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Enhanced Bio-Adhesion and Sustained Delivery of Clotrimazole Encapsulated Solid Lipid Nanoparticles Loaded in Hyaluronic Acid Gel as Anti- Fungal Therapy

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Authors' contributions

This work was carried out in collaboration between all authors. Author MVH performed the study and managed the analyses of the study along with statistical data analysis. Author SV designed the study. Author M. Pooja wrote the final draft of the manuscript. Author M. Paranjothy managed the literature searches. Author RRK helped with the biotechnology work. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Clotrimazole (CTZ) is a broad spectrum anti-fungal drug which faces inability to retain over skin for longer duration and faces systemic side effects. The rationale behind present research effort was to attain sustained release of drug by lipid nanocarriers, enhance absorption by

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prolonged retention of gel and improve the patient compliance by reducing the dosing frequency.
Study Design: CTZ solid lipid nanoparticles (SLN) were prepared by solvent emulsification method and loaded into sodium hyaluronate (Na-Hy) gel.
Methodology: CTZ-SLN were formulated and characterized, loaded into sodium hyaluronate (Na-Hy) gel.. Later assessed for *in vitro* release, *in vitro* bioadhesion, *and in vitro* antifungal activity.
Results: FT-IR and DSC studies revealed no chemical changes or interaction occurred. Optimized CTZ-SLN exhibited the particle size 248 ± 1.31 nm, zeta potential 16.2 mv, entrapment efficiency was 80.7±0.7%, and drug loading 21.8±0.5%. The optimized CTZ SLN loaded Na-Hy gel demonstrated prolonged drug release (upto 24 h) than the conventional dosage form Canesten 1%, which got exhausted within 4 h. CTZ-SLN Na-Hy gel exhibited enhanced *in vitro* bioadhesion and *in vitro* antifungal action compared to conventional dosage form Canesten ointment against a) *Candida albicans* b) *Saccharomyces cerevisiae* c) *Candida glabrata* d) *Candida tropicalis*.
Conclusion: Aforementioned outcomes make the promising applicability of formulated CTZ-SLN Na-Hy gel as a potential drug delivery system for local therapy of vaginal candidiasis and other similar infections.

Keywords: Clotrimazole; solid lipid nanoparticles; sodium hyaluronate gel; vaginal candidiasis.

ABBREVIATIONS

CTZ : Clotrimazole
SLN : Solid lipid nanoparticles
Na-Hy : Sodium hyaluronate

1. INTRODUCTION

Vaginitis is characterized by irritation and inflammation in the vagina, due to the infection in the vulva. Vaginal candidiasis is most prevalent and women of all ages prone to it. Undeniably, 75% of all women are prone to this infection once in their lifetime and 40-45% are prone to multiple episodes in their lifetime [1,2]. Denial or delay of treatment may lead to several complications, pronouncedly in pregnant women. Among the vulvovaginitis cases, infectious vaginitis was reported in about 90% which may be caused by the Candida species or bacterial species [3].

Candida is one of the commensal flora found in the gut, mouth, and genital tracts. They proliferate if alterations in their environment occur. Immuno suppression, antibiotics usage, pregnancy, menstrual cycle, diabetes mellitus may cause alteration in the environment and support the yeast proliferation [4]. Along with the infection, termination of useful microbes from the vaginal flora may occur. Lactic acid generation occurs in the genital tract which is the major metabolite from lactobacillus. And lactic acid has vital role in maintenance of normal vaginal pH of 3.8-4.4. Elevation of pH to 5.5-6.8 may cause proliferation of pathogeneic micro-organisms. Extreme acidic environment obstructs the proliferation of Lactobacillus and cause vaginal candidiasis infections [5].

Most of the vaginal candidiasis cases arises due to *Candida albicans species* but recurrent or chronic form of diseases are caused by the non-*albicans* species [6-8]. Non-*albicans* species have low reactivity to azole antifungal [9]. Researchers consider that alarming rate of vaginal infections is caused by non-*albicans*, (*C. glabrata*, *C. tropicalis* and *C. dubliniensis*) which was witnessed in the recent times [10,11]. It has been reported that azole resistance was attained by the *Candida* species; specifically *C. glabrata* isolates and even long term administration of anti-fungal drugs may lead to recurrent vaginitis [12,13]. Resistance development made the researchers interest towards the exploration of an effective drug moiety [14]. Multi-factorial pathogenesis and increased resistant strains of Candida hinder the exisiting therapies [15,16].

Clotrimazole (CTZ) is classified under class of Imidazole derivatives. It is effective against several species i.e., Isolates of dermatophytes, pathogenic yeasts, and filamentous and dimorphic fungi, as well as some gram-positive bacteria. O-Prasertsawat P et al. [17] have reported by their comparative study that CTZ can be considered as an alternate drug for the vulvovaginal candidiasis therapy. There wasn't any significant differences in the clinical and overall cure rates when compared with Itraconazole and Fluconazole. CTZ (Canesten 1%) can be applicable for the first-line therapy for vulvovaginal candidiasis [18]. These evidences suggest that CTZ can be considered for the treatment of vulvovaginal candidiasis. It is indicated topical treatments i.e 1% cream, applied twice daily & 100 mg, 200 mg or 500 mg pessaries [19,20]. The Long term oral administration of the CTZ (100 mg/kg) for few

weeks resulted in gastrointestinal and hepatic adverse effects and drug interactions i.e interaction with hepatic microsomal enzymes with subsequent interference with its own metabolism. Its low bioavailability and its log *P* value (3.5) indicate that it is highly hydrophobic in nature, and pKa (6.7) permits its transdermal application. The topical administration minimizes the systemic side effects and toxicities and it is beneficial due to direct delivery and targetability at site of action despite low bioavailability hinders the topical delivery [21,22]. However, currently available topical formulations are not capable of retaining the drug on the skin for longer duration so rationalization and development of formulations with longer retention time are essential [23].

Several researchers were successful in overcoming the aforementioned issues by exploiting the solid lipid nanoparticles (SLN) for topical administration. Utilizing the SLN drug delivery system, fungal burden was effectively diminished and exhibited significant therapeutic efficacy when compared with the commercially available formulations [24-27]. These evidences illustrate the success rate and potential of SLN as drug delivery system for topical administration of CTZ. Stratum corneum is the primary site of action for the CTZ where the pathogens reside mainly. The range of Minimum inhibitory concentration (MIC) of CTZ is between 0.001-0.01 µg/ml and fungal inhibitory concentration is between 0.003–0.006 µg/ml. To achieve effective therapeutic action drug concentration should be above the MIC at the site of action. So, in this aspect we adopted SLN to achieve the controlled drug delivery to maintain the drug concentration in deeper layers of the skin [27].

Vaginal Candidiasis is associated with several symptoms like heavy white curd-like vaginal discharge and dryness [28]. To retain the CTZ-SLN at the site of action another medium is necessary for the bypassing the aforementioned problems. This can be overcome by the concept of 'bio-adhesion' because our target is "underlying tissue". So, a suitable medium is necessary for the CTZ-SLN to be occluded in the aqueous environment and protect from the vaginal discharge which may increase the availability of the drug at the target underlying tissue [29].

In this context, Hyaluronic acid (HA) gel was employed in our study as it is characterized by high viscous and adhesion features which may lead to prolonged presence of drug at the site of application [30,31]. HA is a linear chained polysaccharide classified under the family of glycosaminoglycans which are principal constituents of the extra cellular matrix. In physiological pH, it exhibits a polyanionic structure that gives good hydro-coordinating features facilitating the water-retention [32]. Vaginal dryness condition in the vaginal candidiasis can be avoided by the water retention property of HA [33]. So, loading of CTZ-SLN in HA gel tends to achieve sustained release of drug from the lipid shell and vaginal discharge can be avoided. So, the present work focused on developing CTZ-SLN loaded HA gel for the vaginal candidiasis treatment.

2. MATERIALS AND METHODS

2.1 Materials

Palmitic acid and Ethanol- HPLC grade were purchased from SD-Fine chemicals limited, Mumbai, India. Clotrimazole HCl and Poloxamer 188 (F68) were purchased from Sigma-Aldrich, Bangalore, India. Sabouraud Dextrose Agar was purchased from Himedia chemicals Ltd, Mumbai, India. Sodium hyaluronate with a molecular weight of approximately 1300 kDa was a gift sample from Kumar organic products Ltd., Bangalore, India. All other chemicals and reagents used were of analytical grade. Millipore water was utilized for all the studies. The organisms a) *Candida albicans* b) *Saccharomyces cerevisiae* c) *Candida glabrata* d) *Candida tropicalis* was obtained from Institute of Microbial Technology (IMTECH), Chandigarh, India.

2.2 Pre-formulation Studies

2.2.1 Drug-lipid interaction study by differential scanning calorimetry (DSC) analysis

The interaction between CTZ and palmitic acid was determined the melting points using the DSC. Thermograms were recorded for CTZ, palmitic acid, physical mixture and CTZ-SLN. High purity indium metal was employed as standard for instrumental calibration. In nitrogen atmosphere, with a heating rate of 10℃/min, dynamic scans were carried out at temperature range 10-300℃.

2.3 Preparation of CTZ-SLN

CTZ-SLN were prepared by solvent diffusion technique with slight modifications [34]. Palmitic acid and CTZ were dissolved completely in ethanol in a water bath at 70℃. The obtained organic solution was immediately dispersed into aqueous phase containing Poloxamer 188 (F68) under continuous mechanical agitation at 400 rpm in a water bath at 70℃ for 5 minutes. The resultant pre-emulsion (melted lipid droplet) was transferred into an ice bath to solidify the lipid droplets and then brought to room temperature till SLN dispersion was formed. The SLN dispersion was dialyzed in distilled water for 12 hours to remove water-soluble impurities (organic solvents and nonadsorbed surfactants) and subsequently centrifuged (7000 rpm, 5 minutes) to remove unentrapped CTZ and larger lipid particles. The final SLN dispersant was lyophilized.

2.4 Drug Loading and Encapsulation Efficiency

SLNs (100 mg) were extracted with 5 mL of ethanol, diluted with pH 4.5 citrate-phos phate buffer, filtered and analyzed for drug content after suitable dilution, at 263 nm [35].

2.5 *In vitro* Drug Release Studies

Release studies were carried out on prepared formulations in triplicate, employing a basket type dissolution tester-USP XXII (TDT-08L, Electrolab, India) using 600 mL of pH 4.5 citrate phosphate buffer as dissolution medium at 100 rpm at 37 ± 0.5℃ to mimic the vaginal conditions [36-38]. Five ml of the sample was withdrawn at different intervals and analyzed by the UV method at 263 nm [35].

2.6 Determination of Particle Size, Zeta Potential and Morphology

The particle size, zeta potential, and polydispersity index were determined by zetasizer 3000HAS (Malvern Instruments Ltd, Malvern, UK). Before measurement the samples were suspended in deionized water (1 mg/ml). All measurements were carried out at 25℃ and performed in triplicate. The morphology of CTZ-SLNs was determined by scanning electron microscopy (SEM, JEM-200 CX, JEOL, Tokyo, Japan) [39].

2.7 Determination of Drug Entrapment Efficiency and Drug Loading

The quantity of CTZ in SLN was determined by HPLC. About 10 mg prepared SLN was dissolved in 10 ml of ethanol and then centrifuged by a Sigma-3k 30 Centrifuges (Sigma-Aldrich, Seelze, Germany) with 14 000 rpm for 10 min at the temperature of 37℃. Further, extraction by ethanol and filteration using 0.45 mm filter was carried out. The drug content in the supernatant was measured by HPLC method using an mobile phase delivery pump (LC-20 AD; Shimadzu, Japan) of flow rate 1.0 ml min^{-1}, a photodiode array detector (SPDM20A; Shimadzu, Japan) set at 222 nm, a 20 µL loop (Rheodyne) and Phenomenex Gemini C_{18} (250 mm × 4.6 mm) was used. The mobile phase consisted of ethanol and 25 mM of phosphate buffer (pH 4.0) in 80:20 ratio [40]. The encapsulation efficiency and drug loading were calculated by the following formulae.

$$\text{Entrapment efficiency}(\%) = \frac{\text{Weight of drug in SLN}}{\text{Weight of the drug fed initially}} \times 100$$

$$\text{Drug loading}\ (\%) = \frac{\text{Weight of drug in SLN}}{\text{Weight of SLN recovered}} \times 100$$

2.8 Formulation of CTZ-SLN Loaded Na-Hy Gel

Na-Hy gels were prepared by dissolving Sodium hyaluronate with a molecular weight of approximately 1300 kDa [41,42] (2.28% w/v) in HEPES/NaCl buffer (10/115 mM, pH 7.4). CTZ-SLNs were dispersed in the gel. Then gels were triturated using a magnetic stirrer, maintained at room temperature for 1 h and stored at 4℃ for 12h prior to administration or analysis [43].

2.9 *In vitro* Release Studies

The *in vitro* drug release studies were carried out using Franz diffusion cell (Perme Gear Inc., Bethlehem, PA) with donor chamber and water jacketed receptor chamber (20 ml) maintained at 37℃ [44]. Commercial semi-permeable cellophane membrane (Fischer Scientific Co., London, England; pore size 0.45 µm) was utilized as permeation barrier which was rinsed overnight in Simulated Vaginal Fluid (SVF) before the study. 1 gm of gel was applied

carefully on permeation membrane; which was lodged between the donor and receptor compartments. Receptor compartment was maintained with 20 ml SVF, while donor compartment was vacant and uncovered. The conditions of receptor compartment were maintained at 37.5℃ with continuous stirring at rate 25 rpm using a magnetic stirrer. At regular time intervals, 5 ml - Aliquots were withdrawn from receptor compartment and equivalent amount of fresh media (37±5℃) was replaced to maintain the sink conditions. After withdrawal the samples were analyzed by UV-spectrometer at 263 nm. The study was carried out in triplicate.

2.10 Determination of pH

The pH of prepared Na-Hy 2% (w/w) gel was detected using a digital pH meter (Mettler Toledo MP 220, Greifensee, Switzerland) at 25 ℃ in triplicate.

2.11 Determination of Viscosity

Viscosity of Placebo Na-Hy gel and developed CTZ-SLN loaded Na-Hy based gel was evaluated using Brookfield viscometer (DV-II, LV model, Brookfield, USA). The viscosity was determined (n=3) at three different conditions (4℃, 25℃ and 37℃) at different rotational speeds from 0.5-20 rpm with a torque of near to 100%. The samples were equilibrated for 10 min prior to the analysis [45].

2.12 Determination of Spreadability

Wide spreadability is one of the requisite for a gel preparation to meet the ideal qualities. It is evaluated by the extent of area to which gel immediately spreads at site of application. The spreadability was determined by applying 0.5 g gel within a pre-marked circular area of 1 cm diameter on a glass plate with specifications of 5 mm thickness and 15 cm^2 area. And another glass plate with equal dimensions was placed on it; ensure that entrapment of the air bubbles avoided between two slides. Standard weight of 500 g was placed on the upper glass plate for 5 min to spread the gel uniformly. Higher the area of the gel spread over the plate is an indicator of efficient spreadability [46].

2.13 *In vitro* Bioadhesion Studies

In vitro bioadhesive potential of CTZ loaded Na-Hy gel, CTZ-SLN loaded Na-Hy gel was evaluated and compared with placebo Na-Hy gel, In brief, an agar plate (1%, w/w) was prepared in pH 4.5 citrate phosphate buffer (pH = 4.5) and sample (50 mg) was applied at central location and left for 5 min. Then, plate was fixed with USP disintegration test apparatus and made upward downward movements in SVF (pH 4.5) at 37℃. The movement of instrument arm was made in such that the sample on the agar plate was submerged into buffer at the low position and was taken out of solution at the high position. By visual observation, the residence time of samples on plate was determined [47].

2.14 *In vitro* Antifungal Studies

Antifungal activity was evaluated by the cup-plate method using Sabouraud Dextrose Agar plates inoculated with different fungal species a) *Candida albicans* b) *Saccharomyces cerevisiae* c) *Candida glabrata* d) *Candida tropicalis*. A volume of 20 mL of sterilized agar media was dispersed into a sterilized Petri dish and allowed to solidify. Each Petri dish was divided into three sectors, and a bore (6 mm) was made in each sector using a sterile cork borer. Each bore in a different sector was loaded with a placebo polymer (negative control), Clotrimazole pure drug (positive control) and CTZ-SLN loaded gel. Petri dishes were incubated at the temperature of 37 ± 0.5℃ for 24 h to allow the growth of microorganisms. The zone of inhibition produced by the CTZ – SLN loaded Na-Hy gel towards the organism was measured (mm).

3. RESULTS AND DISCUSSION

3.1 Drug-Lipid Interaction Study by Differential Scanning Calorimetry (DSC) Analysis

DSC thermogram of CTZ, Palmitic acid, physical mixture (drug, palmitic acid), and CTZ loaded SLNs are presented in (Fig 1). The DSC thermogram of CTZ and Palmitic acid exhibited a melting endotherm at 148℃ and 63.1℃, respectively. No shift in the melting peaks of physical mixture was observed as compared to melting endotherm of individual ingredient indicating the drug-lipid compatibility. Melting endotherm for CTZ was not formed in thermogram of final formulated CTZ loaded SLNs (SLNs IV), indicating the drug was entirely occluded inside the lipid matrix of SLNs.

3.2 Drug Loading and Encapsulation Efficiency

Encapsulation efficiency ranged from 67 to 80%. Generally the encapsulation efficiency and drug content increases with increasing amounts of polymers in the SLN. Formulation (SLNs IV) showed relatively higher encapsulation efficiency indicating high polymer concentration. It can be inferred from the results that there was a proper distribution of CTZ in the SLNs. During the encapsulation process, mechanical variables cause loss of the final product and hence process yield may not be 100%. Formulation (SLNs IV) showed maximum drug loading of 21.8±0.5%. The results obtained are given in Table 1.

3.3 *In vitro* Drug Release Studies

The release profile of the drug from SLNs clearly indicates that the concentration of polymers slows the release of CTZ from SLNs. At the end of 12 h, *in vitro* drug release from formulation (SLNs IV) was found to be 98.8% in the vaginal environment, as shown in Fig. 2. The total cumulative quantity of the drug released at the end of the 12 h dissolution test was below 100%. This may be in part 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.

3.4 Physico-Chemical Characterization of CTZ-SLN

SEM studies revealed that CTZ-SLN (SLNs IV) were almost uniform-sized; mono-dispersed spherical shaped. It was observed majority of SLN showed smooth surface morphology (Fig. 3).

3.5 Particle Size Distribution

The composition of optimized formulation (SLNs IV) showed particle size 248 ± 1.31 nm (Fig. 4), zeta potential -16.2 mv, drug loading 21.8 ± 0.56% and entrapment efficiency 80.7% which were in good agreement with the predicted values particle size 247.44 nm and entrapment efficiency 82.02%.

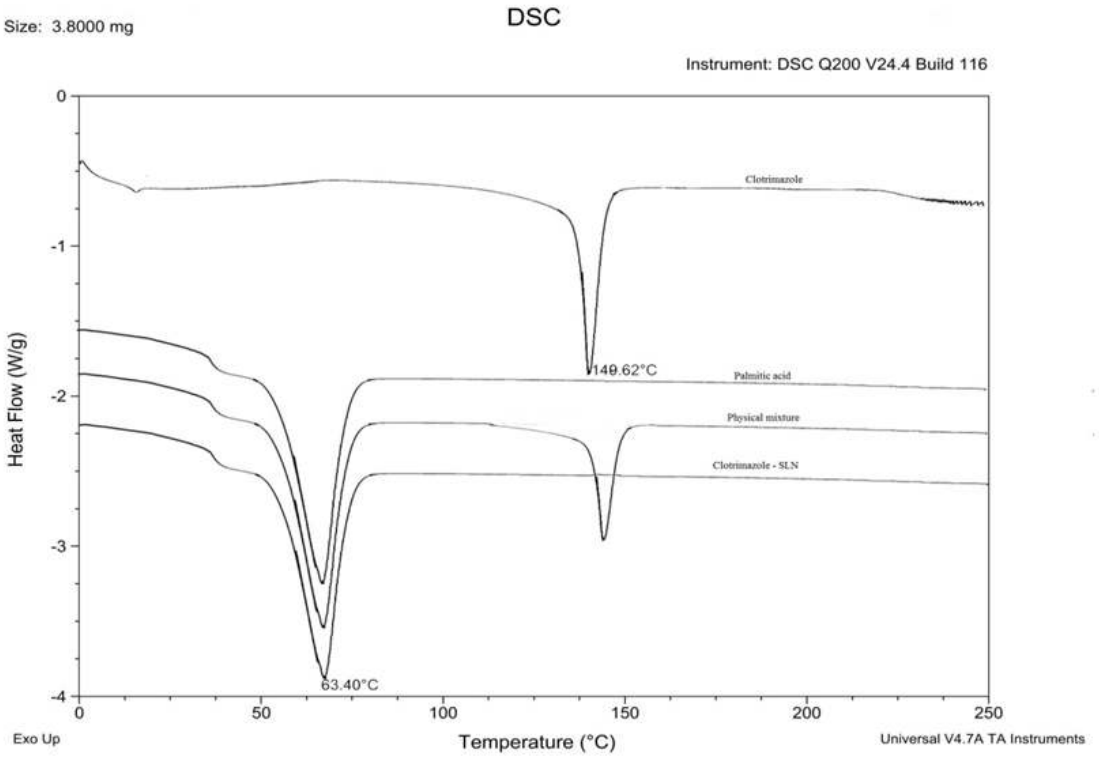

Fig. 1. DSC thermograms of CTZ, Palmitic acid, physical mixture and CTZ-SLN (SLNs IV)

Table 1. Evaluation parameters of CTZ SLNs

Formulation (SLNs)	CTZ (mg)	Palmitic acid (mg)	Poloxamer 188 (F68)	Ethanol (ml)	Actual drug loading (%)[a]	Encapsulation efficacy (%)[a]
I	100	100	10	1	16.6±0.4	67.6±0.7
II	100	200	20	2	18.2±0.9	72.3±0.4
III	100	300	30	3	19.9±0.7	78.8±0.6
IV	100	400	40	4	21.8±0.5	80.7±0.7
V	100	500	50	5	19.6±0.4	79.9±0.7

a Mean ± SD, n=3

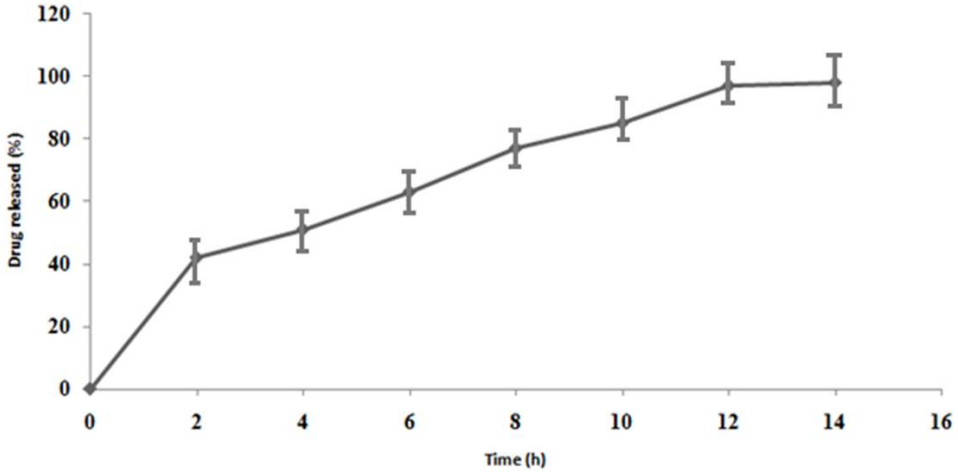

Fig. 2. Drug release profile of CTZ from SLNs (SLNs IV) (mean ± SD, *n* = 3)

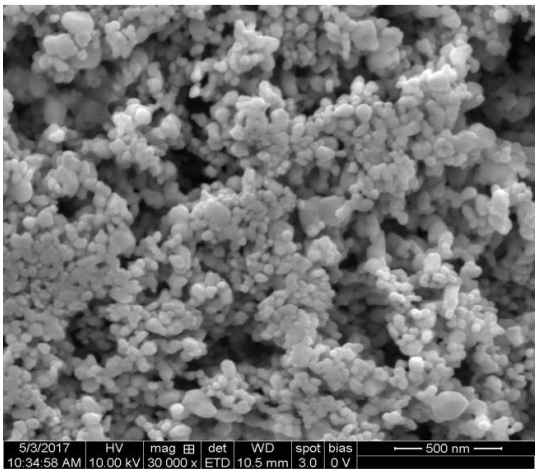

Fig. 3. SEM image of optimized CTZ-loaded SLN

3.6 *In vitro* Release Studies

In vitro drug release studies renders necessary information about the pretended release of the dosage form during in vivo conditions. The results of *in vitro* release studies exhibited prolonged drug release from the developed formulation. CTZ-SLN loaded Na-Hy gel had exhibited prolonged release upto 12h and it was devoid of any burst release. Prolonged release from lipid nanoparticles was due to slow erosion of SLN and accompanied by diffusion of drug into the external environment (Fig. 5). And the Na-Hy gels contribute the standby of the released drug at the site of action and this creates by-stander action of the drug. This creates scope for enhancing vaginal drug delivery and sustains the necessary drug concentration for the vulvovaginal candidiasis therapy.

3.7 Determination of pH

The pH of CTZ-SLN loaded Na-Hy gel was found to be 4.43 which is equivalent to vaginal pH.

3.8 Determination of Viscosity

Viscosity is an important rheological parameter involved in its usage. Higher viscosity hinders the instillation and lower viscosity cause drainage. So, an ideal viscosity gel is necessary to show effective delivery. There wasn't any change or difference in the viscosity between placebo gel,

CTZ Na-Hy gel and CTZ-SLN Na-Hy gel. Presence of either CTZ-naïve or CTZ-SLN didn't hinder the viscosity properties of the Na-Hy gel in different conditions (Table 2).

3.9 Determination of Spreadability

Spreadability is a vital feature of semisolid dosage forms which influence the ease of administration and patient compliance. Ideal gel will spread in a short duration which ultimately enhances the ease of application. Spreadability is calculated by the change in the diameter of earlier drawn circle (1 cm) by the application of weight. In case of Placebo gel exhibited 7.5 cm, CTZ Na-Hy gel of 7.2 cm and CTZ-SLN loaded Na-Hy gel exhibited 7.8 cm the overall elevation in diameter was reflects good spreadability of Na-Hy gel and no variation was observed in both samples.

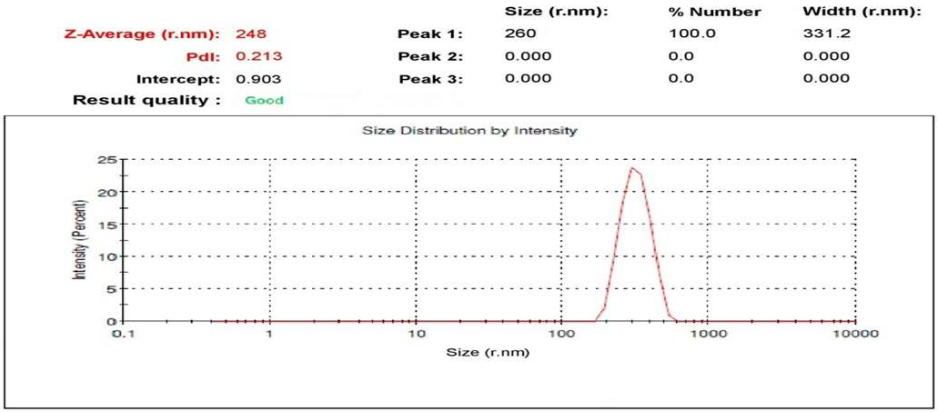

Fig. 4. Particle size distribution analysis report of optimized CTZ-SLN

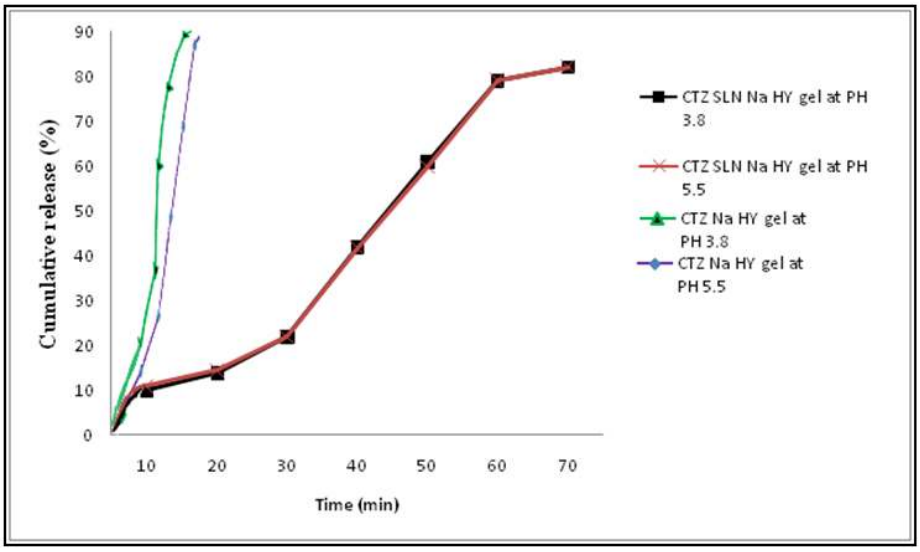

Fig. 5. *In vitro* release behavior assessment of CTZ loaded Na-Hy gel and CTZ-SLN loaded Na-Hy gel at different pH 3.8 and 5.5

Table 2. Determination of viscosity of placebo gel, CTZ Na-Hy gel and CTZ-SLN Na-Hy gel at different conditions

Sample	4°C	25°C	37°C
Placebo Na-Hy gel	88351 cps	786510 cps	782236 cps
CTZ -SLN	89394 cps	784550 cps	791672 cps
CTZ-SLN loaded Na-Hy gel	88716 cps	782400 cps	790149 cps

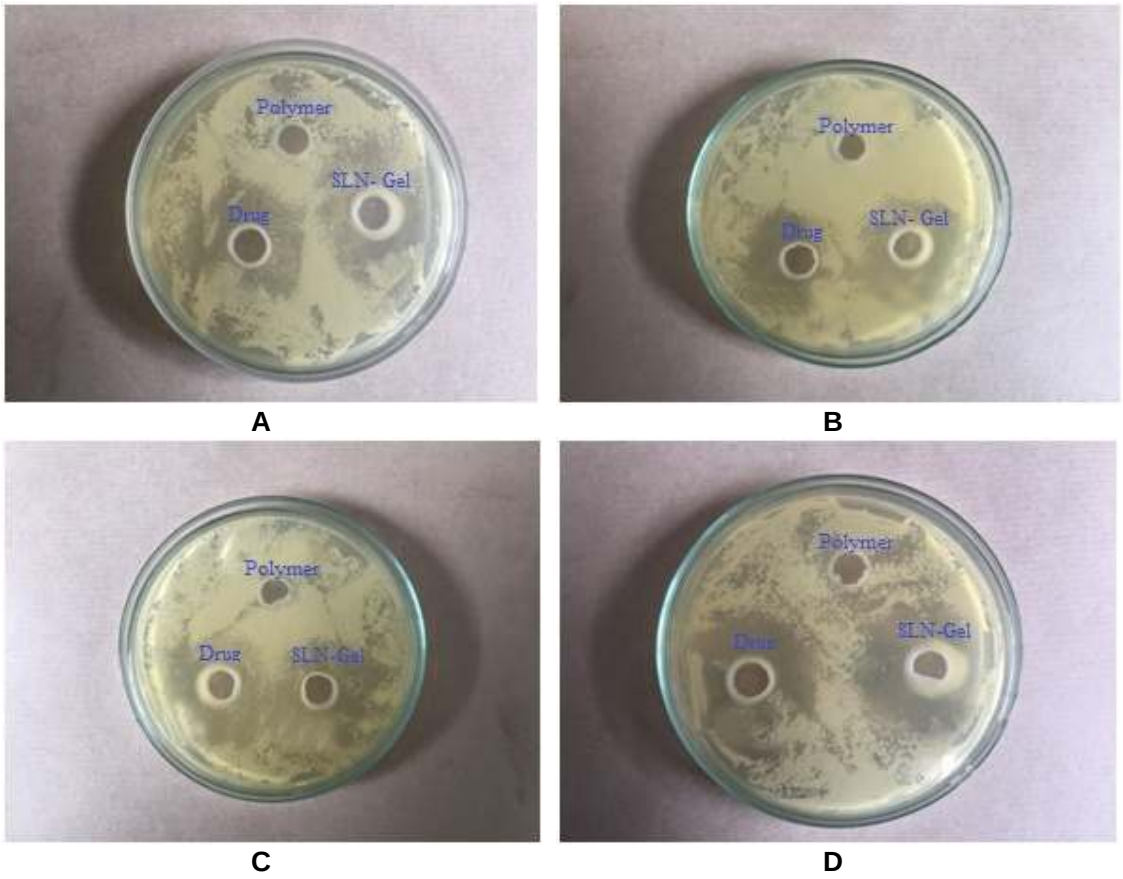

Fig. 6. *In vitro* anti fungal activity of Clotrimazole SLN loaded Na-Hy Gel (SLN-Gel) in comparison to Pure drug (CTZ) using different fungal strains: a) *Candida albicans* b) *Saccharomyces cerevisiae* c) *Candida glabrata* d) *Candida tropicalis* after 24 hrs. [Polymer- SLNs without drug]

3.10 *In vitro* Bioadhesion Studies

Bio-adhesion is the interfacial force held together between two materials (either two biological or synthetic and biological) for certain period of time. When an interaction occurs between any material (polymer/lipid) and epithelial surface is termed as bioadhesion. In the context of bioadhesive dosage forms, bioadhesion is adhesion occurring between soft tissues and polymers, either natural or synthetic. Determination of bioadhesion is crucial to confirm that adhesion caused by the developed Na-Hy gels is adequate for the prolonged retention at the site of administration. And high pressure should not injure the mucous membrane. The bioadhesive potential of CTZ-SLN loaded NA-Hy gel, CTZ loaded gel and placebo Na-Hy gel have exhibited 64±1.8 min, 64±1.2 min and 62±2.7 min respectively (n = 3). Besides, the retention time of CTZ-SLN loaded Na-Hy gel was higher when compared with placebo gel. Presence of Na-Hy gel provided sufficient retention and prolonged action was observed.

3.11 *In vitro* Antifungal Studies

An antifungal study with Sabouraud Dextrose Agar medium showed that the CTZ-SLN loaded gel was able to control (inhibit) the growth of different fungal species a) *Candida albicans* b) *Saccharomyces cerevisiae* c) *Candida glabrata* d) *Candida tropicalis* for more than 24 h. The formulation (SLNs-gel) showed an average zone of inhibition of 19.3 ± 0.5 mm, which was higher compared to the average zone of inhibition of pure Clotrimazole (CTZ) i.e.,14.4 ± 0.4 mm. Also there was no significant effect produced by placebo polymer which implies that the polymer as such has no activity and no interference with the activity of the drug.

4. CONCLUSION

CTZ loaded SLNs were developed using palmitic acid as lipid matrix, Pluronic F-68 as surfactant and ethanol as solvent using solvent injection method. Influencing parameters like surfactant concentration, amount of drug and volume of organic phase were selected for the optimization and carried out by Trial and error basis to develop a CTZ-SLN formulation with minimal particle size and higher entrapment efficiency. Optimized CTZ-SLN (SLNs IV) exhibited good particles which were in good agreement with the predicted values. CTZ-SLN has exhibited sustained release of drug. This kind of release is desirable when the dosage need to be reduced. The *in vitro* bio-adhesive potential was evaluated for CTZ-SLN loaded Na-Hy which was sufficient enough to avoid the drainage of the drug and exhibit bystander action at the site. MIC values of different fungal species a) *Candida albicans* b) *Saccharomyces cerevisiae* c) *Candida glabrata* d) *Candida tropicalis* have revealed good anti fungal activity of the CTZ-SLN loaded Na-Hy gel formulation. Earlier, extensive efforts and research has been focused on developing CTZ-SLN [24-27] to diminish the fungal burden but delivery and patient compliance can be greatly improved by the Na-Hy delivery.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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