# CHAPTER - VII

## ITRACONAZOLE CONTAINING VAGINAL SOFT GEL

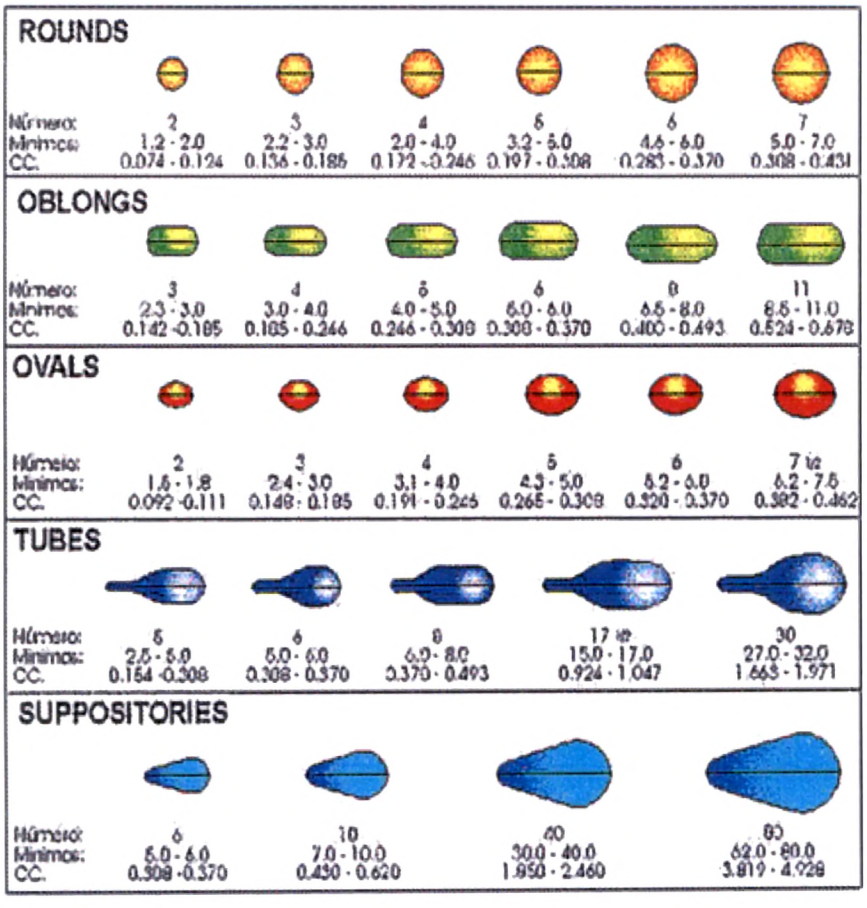
## 7.1 BACKGROUND

In the recent year's soft gelatin or soft elastic gelatin capsules have become a popular dosage form for vaginal delivery of various therapeutic agents. The soft gel (soft gelatin) capsule is a one piece, hermetically sealed shell wall typically filled with the active ingredients in solution, suspension and paste form. The soft gel delivery system permits accurate delivery of a unit dosage (unitary package), which makes it especially important when drug delivered in liquid or semisolid form (**U.S. Patent 5, 919, 481,** 1999). Such uniformity is more difficult to achieve, when total dose of active ingredient incorporated into a solution, cream and gel form which must be measured out prior to each vaginal administration. 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. Usually, commercially available vaginal preparations such as creams, ointments, foams, gels, pessaries, and suppositories requires cleansing of the applicator and reusage during course of treatment, which women may find inconvenient. Soft gel encapsulation further provides the potential to improve patient compliance due to their aesthetic appeal and ease of application (applicator not required for their administration).

The shell gives a unique strength and durability to soft gels and in addition to protects the inner fill materials from atmospheric oxidation. Soft gel delivery system provides several advantages over the other vaginal formulations as mentioned below.

- Provide unit dosage form.
- Offers the possibility of using a liquid, suspension or paste in solid vaginal dosage form.
- Easier to administration due to their soft character.
- Consumer-preferred dosage form.
- Allow product identification, using color and several shapes.
- Allow uniformity, precision and accuracy between dosages.
- Good availability of drug at site of administration and rapid action.
- Elegant and attractive as finished product.
- Mask odours.
- Flexible formulation.
- Immediate or delayed drug delivery.

The shape and size of the capsule are defined depending on the needs of the product as well as market. The different shape and size of softgel capsules are shown in **Fig.** 7.1.




7.1b Different shape and size of softgel

**Fig.** 7.1a Different shape of softgel

**The nature of the capsule shell**

The outer shell of softgel is gelatin matrix consists of gelatin, plasticizer, solvent with optional ingredients such as flavors and colorants.

**Gelatin-** gelatin is bovine, porcine or piscine (fish) origin and comes in a variety of bloom strengths. Higher the bloom strength, more physically stable is the resultant capsule shell. Most oil based fills are encapsulated using bloom strength of 150gm. When polyethylene (PEG) based fills are used, higher bloom strength of gelatin is generally used. Viscosity of gelatin determines manufacturing characteristics of gelatin film. The resultant film is firm, nontacky, nonbrittle and pharmaceutically elegant product.

**Plasticizer-** Glycerin and Sorbitol Special (Pharmaceutical grade) are two most commonly used plasticizers in gelatin shell preparation. Glycerin is generally used with oil based fills. Sorbitol Special is used with PEG based fills. Sorbitol is not soluble in PEG and therefore will not leach out of from the shell. Sorbitol Special is formulated to inhibit sorbitol from crystallizing out in the gelatin shell. The ratio by weight of dry plasticizer to dry gelatin determines the hardness of the gelatin shell.

**Solvent** – Water

**Optional ingredients** – Colors
Flavors
Preservatives
Opacifying agent

**Fill material for soft gelatin formulation**

Softgelatin capsules can be typically used to dispense variety of liquids and solids. The fill material used in a soft gelatin capsule contains a pharmaceuticaly dissolved or dispersed in carrier which is compatible with capsule shell. In addition to soft gelatin capsule, liquid or semisolids (dispersion) fill materials are discussed in US Pat. No. 4,935,243 by Borkan L. *et al.* and US Pat. No. 4,486,412 by shah *et al.*

1. Neat Substance, especially oily liquids
2. Solution Fills: Active dissolved in a carrier
3. Oils such as soybean oil and Miglyol 812

- Polyethylene Glycols: especially PEG 400 -600
- Other solvents: Any other solvent which doesn't degrade or solubilize the gelatin shell, i.e., dimethyl isosorbide, surfactants, diethylene glycol monoethly ether.

4. Optional Ingredients:

-Water or alcohol: up to 10% w/w, if needed for solubility

-Glycerin: 1 to 4% w/w to retard the migration of the glycerin out of the shell into the fill

- Polyvinylpyrrolidone: Up to 10% w/w used in combination with PEG, can increase drug solubility, and also improve stability by inhibiting drug recrystallization.

5. Suspension/paste fills: Active dispersed in a carrier.

Suspended solids must be smaller than 80 mesh size before filling to prevent needles from clogging during filling.

The most widely used vehicles are soybean oil (vegetable oil), mineral oil, liquid paraffin, non ionic surface active agent (plysorbate 80), lecithin, PEG 400 and PEG 600 either alone or in combination. When soybean oil used as suspending agent that simplifies manufacturing process and avoids batch to batch variability. Soybeaan oil with beeswax (4-100%w/w) and lecithin (2-4%w/w) is also commonly used. Lecithin improves material flow and imparts some lubrication during filling with added beeswax to get a good suspension and to avoid creation of non dispersible plug, when hydrophobic drugs are dissolved or dispersed in oily matrix.

## 7.2 MATERIALS

Itraconazole (ITR) was procured as gift sample from Intas Pharmaceutical Limited (Ahmedabad, India). HPMC (hydoxypropylmethyl cellulose) E15 was gifted by Colorcon Asia Pvt. Ltd. (Goa, India). Hydroxypropyl cellulose (HPC) (MW: 1 40 000) was purchased from Innovative Chemicals (Mumbai, India). Xanthan gum and hydroxyethyl cellulose (HEC) were purchased from Himedia (Mumbai, India). Light liquid paraffin, methanol and polyethylene glycol 400 (PEG 400) were purchased from S. D. Fine Chemicals (Mumbai, India). HPLC grade acetonitrile (ACN) and methanol (MeOH) were purchased from Spectrochem Lab. Tetrabutyl ammonium butyl hydrogen sulphate (TBAHS) was purchased from spectrochem pvt. Ltd. (Mumbai, India). Gelatin, glycerin, sorbitol solution 70%, methyl paraben and propyl paraben were procured from Gujarat Liqui Pharmacaps Pvt Ltd., Baroda. Double distilled water was used for preparing mobile phase solutions. All other chemicals used were of analytical grade.

## 7.3 EXPERIMENTAL

### 7.3.1 Preliminary Trial

Preliminary trial was carried out for meaningful selection of the excipients and a suitable carrier (vehicle). Drug and excipients blend is dispersed in a variety of oily vehicle such as vegetable oil and light liquid paraffin (LLP). US Pat. No. 5,071,643 of M. Yu et al. suggested the use of polyethylene glycols (PEG) as vehicle in soft gelatin dosage form. In $ISF_1$ trial, ITR and HPMC E15 were suitably dispersed in soybean oil to obtain a smooth dispersion. Similarly, LLP was added to ITR and HPMC E15 blend in $ISF_2$ trial. But, it was observed that soybean oil and LLP were getting separated from the dispersions in $ISF_1$ and $ISF_2$ respectively. In $ISF_3$, ITR and HPMC E15 were properly dispersed in PEG 400 to get homogeneous dispersions (blend). There was no any separation of contents from carrier as observed in $ISF_1$ and $ISF_2$. To ascertain effect of each polymer in formulation, trials $ISF_4$ to $ISF_7$ (**Table**7.1) were conducted and evaluated for consistency, pH and viscosity of blend. From these trials, the HPMC E15 and xanthan gum was found as most suitable polymer and PEG 400 as vehicle for preparation of blend filled in soft gelatin capsule.

### 7.3.2 Optimization of Formulation

The present study was performed with $3^2$ full factorial designs (Rotthäuser B. *et al.,* 1998) at three different levels for optimization of suitable polymers for soft gel formulation. Two independent factors, amount of HPMC E15 ($X_1$) and amount of xanthan gum ($X_2$) were set at three different levels. Total nine formulations were designed by $3^2$ full factorial designs as shown in **Table**7.2.

**Table** 7.1 Composition of preliminary formulation trials.

| **INGREDIENTS** (mg/soft gel) | $ISF_1$ | $ISF_2$ | $ISF_3$ | $ISF_4$ | **$ISF_5$** | $ISF_6$ | $ISF_7$ |
|---|---|---|---|---|---|---|---|
| ITR | 100 | 100 | 100 | 100 | **100** | 100 | 100 |
| HPMC E15 | 100 | 100 | 100 | 75 | **75** | 0 | 75 |
| HEC | 0 | 0 | 0 | 30 | **0** | 0 | 0 |
| Xanthan gum | 0 | 0 | 0 | 0 | **30** | 75 | 0 |
| HPC | 0 | 0 | 0 | 0 | **0** | 0 | 30 |
| Soybean oil | 300 | 0 | 0 | 0 | **0** | 0 | 0 |
| PEG 400 | 0 | 0 | 300 | 350 | **400** | 400 | 400 |
| LLP | 0 | 300 | 0 | 0 | **0** | 0 | 0 |

**Table** 7.2 Formulation designing by $3^2$ full factorial designs

| Batches | Formulation variables | |
|---|---|---|
| | $X_1$ | $X_2$ |
| $ISF_{51}$ | -1 | -1 |
| $ISF_{52}$ | -1 | 0 |
| $ISF_{53}$ | -1 | 1 |
| $ISF_{54}$ | 0 | -1 |
| $ISF_{55}$ | 0 | 0 |
| $ISF_{56}$ | 0 | 1 |
| $ISF_{57}$ | 1 | -1 |
| $ISF_{58}$ | 1 | 0 |
| $ISF_{59}$ | 1 | 1 |

| Formulation variables | Levels | | |
|---|---|---|---|
| | Low (-1) | Medium (0) | High (+1) |
| $X_1$ = Amount of HPMC E15 (mg) | 35 | 50 | 65 |
| $X_2$ = Amount of Xanthan gum (mg) | 15 | 30 | 45 |

High and low levels of each factor were coded as 1 and -1, respectively, and the mean value as zero. Composition of formulations designed by $3^2$ full factorial designs is shown in **Table**7.3. 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.

**Table**7.3 Composition of formulations designed by $3^2$full factorial designs

| INGREDIENTS (mg/soft gel) | $ISF_{51}$ | $ISF_{52}$ | $ISF_{53}$ | $ISF_{54}$ | $ISF_{55}$ | $ISF_{56}$ | $ISF_{57}$ | $ISF_{58}$ | $ISF_{59}$ |
|---|---|---|---|---|---|---|---|---|---|
| ITR | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| HPMC E15 | 35 | 35 | 35 | 50 | 50 | 50 | 65 | 65 | 65 |
| Xanthan gum | 15 | 30 | 45 | 15 | 30 | 45 | 15 | 30 | 45 |
| PEG 400 | 400 | 400 | 400 | 400 | 400 | 400 | 400 | 400 | 400 |

In case of the desirability function all the responses were combined in one measurement, which gives the possibility to predict optimum value for each of the formulation variable. To

combine all the response variables in one desirability function requires the calculation of the individual desirability function.

### 7.3.3 Preparation of Soft Gel Formulation

#### 7.3.3.1 Gelatin Mass Preparation

Gelatin to be used for capsule shell formation was prepared in gelatin mass reactor (AV pharma machinery, Mumbai). Initially, methyl paraben and propyl paraben were dissolved in water at 75° to 80°C. Gelatin flakes, glycerine, sorbitol solution, mixture of propyl paraben and methyl paraben and water were transferred to gelatin mass reactor and mix for 15. This mixture was heated at 65° to 70°C with continuous stirring until gelatin melted completely. Vacuum was applied in order to remove entrapped air bubble. Then, upload the mass in gelatin storage tank and was kept at 40° to 50°C until fed it to film forming roller.

#### 7.3.3.2 Blend Preparation

Blends that may be capsulated were prepared by mixing of specific amount of ITR and polymers in PEG 400 used as carrier. Solid materials were passed through mesh 80, so as to achieve a smooth and homogenous mixture.

**Table** 7.4 Gelatin shell composition

| Ingredients | %w/w |
|---|---|
| Gelatin | 43.93 |
| Glycerine | 6.00 |
| Sorbitol solution (70%) | 12.00 |
| Methyl paraben | 0.20 |
| Propyl paraben | 0.10 |
| Water | 37.77 |

#### 7.3.3.3 Blend Encapsulation

Encapsulation was carried out by continuous rotary die process using softgel encapsulation machine (Hitech Pharma Equipments, Bhilad, Gujarat) using 20mm oblong cavity rollers. The molten gelatin mass was maintained at 40° to 50°C, when it fed to film forming rollers. Film was formed at 10° to 12°C. The encapsulation process was based on form-fill-seal principle. Gelatin mass temperature, fill temperature, ribbon thickness and fills quantity should be continuously monitored during encapsulation process.

### 7.3.3.4 Drying of Soft Gel Capsules

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.

### 7.3.4 Evaluation of Soft Gel

### 7.3.4.1 Weight Variation

Twenty soft gel capsules were taken throughout the encapsulation process. Content of each soft gel was removed and weighed individually. The percentage weight variation was calculated with respect to actual fill weight.

### 7.3.4.2 Content Uniformity Test

To ensure uniform distribution of ITR in blend, average drug content in soft gel was measured. In this test, ten softgel capsules were randomly selected for samples. Content of each softgel was removed completely and transferred to 100ml volumetric flask. Then, 20ml of water was added and sonicated for 10 minutes to destroy any agglomerates and make upto 100ml with mobile phase. The solution was filtered using whatman filter paper type I. Subsequently, suitable portion of the filtrate was diluted with mobile phase and filtered through 0.45µm membrane filter. After filtration, sample was analyzed by HPLC method as reported in section **5.4.1.1 of chapter V**. The content of ITR was calculated using a preconstructed calibration curve for ITR (5-35 µg/ml) in mobile phase. No polymeric interference was observed under conditions of assay procedure.

### 7.3.4.3 Viscosity and pH Measurement

For studying viscosity and pH, formulation dispersion was prepared with one softgel dispersed in 10 ml each of distilled water and SVF (simulated vaginal fluid). Viscosity of formulation dispersion was determined by Brookfield cone and plate rheometer LVDVIII (Brookfield engineering, Middleboro, MA, USA). Viscosity was measured at 31.2°C by keeping speed 10 RPM and shear rates 20 $sec^{-1}$. The pH of aqueous solution of softgel was measured with pH meter (LABINDIA Pvt. Ltd., New Mumbai). All the parameters were measured in triplicates.

### 7.3.4.4 Moisture Content

Five softgel capsules were weighed accurately and kept in desiccator's containing calcium chloride for 48h. Softgel capsules were removed from desiccator and reweighed until

a constant weight obtained. The percentage of moisture content was calculated as difference between initial weight and final weight with respect to initial weight (Jin-Wook Y. *et al.*, 2006). Moisture content was measured in triplicates.

### 7.3.4.5 Measurement of Swelling Index

Swelling capability of softgel capsule was determined in SVF. Each softgel sample was weighed (Wo) and placed in a preweighed stainless steel basket with 200 mesh aperture. Then, basket containing softgel capsule was submerged into 20 ml medium in glass beaker. Basket was removed from glass beaker at preset time intervals, and reweighed until no further change in weight of softgel capsules (Wt). Swellings index was measured in triplicates. The degree of swelling was calculated as follows. (Gannu R. *et al.*, 2007).

$$\text{Swelling index} = (Wt - Wo) / Wo$$

### 7.3.4.6 Bioadhesion and Retention in Simulated Vaginal Environment

The bioadhesive property of formulation was assessed in simulated vaginal environment (Vermani K. *et al.*, 2002) using a texture analyzer (Instron 1121, UK) equipped with 2.0 kg load cell. Isolated sheep vaginal tube was thawed in normal saline and incised longitudinally. Then, sheep vaginal mucosa was attached to the lower platform of the texture analyzer using a rubber band. Sample was fixed to upper probe with the help of double-sided adhesive tap. Mucosal membrane was kept in contact with sample for five minutes to ensure intimate contact between the tissues and sample. Upper probe of texture analyzer was moved at constant speed of 0.1mm/sec. The force required to detach sample from the tissue surface was determined as bioadhesive strength.

Retention of property of ITR formulation in simulated vaginal environment was studied by an in-vitro method based on the principle of measuring weight of formulation dispersions falling down (or retained) as function of time. The excised and clean sheep vaginal tube was vertically suspended in the glass cell. The ends of the vaginal tube were averted on the tapering of the upper and lower ends of the glass cell and crimped using rubber bands. Dispersion of vaginal formulation (content of one softgel dispersed in 4ml) placed inside a vertically suspended isolated sheep vaginal tube was allowed to fall under influence of gravity. The weight of formulation falling down was recorded as percentage leakout and percent retained as function of time.

### 7.3.4.7 Drug Release Study

The release of ITR from vaginal soft gel was studied using small volume dissolution medium (100ml) maintained at 37±2°C (Garg S. *et al.,* 2005). In this study, phosphate buffer (pH 4.5) was used as a dissolution medium. A soft gel was placed into a 100 ml glass beaker (having 4cm i.d.) that is filled with dissolution medium. Allow to stand for 5min to stick softgel capsule in one corner of glass beaker. Then, stirred on a magnetic stirrer at 300RPM by placing magnetic bead opposite side to softgel in glass beaker. Samples (5 ml) were withdrawn periodically at 1, 2, 3, 4, 5, 6, 7, 8h and were compensated with equal volume of fresh dissolution medium. The sample dilution was made upto 100ml with mobile phase and filtered using whatman filter paper type I. Subsequently, suitable portion of the filtrate was diluted with mobile phase and filtered through 0.45µm membrane filter. Sample was analyzed by HPLC method as reported in section 5.4.1.1 of chapter V. The cumulative percentage of drug released was plotted against time in order to obtain the release profile.

### 7.3.5 Stability Studies

Stability studies of optimum formulation were performed according to the ICH guidelines. Samples were individually sealed in sachets prepared from polyethylene laminated alluminium foil and stored at intermediate stability condition of 30±2°C and 65±5% RH. The accelerated stability studies were performed at 40±2°C and 75±5% RH. A visual inspection (for change in color and odor), pH of formulation dispersion, assay of ITR and retention properties were determined periodically at the end of 1, 2, 3 and 6 months of the stability study.

## 7.4 RESULTS AND DISCUSSION

### 7.4.1 Analytical Method

The chromatographic separation of ITR was achieved with reverse-phase C18 column (250mm×4.6mm i.d., 5µm particle size), using a mixture of MeOH, ACN and 0.01N TBAHS in the ratio of 70:20:10 (%v/v) at a flow rate of 0.7 ml/min with UV detection at 261 nm and quantified based on drug peak area. The development of this method was described in section **5.4.1.1 of chapter V**. This method was used for estimation of ITR in softgel capsules.

### 7.4.2 Preliminary Experiments

Preliminary trial was carried out in order to select a suitable carrier in which drug and polymers can incorporate. In trial $ISF_1$ to $ISF_3$, different vehicle such as soybean oil, LLP and PEG 400 were explored for preparation of encapsulation material blend.

Consistency of 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. As a progressive hydration approach to solid bioadhesive delivery system, the product absorbs moisture, outside becomes a gel and adhere to mucosal surface for prolong period of time and prevent expulsion of formulation. This will possible in case of soft gelatin dosage form, if liquid vehicle used in preparation of encapsulation blend is hydrophilic in nature. According to literature survey, PEG 400 is also used as good vehicle for preparation of encapsulation blend. In trial $ISF_3$, 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. The consistency of dispersion was adjusted by adding appropriate amount PEG 400. Viscosity and dispersibility of formulation in vaginal environment after administration governs the spreading and retention of the formulations which is essential to achieve desired efficacy. Therefore, a variety of water-soluble polymers such as HEC, HPC and xanthan gum were investigated with HPMC E15 in trial $ISF_4$ to $ISF_7$. Trial $ISF_4$ and $ISF_5$ were taken by incorporating HEC and xanthan gum in $ISF_3$ composition respectively. Encapsulation blend of $ISF_5$ produced more viscosity upon dispersed in SVF than $ISF_4$ blend. Encapsulation blend should be sufficient viscous so that it cannot be readily expelled from vaginal cavity immediately after bursting of outer shell. Significant difference was observed in $ISF_5$ and $ISF_6$ with respect to consistency of encapsulation material blend. Xanthan gum and HPMC E15 were also responsible for imparting bioadhesion property to the formulation (Vermani K. *et al.*, 2002). Hence, HPMC E15 premium and xanthan gum was selected for preparation of blend that is encapsulated in soft gel.

### 7.4.3 Evaluation of Soft Gel Capsules

#### 7.4.3.1 Weight Variation

Weight variation of soft gel contents were 1.93% calculated with respect to actual fill weight (theoretical fill weight).

#### 7.4.3.2 Content Uniformity

Ten softgel capsules were randomly selected for content uniformity test. Softgel content was estimated by HPLC method. Average drug content was found between 96.14 to 103.17% of ITR per softgel capsule (**Table** 7.6).

**Table** 7.5 Fill weight of twenty softgel capsule

| Softgel capsule | Fill weight (mg) | Softgel capsule | Fill weight (mg) |
|---|---|---|---|
| **1** | 632 | **11** | 619 |
| **2** | 622 | **12** | 598 |
| **3** | 605 | **13** | 627 |
| **4** | 593 | **14** | 617 |
| **5** | 619 | **15** | 629 |
| **6** | 581 | **16** | 604 |
| **7** | 603 | **17** | 599 |
| **8** | 621 | **18** | 601 |
| **9** | 594 | **19** | 607 |
| **10** | 610 | **20** | 596 |

**Table** 7.6 Assay content of softgel capsule

| Softgel capsule | Fill weight (mg) | % of ITR per softgel | Softgel capsule | Fill weight (mg) | % of ITR per softgel |
|---|---|---|---|---|---|
| **1** | 621 | 101.35 | **6** | 625 | 103.17 |
| **2** | 599 | 98.23 | **7** | 606 | 99.01 |
| **3** | 608 | 99.53 | **8** | 597 | 97.71 |
| **4** | 591 | 96.14 | **9** | 612 | 100.57 |
| **5** | 619 | 101.87 | **10** | 595 | 97.19 |

### 7.4.3.3 Viscosity and pH of Formulation Dispersion

Assuming, there is sufficient vaginal moisture to dissolve gelatin shell and fill material of softgel dispersed in vaginal fluid after administration. Viscosity of formulation dispersion governs the retention of formulations, which in turn are important for the desired efficacy (Owen D.H. *et al.*, 2000). The viscosity of formulation dispersion was found to be more in SVF (65.8 7 ± 2.80) as compared to water (19.04 ± 1.95). Higher viscosity of formulation dispersion in SVF may reduce leakage and expulsion from vaginal cavity. Also, pH of the formulation dispersion is an important aspect as they can impact on the acidic pH of the female genital tract and normal microflora. The pH of ITR softgel was found to be alkaline (7.79 ± 0.09) in water and acidic (5.12 ± 0.07) in SVF reveals that vaginal pH as well as micro flora may remains unaffected after administration of softgel.

### 7.4.3.4 Moisture Content

The moisture content of the softgel was found to be 6.77± 0.38% (w/w). It was found satisfactory as it is between 6 to 10% which is allowable limit for softgel capsule depending

on the gelatin formula used. The little amount of moisture content in formulations helps them to remain stable and prevent from being a completely dry and hard.

### 7.4.3.5 Swelling Index

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 the property to get hydrated by absorbing large amount of water. Hence, the swelling capability of softgel depends on the amount of these variables present in the formulation. The effect of various compositions on the swelling index of the softgel is shown in **Fig** 7.3. Swelling index increases with time due to increase in hydration phenomenon of fill material of softgel. Maximum swelling was seen with softgel containing high concentration of HPMC E15 and xanthan gum. Increment in swelling capability of softgel is more with increase in xanthan gum concentration than HPMC E15 concentration.

### 7.4.3.6 Bioadhesive Strength

Bioadhesion strength of formulation is very important aspect for maintaining high drug levels at the site of administration and prevents expulsion of formulation. Literature review indicates that xanthan gum has very good bioadhesive property (Nakamura *et al.*, 1996 & Sinha VR *et al.*, 2002). Xanthan gum is hydrophilic polymer which tends to hydrate by absorbing water from mucosal surface. This polymer form viscous liquids when hydrated with water and may lead to adhesive interaction with mucosal surface. Bioadhesive strength of softgels with various compositions ($ISF_{51}$-$ISF_{59}$) is shown in **Fig.**7.4. Amongst nine softgel, $ISF_{59}$ showed good bioadhesion (0.490 ± 0.017 N) under simulated vaginal environment. Thus, bioadhesive strength of softgel enhance with increased in concentration of xanthan gum.

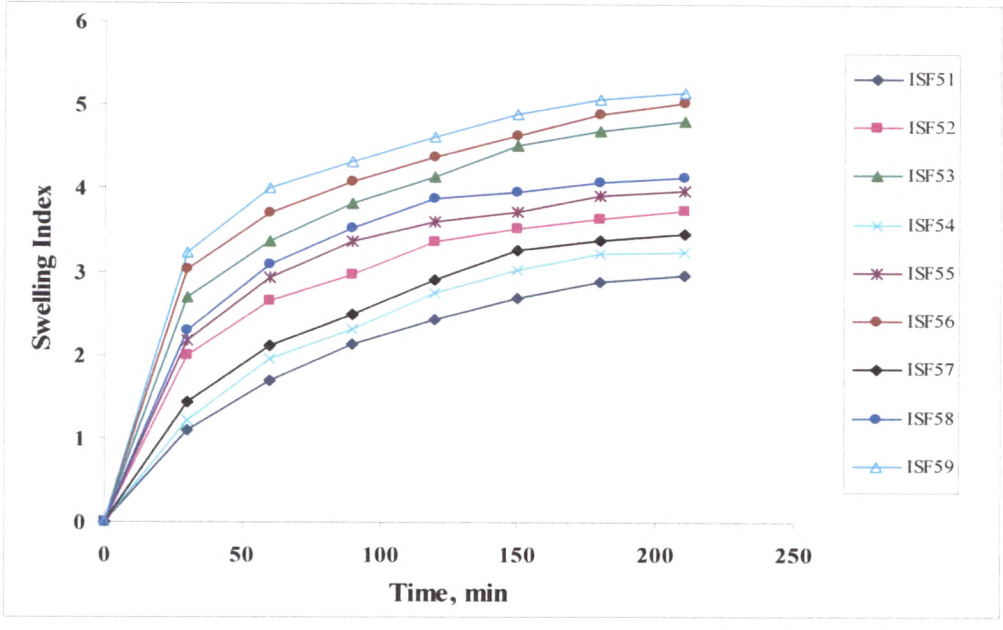



**Fig**7.3. Swelling index of the softgels with various compositions ($ISF_{51}$- $ISF_{59}$)

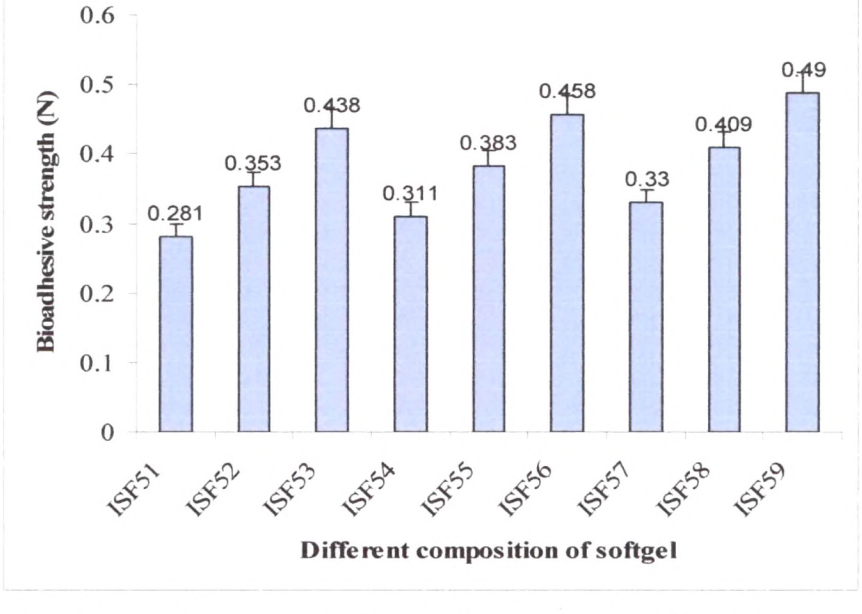



**Fig.**7.4 Bioadhesive strength of softgels with various compositions ($ISF_{51}$- $ISF_{59}$)

### 7.4.3.7 Retention Characteristic of Softgel in Simulated Vaginal Environment

Retention of a dosage form in vaginal cavity for prolonged intervals is desirable for therapeutic efficacy 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. In order to measure retention behavior of softgel, weight of formulation

dispersions falling down (or retained) from vertically suspended isolated sheep vaginal tube was recorded as function of time. $ISF_{59}$ showed very good retention (> 7 hours) in the ex vivo experiment. Amount of formulation dispersions retained in vaginal tube at predetermined time interval for each softgel are shown in **Fig.** 7.5. Time required for entire removal of formulation dispersions from vaginal mucosa varied with different compositions of softgel. A softgel with less polymeric concentration was falling down rapidly from vaginal tube. An interesting observation of this study was residence time of formulation dispersion increased as the ratio of HPMC to xanthan gum in softgel increased. This indicates retention property of softgel can be controlled by varying HPMC/xanthan gum ratio.

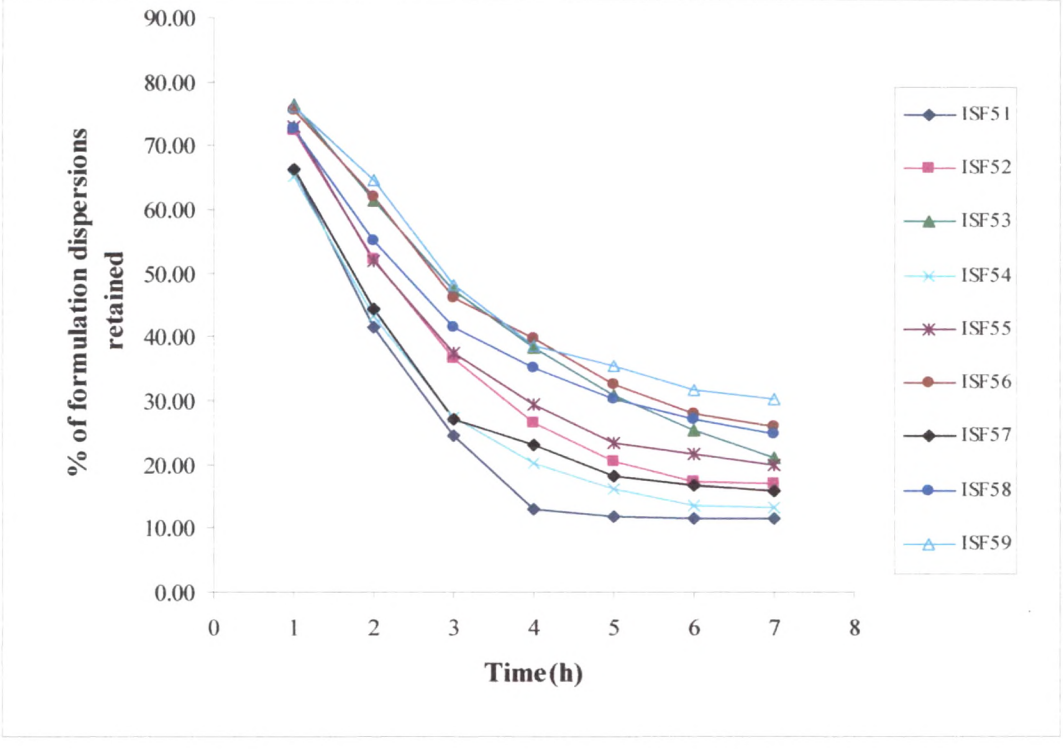



**Fig.** 7.5. Percentage retention of formulation dispersions in vertically suspended isolated sheep vaginal tube as a function of time.

### 7.4.3.8 Drug Release Study

A sample collected at different time intervals was analyzed by HPLC method and percentage of ITR released was calculated using a preconstructed calibration curve. **Table** 7.7. Shows the cumulative percent release profiles of softgel formulation $ISF_{51}$ to $ISF_{59}$. An interesting finding of these studies was the drug release retarded as the concentration of xanthan gum increase in the formulation. This may due to xanthan gum swelled rapidly in contact with SVF and formed rigid gel which is ultimately responsible for retarding drug release.

**Table** 7.7. Cumulative percent release profiles of softgel formulation $ISF_{51}$ to $ISF_{59}$

| Time (hr) | Cumulative Percentage release of Itraconazole, Mean ± SD ( n = 3) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | $ISF_{51}$ | $ISF_{52}$ | $ISF_{53}$ | $ISF_{54}$ | $ISF_{55}$ | $ISF_{56}$ | $ISF_{57}$ | $ISF_{58}$ | $ISF_{59}$ |
| 1 | 37.65±0.26 | 34.26±0.19 | 29.70±0.21 | 40.51±0.0.24 | 37.00±0.20 | 32.44±0.29 | 42.21±0.24 | 38.69±0.27 | 34.78±0.21 |
| 2 | 82.97±0.11 | 70.3±0.14 | 61.00±0.23 | 85.33±0.12 | 72.93±0.14 | 63.77±0.21 | 87.97±0.13 | 75.16±0.14 | 65.74±0.25 |
| 3 | 98.14±0.12 | 89.55±0.11 | 78.38±0.15 | 100.64±0.08 | 92.57±0.17 | 85.44±0.17 | 98.45±0.15 | 94.28±0.17 | 88.20±0.19 |
| 4 | - | 94.82±0.13 | 89.49±0.11 | - | 98.75±0.09 | 93.86±0.12 | - | 97.21±0.10 | 95.84±0.08 |
| 5 | - | 97.08±0.10 | 96.51±0.13 | - | - | 99.68±0.13 | - | - | 97.87±0.10 |

Excipients did not show any interference peak at retention time corresponds to ITR peak (**Fig.** 7.6) suggest the specificity of method. It was confirmed that gelatin was not interfered during quantification of ITR from dissolution sample.

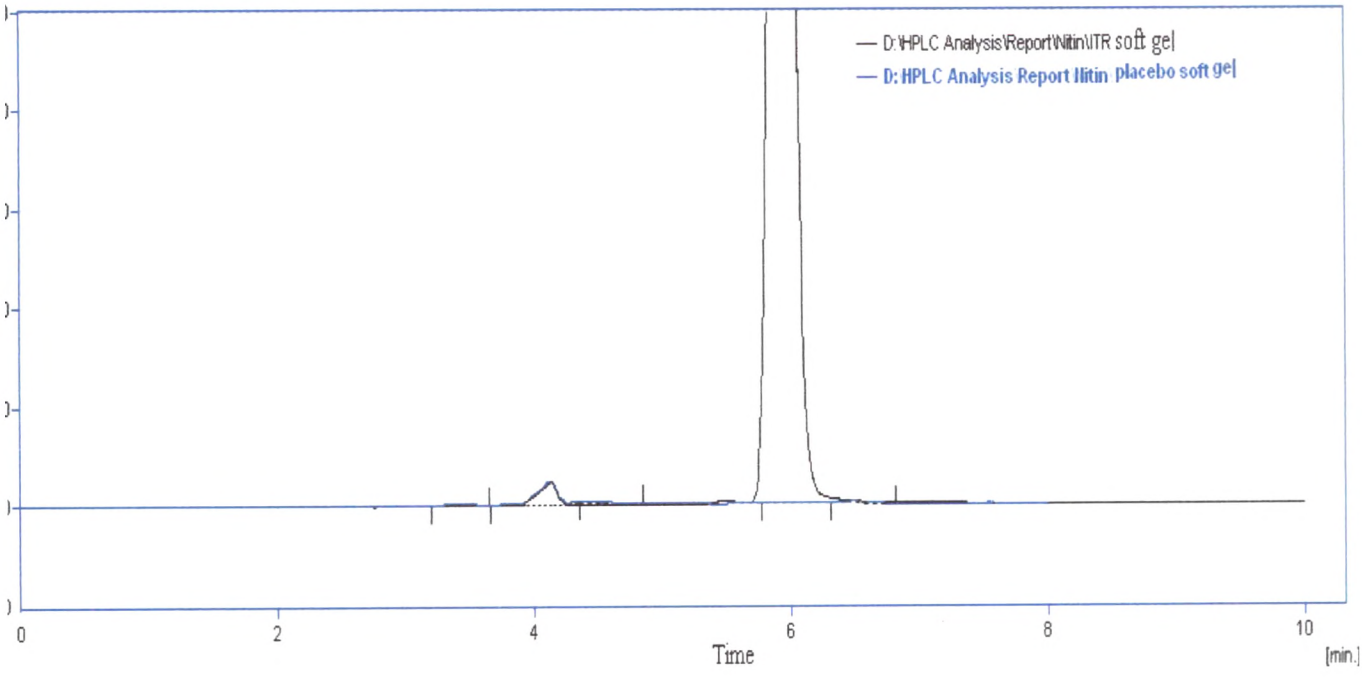



**Fig.** 7.6 Overlay chromatogram of placebo and ITR softgel

### 7.4.4 Optimization of Formulation Responses using Desirability Function

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 $\mathbf{Y_{5hr}}$. The individual desirability for each response variable was calculated and combined in one measurement. The calculation of the desirability function for swelling index was carried out using the following equations:

$$d_1 = 0 \quad for\ Y_i < Y_{min}$$
$$d_1 = 1 \quad for\ Y_{min} < Y_i < Y_{max}$$
$$d_1 = 0 \quad for\ Y_i > Y_{max}$$

Where, $d_1$ is individual desirability of swelling index and $Y_i$ is the experimental results. The values of $Y_{min}$ and $Y_{max}$ for swelling index were 2.89 and 5.09 respectively.

In addition, optimum softgel formulation should possess good retention characteristics over vaginal mucosa. The desirability functions of Rt and $Y_{5hr}$ responses was calculated using the following equation:

$$d_2 \text{ or } d_3 = \frac{Y_i - Y_{min}}{Y_{target} - Y_{min}} \quad for\ Y_i < Y_{target}$$
$$d_2 \text{ or } d_3 = 1 \quad for\ Y_i > Y_{target}$$

Where $d_2$ is individual desirability of Rt and $d_3$ is the individual desirability of $Y_{5h}$. The values of $Y_{target}$ and $Y_{min}$ for Rt are 8.0 and 2.0. For $Y_{5h}$, values of $Y_{target}$ and $Y_{min}$ are 50.00 and 11.9. $Y_i$ is the experimental result. The overall desirability values were calculated from individual values by using the following equation:

$$D = (d_1 d_2 d_3)^{1/3}$$

**Table** 7.8 Experimental result of response variables and overall desirability of formulations designed by $3^2$ full factorial designs.

| Batches | Variables | | Response values* | | | Overall Desirability |
|---|---|---|---|---|---|---|
| | $X_1$ | $X_2$ | Swelling index | [a]Rt(h) | $Y_{5hr}$ | |
| $ISF_{51}$ | -1 | -1 | 2.89 | 2 | 11.93 | 0.00 |
| $ISF_{52}$ | -1 | 0 | 3.66 | 4 | 20.61 | 0.43 |
| $ISF_{53}$ | -1 | 1 | 4.71 | 6 | 30.94 | 0.74 |
| $ISF_{54}$ | 0 | -1 | 3.22 | 3 | 16.09 | 0.27 |
| $ISF_{55}$ | 0 | 0 | 3.92 | 4 | 23.31 | 0.49 |
| $ISF_{56}$ | 0 | 1 | 4.91 | 7 | 32.50 | 0.81 |
| $ISF_{57}$ | 1 | -1 | 3.39 | 3 | 19.00 | 0.34 |
| $ISF_{58}$ | 1 | 0 | 4.09 | 6 | 30.31 | 0.73 |
| **$ISF_{59}$** | **1** | **1** | **5.09** | **7** | **35.62** | **0.85** |

* Average of three experiments, [a] RT at 25% formulation retained

Experimental result of response variables and overall desirability of formulations designed by $3^2$ full factorial designs are shown in **Table** 7.8.

Batch **$ISF_{59}$** showed highest overall desirability of 0.85. Therefore, this batch was considered to be optimized batch and values of formulation variables of this batch were considered to be optimum values for softgel capsule dosage form. Excipients used in softgels are GRAS listed and approved for vaginal use (Garg S. *et al.,* 2001). The optimized composition of softgel capsule containing ITR is given in **Table** 7.9.

**Table** 7.9 Optimized composition of softgel formulation containing Itraconazole.

| Formulation Variables | Optimum Value (mg per softgel capsule) |
|---|---|
| ITR | 100 |
| HPMC E15 | 65 |
| Xanthan gum | 45 |
| PEG 400 | 400 |

The prediction profiles were obtained for the measured responses using JMP 5.1, statistical discovery software. The relationship between formulation variables and dependent response value of ITR softgel can be further explained by using prediction profile as shown in **Fig.**7.7. Among tested variables, the xanthan gum concentration seems to be the most prominent factor in determining response value of softgel. An interesting observation of these profiles was improved swelling index, bioadhesion, and $Y_{5h}$ as the concentration of xanthan gum increased.

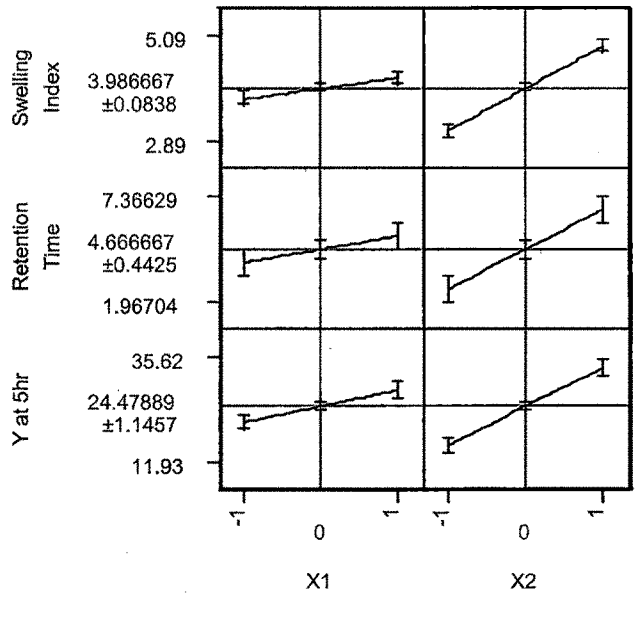



**Fig.**7.7 Predictions profile for all the dependent variables against independent variables.

### 7.4.5 Stability Studies

Stability samples were analyzed at 0, 1, 2, 3 and 6 months time intervals for various physical properties such as brittleness or softening, color fading, pH, moisture content and assay of ITR as per the procedure described in method section. The result of the stability study of ITR softgel is shown in **Table** 7.10. Prior to testing, capsules should be equilibrated to known atmospheric conditions. The physical stability of softgel capsules is associated primarily with the pick up or loss of water by the capsule shell. The results of accelerated storage condition demonstrate the influence of relative humidity (75% RH) on the capsule shell. Some capsule shell became more soften due to effects of temperature and humidity indicates necessity of proper storage and packaging conditions. This observation was anticipated due to the higher moisture content of the softgel stored at the higher relative humidity. But, assay of content remains unaffected at accelerated storage condition. The compound was stable in all storage conditions upto 6 months. No differences were observed in the assay, pH of formulation dispersion and formulation color change with softgel capsule suggesting compatibility of the softgel shell with the compound and the formulation. The pH of polymeric dispersion of ITR softgel in SVF was found acidic (5.07 ± 0.05) suggesting that vaginal pH and microflora remains unaffected.

**Table** 7.10 Results of the stability studies of ITR softgel capsule.

| **Sample storage condition** | **Testing interval (Month)** | **pH of polymeric dispersion** mean ± S.D (n=3) | **Moisture content (%)** | **Content of softgel (%)** mean ± S.D (n=3) |
|---|---|---|---|---|
| - | 0 | 5.07 ± 0.05 | 7.04±0.21 | 99.41±1.20 |
| Intermediate condition (30±2°C and 65±5% RH) | 1 | 5.14 ± 0.04 | 6.78±0.27 | 100.38±1.72 |
| | 2 | 5.10 ± 0.08 | 6.94±0.14 | 98.12±1.43 |
| | 3 | 5.17 ± 0.10 | 7.14±0.17 | 98.65±1.11 |
| | 6 | 5.08 ± 0.07 | 6.99±0.22 | 97.65±1.26 |
| Accelerated stability conditions (40±2°C and 75±5% RH) | 1 | 5.03 ± 0.11 | 6.87±0.18 | 99.20±1.58 |
| | 2 | 5.13 ± 0.08 | 7.16±0.15 | 98.27±1.80 |
| | 3 | 5.07 ± 0.10 | 7.32±0.10 | 97.34±1.48 |
| | 6 | 5.15 ± 0.06 | 7.39±0.17 | 97.86±1.36 |

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