



CHAPTER - X

SUMMARY AND CONCLUSION

10.1 SUMMARY

Vaginal drug delivery has received considerable attention during last few years for noninvasive delivery of various therapeutically active compounds. Vaginal drug delivery system offers a lot of advantages for local and systemic treatment. In the recent year, research has been focused on the development of novel vaginal drug delivery system for the prevention of undesirable conception, vaginal infections, sexual transmitted diseases (STDs) and cure of other gynecological conditions. These innovative delivery systems also may enables to extend product life cycle and remains competitive in the market place.

This thesis describes the development and characterization of bioadhesive vaginal formulations for vaginal delivery of itraconazole (ITR) and clindamycin phosphate (CL). Introduction of vaginal drug delivery with respect to vaginal anatomy, physiological and pathological consideration, physiochemical factors associated with the drug, factors associated with the dosage form, carriers for drug delivery and experimental models is discussed in **chapter I**.

Chapter II describes the significance of present investigation. Bacterial vaginosis and vulvovaginal candidiasis are the two most common forms of vaginitis in female patients. Vaginitis is one of the most frequent genital infections occurring in women of all age groups. Bacterial vaginosis has been linked with an increased risk of STD/HIV acquisition. About 75% of women experience an acute episode of vaginal candidiasis once in their lifetime, most commonly during pregnancy or after treatment with antibiotics.

The increasing incidence of vaginitis has highlighted an urgent need of establishing therapeutic strategies that can fulfill certain functions such as successful eradication of the infectious agent, short-term treatment, achievement of high drug levels at the target site, the avoidance of the first-pass metabolism and safety.

Itraconazole is a new orally active triazole antifungal drug with a broad spectrum of activity. This agent appears to be an attractive alternative for treatment of vaginal candidiasis because of its enhanced activity against *Candida species*, leading to short courses of therapy. Currently, two antibiotics, metronidazole and clindamycin phosphate are most commonly used for treatment of bacterial vaginosis. But, use of metronidazole associated with multiple side effects including gastrointestinal side effects. Hence, CL and ITR were selected as model drug in present investigation for development of bioadhesive vaginal formulations.

The perceived convenience of oral medications is often associated with risk of systemic adverse drug reaction and potential to interact with concomitant medication. From such perspective, topical treatment could represent a rational choice to treat localized infections and is often recommended by various healthcare practitioners. The vast majority of gels, foams, creams, suppositories, and tablets are presently used as vaginal delivery systems which breakdown immediately later than insertion into the vaginal cavity and have minimal bioadherence to the vaginal walls. They are also associated with problems like messiness and leakage causing inconvenience to users, leading to poor patient compliance. Therefore, it has been difficult to achieve optimal potential effectiveness of the therapeutic agent with the existing vaginal delivery system. It has highlighted urgent need of adequate delivery system aimed to hold formulation closed to vaginal mucosa for long time and achievement of high drug levels at the infectious site. Bioadhesive formulations can adhere to vaginal mucosa in order to bring drug in contact with vaginal mucosa for sufficient period of time and prevent expulsion of formulation. Thus, bioadhesive vaginal drug delivery system (BVDDS) is become best alternative to overcome limitations associated with existing vaginal delivery system. The vagina is a highly suitable site for delivery of bioadhesive formulations.

Usually, commercially available vaginal preparations such as creams, ointments, foams, gels, pessaries, and suppositories requires cleansing of the applicator and reuse during course of treatment, which women may find inconvenient. Hence, we selected **polymeric film** and **soft gel formulation** for the vaginal administration due largely to the fact that it was easy to use (applicator not required for their administration), and did not produce a lot of discharge. **Chapter III** describes the profiles of drugs used in this study.

Chapter IV describes preparation and characterization of solid dispersions of ITR. ITR possess very poor water solubility ($\sim 4 \mu\text{g/ml}$ at acidic pH) that makes it difficult to formulate in bioadhesive vaginal delivery system. One approach, which has been applied for producing BVDDS of ITR, is use of solid dispersion of ITR (SDITR) and hydroxypropyl methylcellulose (HPMC) E15 in which drug particles are homogeneously distributed throughout the hydrophilic polymer. **SDITR** can improve wettability of ITR which may help in development of bioadhesive delivery system and dispersing ITR throughout the vaginal cavity. Also, SDITR forms colloidal dispersion in simulated vaginal fluid (SVF) is essential for local action in vaginal cavity.

Solvent evaporation method was used for the preparation of SDITR. The results showed that the greater extent of solubility was obtained with spray drying techniques as compared to conventional solvent evaporation method. SDITR were investigated using various polymers

such as PVP K30, d-manitol, PEG 6000, d-sorbitol, chitosan, HPMC E15 premium, and polyvinyl alcohol. HPMC E15 premium was selected for preparation of solid dispersions. The prepared solid dispersions were characterized by differential scanning calorimetry and X-ray diffraction technique. DSC thermogram of solid dispersions shows complete absence of ITR peak which be a sign of formation of an amorphous SDITR. The XRD results of SDITR indicate the transition of ITR from crystalline to amorphous state, results in enhance solubility of drug in SVF. ITR content of solid dispersions was found in range of 97.20 to 103.45% suggest the preparation of SDITR with high content uniformity. An *in vitro* antifungal activity exerted by ITR alone and SDITR was performed by cup plate method. The higher antifungal activity was observed with solid dispersion. This may be due to get better solubility of ITR in SVF. The results of the stability study showed that there is no significant change observed in color, and assay of ITR in solid dispersions stored at intermediate and accelerated stability conditions. Also no recrystallization of the ITR was observed in this time period suggesting their physically stability under specified storage condition.

Chapter V describes the development and evaluation of bioadhesive vaginal film of ITR. HPLC method was developed for evaluation of ITR content in film and softgel. The proposed method was evaluated for linearity, specificity, precision, and accuracy. The results revealed that the analytical methods were selective, and accurate with high precision. The method could also estimate the ITR in presence of other constituents of film and softgel without any interference suggests their specificity.

Solvent evaporation method was used for the preparation of the film. Attempts were made to prepare film formulation using ITR alone (instead of solid dispersions), but it was not successful. This may due to very less aqueous solubility ($\sim 4 \mu\text{g/ml}$ at acidic pH) of ITR. Solid dispersions can improve wettability of ITR and produce homogenous aqueous dispersions. Therefore, SDITR has been used for development of film formulation in present investigation. In preliminary experiments, different water soluble plasticizer and polymers were explored for development of bioadhesive film. Film prepared using glycerol and d-sorbitol as plasticizer, could not remove from casting surface. Physical characteristics and other mechanical properties of ITR film prepared from HPC and PEG 400 was found acceptable. Also, HPC is helpful to improve bioadhesion property of the film formulation. Hence, these were selected as formulation variables and effect of different levels was studied by **3^2 full factorial designs**. In the present work, total nine formulations were designed from 3^2 full factorial designs in a fully randomized order. The dependent variables to be measured are tensile strength (TS), percentage elongation at break (%EB) and % drug retained on the

vaginal mucosa up to 8 h (Y_{8h}). Films were also evaluated for various aesthetic (appearance, odor, color, flexibility and peelability) and physicydynamic properties such as moisture content, pH and viscosity of polymeric dispersion, swelling index, film thickness, content uniformity and bioadhesion.

Drug-excipients compatibility was studied by thermal (DSC) and isothermal (HPLC) methods. DSC thermogram of film formulation shows complete absence of endothermic peak which indicates no risk of transforming physical state of ITR from an amorphous to crystalline state during preparation of film. The results obtained from thermal and isothermal testing suggest the compatibility between ITR and excipients to be used for preparation of film. The mechanical properties %EB and TS of all the design films were found in range of 51.42–70.08% and 6.99–10.46 N/mm² respectively, suggesting their “soft” and “tough” nature. An interesting finding was decrease in TS and increase in %EB of film as a function of plasticizer by weakening the intermolecular interactions between polymer chains.

The **desirability function** was used for optimization of formulation composition by combining all the responses in one desirability function. Desirability function was calculated for TS, %EB and % drug retained on vaginal mucosa at 8h. Batch ITRF₆₅ showed highest overall desirability of 0.92. Therefore, this batch was considered to be optimized batch and values of formulation variables of this batch were considered to be optimum values for film formulation. Excipients used in films are **GRAS** listed and approved for vaginal use.

A newly developed ITR films are colorless, odorless, flexible, uniform and possesses smooth surface. Average drug content was found between 94.5 to 98.9% of label claimed of ITR per film (2.5 cm × 2.5 cm). The viscosity of polymeric dispersion of film was found to be more in SVF (7.84 ± 0.25) as compared to water (7.57 ± 0.31) which governs the spreading and retention characteristics of the formulations. The pH of ITR film was found to be slightly acidic (4.90 ± 0.04) in SVF and alkaline (7.04 ± 0.07) in water reveals that vaginal pH as well as micro flora may remains unaffected after administration of bioadhesive film. The little amount (7.66 ± 0.51 %w/w) of moisture content in film helps them to remain stable and prevent from being a completely dry and brittle film. The **scanning electron photomicrographs** of the film at 1000X magnification confirm that film surface was free from any scratches or transverse striations. The smooth surface of film may reduce chance of mechanical injury during insertion in vaginal cavity and improving patient compliance. The swelling capability of polymer is prerequisite for its bioadhesiveness. Maximum swelling was seen with film containing high content of HPC.

HPC has very good bioadhesive property. This may be explained by the fact that the HPC contain a large number of hydroxyl groups that provide the ability to form hydrogen

bonds with the mucosal membrane. Therefore, the bioadhesive strength of film was enhanced with increased in content of HPC. ITRF₆₅ showed good bioadhesion (0.368 ± 0.02 N) under simulated vaginal environment. **Retention behavior of film was studied using simulated dynamic vaginal system which mimics the physiodynamic conditions of vagina.** Simulated dynamic vaginal system consisted of closed glass cell with 30° angle slope and flow rate pump. Initially, the film softened on the vaginal mucosa after absorbing SVF and became a swollen structure, helping it to adhere to the vaginal mucosa. Film would form bioadhesive layer over vaginal mucosa which may help to achieve high drug levels at infectious site for longer time. Interesting findings of *ex vivo* retention studies is the residence time of film increased as the ratio of HPC to PEG 400 in film increased.

***In vitro* antifungal activities** exerted by ITR alone and their film formulations against *C. albicans* were performed by cup plate method. The results of this study manifested that mean diameter of zone of inhibition obtained with the film formulation was larger than that of ITR alone. However, ITR have inhibitory effect against *Candida albicans*, it should not affect the growth of normal vaginal flora (lactobacillus). Lactobacillus acidophilus is considered normal vaginal flora which help to maintain acidic environment of vaginal cavity. If the growth of lactobacilli is reduced, the pH may rises to a level between 4.5 and 7.0 which favor the growth of anaerobic or facultative anaerobic organisms already present in vaginal cavity. Therefore, Lactobacillus inhibition study was performed for all the developed bioadhesive vaginal formulations. *In vitro* activities exerted by ITR alone, placebo film and their bioadhesive film against lactobacillus acidophilus could be estimated by using cup plate method. The results of these studies point out that ITR and bioadhesive polymers did not significantly affect the growth of lactobacillus. It confirms that bioadhesive polymers could safely use for preparation of ITR film without affecting normal vaginal flora.

Improvement in the therapeutic efficacy of ITR, when delivered as bioadhesive formulation could be convincingly explained by assuming prolonged residence time of film on vaginal mucosal surface. Such hypothesis was supported by the results obtained from in vivo studies that successful clearance of *C. albicans* was achieved only in rats which receive film formulation. It was making the therapeutic benefit of ITR containing bioadhesive film significantly higher ($P < 0.041$) than that exerted by ITR alone. The results obtained from histopathological study reveals that repeatate use of bioadhesive film did not alter the morphology of vaginal mucosa. Also, films were found physically and chemically stable under accelerated conditions suggesting their suitability for tropical climates.

Chapter VI describes the development and evaluation of clindamycin phosphate containing bioadhesive vaginal film. CL was not analyzed by UV spectrophotometric method because of absence of chromohperic group in their chemical structure. Hence, **HPLC method** was developed for estimation of CL from its film and softgel formulations. Different experimental variables were studied in order to achieve optimum separation. Mobile phase composition of ACN: 25mM NaH₂PO₄ buffer of 45:55, v/v adjusted to pH 4.75 was finally recommended for chromatographic separation of CL. The best sensitivity of the method was obtained at 205 nm with mobile phase flow rate of 0.6 mL/min. The proposed method was also found very accurate and précised for quantification of CL. Excipients did not show any interference peak at retention time corresponds to CL peak suggest the specificity of method.

As the desired product must dissolve in vaginal fluid, only water soluble polymers and plasticizers were explored for preparation of CL films. In preliminary trial, it was concluded that film containing only HPMC or HPC and CL with plasticizer could not formed film of desired characteristics, necessitating the addition of another film forming polymer to improve physicodynamic properties of CL film. Films obtained with HPC, xanthan gum and PEG 400 has acceptable physical characteristics. Hence, HPC and xanthan gum was selected as formulation variables for further optimizations of the formulation. Drug-excipients compatibility was studied by **thermal (DSC) and isothermal (HPLC) method**. DSC thermogram of pure CL did not showed any sharp endotherms. This may due to combustions of the compound instead of melting at higher temperature. Hence, an isothermal testing method was carried out for drug excipient compatibility study. The results of isothermal testing confirmed the compatibility between CL and excipeints to be used for preparation of film.

In present work, **3² full factorial designs** was used to study all the possible combinations of all factors at all levels (two factors, three levels). Two independent factors i.e. concentration of HPC and xanthan gum were set at three different levels. Level of independent variables was decided from the results of the preliminary experiments. Three response variables such as % EB, TS and Y_{8h} were selected for systematic optimization studies. **Desirability function** was utilized to find out optimum level of HPC and xanthan gum out of nine batches. It was calculated for TS, %EB and Y_{8h}. Batch F₈₆ showed highest overall desirability of 0.99. Therefore, the values of independent variables of batch F₈₆ were considered to be optimum values for bioadhesive vaginal film. The prediction profiles were obtained for measured responses suggest, the xanthan gum concentration seems to be the most prominent factor in determining response value of film. Contour plots were obtained for the measured responses based on the model using sigma plot software. The contour lines

indicate that the addition of higher amount of HPC resulted a higher TS and lower % EB, while increase amount of xanthan gum resulted a lower TS and higher % EB. Therefore, the optimum level of xanthan gum was desired because greater amounts of xanthan gum resulted in lower TS and higher % EB that made films more soft and difficult to handle.

A newly developed CL films are odorless, flexible, very thin ($0.29 \pm 0.02\text{mm}$, Mean \pm SD, $n = 5$), and possess smooth surface. These features of films may reduce chance of mechanical injury during administration and improving patient compliance. Also, **scanning electron micrograph** of CL film captured at 150X, 1000X and 5000X confirm that film surface was free from any scratches or transverse striations. It contain small amount (8.40 ± 0.50 % w/w) of moisture that may be prevent them from being a completely dry. A dose of 100mg of CL was incorporated in 2.5×2.5 sq. cm surface area of film. Average drug content was found to be 97.23 ± 3.59 (Mean \pm SD, $n = 5$) percentage per unit of film ($2.5 \text{ cm} \times 2.5 \text{ cm}$). The pH of film was found to be slightly acidic (4.39 ± 0.04) in SVF that favors the growth of normal vaginal micro flora and maintain healthy vaginal environment. Higher viscosity of polymeric aqueous solution of film in SVF may improve retention of film in vaginal cavity. Maximum swelling was seen with film containing high concentration of HPC and xanthan gum. Rapid swellability of HPC and xanthan gum may prevent the fast erosion of polymeric film. Retention behavior of CL film was also studied using simulated dynamic vaginal system. Initially film gets swollen due to the fluid present at the site of administration and then form smooth, homogenous, viscous and bioadhesive gel/solution which has capability to retain in vaginal cavity for prolong period of time.

Lactobacillus inhibition study was performed by cup plate method. F₈₆ film and CL bulk powder at a concentration of 10mg per ml showed inhibitory effect on growth of *Lactobacillus acidophilus* after 24h, **but after 48h incubation Lactobacillus growth resumed and was comparable to that of the control.** The results were in agreement with previous clinical studies of intravaginal clindamycin showed initial suppression of lactobacilli growth. But growth and dominance of *Lactobacillus* was restored in a month. Stability studies of developed films were performed according to the ICH guidelines for zones IV countries like India. Overall results of the stability studies indicated that there is no significant change in assay, pH of polymeric dispersion, and mechanical properties of CL films which were stored at different stability conditions.

Chapter VII describes the development and evaluation of ITR containing vaginal soft gel. In present investigation ITR formulated in softgel capsule form. Because of their soft and elastic character, some patients view these capsules as easy to deliver in vaginal cavity as compared

to conventional tablets or hard gelatin capsule. These features of soft gel may reduce chance of mechanical injury during administration and improving patient compliance. They can easily scale up using existing softgel manufacturing facilities. Therefore, attempt was also made to formulate ITR in softgel.

In preliminary trial, different vehicle such as soybean oil, LLP and PEG 400 were explored for preparation of encapsulation material blend. Consistency of the blend is very crucial parameter for avoiding weight variation issues on large scale manufacturing. But soybean oil and LLP produced blend with lack of consistency. Also, soybean oil and LLP impaired bioadhesive property of prepared blend which may be due to its hydrophobic nature. But, ITR was homogeneously dispersed in PEG 400 and produced consistent encapsulation blend which may be due to hydrophilic nature of both PEG 400 and HPMC E15. Encapsulation blend should be sufficient viscous so that it cannot be readily expelled from vaginal cavity immediately after bursting of outer shell. Xanthan gum and HPMC E15 could enhance bioadhesion property of the formulation. Hence, HPMC E15 premium and xanthan gum was selected for preparation of blend that is encapsulated in soft gel. These formulation variables were further optimized by **3² full factorial designs** at three level and total nine formulations ISF₅₁ to ISF₅₉ were designed. Gelatin mass was prepared in gelatin mass reactor (AV pharma machinery, Mumbai) and kept at 40° to 50°C until fed it to film forming roller. All solid materials were passed through mesh 80 before blend preparation, so as to achieve a smooth and homogenous blend. Encapsulation was carried out by **continuous rotary die process** using softgel encapsulation machine (Hitech Pharma Equipments, Bhilad, Gujarat) using 20mm oblong cavity rollers. Softgel capsules were spread onto trays and kept under controlled temperature (26° to 27°C) and humidity (20% to 22%) for 48h to complete the drying activity. Soft gel capsules were evaluated for weight variation, content uniformity, pH and viscosity of encapsulation blend, moisture content, swelling index, drug release, retention and bioadhesive properties.

Average drug content was found between 96.14 to 103.17% that signify the uniform distribution of ITR in each softgel during encapsulation process. Moisture content was found satisfactory as it is between 6 to 10% which is allowable limit for softgel capsule. The study of swelling behavior of formulation was essential to understand its bioadhesive characteristics. Adhesion occurs shortly after the beginning of swelling but the bond formed is not very strong. Xanthan gum and HPMC E15 has property to get hydrated by absorbing large amount of water. Therefore, maximum swelling was seen with softgel containing high concentration of HPMC E15 and xanthan gum. Amongst nine softgel, ISF₅₉ showed good bioadhesion (0.490 ± 0.017 N) under simulated vaginal environment. This may due to higher

concentration of xanthan gum. Retention of a dosage form in vaginal cavity for prolonged intervals is desirable to achieve optimal potential effectiveness of the therapeutic agent and minimizes the need of frequent dosing intervals. Polymeric content of softgel forms viscous liquid when hydrated with water that increases their retention time in vaginal tube. ISF₅₉ showed very good retention (> 7 hours) in the **ex vivo experiment**. An interesting observation of these studies was residence time of formulation dispersion increased as the ratio of HPMC to xanthan gum in softgel increased. The **release of ITR** from vaginal soft gel was studied using small volume dissolution medium (100ml) maintained at 37±2°C. In this study, phosphate buffer (pH 4.5) was used as a dissolution medium. An interesting finding of these studies was the drug release is retarded as the concentration of xanthan gum increase in the formulation. This may due to xanthan gum swelled rapidly in contact with water and formed rigid gel and it is ultimately responsible for retarding drug release. **Desirability function** was utilized to find out optimum level of HPMC and xanthan gum out of nine batches. Desirability function was calculated for swelling index, retention time and % drug retained in vaginal tube at 5h. Batch ISF₅₉ showed highest overall desirability of 0.85. Therefore, the values of formulation variables of this batch were considered to be optimum values for softgel capsule dosage form. The prediction profiles were obtained for the measured responses using JMP 5.1, statistical discovery software. An interesting observation of these profiles was improved swelling index, bioadhesion, and Y_{8h} as the concentration of xanthan gum increased. The results of the stability study showed that there is no any significant change in the assay, pH and moisture content of softgel capsule at intermediate and accelerated study after 6 months.

Chapter VIII describes the development and evaluation of vaginal soft gel of CL for effective treatment of bacterial vaginosis. Smooth surface and enough flexibility of softgel which may help to minimize mechanical injury during insertion into vaginal cavity. Also, solid dosage forms are more preferred by women in different region of the world due to their aesthetic appeal and ease of application (applicator not required for their administration). Hence, attempts were also made to formulate CL in softgel capsule form.

Initially, soybean oil, LLP and PEG 400 were investigated as vehicle for preparation of encapsulation material blend. Inconsistency of encapsulation blends was observed in batch SF₁ and SF₂ because of soybean oil and LLP were getting separated from the dispersions. In SF₃ trial, CL and excipients were evenly dispersed in PEG 400 and produced consistent encapsulation blend. This may be due to hydrophilic nature of vehicle and other excipients.

Hydrophilic nature of encapsulation blend may favors the adhesion of formulation to mucosal surface and prevent expulsion of formulation. Viscosity of formulation in vaginal environment governs the retention characteristics of the softgel which is essential to get desired therapeutic effect. Therefore, a variety of water-soluble polymers such as HEC, HPC HPMC K15 and xanthan gum were investigated alone or in combination (trial SF₄ to SF₈). The viscosity of batch SF₇ was acceptable i.e. supported by their good consistency. Hence, xanthan gum, HPMC K15 and PEG 400 was selected for preparation of encapsulation blend. **Factorial experimental design and desirability function** have been proved to be a useful approach for the optimization of the formulations. In the present study, total nine formulations SF₇₁ to SF₇₉ were designed by 3² full factorial designs. All the designed formulations were evaluated for swelling index, retention time (Rt) and % drug retained in vaginal tube at 5h (Y_{5hr}) which are designated as response variables. Amongst the designed formulations, SF₇₉ showed highest overall desirability of 0.94. Therefore, level of formulation variables of this batch was considered to be optimized values for softgel capsule dosage form.

Soft gel capsules were evaluated for weight variation, moisture content, and content uniformity. During encapsulation process, twenty soft gel capsules were collected randomly for weight variation testing. The weight variation was found less than 2% with respect to theoretical weight. Softgel content was estimated by HPLC method. Average drug content was found between 96.64 to 102.86% per softgel capsule.

Rheological, bioadhesive and retention properties were studied and compared with that of clingen softgel (marketed formulation). Dispersions of CL softgel (SF₇₉) in SVF were found to possess higher viscosity than that of clingen softgel. Higher viscosity in SVF may prevent leakage and expulsion of formulation from vaginal cavity. This property of SF₇₉ will translate to longer retention and duration of action in vagina. Bioadhesive strength of CL softgel were compared with clingen softgel and was found significant greater ($p < 0.05$) for SF₇₉. Retention study was performed using vertically suspended isolated sheep vaginal tube, the formulation dispersion retained in the vaginal tube was higher than the marketed softgels at all time points. Overall results of retention study suggested that SF₇₉ possessed better retention characteristics than marketed softgels. In drug release study, optimized formulation (SF₇₉) showed release of CL from softgel up to 7h. This may due to HPMC K15 and xanthan gum swelled rapidly in contact with water and formed viscous gel which retards drug release. But, market formulation showed burst release of CL within 3h which may be due to lack of polymeric concentration in fill material blend (i.e. filled in softgel capsules). During stability studies, no significant change in assay and other properties such as physical appearance,

nature of capsule shell, moisture and pH of formulation was observed at different storage conditions.

Chapter IX describes cell viability studies of bioadhesive vaginal formulations. High dose and greater retention time of the bioadhesive vaginal formulation might produce toxicity for the vaginal epithelium cells. Therefore, the optimized bioadhesive vaginal formulations were next tested for cell viability (cytotoxicity). This study may also assure the biocompatibility of bioadhesive formulations for their intended use. We used MTT assay in our experiments to measure cell viability.

Cell lines procured from NCCS, Pune were found free from any kind of bacterial and fungal contamination. Percentage viability of cell lines was studied by Trypan blue dye exclusion technique. The **% viability of HeLa cell line** was found between **72-78%** which is most suitable to perform cytotoxicity studies. Cell viability study was performed with all the drugs individually and their formulations (film and softgel) and placebo. The optical density (OD) was measured at 570nm with a 96-well multiscanner ELISA reader with DMSO serving as blank.

Overall results of cell viability demonstrate that ITR and CL in either bulk or in films/softgels have excellent safety profile. Therefore, all the developed formulations can be safely used intravaginally without affecting cell viability of vaginal mucosa.

CONCLUSION

In present research work, bioadhesive formulations have been developed for intravaginal delivery of ITR and CL. Using the biocompatible materials which are GRAS listed and approved by the Food and Drug Administration (FDA) for vaginal use, polymeric films of each drug (2.5 cm × 2.5 cm containing 100mg drug) with acceptable aesthetic, physical and mechanical properties were formulated. Our results showed that ITR and CL could be successfully formulated in polymeric films along with good bioadhesive properties which favor prolong retention characteristics of formulation and can also greatly minimize the drawbacks once associated with conventional vaginal drug delivery systems. ITR and CL are also formulated in softgel capsule form. Smooth surface and enough flexibility of softgel capsules may help to minimize mechanical injury during insertion into vaginal cavity and improving patient compliance. Encapsulation blend of softgels produced enough viscosity in SVF which may prevent leakage and expulsion of formulation from vaginal cavity and also, there is no risk of burst release. Various performance characteristics and safety of the formulations were studied *in vitro* and *ex vivo*. They did not significantly affect the normal vaginal microflora (Lactobacilli) and possess excellent safety profile. Hence, this extraordinary technology may be explored as an effective tool in clinical management of BV and Vulvovaginal candidiasis as to achieve both efficiency and patient compliance. Also, this novel delivery system (BVDDS) can use as effective alternative for intravaginal delivery of microbicides and other therapeutic agents without any interruption to women's daily routine.