CHAPTER - VIII

CLINDAMYCIN PHOSPHATE CONTAINING VAGINAL SOFT GEL

8.1 INTRODUCTION

Smooth surface of softgel capsules 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**). They can easily scale up using existing softgel manufacturing facilities. Hence, attempts were also made to formulate CL in softgel capsule form.

8.2 MATERIALS

Clindamycin phosphate (CL) was procured as gift sample from GO-ISH remedies Limited (Baddi, H.P., India). HPMC (hydoxypropylmethyl cellulose) K15 was gifted by Colorcon Asia Pvt. Ltd. (Goa, India). Xanthan gum and hydroxyethyl cellulose (HEC) were purchased from Himedia (Mumbai, India). Hydroxypropyl cellulose (HPC) (MW: 1 40 000) was purchased from Innovative Chemicals (Mumbai, India). Light liquid paraffin, Polyethylene glycol 400 (PEG 400) and sodium dihydrogen phosphate (NaH_2PO_4) was purchased from S. D. Fine Chemicals (Mumbai, India). HPLC grade acetonitrile (ACN) and methanol (MeOH) were purchased from Spectrochem Lab (Mumbai, India). Light liquid paraffin was purchased from Qualigens fine chemicals (Mumbai, India). Gelatine, glycerin, sorbitol 70% solution, Methyl paraben and propyl paraben waere procured from Gujarat Liqui Pharmacaps Pvt Ltd, Baroda. Double distilled water was used for preparation of mobile phase solutions. Gelatin, glycerin, sorbitol solution 70%, methyl paraben and propyl paraben were procured from Gujarat Liqui Pharmacaps Pvt Ltd., Baroda. All other chemicals and solvent used were of analytical grade. All in vitro tests was carried out in simulated vaginal fluid (SVF).

8.3 EXPERIMENTAL

8.3.1 Analytical Method

In present study, HPLC method (Shimadzu SPD-20A, LC-20AT, Japan) was used for estimation of CL from their softgel formulation. The development and optimization of proposed method was discussed in **section 6.4.1.3 of chapter VI**. Furthermore, specificity of this method was checked by dissolving placebo softgel in mobile phase and injecting the filtered portion.

8.3.2 Preliminary Trial

In preliminary trial, vegetable oil, light liquid paraffin (LLP) and PEG 400 were tried for

preparation of fill material blend that is filled in the soft gelatin capsule. In SF_1 trial, soybean oil was used as vehicle for preparation of fill material blend. Similarly, CL and HPC were suitably dispersed in LLP to obtain a smooth dispersion in SF_2 trial. But, it was observed that soybean oil and LLP were getting separated from the dispersions in SF_1 and SF_2 respectively. Literature review also indicates use of hydrophilic polyethylene glycols as a vehicle in soft gelatin dosage form (M. Yu *et al.*, US Pat. No. 5,071,643). Therefore, trial SF_3 was carried out using PEG 400 as vehicle to get homogeneous blend. No separation of contents from carrier was observed. In order to study the effect of each polymer in formulation, trials SF_4 to SF_8 (**Table** 8.1) were conducted. They were evaluated for consistency, pH and viscosity of blend. From these trials, SF_7 (HPMC K15 and xanthan gum dispersed in PEG 400) was found most suitable and selected for further optimizations by 3^2 factorial design.

Table 8.1 Composition of preliminary softgel formulation trials.

INGREDIENTS (mg/soft gel)	SF_1	SF_2	SF_3	SF_4	SF_5	SF_6	SF_7	SF_8
CL	100	100	100	100	100	100	100	100
HPC	100	100	100	60	60	60	0	0
Xanthan gum	0	0	0	30	0	00	60	60
HEC	0	0	0	0	30	00	0	30
HPMC K15	0	0	0	0	0	30	30	0
Soybean	350	0	0	0	0	0	0	0
PEG 400	0	0	350	350	400	400	400	400
LLP	0	350	0	0	0	0	0	0

8.3.3 Experimental Design and Application of the Desirability Function

Factorial experimental design and desirability function are useful approach for the optimization of the formulations (Rane Y.M. et al 2007). In the present study, total nine

formulations were designed by 3^2 full factorial designs (**Table** 8.2). Two formulation variables i.e. concentration of xanthan gum (X_1) and concentration of HPMC K15 (X_2) were set at three different levels for optimization of soft gel formulation. Level of formulation variables was decided from the results of the preliminary experiments. High and low levels of each factor were coded as 1 and -1, respectively, and the mean value as zero. All the designed formulations were evaluated for swelling index, retention time (Rt) and % of drug retained in vaginal tube at 5h (Y_{5hr}) which are designated as response variables. Composition of formulations designed by 3^2 full factorial designs is shown in **Table** 8.3.

Desirability function was applicable to predict the optimum value for each of the formulation variable (Rotthäuser B. *et al.,* 1998). In case of the desirability function, all the formulation responses were combined in one measurement. To combine all the response variables in one desirability function requires the calculation of the individual desirability function.

Table 8.2 Formulation designing by 3^2 full factorial designs

Batches	**Formulation variables**	
	X_1	**X_2**
SF_{71}	-1	-1
SF_{72}	-1	0
SF_{73}	-1	1
SF_{74}	0	-1
SF_{75}	0	0
SF_{76}	0	1
SF_{77}	1	-1
SF_{78}	1	0
SF_{79}	1	1

Formulation variables	**Levels**		
	Low (-1)	**Medium (0)**	**High (+1)**
X_1 = Amount of Xanthan gum (mg)	25	40	55
X_2 = Amount of HPMC K15 (mg)	5	15	25

Table 8.3 Composition of formulations designed by 3^2full factorial designs

INGREDIENTS (mg/soft gel)	SF_{71}	SF_{72}	SF_{73}	SF_{74}	SF_{75}	SF_{76}	SF_{77}	SF_{78}	SF_{79}
Clindamycin phosphate	100	100	100	100	100	100	100	100	100
Xanthan gum	25	25	25	40	40	40	55	55	55
HPMC K15	5	15	25	5	15	25	5	15	25
PEG 400	400	400	400	400	400	400	400	400	400

8.3.4 Preparation of Soft Gel Formulation

8.3.4.1 Preparation of Fill Material (Blend)

All the required ingredients of the formulation were weighed accurately. Solid materials were passed through mesh 80, so as to achieve a smooth and homogenous mixture. The mixture of solid materials were dispersed in PEG 400 and continuously stirred for 30 min using magnetic stirrer (Remi equipments Ltd., Mumbai, India)

8.3.4.2 Preparation of shell (gelatin) mass

The shell-forming composition was prepared in gelatin mass reactor (AV pharma machinery, Mumbai). It is a closed, evacuated and temperature-controlled vessel. Initially, methyl paraben and propyl paraben were dissolved in water at 75° to 80°C. Charge the material in the following sequence (as shown in **Table** 8.4) with both stirrers 'ON'. This mixture was heated at 65° to 70°C with continuous stirring until a homogenous molten blend is obtained. Vacuum was applied in order to remove air. Molten mass of gelatin was kept at 40° to 50°C until fed it to film forming roller.

Table 8.4 Gelatin shell composition.

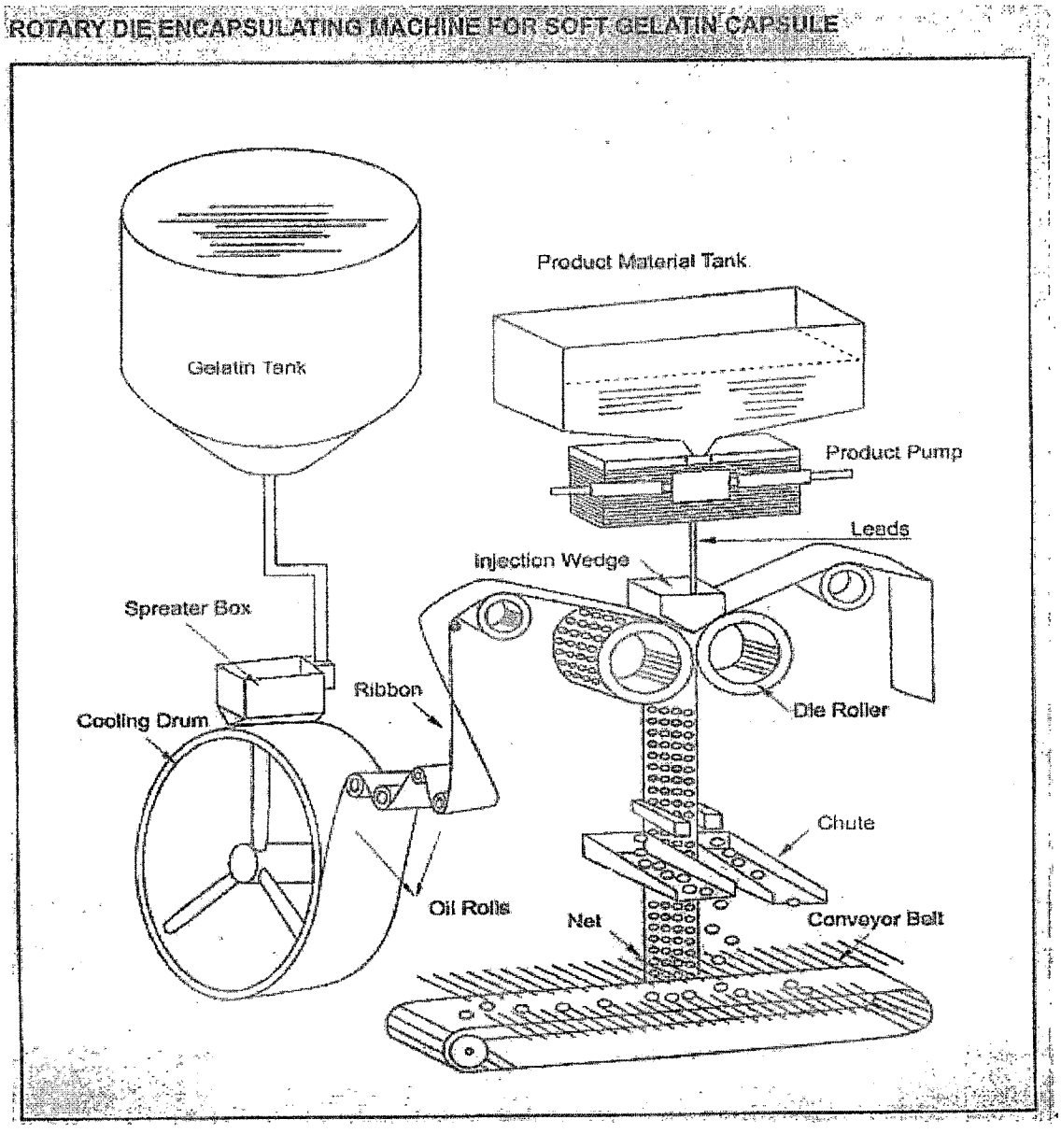
Ingredients	%w/w
Water	37.77
Glycerine	6.00
Sorbitol solution (70%)	12.00
Methyl paraben	0.20
Propyl paraben	0.10
Gelatin	43.93

8.3.4.3 Encapsulation of Fill Material

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 15° to 20°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.

8.3.4.4 Drying of soft gel capsules

Softgel capsules were dried at controlled temperature (26° to 27°C) and relative humidity (20% to 22%RH) for 48 h.



8.3.5 Evaluation of Vaginal Soft Gel Capsules

8.3.5.1 Weight Variation

During encapsulation process, twenty soft gel capsules were collected randomly. Content of each soft gel was removed and weighed individually. The percentage weight variation was calculated with respect to actual fill weight.

8.3.5.2 Content Uniformity

Ten softgel capsules were randomly selected for determination of average drug content of softgel. Content of each softgel was removed completely and transferred to 100ml volumetric flask. Add 25ml of water to dissolve softgel content followed by sonication for 10min to demolish any agglomerates and made upto 100ml with mobile phase (ACN: 25mM NaH_2PO_4 buffer of 45:55 v/v adjusted to pH 4.75). 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.

8.3.5.3 Viscosity and pH Measurement

Viscosity of formulation dispersion was determined by Brookfield cone and plate rheometer LVDVIII (Brookfield engineering, Middleboro, MA, USA). Formulation dispersion was prepared by dissolving one softgel in 10 ml each of distilled water and SVF (simulated vaginal fluid). Viscosity was measured at 33.4°C by keeping speed 20 RPM and shear rates 40 sec^{-1}. The pH of the softgel dispersion has got influence not only on the vaginal pH but also on normal micro flora of vagina. The pH of aqueous solution of softgel was measured with pH meter (LABINDIA Pvt. Ltd., New Mumbai). All the parameters were measured in triplicates.

8.3.5.4 Moisture content

Softgel capsules were weighed accurately and placed in desiccator's containing calcium chloride for 48h. The final weight was noted, when there was no further change in weight of softgel capsule. 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). This test was performed in triplicates.

8.3.5.5 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 in 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).

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

8.3.5.6 Bioadhesion and Ex Vivo Retention in Simulated Vaginal Environment

The bioadhesive properties of softgel capsule were assessed on sheep vaginal mucosa (Vermani K. *et al.*, 2002) using a texture analyzer (Instron 1121, UK). For experiments, the vaginal tube (thawed in normal saline with 0.1% w/v sodium azide preservative) incised longitudinally and held on 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. The vaginal mucosa was moistened with simulated vaginal fluid. 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 property of CL softgel was studied by an in-vitro method based on the measurement 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) was placed inside a vertically suspended isolated sheep vaginal tube and 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. This test was also performed for marketed formulation of CL (Clingen Soft Gel).

8.3.5.7 Drug release study

In vitro drug release studies were carried out 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. The ready to use softgel capsule containing 100mg CL was used in this test. A soft gel was placed into a glass beaker (having 4cm i.d.) that is filled with dissolution medium (100 ml). Stand for 5min that allowed soft gel to stick in one corner of glass beaker. Then, stirred on a magnetic stirrer at 300RPM by placing magnetic bead opposite side to soft gel in glass beaker. From that, 5ml of the sample was withdrawn at different time intervals (1, 2, 3,

4, 5, 6, 7 and 8h) and were compensated with equal volume of fresh dissolution medium. Then, sample was filtered through Whatman filter paper type I and appropriate dilutions were done with mobile phase and subsequently filtered through a 0.45µm membrane filter. Samples were analyzed by HPLC method. The cumulative percentage of drug released was plotted against time in order to obtain the release profile. The drug release study was also performed for marketed formulation of CL.

8.3.6 Stability studies

Softgel capsule were subjected to stability testing according to the ICH guidelines. Samples were individually sealed in sachets prepared from polyethylene laminated alluminium foil and stored at intermediate stability condition (30±2°C and 65±5% RH) and accelerated stability condition (40±2°C and 75±5% RH). The samples of softgel were examined for physical appearance, pH, moisture content and assay of CL at interval of 1, 2, 3and 6 months of the stability study.

8.4 RESULTS AND DISCUSSION

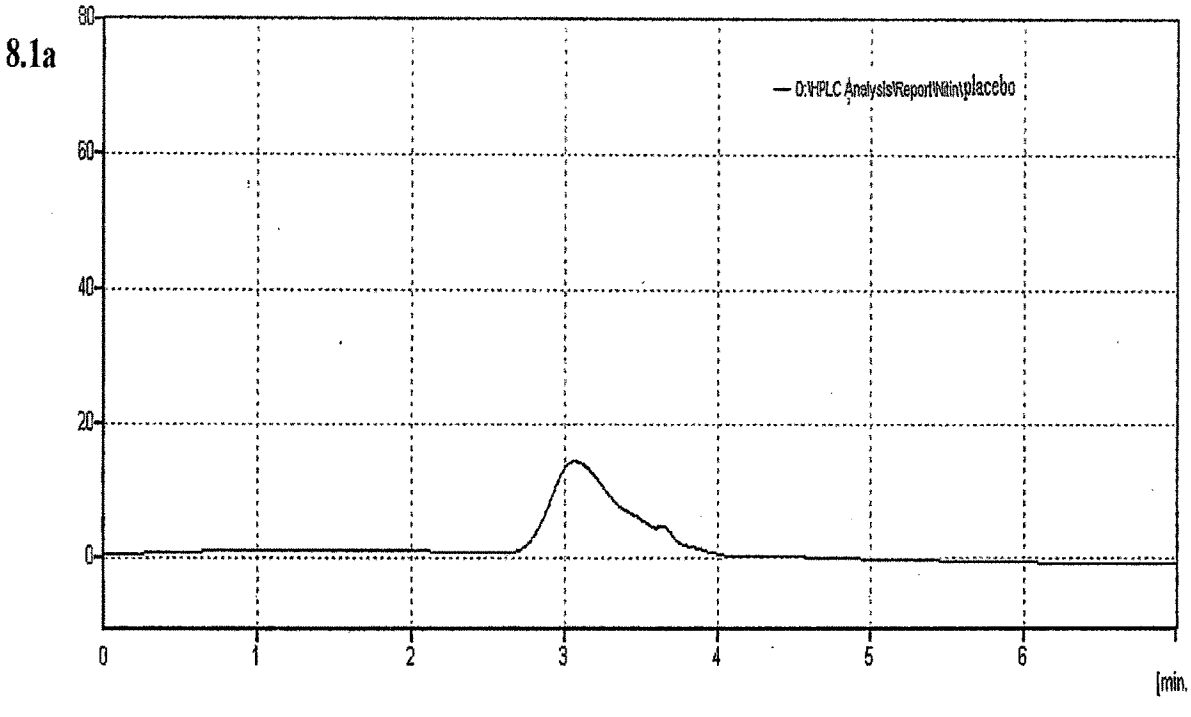
8.4.1 Analytical Method

The chromatographic separation of CL was achieved with reverse-phase C18 column (250mm×4.6mm i.d., 5µm particle size), using a mixture of acetonitrile (ACN):25mM phosphate buffer (25mM NaH_2PO_4 buffer, pH 4.75) in the ratio of 45:55 (%v/v) at a flow rate of 0.6 ml/min with UV detection at 205 nm and quantified with respected to drug peak area. The calibration curve was linear (correlation coefficient of 0.9992) in the concentration range of 10–70 µg/ml. This method was used for estimation of CL in softgel capsules. The specificity of method was confirmed from **Fig.** 8.1b as excipients did not shows any interference peak at retention time corresponds to CL peak.

8.4.2 Preliminary Experiments

In preliminary experiment from SF_1 to SF_3, soybean oil, LLP and PEG 400 were explored as vehicle for preparation of material blend which is encapsulated in softgel capsules. Consistency of the blend is very crucial parameter for avoiding weight variation issues on large scale manufacturing. As the consistency of blend depends on the type of vehicle, batch SF1 and SF_2 were found unacceptable because of soybean oil and LLP were getting separated from the dispersions. Also, soybean oil and LLP impaired bioadhesive property of prepared blend which may be due to its hydrophobic nature. In trial SF_3, CL was homogeneously

dispersed in PEG 400 and produced consistent encapsulation blend. This may be due to hydrophilic nature of vehicle (PEG 400) and polymer (HPC) used for blend preparation.



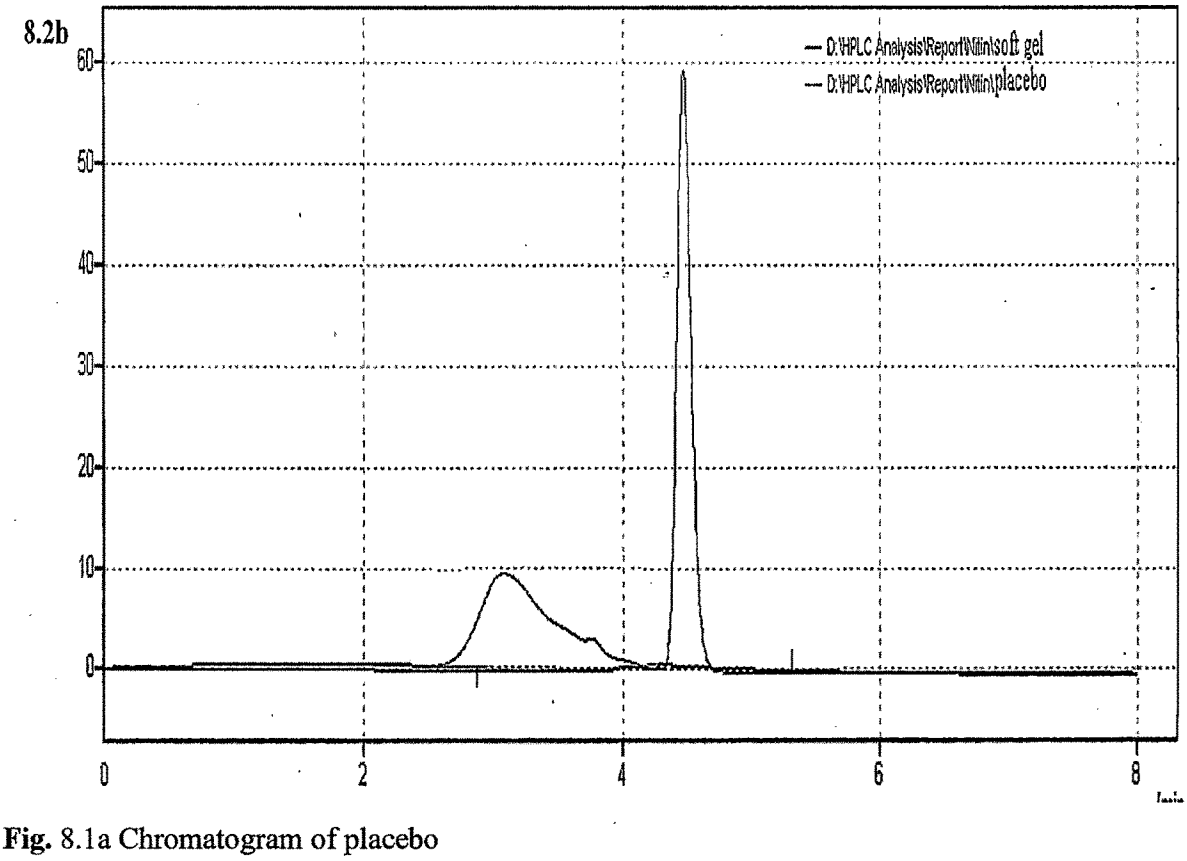


Fig. 8.1a Chromatogram of placebo

8.1b Overlay chromatogram of placebo and CL containing softgel formulation

Hydrophilic nature of encapsulation blend may favors the adhesion of formulation to mucosal surface and prevent expulsion of formulation. The consistency of blend was adjusted by adding suitable concentration of polymers. Viscosity is one important parameter which provides vital information during the optimization of the softgel formulation. After administration, viscosity and dispersibility of formulation in vaginal environment governs the spreading and retention character of the formulations which is essential to achieve desired efficacy. Therefore, a variety of water-soluble polymers such as HEC, HPC, HPMC K15, and xanthan gum were investigated alone or in combination (trial SF_4 to SF_8). Trial SF_4 to SF_6 were taken by incorporating HEC, xanthan gum and HPMC K15 along with HPC respectively. While HPMC K15 and HEC incorporated along with xanthan gum in SF_7 and SF_8 respectively. The results of evaluation of CL softgel batches from SF_1 to SF_8 are shown in **Table.** 8.5. The consistency of the blends were assessed from less (+) to moderate (++) to good (+++). The viscosity of the batches SF_1 and SF_2 was law because of its fluid like consistency. While the viscosity of the batches from SF_3 to SF_8 were found higher because of drug was incorporated with hydrophilic polymers and vehicle. pH of all the batches (in SVF) was measured with pH meter (LABINDIA Pvt. Ltd., New Mumbai) and found satisfactorily as it is between 2.5 and 7.5. Higher acidic pH of the preparation may causes hydrolysis and leakage of the gelatin shell, while alkaline pH of preparation may tan the gelatin and thus affect the solubility of the shell.

Table 8.5 Evaluation of preliminary experiments

Parameters	SF_1	SF_2	SF_3	SF_4	SF_5	SF_6	SF_7	SF_8
Consistency	+	+	++	++	++	++	+++	+++
pH	6.36	6.05	5.39	5.46	5.42	5.37	5.44	5.40
Viscosity(cp)	21.56	17.38	34.22	49.67	42.16	53.24	62.11	58.63

Encapsulation blend should be sufficient viscous which can prevent formulation expelled out from vaginal cavity immediately after bursting of outer shell. The consistency and viscosity of the softgels are related to each other because both are dependent on type and concentration of polymers and vehicles. The viscosity of batch **SF_7** was acceptable i.e. supported by their good consistency. Xanthan gum and HPMC K15 were also responsible for imparting bioadhesion property to the formulation (Vermani K. *et al.*, 2002). Hence, encapsulation blend (**SF_7**) containing xanthan gum, HPMC K15 and PEG 400 was acceptable and selected for further optimizations of the softgel formulation.

8.4.3 Evaluation of Vaginal Soft Gel Capsules

8.4.3.1 Weight Variation

Weight variation of soft gel contents were found 1.73 % which was calculated with respect to theoretical fill weight.

Table 8.6 Fill weight of twenty softgel capsule

Softgel capsule	Fill weight (mg)	Softgel capsule	Fill weight (mg)
1	592	**11**	597
2	603	**12**	568
3	574	**13**	571
4	586	**14**	582
5	566	**15**	599
6	594	**16**	591
7	582	**17**	567
8	569	**18**	574
9	562	**19**	596
10	576	**20**	578

8.4.3.2 Content uniformity

Softgel content was estimated by HPLC method. Average drug content was found between 96.64 to 102.86% per softgel capsule.

8.4.3.3 Viscosity and pH of Formulation Dispersion

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 optimized formulation (SF_{79}) was found to be 138.02±3.32(cp) & 41.84±2.11(cp) in SVF and in water respectively. While viscosity of clingen softgel (market formulation) in SVF was 52±2.83(cp) found. Dispersions of CL softgel 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. pH of the formulation dispersion is also one of the important aspects as it impact on the gelatin shell property and microflora (normal vaginal flora). The pH of CL softgel was found to be alkaline (7.39 ± 0.06) in water and acidic (5.47 ± 0.04) in SVF reveals that vaginal pH as well as micro flora may remains unaffected after administration of softgel.

8.4.3.4 Moisture Content

The moisture content of the softgel was found 7.72±0.57 % (w/w) which is within allowable limit (6-10%w/w) for softgel capsule. It is depending on the gelatin formula used. The small amount of moisture content in softgel helps them to remain stable and prevent from being a completely dry and hard.

8.4.3.5 Swelling Index

The effect of various compositions on swelling index of the softgel is shown in **Fig** 8.2. Formulation variables such as HPMC K15 and xanthan gum had significant effect on swelling index because they tends to get hydrated by absorbing large amount of water. Hence, maximum swelling was seen with softgel (SF_{79}) containing high concentration of HPMC K15 and xanthan gum. Swelling index increases with time due to increase in hydration phenomenon of fill material of softgel. The swelling capability of polymer was crucial for its bioadhesive behavior. Adhesion occurs shortly after the beginning of swelling.

8.4.3.6 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). Bioadhesive strength of soft gels with various compositions (SF_{71}-SF_{79}) is shown in **Fig.**8.3. Xanthan gum and HPMC K15 are formulation variables which forms viscous fluid, when hydrated in presence of SVF and may lead to adhesive interaction with mucosal surface. Thus, bioadhesive strength of softgels was increased with increasing concentration of polymeric compositions. Amongst nine softgel, SF_{79} showed good bioadhesion (0.690 ± 0.012 N) under simulated vaginal environment. Bioadhesive strength of SF_{79} (optimized) were compared with marketed product and was found significantly greater ($p<0.05$) than marketed product (Clingen softgel).

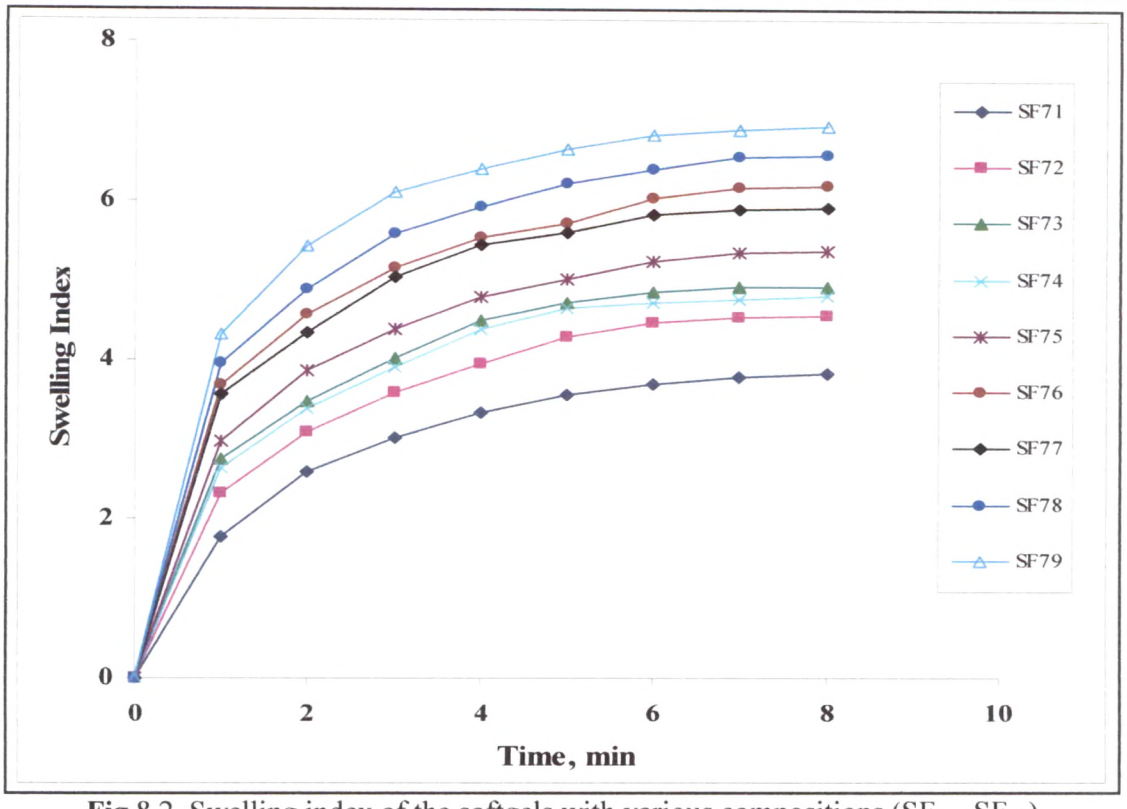


Fig 8.2. Swelling index of the softgels with various compositions (SF_{71}- SF_{79})

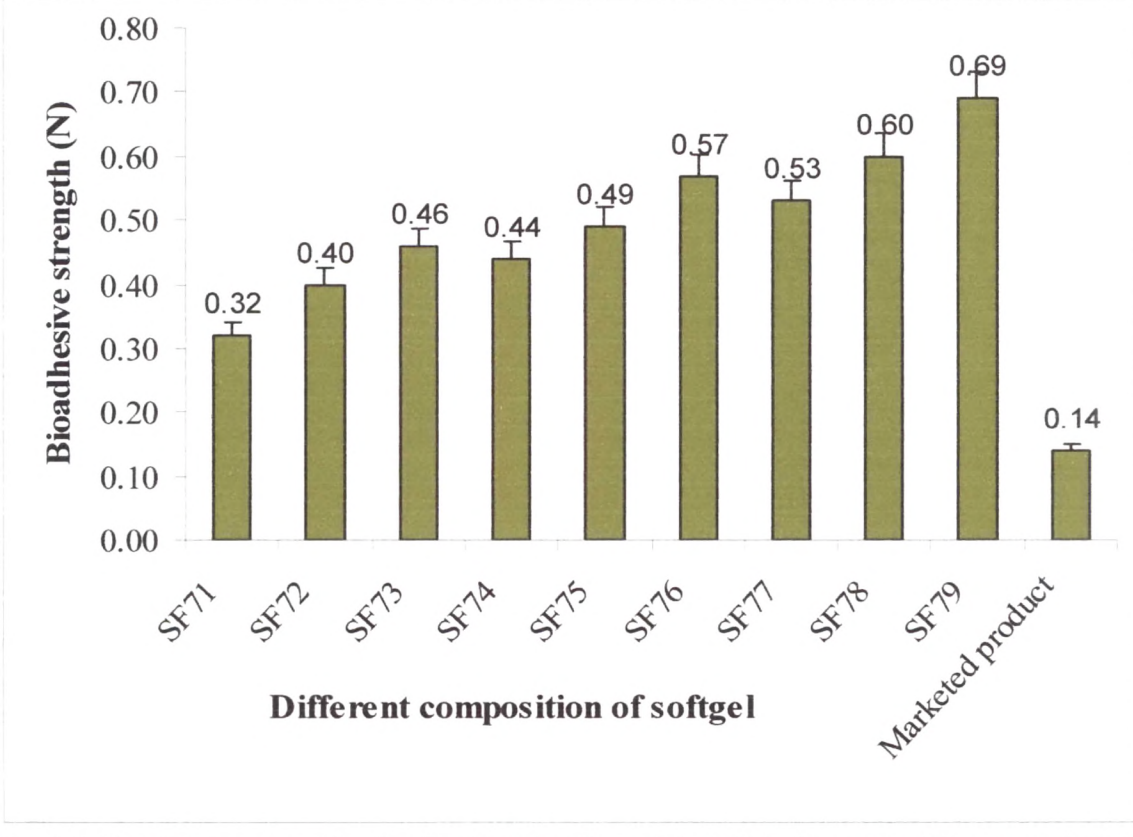


Fig. 8.3 Bioadhesive strength of softgel batches from $SF_{71}$to SF_{79} in comparision to marketed vaginal softgels.

8.4.3.7 Retention Characteristic of Softgel in Simulated Vaginal Environment

In addition to the desirable performance characteristic, the developed softgels possess prolonged retention in vaginal cavity. In order to measure retention characteristic of softgel, weight of formulation dispersion falling down (or retained) from vertically suspended isolated sheep vaginal tube was recorded as a function of time. Amount of formulation dispersions retained in vaginal tube at predetermined time interval for each softgel are shown in **Table.** 8.7. SF_{79} showed very good retention (> 10h) during the ex vivo studies. A softgel with less polymeric concentration was falling down rapidly from vaginal tube. Results of *ex vivo* studies shows that the retention time of formulation dispersion increased as the ratio of xanthan gum to HPMC K15 in softgel increased. This reveals that retention property of softgel can be controlled by varying xanthan gum/HPMC K15 ratio. Polymeric composition of softgel was hydrated with water and forms viscous liquids that may improve retention characteristic of softgel in isolated vaginal tube. In this experiment, dispersion of SF_{79} retained in vaginal tube was higher than the marketed products at all time points suggesting its better retention as compared to marketed product (as see in **Fig.** 8.4).

Table 8.7 Percentage retention of formulation dispersions (SF_{71}- SF_{79}) from vertically suspended isolated sheep vaginal tube as a function of time.

Time	Percentage retention of formulation dispersions, Mean ± SD (n = 3)								
(hr)	SF_{71}	SF_{72}	SF_{73}	SF_{74}	SF_{75}	SF_{76}	SF_{77}	SF_{78}	SF_{79}
1	49.00 ±0.71	54.21 ±0.83	59.63 ±0.78	56.68 ±0.84	62.36 ±0.93	66.71 ±0.86	64.91 ±0.93	68.47 ±0.88	73.82 ±0.64
2	36.42 ±0.59	39.37 ±0.64	46.74 ±0.82	42.66 ±0.59	48.1 ±0.74	53.49 ±0.92	51.76 ±0.77	57.26 ±1.12	61.14 ±0.71
3	29.12 ±0.83	32.48 ±0.59	38.96 ±0.74	35.53 ±0.79	41.84 ±0.88	47.23 ±0.60	45.57 ±0.95	49.69 ±0.91	54.38 ±0.65
4	24.34 ±1.10	28.11 ±0.71	32.84 ±0.62	31.42 ±0.86	35.72 ±0.67	42.86 ±0.73	40.16 ±0.84	45.93 ±0.86	49.10 ±0.87
5	20.93 ±0.73	24.57 ±0.89	27.89 ±0.68	27.14 ±1.12	31.33 ±0.75	38.67 ±0.68	36.23 ±0.66	41.78 ±0.63	44.84 ±0.94
6	18.37 ±0.62	22.12 ±0.92	25.45 ±0.75	24.76 ±0.91	29.68 ±0.69	36.11 ±0.81	33.20 ±0.70	37.49 ±0.72	41.63 ±0.78
7	17.32 ±0.67	19.81 ±0.55	23.71 ±0.63	22.39 ±0.57	27.39 ±0.64	33.65 ±0.61	31.89 ±0.72	36.15 ±0.58	39.47 ±0.66

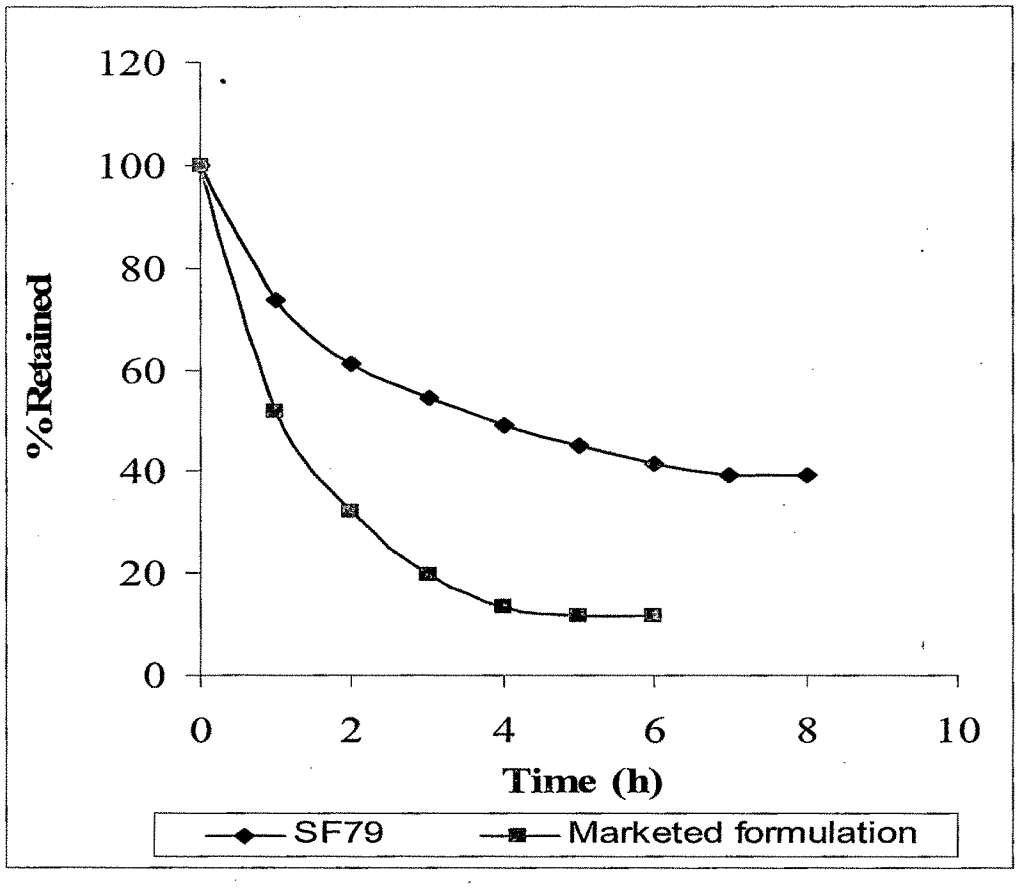


Fig. 8.4 Retention of SF_{79} softgel in comparision with marketed softgels (Clingen).

8.4.3.8 Drug Release Study

In order to study drug release characteristics of softgel formulations, the samples collected at predetermined time interval were analyzed by HPLC method and percentage of CL release was calculated using a preconstructed calibration curve. The release rate profile was drawn as the cumulative percentage drug release from the vaginal soft gel vs. time. Drug release characteristic of SF_{79} (i.e. optimized formulation) and Clingen softgel (market formulation) was described as function of time as shown in **Fig.** 8.5. As can be seen from **Fig.** 8.5., more than 90% of total CL loaded in the softgel was released within 7.0h and 3.0h from SF_{79} and marketed formulation respectively. In optimized formulation (SF_{79}), the burst release of CL from the softgel was observed within first 3h, and then gradually increased up to 7h. This may due to HPMC K15 and xanthan gum swelled rapidly in contact with water and formed rigid gel and it is ultimately responsible for retarding 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).

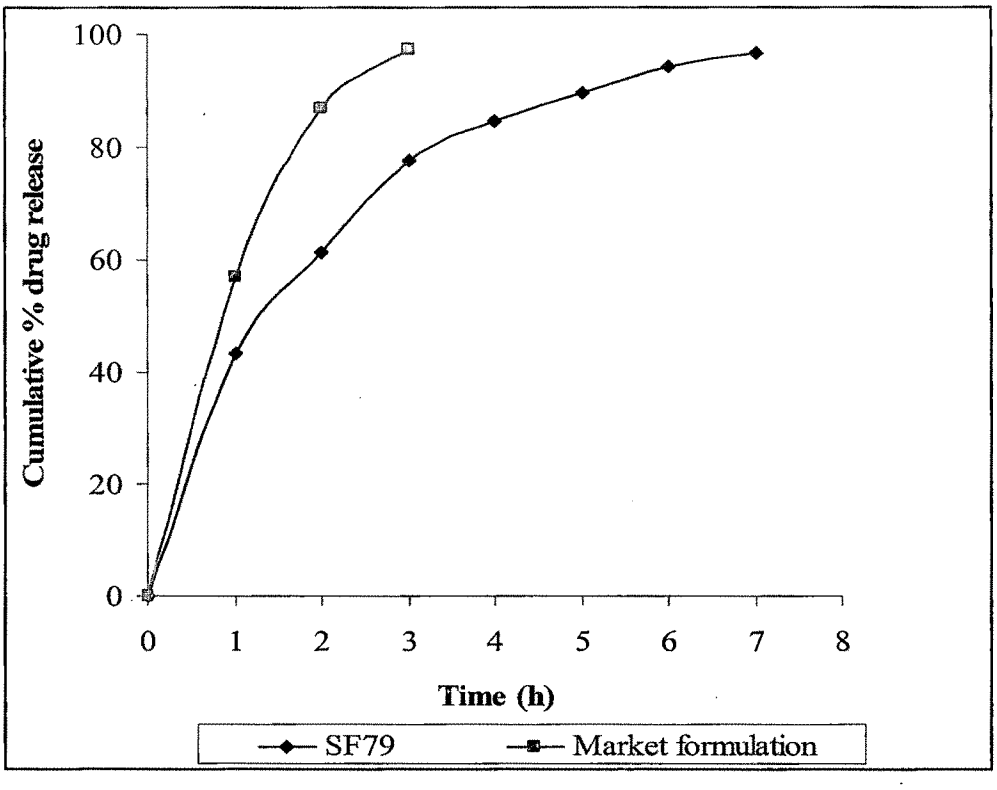


Fig. 8.5 The release profiles of CL from SF_{79} and market formulation.

8.4.4 Optimization of Formulation Responses using Desirability Function

Optimum level of formulation variables such as HPMC and xanthan gum out of nine batches were decided by application of desirability function. In addition, the desirability function was calculated individually for all the formulation responses such as swelling index, retention time (upto 40% formulation retained),Y_{5hr} and combined in one measurement.

In this study, range of values of swelling index of produced formulations was selected for desirability calculation. Therefore, the formulations that have its value within the range of 4.0-7.0 have a desirability of 1, while the formulations which have values out of this range have a desirability of zero. 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 3.57 and 6.65 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 3.0. For Y_{5h}, values of Y_{target} and Y_{min} are 50.00 and 20.93. 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 8.8 Experimental result of response variables and overall desirability of formulations designed by 3^2 full factorial designs.

Batches	Formulation Variables		Response variables*			Overall Desirability
	X_1	X_2	Swelling index	[a]Rt(h)	Y_{5hr}	
SF_{71}	-1	-1	3.57	3.0	20.93	0.00
SF_{72}	-1	0	4.29	4.0	24.57	0.31
SF_{73}	-1	1	4.73	6.0	27.89	0.52
SF_{74}	0	-1	4.65	5.0	27.14	0.45
SF_{75}	0	0	5.03	8.0	31.33	0.71
SF_{76}	0	1	5.72	8.0	38.67	0.85
SF_{77}	1	-1	5.61	8.0	36.23	0.81
SF_{78}	1	0	6.23	8.0	41.78	0.90
SF_{79}	**1**	**1**	**6.65**	**8.0**	**44.84**	**0.94**

* 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** 8.8. Amongst the designed formulations, SF_{79} showed highest overall desirability of 0.94. Hence it was said to be optimum. Therefore, level of formulation variables of this batch was considered to be optimized values for softgel capsule dosage form. Excipients used in softgel are GRAS listed and approved for vaginal use (Garg S. *et al.* 2001). The optimized composition of softgel capsule containing CL is given in **Table** 8.9.

Table 8.9 Optimized composition of softgel formulation containing CL.

Formulation variables	**Optimum value (mg per softgel capsule)**
Clindamycin phosphate	100
HPMC K15	25
Xanthan gum	55
PEG 400	400

7.4.5 Stability Studies

Stability samples were analyzed for various physical properties such as brittleness or softening, color fading, pH of dispersion, moisture content and assay of CL as per the procedure described in method section. The result of the stability study of CL softgel is shown in Table 8.10. Prior to testing, softgel capsules should be equilibrated to known atmospheric conditions. There was no significant change in assay and other properties such as physical appearance, nature of capsule shell, moisture and pH of formulation observed at intermediate as well as accelerated conditions. These observations indicate that prepared softgel (SF_{79}) is stable up to 6 months under the given storage conditions.

Table 8.10 Results of the stability studies of CL 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	5.43 ± 0.07	7.94±0.32	97.79 ±1.34
Intermediate condition (30±2°C and 65±5% RH)	1	5.39 ± 0.10	7.78±0.46	99.43 ±1.19
	2	5.48 ± 0.06	7.62±0.19	96.68±1.46
	3	5.43 ± 0.09	7.98 ±0.36	98.26 ±1.64
	6	5.51 ± 0.07	8.08±0.27	99.11 ±1.37
Accelerated stability conditions (40±2°C and 75±5% RH)	1	5.44 ± 0.08	7.83±0.29	96.44±1.36
	2	5.49 ± 0.05	7.99 ±0.47	98.87 ±1.49
	3	5.41 ± 0.12	8.12±0.53	99.12 ±1.54
	6	5.46 ± 0.09	8.04±0.42	97.78±1.66

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