The nanogel dispersion is stabilised with a water-soluble nonionic polymer such as hydroxypropylmethylcellulose or ethylcellulose.[10] Interactions between the polymer matrix and the drug (electrostatic, hydrophobic van der Waals interactions) can cause phase separation of the drug-loaded nanogel, which can be hampered by dispersing the hydrophilic polymer. The dispersed hydrophilic polymer exposes to the skin surface by forming a protective barrier that surrounds the nanogel, allowing the drug particles to remain dispersed in the gel matrix.[11] Modified natural biopolymers with high content functional groups and superfunctional cross linkers are used to make biopolymer-based nanogels. Chemical cross-linking, photopolymerization, chemical-based cross-linking, and other innovative strategies have all been adopted to achieve self-assembly and cross-linking of hydrophilic block copolymers. Block polymers are used between the inner and outer layers of nanogels to regulate drug release from the polymer matrix.[12,13] The adaptability of these architectures allows the integration of a wide range of guest molecules by appropriately modifying the materials used for construction while maintaining gel-like behaviour, from inorganic nanoparticles to biopolymers such as proteins and DNA. Modified with a ligand to allow receptor-mediated drug delivery at the site of action for target-specific or cell-specific drug delivery. Nanogels filled with drugs or biological agents can overcome biological barriers and release therapeutic agents into cells. In recent years, nanogels have been widely applied in the fields of...
biotechnology to address genetics, enzyme immobilisation, and protein synthesis, proving to be a valuable tool for the development of new therapeutic systems in medicine.\textsuperscript{[14]}

Clotrimazole, a synthetic imidazole derivative, is mostly used locally to treat yeast and dermatophyte infections of the vaginal and skin. It works well against Candida spp., Trichophyton spp., Microsporum spp., and Malassezia furfur in vitro (Pityrosporum orbiculare). It also shows some in vitro activity against certain Gram-positive bacteria, as well as activity against Trichomonas spp. at very high concentrations. (phylis)

Clotrimazole is a broad spectrum antifungal drug used primarily for the treatment of Candida albicans and other fungi. Clotrimazole is a local treatment for athlete’s foot (athlete’s foot). It is a synthetic azole antifungal agent. Clotrimazole is a well-tolerated drug with few side effects, but some patients are refractory to treatment especially those with immunodeficiency \textsuperscript{[15]}

Clotrimazole is a lipophilic drug with a log k o / w of 4.1 and slow solubility in water. Studies have been conducted to improve the solubility of clotrimazole using microcapsules, liposomes, suspensions containing HPMC and nanospheres, cyclodextrin inclusion complexes, and solid dispersion techniques using mannitol as a carrier. I am. Continuing this research, our goal is to learn more about the effects of water-soluble polymers [natural and synthetic] on the skin using nanogel formulations.\textsuperscript{[16]}

**MATERIALS**

Γ-Cyclodextrin was purchased from ANALAB Fine Chemicals (Mumbai, India), Hydroxypropyl methylcellulose was purchased from LobaChemie Pvt. Ltd (Mumbai, India), Span80, Ethylene Glycol Diglycidyl Ether, Dichloromethane was purchased from TCI Chemicals Pvt. Ltd (India), Clotrimazole was gifted by Mylan Pharmaceuticals (Mumbai, India). All of the other compounds were analytical grade and were utilised just as they were ANALAB Fine Chemicals (Mumbai, India) provided cyclodextrin, LobaChemie Pvt. Ltd (Mumbai, India) provided hydroxypropyl methylcellulose, TCI Chemicals Pvt. Ltd (India) provided Span80, Ethylene Glycol Diglycidyl Ether, Dichloromethane, and Mylan Pharmaceuticals (Mumbai, India) gifted Clotrimazole (Mumbai, India). The other substances were all analytical grade and used just as they were.

**METHODS**

Cyclodextrin derivative selection

The best-fit derivative of cyclodextrin with clotrimazole was determined using SchrodingerTM molecular docking software version 9.0. The best fit was determined using the glide score and hydrogen bonding parameter. The cyclodextrin derivative was chosen based on a supramolecular synthon method that shows the capability of forming hydrogen bonds with clotrimazole. The structures of the following cyclodextrin derivatives were found from the PubChem database: γ-cyclodextrin (PubChem CID: 71597046), β-cyclodextrin (PubChem CID: 444041), and 2HP—cyclodextrin (PubChem CID: 56972821). Low Glide Score [Binding energy (kcal/mol)] was used to choose a cyclodextrin derivative.

Pure CTZ was studied for solubility in simulated vaginal fluid and water at various pH levels.\textsuperscript{[17]} 0.2M NaOH and 0.2M HCL were used to alter the pH of the prepared simulated vaginal fluid and water. In a conical flask, an excess of pure medication was introduced to 10mL simulated vaginal fluid and water with a pH range of 3.5 to 7.5. To facilitate solubilization, the mixture was maintained on a mechanical shaker (Remi, Mumbai, India) for 48 hours at 37°C and 80 rpm. To achieve equilibrium, the samples were held at room temperature for 24 hours. After filtering the material with a Whatman filter (0.45m), the filtrate was examined using a spectrophotometer (Jasco V-550, Japan). The same process was used to investigate the solubility enhancement of generated CTZ nanogel at pH 3.5, 4.5, 5.5, 6.5, and 7.5 (Simulated vaginal fluid).

Phase solubility studies of Clotrimazole in SVF:

In the SVF medium, a phase solubility analysis of CTZ was carried out using the Higuchi and Corners method. In 10 mL simulated vaginal fluid at pH 4.5, an excess of CTZ (200mg) was added to an increasing concentration of gamma-cyclodextrin (γ-CD) (10, 40, 70, 100, 130, 160g/mL). To facilitate solubilization, the mixture was maintained on a mechanical shaker (Remi, Mumbai, India) for 48 hours at 37°C and 80 rpm. To achieve equilibrium, the samples were held at room temperature for 24 hours. After that, the sample was filtered through a 0.45m Whatman filter, and the filtrate was examined using a spectrophotometer (Jasco V-550, Japan). Using the Higuchi equation, the type of inclusion complex and the stability constant (ks) were calculated from the phase solubility diagram.

\[ ks = \text{slope} / So \text{ (1-slope)} \] \text{........................................... (1)}

Where ks is stability constant (M-1), So is the intrinsic solubility of CTZ. The slope is obtained from the linear line equation of the phase diagram.

FTIR study of the pure clotrimazole, dried powder of the CTZ—CD inclusion complex, unloaded γ-CD-nanogel, and clotrimazole loaded γ-CD-nanogel were all subjected to FTIR analysis. To access the conformation of the drug in the complex and nanogel, 5mg of each sample was weighed on an analytical balance (Mettler Toledo, H51, Switzerland) and placed on the prism of FTIR (QATAR-SIRSpirit), and the spectrum was scanned across a frequency mid-IR area 400–4000cm-1.

Preparation of nanogel

The nanogel was made using an emulsion-solvent evaporation process, as described below.
Preparation of aqueous phase:
4 mL of ethylene glycol diglycidyl ether (EGDE) was added to a 10 mL (20% w/w) solution of γ-CD in 0.2M NaOH and agitated for 5 minutes. HPMC solution in 0.2M NaOH (1 percent, 2 percent w/w) was added to this 10 ml batch, which was heated at 60°C for 25 minutes.

Preparation of organic phase:
The organic phase is made up of Span 80 (0, 0.5, 1, and 2% w/v) solutions in the organic solvent dichloromethane.

Preparation of w/o emulsion:
An ultra-turra T 25 (Janke and Kunkel, Ink-Laborteknik, Germany) was used to homogenize 14 ml of the aqueous phase with 20 ml of the organic phase at 4000 rpm for 8 minutes. The emulsion was maintained at 60°C for 30 minutes on a magnetic stirrer (100 revolutions per minute). The emulsions were immediately placed into 100 mL of distilled water and agitated at 60°C for 3 hours to allow the dichloromethane to evaporate completely and create a colloidal dispersion.

Dialysis of nanogel dispersion:
Each colloidal system was divided into 50 mL dialysis bags (MWCO 12—14 KDa), which were subsequently placed into water-filled beakers. To remove contaminants, the water in the beakers was refreshed every 12 hours. The colloidal dispersion was maintained at -40°C in a deep freezer before being lyophilized (Operon, FDB-5503, Korea) to generate an unloaded γ-CD-nanogel powder.

Evaluation of unloaded gamma CD nanogel:

Determination of particle size and polydispersity index:
A particle size analyzer was used to determine the particle size and PDI of freeze-dried unloaded nanogel (Malvern Zetasizer ZS 90 UK). To assess particle size and PDI, freeze-dried nanogel powder was diluted with distilled water. [18]

Determination of swelling ratio:
The prepared unloaded γ-CD-swelling nanogel's ratio was calculated. [19, 20] Approximately 0.2 gm of unloaded γ-CD-nanogel was soaked in 100 mL of water for 2 hours, 4 hours, 6 hours, and 8 hours, and then reweighed after carefully removing excess water with filter paper. The swelling ratio of the unloaded γ-CD-nanogel was computed using the formula below at each time point.

Differential Scanning Calorimetric studies of unloaded/loaded Gamma CD-nanogel:
On a DSC-821 (Mettler Toledo DSC, Switzerland) with a thermal analyzer, differential scanning calorimetric (DSC) measurements were taken. [21] Pure CTZ, HPMC, γ-CD, unloaded γ-CD-nanogel, and CTZ loaded γ-CD-nanogel (5 mg each) were accurately weighed and deposited in sealed aluminum pans before being heated under nitrogen flow (20 mL/min) at a scanning rate of 10°C/min from 25 to 280°C smashing an empty aluminum pan as a control.

Method of drug loading onto freeze dried gamma CD nanogel:
According to the results of the phase solubility investigation, CTZ-CD inclusion complex formation was stable at 1:1 molar ratio, and all freeze-dried nanogel batches were loaded with CTZ in 1:1 molar ratio, with the molecular weights of CTZ and γ-CD being 344.83 gm/mol and 1297 gm/mol, respectively. Clotrimazole acetone solution (10mL) was produced. For 60 minutes, the generated solution was treated in an ultrasonic bath at 25°C. The samples were processed in an ultrasonic bath at 25°C for 60 minutes after adding an aqueous solution of freeze-dried nanogel to the drug solution. Acetone was evaporated to make a gel with a Rota vapor R-210 and Vacuum Controller V-850 (Buchi, Switzerland). [22]

Evaluation of CTZ-loaded gamma-CD nanogel

Drug content and entrapment efficiency:
The CTZ-loaded γ-CD-drug nanogel content and entrapment efficiency were assessed. 0.5 gm of CTZ-loaded CD-nanogel was properly weighed and transferred to a 100ml conical flask, followed by 50mL of HPLC grade methanol and sonication for 10 minutes. The concentration of CTZ was obtained by filtering and analyzing the following colloidal dispersion with a spectrophotometer (Jasco V-550, Japan). The drug content and entrapment efficiency were calculated using the equation below.

Determination of particle size, polydispersity index (PDI), and zeta potential
A particle size analyzer (Malvern Zetasizer ZS 90, Worcestershire, UK) was used to determine the particle size, PDI, and zeta potential of CTZ-loaded nanogel, as detailed in the previous section of the approach. [23]
Optimized formulation:
The optimal formulation was chosen based on the results of drug content, entrapment efficiency, particle size, PDI, and zeta potential, and was then tested for mucoadhesion force and drug release in SVF.

Mucoadhesion test:
The improved formulation's mucoadhesion test was carried out in triplicate using a goat vaginal mucosal membrane from a slaughterhouse that was kept in simulated vaginal fluid with a pH of 4.5. The mucoadhesive force was measured using a Brookfield CT3 texture analyzer (manufactured in the USA) and an appropriate probe (g).[24]

Drug release:
The Franz diffusion cell (SES GmbH, Analyze system, Germany) method was used to test the drug release of all of the produced nanogel batches. 2gm of γ-CD nanogel was applied on a semi-permeable membrane with a diameter of 25mm and a pore size of 0.45m, which was then installed on the donor compartment's glass fibre. Simulated vaginal fluid with a pH of 4.5 was put in the receptor compartment. Approximately 0.5mL of the material was withdrawn at regular intervals up to 6 hours while the sink condition was maintained. SVF was used to dilute each 0.5mL of the sample to a total of 10 mL. A spectrophotometer was used to evaluate the sample at a maximum wavelength of 280 nm. A graph of cumulative drug release vs. time was created. The improved batch F8's drug release was compared to Carbopols.

Drug release mathematical modelling:
DDSolver™ was used to undertake mathematical modelling of the drug release.[25,26,27] r2 (correlation coefficient), AIC (Akaike information criterion), and RMSE (root mean squared error) were used as selection factors to see which model best fit the dissolution data. In addition, the slope of the line equation created by plotting cumulative drug release vs. time was used to quantify drug flow (J).

Antifungal Efficacy Studies:
Antifungal drug efficacy of the formulation was performed using the cup plate method. The fungi Candida albicans (NCIM No.3674) was received from the National Collection of Industrial Microorganism, Pune. The fungal culture was revived using the streak plate technique. For the revive process, the fungi were streaked on the Sabouraud Dextrose medium containing 2% of agar and to grow at 30°C for a day. The grown culture was then poured into the five sterilised Petri plates in aseptic condition and mixed with the 20ml sterilized SDA media in each 4 petri plates. After solidification of the media, each petri plates were bored using a cup borer to form the wells. 2 wells were bored in each petri plate. Formulations were added to the well. Zone of inhibition was recorded after 24 hrs.

In vivo vaginal permeation study:
Research of in vivo vaginal permeation: Female Wistar rats weighing 200–220 g were used in the study of in vivo vaginal permeation of the formulated formulation. According to the strict criteria of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India, the protocol was authorised by the institutional animal ethical committee. A trained individual carried out every step of the animal handling process. The rats were housed in isolation at a temperature of 25 °C with a 12 h light/dark cycle and were provided access to food and water at will. There were four groups of rats. Six animals were placed in the first group, which acted as the control group, and received no treatment. Examining vaginal permeability in real time: With 6 rats in the control group and 18 rats in groups 2, 3, and 4, the second group received CTZ dispersion, the third group received plain CTZ in situ gel, and the fourth group received CTZ-γ-CD nanogel. Rats' vaginal smears were checked beforehand to determine the oestrus cycle stage, and only rats in the proestrus or estrus phase were chosen for the experiment. Before the investigation, the rats were starved for a whole night. Six sub-groups of rats from each group were created to examine the drug's vaginal permeation throughout time intervals of 1, 2, 4, 8, and 48 hours. The rats were housed around the thorax with its ventral surface facing up throughout the administration of in situ gelling formulations. A 0.1 ml dose of an in situ gelling formulation with a CTZ dose of 2.5 mg/Kg of body weight was gently injected into the vagina at a depth of 1 to 2 mm using the soft plastic tip of a micropipette. To guarantee that the formulation turned into gel, the vaginal specimen was weighed, cleaned with sterile saline, wiped dry, and then sliced with a cutter. The sliced tissue was homogenised for 10 minutes at 8000 rpm with previously cooled dichloromethane (5 ml) in an ice bath to extract the medication. The resulting mixture was centrifuged at 10,000 rpm for 10 minutes in a cooling centrifuge (Remi, CM 12 plus, India). Amlodipine (50 g/ml) was added to the vaginal tissue extract (0.5 ml), and the solution was evaporated to dryness in a vacuum oven at 40 °C. A 60:40 mixture of acetonitrile and 30 mM phosphate buffer (pH 4.0) in 1 ml of mobile phase was used to reconstitute the residue before being vortexed and centrifuged. A previously created and approved reverse phase HPLC method was used to determine the drug content after the supernatant had been filtered using syringe-driven filters with a 0.22 m pore size. The HPLC quaternary gradient system (Lachrom 2000) with a Neosphere C18 column (150 X 4.6 mm, 5.0 m), UV visible detector (L-7400), and Merck Hitachi L-7100 pump was employed. The linearity for CTZ was observed in concentration range of 0.5 to 10µg/ml. Pharmacokinetic parameters like maximum peak concentration of the drug in tissue (Cmax), area under the tissue concentration-time curve (AUC 0→24) were determined using PK Solver software in Microsoft Excel. All results were presented as mean ± standard deviation values.[28]

RESULTS:
Selection of Cyclodextrin derivative:
Docking experiments for CTZ-γ-cyclodextrin, CTZ-β-cyclodextrin, and HP-β-cyclodextrin using SchrodingerTM molecular docking software revealed glide scores of -2.85 kcal/mol, -2.61 kcal/mol, and 0 kcal/mol, respectively, and bond lengths of 2.4, 2.12, and 0 kcal/mol. Docking experiments with HP-β-cyclodextrin, on the other hand, revealed no interaction. γ-cyclodextrin was chosen as the best fit for clotrimazole due to its low glide score and bond length parameter.

Solubility studies of CTZ at different pH conditions and different medium:
For the pure medication in water and simulated vaginal fluid, pH solubility studies were conducted at pH 3.5, 4.5, 5.5, 6.5, and 7.5. The same research was done in SVF with CTZ loaded nanogel. The solubility of pure Clotrimazole in water at various pH levels was found to be in the range of 0.05207 to 0.01189 mg/ml, while the solubility in SVF was found to be in the range of 0.5008 to 0.7858 mg/ml, as shown in fig. 2. The blend of alcohol, acids, and bovine serum albumin contained in SVF may aid in medication solubilization, resulting in higher drug solubility in SVF compared to water. The solubility of Clotrimazole-loaded nanogel ranged from 0.6931 to 1.4417 mg/ml. The solubility of CTZ loaded nanogel increased as compared to pure Clotrimazole in water 3.5 to 7.5 pH by 21.16, 15.45, 7.49,6.07, 16.63 folds.
Fig. 2: Solubility study of pure Clotrimazole and CTZ loaded γ-CD nanogel in various mediums at different pH conditions

**Phase solubility study:**
In SVF, a phase solubility investigation of CTZ with γ-CD was carried out. Figure 3 shows the curve of CTZ solubility as a function of increasing γ-CD concentrations. The solubility of CTZ increases as the concentration of γ-CD increases, as can be shown. A linear relationship is indicated by a line equation with a $r^2$ value of 0.998. In addition, the phase solubility diagram displayed an AL type curve, indicating the production of a 1:1 stoichiometric complex. A stable complex formation is indicated by the stability constant of 545 M⁻¹.

**Fig. 3: Phase solubility diagram of CTZ with γ-CD in SVF (pH 4.5) at 37°C**

**FTIR study:**
Figure 4 shows The characteristic peaks of pure CTZ like C-N stretching at 1313.57 cm⁻¹, C-H stretching at 756cm⁻¹, C=C stretching at 1633cm⁻¹, N-H stretching at 1653 cm⁻¹. The frequencies of gamma CD were observed at 3271 cm⁻¹, 2926 cm⁻¹, 1019 cm⁻¹, 997 cm⁻¹. The loaded nanogel shows the disappearance of the drug peak at 1313 cm⁻¹. The unloaded drug does not show any drug peak. This gave confirmation of FTIR study.
Fig. 4: FTIR of pure CTZ, γ -CD, CTZ loaded γ -CD nanogel, unloaded γ -CD nanogel

**Preparation of freeze dried nanogel:**
Table 1 shows the formula for preparing nanogel batches. Various concentrations of HPMC and Span 80 were used to make nanogel batches.

<table>
<thead>
<tr>
<th>γ -CD (%w/w)</th>
<th>HPMC(%w/w)</th>
<th>SPAN 80 (%w/v)</th>
<th>BATCH</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>1</td>
<td>0</td>
<td>F</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5</td>
<td>F2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>F3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>F4</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>0</td>
<td>F5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5</td>
<td>F6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>F7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>F8</td>
</tr>
</tbody>
</table>

**Percentage yield, particle size, PDI analysis of unloaded nanogel:**
Table 2 shows the percentage yield, particle size, and PDI analysis of produced nanogel. The yield ranged from 46.74 to 54.44 percent, with batch F1 having the lowest percentage yield of 46.74 percent and batch F8 having the greatest percentage yield of 54.44 percent. The percentage yield of the nanogel increases when the concentration of HPMC and Span 80 increases, according to the results.

<table>
<thead>
<tr>
<th>Sr no.</th>
<th>Batch</th>
<th>Percentage yield</th>
<th>Particle size (nm)</th>
<th>PDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F1</td>
<td>33(±0.097)</td>
<td>323.71(±0.102)</td>
<td>0.672(±0.04)</td>
</tr>
<tr>
<td>2</td>
<td>F2</td>
<td>36(±0.076)</td>
<td>298.64(±2.01)</td>
<td>0.544(±0.012)</td>
</tr>
<tr>
<td>3</td>
<td>F3</td>
<td>37(±0.055)</td>
<td>263.01(±0.617)</td>
<td>0.413(±0.032)</td>
</tr>
<tr>
<td>4</td>
<td>F4</td>
<td>39(±0.12)</td>
<td>254.49(±0.974)</td>
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<td>5</td>
<td>F5</td>
<td>36(±0.10)</td>
<td>311.8(±1.651)</td>
<td>0.576(±0.036)</td>
</tr>
<tr>
<td>6</td>
<td>F6</td>
<td>39(±0.079)</td>
<td>287.93(±0.0997)</td>
<td>0.369(±0.097)</td>
</tr>
<tr>
<td>7</td>
<td>F7</td>
<td>40(±0.068)</td>
<td>255.11(±1.03)</td>
<td>0.437(±0.085)</td>
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<td>8</td>
<td>F8</td>
<td>42(±0.094)</td>
<td>211.6(±0.87)</td>
<td>0.382(±0.053)</td>
</tr>
</tbody>
</table>

**Determination of swelling ratio:** Table 3 shows the swelling ratios of various testing batches. The lowest swelling ratios were found in batches F4 and F8. The concentration of HPMC in the nanogel could explain this outcome. Because HPMC acts as a barrier for water to penetrate into the unloaded -CD nanogel, the ratio decreased as the amount of HPMC increased.

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</table>
Table 3: SWELLING RATIO OF UNLOADED Γ-CD NANOGEL

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>batch</th>
<th>2 hours</th>
<th>4 hours</th>
<th>6 hours</th>
<th>8 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F1</td>
<td>401(±1.019)</td>
<td>438(±1.778)</td>
<td>491(±0.811)</td>
<td>510(±0.632)</td>
</tr>
<tr>
<td>2</td>
<td>F2</td>
<td>398(±1.401)</td>
<td>415(±1.03)</td>
<td>453(±0.452)</td>
<td>460(±1.261)</td>
</tr>
<tr>
<td>3</td>
<td>F3</td>
<td>355(±0.702)</td>
<td>380(±0.111)</td>
<td>398(±0.234)</td>
<td>488(±0.346)</td>
</tr>
<tr>
<td>4</td>
<td>F4</td>
<td>305(±1.504)</td>
<td>360(±0.850)</td>
<td>391(±0.121)</td>
<td>475(±0.217)</td>
</tr>
<tr>
<td>5</td>
<td>F5</td>
<td>295(±0.702)</td>
<td>355(±0.611)</td>
<td>386(±1.042)</td>
<td>445(±1.083)</td>
</tr>
<tr>
<td>6</td>
<td>F6</td>
<td>285(±0.750)</td>
<td>365(±1.345)</td>
<td>410(±0.623)</td>
<td>435(±0.463)</td>
</tr>
<tr>
<td>7</td>
<td>F7</td>
<td>220(±1.021)</td>
<td>350(±1.113)</td>
<td>381(±0.327)</td>
<td>415(±0.747)</td>
</tr>
<tr>
<td>8</td>
<td>F8</td>
<td>136(±1.171)</td>
<td>254(±0.8)</td>
<td>311(±0.785)</td>
<td>388(±0.834)</td>
</tr>
</tbody>
</table>

DSC studies of nanogel:
Figure 5 shows a DSC overlay of pure Clotrimazole, HPMC, γ-CD, unloaded γ-CD nanogel, and Clotrimazole-loaded γ-CD nanogel. Pure CTZ has a pronounced endothermic peak at 147.05°C, which corresponds to the drug's melting temperature of 148°C. The absence of this high endothermic peak in loaded nanogel confirms drug trapping in the γ-CD cavity.

Evaluation of CTZ loaded gamma γ-CD nanogel:
CTZ-loaded γ-CD Nanogel drug content, entrapment efficiency, particle size, polydispersity index, and zeta potential.

Table 4 shows the drug concentration, entrapment efficiency, particle size, polydispersity index, and zeta potential of the CTZ-loaded γ-CD nanogel. It’s clear that when the concentration of HPMC and Span 80 rises, so does the drug content and entrapment efficiency. Batch F8 had the highest (percent) drug content of 64.73 and the highest (percent) entrapment efficiency of 94.49, whereas batch F3 had the lowest (percent) drug content of 56.32 and the lowest (percent) entrapment efficiency of 84.51. In comparison to Span80, HPMC plays a significant influence in boosting or lowering drug content and entrapment efficiency. Batch F8 had the smallest particle size, measuring 303 nm, with a PDI of 0.395 and a zeta potential of -1.33 mV, while batch F1 had the largest particle size, measuring 425 nm with a PDI of 0.606 and a zeta potential of -13.8 mV. As the concentration of Span 80 increased, the particle size reduced because Span 80 acts as an emulsifier/stabilizer, reducing droplet size and increasing zeta potential.

Table 4: % DRUG CONTENT, ENTRAPMENT EFFICIENCY, PARTICLE SIZE, PDI AND ZETA POTENTIAL OF CTZ-LOADED NANOGEL

<table>
<thead>
<tr>
<th>Batch</th>
<th>(%) Drug content (±SD)</th>
<th>(%) Entrapment efficiency</th>
<th>Particle size (nm) (±SD)</th>
<th>PDI (±SD)</th>
<th>Zeta potential (mV) (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>56.69(±0.036)</td>
<td>84.51(±0.007)</td>
<td>425(±0.577)</td>
<td>0.606(±0.061)</td>
<td>-0.65(±0.002)</td>
</tr>
<tr>
<td>F2</td>
<td>61.47(±0.290)</td>
<td>92.86(±0.141)</td>
<td>427(±1.577)</td>
<td>0.418(±0.147)</td>
<td>-1.03(±0.009)</td>
</tr>
<tr>
<td>F3</td>
<td>56.32(±0.399)</td>
<td>87.17(±0.017)</td>
<td>494(±0.513)</td>
<td>0.292(±0.180)</td>
<td>-0.73(±0.004)</td>
</tr>
<tr>
<td>F4</td>
<td>64.14(±0.490)</td>
<td>93.01(±0.082)</td>
<td>596(±0.923)</td>
<td>0.360(±0.112)</td>
<td>-1.12(±0.0065)</td>
</tr>
<tr>
<td>F5</td>
<td>60% (±0.620)</td>
<td>87.04% (±0.93)</td>
<td>496(±1.311)</td>
<td>0.207(±0.126)</td>
<td>-0.91(±0.0025)</td>
</tr>
<tr>
<td>F6</td>
<td>58.52% (±0.130)</td>
<td>92.43% (±0.96)</td>
<td>492(±1.216)</td>
<td>0.301(±0.251)</td>
<td>-0.93(±0.0078)</td>
</tr>
<tr>
<td>F7</td>
<td>57.37% (±0.28)</td>
<td>91.18% (±0.0106)</td>
<td>482(±1.058)</td>
<td>0.276(±0.189)</td>
<td>-1.16(±0.006)</td>
</tr>
<tr>
<td>F8</td>
<td>64.73% (±0.273)</td>
<td>94.49% (±0.007)</td>
<td>303(±0.650)</td>
<td>0.395(±0.125)</td>
<td>-1.33(±0.0031)</td>
</tr>
</tbody>
</table>

n=3 (±SD)
Selection of optimized batch:
Batch F8 was chosen as an optimum batch for mucoadhesion and drug release based on particle size, PDI, zeta potential, (percent) drug content, and (percent) entrapment efficiency of CTZ-loaded γ-CD nanogel.

Mucoadhesion and drug release study of selected nanogel batch:
An optimized F8 batch’s mucoadhesion was found to be between 12.8 g and 14.9g. Figure 6 depicts a plot of percent cumulative release versus time. The drug release of all batches at the end of 6 hours varies, with batch F8 releasing the most at 79.67 percent and batch F1 releasing the least at 20.2 percent. This can be related to the particle size of the produced nanogel, which is dependent on the concentration of Span 80. (0 percent in F1 and 2 percent in F8 batch). It can be seen that the drug release from the nanosized gel is influenced not only by the concentration of Span 80, but also by the concentration of HPMC. There was an increase in drug release.

In comparison to nanogel batches containing 1 percent HPMC, batches containing 2 percent HPMC showed increased drug release. The drug release of the F8 nanogel batch was compared to that of a carbopol gel containing CTZ. The F8 batch released more drugs than the carbopol gel. According to the selection criteria (r², AIC, RMSE) listed in table 5, mathematical modelling was used to investigate the mechanism of drug release. The Korsmeyer-Peppas model indicated that drug release from the polymeric system followed the Korsmeyer-Peppas paradigm. Furthermore, the first-order model was shown to be the second-best match, implying that drug release is influenced by its concentration. The Korsmeyer-Peppas model had a r² of 0.9984, an AIC of 18.9194, and an RMSE of 1.2982. The drug flux was calculated to be 1.3596 ug/cm²/h using the slope of the linear portion.

| TABLE 5: DRUG RELEASE MATHEMATICAL MODELS OF BATCH F8 |
|----------|-----|-----|
| MODEL    | R²  | RMSE| AIC   |
| Zero order | 0.8979 | 1.3603 | 18.8505 |
| First order | 0.9719 | 6.5709 | 40.8994 |
| Korsmeyer-Peppas model | 0.9984 | 1.2982 | 18.9194 |
| Hickson-Crowell | 0.9500 | 10.7955 | 47.8501 |
| Higuchi | 0.8652 | 4.9263 | 36.8667 |

Antifungal Efficacy Studies:
The final formulation showed the highest zone of inhibition which is of the standard zone of inhibition. Placebo-containing formulation showed the least zone of inhibition which is of the standard ZOI. The observations are indicating that the formulation is having antifungal efficacy. The results of the antifungal efficacy test are shown in the (Table 6)
### Table 6: ANTIFUNGAL EFFICACY STUDY RESULTS

<table>
<thead>
<tr>
<th>Sample</th>
<th>Zone of inhibition(mm)</th>
<th>%Zone of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>18</td>
<td>100</td>
</tr>
<tr>
<td>Formulation</td>
<td>17.3</td>
<td>96.11</td>
</tr>
<tr>
<td>No drug</td>
<td>1.9</td>
<td>10.55</td>
</tr>
</tbody>
</table>

In vivo vaginal tissue uptake:

In order to study the effect of gamma-CD complex on drug penetration in vagina, the in vivo vaginal tissue uptake study was conducted in Wistar rats. Fig 8 compares the in vivo performance of CTZ-gamma-CD nanogel (VM1) to that of plain nanogel of CTZ without gamma-CD, administered intravaginally. The CTZ penetrated per g of vaginal tissue was determined after single dose administration of intravaginal formulations by reverse phase HPLC method. The linearity for CTZ was found over the range, 0.5 to 10 µg/ml. Immediately after administration of CTZ-gamma-CD nanogel, plain CTZ nanogel and CTZ dispersion (at t=0), the CTZ concentration (C0) was 5.73 µg/g, 4.22 µg/g and 2.96 µg/g respectively (Table 7). For CTZ dispersion, maximum concentration (Cmax) of 3.54 µg/g was observed in 6h. The concentration of CTZ was beyond detection limit after 48 h. This could be due to removal of the dispersion from the vagina due to its self cleansing action. The CTZ plain nanogel reached maximum concentration...
(Cmax) in vagina(4.03 µg/g) in 6h. There was further decrease in concentration of CTZ to 3.98 µg/g at the end of 48 h. For CTZ-gamma-CD gel, the maximum concentration, Cmax (11.13µg/g) reached in 4h. The faster CTZ penetration in case of gamma-CD gel is attributed to increase in solubility of CTZ in presence of gamma-CD and increase in drug uptake by virtue of gamma-CD. Total vaginal uptake of CTZ was 202.13 µg.g1.h and 181.80 µg.g450 1.h when administered as CTZ-gamma-CD nanogel and CTZ nanogel respectively.

Table 7 : PHARMACOKINETIC PARAMETERS FOR VAGINAL DISTRIBUTION

<table>
<thead>
<tr>
<th>Vaginal Pharmacokinetic parameters</th>
<th>Carbopol gel with CTZ</th>
<th>CTZ loaded nanogel</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax</td>
<td>4.03µg/g</td>
<td>11.13µg/g</td>
</tr>
<tr>
<td>AUC0-24</td>
<td>181.87µg.g.h</td>
<td>391.61µg.g.h</td>
</tr>
</tbody>
</table>

Fig. 8: In vivo vaginal tissue uptake study

DISCUSSION:
As previously mentioned, cyclodextrin belongs to a type of drug carriers that enhances the solubility of pharmaceuticals and extends their benefits through the manipulation of behaviour, targeting, and API loading. However, the correct cyclodextrin selection must be done in order to effectively benefit the target medicine. An empirical scoring method that comes close to the ligand binding free energy is called the glide score. There are several terminology in it, such as contributions to force fields (electrostatic, van der Waals), as well as terms for rewarding or punishing interactions that are known to affect ligand binding. It has been enhanced for database enrichment, binding affinity prediction, and docking precision. More negative value indicate tighter binders since it simulates binding free energy. Results with γ-cyclodextrin were quite good, and it was chosen as the compound that fit clotrimazole the best.\(^{[29]}\) Phase solubility tests also showed that γ-cyclodextrin and stable stoichiometric compound formation. Copolymers were used to chemically cross-link γ-cyclodextrin to create nanogel. In this work, HPMC was chosen as the polymer, and ethylene glycol diglycidyl ether (EGDE) served as the crosslinker. In the presence of a mild alkaline condition (0.2M NaOH) and a high temperature of 60°C, the -OH groups found in the CD and HPMC react with the epoxide groups of EGDE (non-toxic cross-linker) to generate a strongly cross-linked complex. In the case of CD, -OH groups at C2 of the backbone linkage and -OH groups at C2 and C6 of the branch react, as do the -OH groups at C2 and C6 of HPMC. Studies using FTIR and DSC verified that the medication was present in the γ-CD cavity. The concentration of Span 80 had an impact on the nanogel's generated particle size. As Span 80 acts as an emulsifier and stabiliser, it reduces the size of the droplets, increases zeta potential, and results in a decrease in particle size. A medicine must be solubilized in vaginal fluid in order to be absorbed locally or systemically through the vaginal mucosa. Since there isn't much fluid available in the vaginal cavity, a medicine like clotrimazole's poor aqueous solubility is one of the challenges that must be overcome. In comparison to a pure drug, Clotrimazole's solubility in nanogel formulation increased in SVF. This outcome can be attributed to the drug's greater solubilization due to its nano-size and inclusion complex with cyclodextrin. The effectiveness of any vaginal dose form or device is directly correlated with how long it stays in the lumen. The dosage form has a high likelihood of leaking or washing out before it has an opportunity to act therapeutically. According to drug release data, the drug will release at its peak concentration six hours before any possibility of infection arises.

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Compliance with ethical standards:
Conflict of interests: The authors declare that they have no conflict of interests.
Statement of Human and Animal Rights: Approval from Ethical Committee in accordance with "Principles of Laboratory Animal Care".
Statement of Informed Consent: Not applicable

Abbreviation:
BCS: Biopharmaceutical classification system; CTZ: Clotrimazole; γ-CD: Gamma-Cyclodextrin; DSC: Differential scanning calorimetry; r2: correlation coefficient; RMSE: Root-mean-square deviation; FTIR: Fourier-transform infrared spectroscopy; HPMC: Hydroxypropyl methylcellulose; EGDE: Ethylene glycol diglycidyl ether; SVF: Simulated vaginal fluid; API: Active pharmaceutical ingredient.

REFERENCES:


Figure legends:
Fig. 1: Molecular docking of Clotrimazole with γ-cyclodextrin
Fig. 2: Solubility study of pure Clotrimazole and CTZ loaded γ-CD nanogel in various mediums at different pH conditions
Fig. 3: Phase solubility diagram of CTZ with γ-CD in SVF (pH 4.5) at 37°C
Fig. 4: FTIR of pure CTZ, γ-CD, CTZ loaded γ-CD nanogel, unloaded γ-CD nanogel
Fig. 5: DSC overlay of Clotrimazole, HPMC, γ-CD, CTZ loaded γ-CD nanogel, unloaded - γ-CD nanogel
Fig. 6: (%) Cumulative drug release vs. time profile of Clotrimazole nanogel of all batches
Fig. 7: Antifungal efficacy study
Fig. 8: In vivo vaginal tissue uptake study

Table legends:
Table 1: γ-CD nanogel batches preparation
Table 2: Percentage yield, particle size, PDI, analysis of unloaded nanogel batches.
Table 3: Swelling ratio of unloaded γ-CD nanogel
Table 4: Drug content, Entrapment efficiency, Particle size, PDI and Zeta potential of CTZ-loaded nanogel
Table 5: Drug release mathematical models of batch F8
Table 6: Antifungal efficacy study results
Table 7: Pharmacokinetic parameters for vaginal distribution.