CHAPTER - VI

CLINDAMYCIN PHOSPHATE CONTAINING BIOADHESIVE FILM FOR VAGINAL DELIVERY

6.1 INTRODUCTION

Bacterial vaginosis (BV) is considered a common vaginal disorder in women of reproductive age. The normal vaginal microflora consists of lactobacilli, while the disturbed vaginal microflora is characterized by the overgrowth of *Gardnerella vaginalis* and other anaerobic bacteria like *Mobiluncus* spp., *Mycoplasma hominis* and *Prevotella* spp. The interest in BV has increased lately because of evidence on this disorder, such as amniotic fluid infection, clinical chorioamnionitis, premature rupture of membranes (PROM), preterm delivery, low birth weight and postpartum endometritis. (Majeroni B.A. *et al.,* 1998). Non-pregnant women with BV have been reported to get post-abortion pelvic inflammatory disease (Eschenbach DA. *et al.,* 1993), post-hysterectomy vaginal cuff cellulitis and plasma cell endometritis. Several publications reports mentioned about an altered vaginal microflora being linked to an increased susceptibility to the acquisition of HIV and other sexually transmitted infectious agents such as *Neisseria gonorrhoeae* and *Chlamydia trachomatis* (Joesoef M.R. *et al.,* 1996). In US alone, bacterial vaginitis accounts for an anticipated **10 million office visits** per year (Schwebke J. R., 2003). In US, bacterial vaginosis is diagnosed more frequently than vulvovaginal candidiasis and UK has also higher occurrence of vulvovaginal candidiasis. It has been reported that BV is present in approximately 15% of private gynecologic patients, 10–30% of pregnant women, and 12–61% of women seen at clinics for sexually transmitted diseases. Although BV is curable, but if left untreated it can causes complications ranging from pelvic infection to miscarriage to increased susceptibility to sexually transmitted diseases.

Now days, bacterial vaginitis is most commonly treated with one of two available antibiotics which may be administered either systemically or vaginally in form of various preparation (Paavonen J. *et al.,* 2000). Both metronidazole and clindamycin phosphate (CL) have a long history for excellent coverage against the common pathogens implicated in bacterial vaginosis (Greaves W.L. *et al.,* 1988). However, a number of studies have shown that intravaginal CL is more effective and better tolerated than metronidazole for bacterial vaginosis treatment (Nagaraja P., 2008). The perceived convenience of oral medications is often associates with risk of systemic adverse drug reaction and potential to interact with concomitant medication (Mikamo H. *et al.,* 1997). From such perspective, topical treatment could represent a rational choice to treat localized infections and is often recommended by various healthcare practitioners. Criticisms for the vaginal delivery of existing medications have often included the inconvenience of multiple days of dosing, associated with the messiness and product leakage from the vaginal cavity. However, available vaginal

formulations possess poor bioadhesive properties as describe in **section 5.2 of chapter V**. Therefore, it has been difficult to achieve optimal potential effectiveness of therapeutic agent with the existing delivery system. These poor bioadhesive properties of existing vaginal formulations emphasizes the need of bioadhesive vaginal delivery system which aimed to hold formulation closed to vaginal mucosa for long time and prevent expulsion of formulation.

Hence, efforts were made to develop bioadhesive film formulation (BFF) for CL. The vaginal films were targeted to dissolve in contact with fluids to form a smooth, viscous and bioadhesive gel. The films should also possess aesthetic appeal such as flexibility, softness and free of any sharp edges to avoid mechanical injuries during insertion. These desired features of films are expected to facilitate ease of administration, user convenience after administration and result in immediate formation of smooth and bioadhesive dispersion that could be retained in the vaginal cavity for prolonged intervals.

6.2 MATERIALS

CL was procured as gift sample from GO-ISH remedies Limited (Baddi, H.P., India). Hydroxypropyl methylcellulose E15 (HPMC E15) was gifted by Colorcon Asia Pvt. Ltd. (Goa, India). Hydroxypropyl cellulose (HPC) was purchased from Innovative Chemicals (Mumbai, India). MRS broth, xanthan gum and hydroxyethyl cellulose (HEC) was purchased from Himedia (Mumbai, India). Polyethylene glycol 400 (PEG 400) and sodium dihydrogen phosphate (NaH_2PO_4) was purchased from S. D. Fine Chemicals (Mumbai, India). Acetonitrile (HPLC grade) was purchased from Spectrochem Lab. (Mumbai, India). Double distilled water was used for preparing mobile phase solutions. All other chemicals and solvent used were of analytical grade. All in vitro tests was carried out in simulated vaginal fluid (SVF).

6.3 EXPERIMENTAL

6.3.1 Development and Validation of Analytical Method (HPLC)

6.3.1.1 Instrument and software

A HPLC (Shimadzu, Kyoto, Japan) was composed of a LC-20AT Prominence solvent delivery module, a manual rheodyne injector with a 20µl fixed loop and a SPD-20A Prominence UV–visible detector. Separation was performed on a Hypersil BDS C18 column (particle size 5µm; 250mm×4.6mm i.d.) at an ambient temperature. Chromatographic data

were recorded and processed using a Spinchrom Chromatographic Station® CFR Version 2.4.0.195 (Spinchrom Pvt. Ltd., Chennai, India).

6.3.1.2 Preparation of Standard Solutions

A stock solutions of CL (1000 µg/ml) was prepared in SVF (pH 4.20) and were stored at 2–8 °C until used. Aliquot of this solution was diluted stepwise with the mobile phase to obtain concentration in range of 10 – 70 µg/ml of CL. These samples were used for preparation of calibration curve.

6.3.1.3 Sample Preparation

Vaginal film was accurately weighed and transferred into a 100 ml volumetric flask. About 70–80 ml of SVF was added to it and was allowed to stir on magnetic stirrer (Remi equipments ltd., Mumbai, India) for 30 min followed by sonication for 5 minute. Volume was made up to 100 ml with SVF. Appropriate dilutions were done with mobile phase and subsequently filtered through a 0.45µm membrane filter to remove any particulate matter.

6.3.1.4 Optimization of Chromatographic Conditions

Different experimental variables were studied in order to achieve optimum separation. Hence, a number of preliminary trials were conducted with different combinations of different organic solvents and buffers at various pH, and flow rate to check the retention time, shape, resolution, and tailing factor of peak. From these trials, the mobile phase in combination of ACN and 25mM NaH_2PO_4 buffer (in acidic pH range) was found to be most suitable for chromatographic separation of CL. In order to achieve best chromatographic separation, following chromatographic conditions were studied: (i) Mobile phase composition varied at 55:45, 50:50, and 45:55v/v with pH and flow rate kept constant at 4.75 and 0.5 ml/min, respectively. (ii) pH of mobile phase was varied at 4.0, 4.75, and 5.50 keeping the composition of ACN: 25 mM NaH_2PO_4 buffer 50:50 and flow rate of 0.5 ml/min fixed. (iii) Flow rate was varied 0.4, 0.5, and 0.6 ml/min with mobile phase composition and pH maintained at 50:50 and 4.75, respectively. However, the effects of different level of all these three factors were systematically addressed on system suitability parameters such as theoretical plates, retention time, asymmetry, and capacity factor etc.

6.3.1.5 Validation of the Proposed Method

The objective of validation of an analytical procedure is to verify that the characteristics of

the method satisfies the requirements of the application domain. The proposed method was validated as per ICH guidelines for linearity, specificity, precision, and accuracy (Walfish S., 2006).

Linearity and Range: The linearity of an analytical method is its ability within a definite range to obtain results directly proportional to the concentrations of an analyte in the sample. The concentration of standard solution of CL in the range of 10–70 µg/ml was used for the construction of the calibration curves. The calibration curve for HPLC analysis was constructed by plotting the peak area of drug against drug concentration. The calibration plot was generated by replicate analysis (n = 5) at all concentration levels and the linear relationship was calculated using the least square method within Microsoft Excel® program.

Accuracy and Precision: In order to establish the accuracy of the proposed method, recovery experiments were carried out by the standard addition method at 80, 100 and 120% (recovery study). The known amount of the pure drug was added to pharmaceutical formulation and mixture was analyzed by the proposed method. The experiment was repeated in triplicate and the percentage recovery was calculated.

Both intraday precision (repeatability) and interday precision (reproducibility) were determined using solutions containing lowest, intermediate, and highest concentrations of the calibration curve, i.e. 20, 50, and 70 µg/ml. Three injections at each of the specified concentration levels were injected within the same day for intraday precision, and over a period of 3 days (one injection per day) for reproducibility. Mean and relative standard deviation were calculated and used to judge precision of the method.

Specificity: The specificity criterion tries to demonstrate that the result of the method is not affected by the presence of excipients. The specificity of the method was determined by comparing the chromatogram obtained from the formulation sample with chromatogram of pure CL.

Sensitivity: The slope of the calibration line can provide the instrumental response sensitivity because a method with a large slope is better able to discriminate between minute differences in analyte content. LOQ and LOD were determined using following equation:

$$\text{LOQ or LOD} = \frac{ksB}{S}$$

Where k is a constant (10 for LOQ and 3 for LOD), sB is the standard deviation of y-intercepts of regression lines, and S is the slope of calibration curve.

6.3.2 Formulation Development and Optimization

6.3.2.1 Selection of Film Forming Polymer and Plasticizer

A preliminary trial was carried out to search suitable excipients for preparation of polymeric film. As the desired product must dissolve in vaginal fluid, only water soluble polymers and plasticizers were explored for preparation of CL films formulation. Different water soluble polymers such as HPMC, HEC, HPC, guar gum, sodium alginate and xanthan gum were investigated for preparation of polymeric film. Different water soluble plasticizer such as glycerol, d- sorbitol and PEG 400 were also explored for development of bioadhesive film (BF).

To ascertain the effect of each polymer and plasticizer on the physical characteristics of film, trials F_1 to F_{15} (**Table** 6.1) were conducted. Polymeric film prepared in the preliminary trials was physically characterized. From these trials, the combination of HPC, xanthan gum and PEG 400 was found most suitable composition for preparation of CL film and selected for further optimization of the film formulation.

Table 6.1 Composition of preliminary formulation trials.

Ingredients (mg/ film of 2.5 cm × 2.5 cm)	**F_1**	**F_2**	**F_3**	**F_4**	**F_5**	**F_6**	**F_7**	**F_8**	**F_9**	**F_{10}**	**F_{11}**	**F_{12}**	**F_{13}**	**F_{14}**	**F_{15}**
Clindamycin phosphate	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
d- sorbitol	50	0	0	50	0	0	0	0	0	0	0	50	0	50	0
Glycerol	0	50	0	0	50	0	0	0	0	0	0	0	0	0	0
PEG 400	0	0	50	0	0	50	50	50	50	50	50	0	50	0	100
HPMC	100	100	100	0	0	0	50	0	0	0	0	0	0	0	0
HPC	0	0	0	100	100	100	100	100	100	100	100	0	0	0	0
HEC	0	0	0	0	0	0	0	0	50	0	0	0	0	0	0
Xanthan gum	0	0	0	0	0	0	0	50	0	0	0	0	0	0	0
Guar Gum	0	0	0	0	0	0	0	0	0	50	0	100	100	0	0
Sodium Alginate	0	0	0	0	0	0	0	0	0	0	50	0	0	100	100

6.3.2.2 Drug Excipients Compatibility Study

Excipients having promising role in development of formulation were subjected to thermal (DSC) and isothermal stress analysis (HPLC) to ensure their compatibility with CL. For thermal analysis, CL, excipients, and their physical mixture were characterized by

differential scanning calorimeter (DSC 60, Shimadzu, Japan). Instrument was calibrated using indium as the reference standard. Samples were crimped in aluminum pans and analyzed at a nitrogen flow of 30 ml/min and heating rate of 15°C/min from 35°C to 300°C. For isothermal stress testing pure drug, CL film and their placebo were store at stress conditions (50°C) for 3 weeks. Samples were examined periodically for any unusual changes in color and physical form. After 3 weeks of storage, the samples were analyzed quantitatively for content of CL by HPLC. Refrigerator stored samples were considered as controls.

6.3.2.3 Factorial Design and the Desirability Function

Factorial experimental design, contour plots, prediction profile and desirability function have been proved to be a useful approach for optimization of the formulations (Paterakis PG *et al.*, 2002). The independent variables selected for predefined screening were amount of HPC (X_1) and amount of xanthan gum (X_2). Whereas mechanical properties of film such as % elongation at break (%EB) and tensile strength (TS), and % drug retained on the vaginal mucosa up to 8 h (Y_{8h}) were designated as response variables.

In the present research, 3^2 full factorial designs was used to study all the possible combinations of all factors at all levels, (two factors, three levels) and were conducted in a fully randomized order. Total nine formulations were designed by 3^2 full factorial designs (see **Table** 6.2). 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 preliminary experiments. High and low levels of each factor were coded as 1 and -1, respectively, and the mean value as zero. Compositions of formulations designed by 3^2 full factorial designs are shown in **Table** 6.3.

The study of desirability function is best approach for optimization of formulation composition (Rane Y.M. *et al.* 2007). In case of desirability function all the responses were combined in one measurement, this gives the possibility to predict the optimum levels for each of independent variable. To combine all the responses in one desirability function requires the calculation of individual desirability function. The individual desirability for each response was calculated using the following methods.

In this study, there were no specific requirements for TS of optimum formulation. Therefore, range of TS values of produced formulations was selected. As moderate TS was desired, the formulations that have its value within the range of 3.0-5.0 have a desirability of 1, while the formulations which have values out of this range have a desirability of zero.

Table 6.2 Formulation designing by 3^2 full factorial designs

Batches	Formulation variables	
	X_1	X_2
F_{81}	-1	-1
F_{82}	-1	0
F_{83}	-1	1
F_{84}	0	-1
F_{85}	0	0
F_{86}	0	1
F_{87}	1	-1
F_{88}	1	0
F_{89}	1	1

Formulation variables	Levels		
	Low (-1)	Medium (0)	High (+1)
X_1 = Amount of HPC (mg)	50	75	100
X_2 = Amount of xanthan gum (mg)	40	60	80

Table 6.3 Compositions of formulations designed by 3^2full factorial designs

INGREDIENTS (mg/ film of 2.5 cm × 2.5 cm)	F_{81}	F_{82}	F_{83}	F_{84}	F_{85}	F_{86}	F_{87}	F_{88}	F_{89}
Clindamycin phosphate	100	100	100	100	100	100	100	100	100
HPC	50	50	50	75	75	75	100	100	100
Xanthan gum	40	60	80	40	60	80	40	60	80
PEG 400	50	50	50	50	50	50	50	50	50

The calculation of the desirability function for TS 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 TS and Y_i is the experimental results. The values of Y_{min} and Y_{max} for TS were 3.37 and 4.73 respectively.

In addition, optimum vaginal film formulation should possess high % EB and Y_{8h}. Therefore, the desirability function of these 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 % EB and d_3 is the individual desirability of Y_{8h}. The values of Y_{target} and Y_{min} for % EB are 91.35 and 72.21. For Y_{8h}, values of Y_{target} and Y_{min} are 13.48 and 6.18. Y_i is the experimental result. The overall desirability values were calculated from individual values by using the following equation:

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

6.3.3 Preparation of Films

Films were prepared by solvent evaporation technique. Polymeric aqueous solution was prepared by dissolving specific amount of CL, plasticizer and film forming polymers in distilled water and was allowed to stir on magnetic stirrer (Remi equipments ltd., Mumbai, India) for 6 hours followed by sonication for 30 minutes. Allow to stand for 1h to remove all the entrapped air bubbles. Then, polymeric aqueous solution was uniformly spread onto a glass plate of defined area (50 cm^2) and dried in vaccum oven at 50 °C for 20 h. Dried films were removed from glass surface, cut into pieces of definite size (2.5 cm ×2.5 cm) and individually sealed in sachets prepared from polyethylene laminated alluminium foil. These films were stored at temperature of 30 ± 2°C and relative humidity 60 ± 5% until further analysis.

6.3.4 Physical Characteristics of Films

BFF was evaluated pharmaceutically for various aesthetic (appearance, odor, color, flexibility and peelability) and physicodynamic properties such as pH and viscosity of polymeric dispersion, TS, %EB, moisture content, film thickness, swelling index, content uniformity, bioadhesion and % drug retention on vaginal mucosa (Repka A.M. *et. al.,* 2000).

Thickness of each sample was measured using a thickness tester (Model 110, 0.01 mm capacity, Mitutoyo Manufacturing Corporation Ltd., Japan) at five different locations (four corners and center) and mean thickness was calculated. Samples with air bubbles, nicks, or tears and having mean thickness variations of greater than 5% were excluded from

analysis. Viscosity of polymeric dispersion was determined by Brookfield cone and plate rheometer LVDVIII (Brookfield engineering, Middleboro, MA, USA). Viscosity of BF was measured at 29°C at 20 RPM and shear rates 40 sec^{-1}. The pH of aqueous solution of film was measured with pH meter (LABINDIA Pvt. Ltd., New Mumbai). For viscosity and pH measurement, one unit of film (2.5 cm ×2.5 cm in size) was dissolved in 10ml of distilled water and SVF. All the parameters were measured in triplicates.

6.3.5. Scanning Electron Microscopy (SEM) Studies

Morphological characterization of film was carried out by SEM studies. Surface feature of the film exposed to vaginal mucosa was observed by scanning electron microscope (Model JSM 5610LV, Jeol, Japan). The samples were attached to slab surfaces with double-sided adhesive tapes and scanning electron photomicrograph was taken at 150X, 1000X and 5000X magnification.

6.3.6. Mechanical Properties

A calibrated Instron universal testing instrument (Model 1121, Instron Limited, UK) equipped with a 100 kg load cell was used for measurement of mechanical properties of film. Film was cut into narrow strips in dimension of 40 mm × 10 mm. Film strip free from air bubbles was placed between two clamps positioned at a distance of 10 mm in same plane. Lower clamp was fixed and strip was pulled by top clamp at a rate of 100 mm/min during measurement. The force and elongation at a moment of break were recorded. TS and % EB was calculated by using following equations (Peh K.K. *et al.,* 1999; Aulton M.E. *et al.,* 1981).

$$Tensile\ strength = \frac{Force\ at\ break\ (N)}{Initial\ cross\ sectional\ area\ of\ the\ sample\ (mm^2)}$$

$$\%\ Elongation\ at\ break\ (\%mm^{-2}) = \frac{Increase\ in\ length}{Original\ length} \times \frac{100}{cross-sectional\ area\ (mm^2)}$$

Results from film samples, which broke at and not between the clamps were not included in calculations. Measurements were run in triplicates for each film.

6.3.7. Average Drug Content of Films

Average drug content in film was measured in order to confirm uniform distribution of CL in BF. In addition, samples (2.5 cm ×2.5 cm) were collected from centre and four corners within film, weighed and dissolved in 100ml of SVF. An aqueous samples were

subsequently filtered through a 0.45μm membrane filter, followed by dilution with mobile phase consisted of 25mM phosphate buffer (pH 4.74) and ACN (50:50, v/v). The samples were analyzed by HPLC method. The content of CL was calculated using a preconstructed calibration curve for CL. No polymeric interference was observed under the conditions of assay procedure.

6.3.8. Measurement of Film Swelling Capability

This study was carried out by a new and convenient method using stainless steel basket. Swelling capability of film was performed in simulated vaginal environment using SVF as media. Each film sample with surface area 2.5 cm × 2.5 cm was weighed and placed in a preweighed stainless steel basket with 200 mesh aperture. Then, basket containing film sample was submerged into 20 ml SVF medium in glass beaker. The basket was removed from beaker at preset time intervals; excess of SVF was soaked by tissue paper and reweighed until no further change in weight of film. Each measurement was repeated in triplicate. The swelling capability was expressed as swelling index (Deshpande A.A. *et al.*, 1997) and it was calculated by following equation:

$$\text{Swelling capability (\%)} = \left[\frac{Wt - Wo}{Wo}\right] \times 100$$

Where, W_t = weight of film at time t, and Wo = initial weight of film.

6.3.9. Moisture Content

The films (2.5 cm ×2.5 cm) were weighed accurately and placed in desiccator's containing calcium chloride for 24 h. Films were removed from desiccators and reweighed until a constant weight obtained. The percentage of moisture content was calculated as difference between initial weight and constant weight of film with respect to initial weight. Each measurement was repeated in triplicate.

6.3.10. In Vitro Bioadhesion Evaluation

Vaginal mucosa of different animals including pig, monkey, cow and sheep has been used as model biological tissues for evaluation of bioadhesion. (Castle P.E. *et al.*, 1998, Dobaria N. *et al.*, 2007) In this study, sheep vaginal mucosa was used. The bioadhesive property of film was assessed in simulated vaginal environment (Lei W., 2008) using a texture analyzer equipped with 2.0 kg load cell (Model 1121, Instron Limited, UK). For experiments, vaginal tube (which was freshly collected from local slaughter house) was incised longitudinally and holds on lower platform of texture analyzer. Film was fixed on

upper probe with help of double-sided adhesive tap. Vaginal mucosa was moistened with SVF and kept in contact with film for 5 min to allow formation of adhesive bond. Then, upper probe of texture analyzer was moved at speed of 0.1mm/sec. The force required to detach film from tissue surface was determined as bioadhesive strength. For measurement of bioadhesive strength, experiment was repeated three times.

6.3.11 Ex Vivo Retention Study

This study was performed by a new model using simulated dynamic vaginal system. Simulated dynamic vaginal system consisted of closed glass cell with 30° angle slope and flow rate pump as describe in **section 5.3.11 of chapter V**. This study involved amount of CL falling down (or retained on mucosa) was measured as function of time. Sheep vaginal tube was obtained from local slaughterhouse immediately after sacrifice of animal. Before commencement of the experiments, supporting tissues was removed from sheep vaginal tube and thawed in normal saline. Sheep vaginal mucosa was cut into 5 cm × 5 cm pieces and mounted on simulated dynamic vaginal system with mucosal side up. Then, BFF (2.5cm ×2.5cm) was placed on mucosal membrane and SVF was applied on films with a flow rate of 5 ml/hr. At predetermined time intervals, perfused SVF was collected into a receiver beaker for 8h and was analyzed by HPLC method. Amount of drug retained on vaginal mucosa was calculated as difference between initial drug content of film and amount of drug collected into a receiver beaker at predetermined time intervals.

6.3.12. *In Vitro* Lactobacillus Inhibition

Lactobacillus acidophilus growth against placebo film and BFF was estimated by using cup plate method (E-aithy H.M. *et. al.,* 2002). The bacterial strains of lactobacillus acidophilus were obtained from MTCC (Microbial Type Culture Collection, Chandigarh, India) and subcultured in MRS broth for two to three times before commencement of experiment. *In vitro* activities exerted by CL, placebo film and BFF against lactobacillus acidophilus was determined by same method as described previously in **section 5.4.12 of chapter V.**

6.3.13. Stability Studies

Stability studies of developed films were performed according to the ICH guidelines for zones IV countries like India. The developed films were individually sealed in sachets prepared from polyethylene laminated alluminium foil. For intermediate stability studies,

films were stored at 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 polymeric dispersion, mechanical properties of film and CL content estimation was carried out periodically at the end of 1, 2, 3 and 6 months of the stability study.

6.4 RESULTS AND DISCUSSION

6.4.1 Analytical Method Development

6.4.1.1 HPLC Method

CL was not analyzed by UV spectrophotometrically because absence of chromohperic group in their chemical structure. Therefore, HPLC method was developed for estimation of CL from their formulations. HPLC is most popular mode of chromatography. Almost 90% of all analysis of low-molecular-weight samples were carried out using RP HPLC (Sethi P.D., 2001). One of the main drivers for its enormous popularity is its ability to discriminate very closely related compounds and the ease of variation of retention and selectivity.

6.4.1.2 Optimization of Chromatographic Conditions

With the aim of the optimization of mobile phase composition, the ratio of ACN: 25mM NaH_2PO_4 buffer was studied at 55:45, 50:50, and 45:50v/v levels and the remaining two factors kept constant, i.e. flow rate and pH of mobile phase. The chromatographic parameters such as retention time, capacity factor, theoretical plates and asymmetry value at different composition of mobile phase are enumerated in **Table** 6.4. Usually, the retention time decrease with increase in concentration of organic solvent is mainly due to a decrease in the distance between the solute molecule and the terminal carbon atoms (C18) in the ODS ligand (Ban K. *et al.*, 2001). Similar findings were observed in the present study with increasing ACN concentration in mobile phase. Minimum retention time is one of the desirable criteria. But, the asymmetry was found to be greater than 1.5 with mobile phase composition 55:45 and 50:50 which indicates tailing of peaks. However, the asymmetry value at 45:55 v/v was found to be 1.3 and so was considered to be optimum.

The effect of mobile phase pH on chromatographic separation of CL was studied at 4.0, 4.75 and 5.50 with keeping fixed mobile phase composition and flow rate. The chromatographic responses obtained at different pH are given in **Table** 6.4. Fronting was observed at mobile phase pH 4.0. But, asymmetry was found below 1.25 with mobile phase pH 4.75. It is desirable criteria for optimum chromatographic parameters.

The results of the chromatographic parameters at different flow rate are listed in **Table** 6.4 indicates that the change in flow rate had no significant effect on peak asymmetry and their shape. But, it could be observed that retention time decreases as the flow rate increases. Theoretical plates were found highest at flow rate 0.6ml/min with asymmetry of less than 1.3.

Table 6.4 Effect of different mobile phase compositions, pH and flow rate on various chromatographic parameters.

Variables	Values	Chromatographic parameters			
		Retention time (*R*t, min)	**Asymmetry,** (*A*s)	**Capacity factor,** (*k'*)	**Theoretical plates** (mm)
Mobile phase composition(v/v) (ACN:25mM NaH_2PO_4 Buffer)	55:45	4.57	1.297	1.47	5813
	50:50	4.72	1.263	1.54	5902
	45:55	4.91	1.270	1.64	6619
pH	4.0	4.74	1.231	1.54	5006
	4.75	4.72	1.263	1.54	5902
	5.5	4.81	1.351	1.53	5855
Flow rate (ml/min.)	0.5	4.72	1.263	1.54	5902
	0.6	4.27	1.273	1.91	6154
	0.7	3.98	1.333	1.12	5174

6.4.1.3 Proposed Chromatographic Method

Mobile phase composition of ACN: 25mM NaH_2PO_4 buffer of 45:55, v/v adjusted to pH 4.75 was finally recommended for chromatographic separation of CL. The mobile phase was prepared freshly everyday. The mobile phase was premixed and filtered through a 0.2 μm membrane filter to remove any particulate matter. The mobile phase was degassed by sonication before use. Prior to injecting solutions, the column was saturated for at least 45 min with 0.3 ml/min flow rate of mobile phase. The best sensitivity of the method was obtained at 205 nm with mobile phase flow rate of 0.6 ml/min. Hence, the UV detector was set at a wavelength of 205 nm. Typical chromatogram at the optimized condition gives sharp and symmetric peak with retention time of 4.54± 0.07 min.

6.4.1.4 Validation of Proposed Method

Experimental set (10, 20, 30, 40, 50, 60 and 70µg/ml) was analyzed five times by using proposed HPLC method. Peak area response of five experimental set is enumerated in **Table** 6.5. The calibration curves for CL (**Fig.** 6.1a) were constructed by plotting the area of the peaks vs. concentration; this was found to be linear over an analytical range of 10-70 µg/ml. A linear regression by the least squares method was then applied. The calculated value for the determination of coefficient (r^2 = 0.9992) indicates strong linear relationship between the variables. **Fig.** 6.1b represents overlay HPLC chromatograms of CL for calibration curve.

Table 6.5 Measurement of peak area of CL in different experimental set by HPLC method

Concentration (µg/ml)	Experiment Sets					Mean	SD	%RSD
	I	II	III	IV	V			
10	87.65	90.44	89.8	91.26	92.47	90.32	1.80	1.99
20	168.14	164.96	162.18	167.2	166.59	165.81	2.34	1.41
30	248	259.95	252	255	250.42	253.07	4.61	1.82
40	332.61	338	337.8	341.46	338.27	337.63	3.18	0.94
50	435.21	437.28	437.42	439.8	432.69	436.48	2.67	0.61
60	529.31	513.78	517.97	523.74	522	521.36	5.88	1.13
70	612.59	606.23	608.74	614.66	606.11	609.67	3.83	0.63

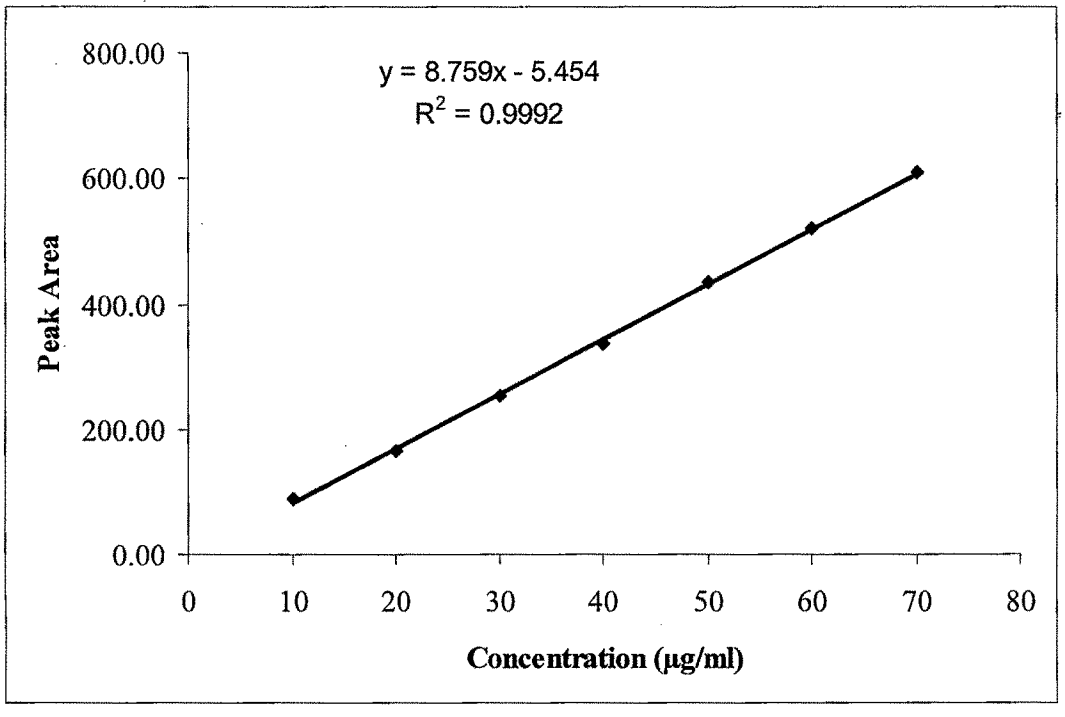

Fig. 6.1a Calibration curves for CL

Spectral and statistical data for estimation of CL by proposed HPLC method are illustrated in **Table** 6.6. The LOD and LOQ were obtained 2.36 and 7.88 µg/ml, respectively. The sensitivity of proposed method was found to be adequate for perform assay of formulations.

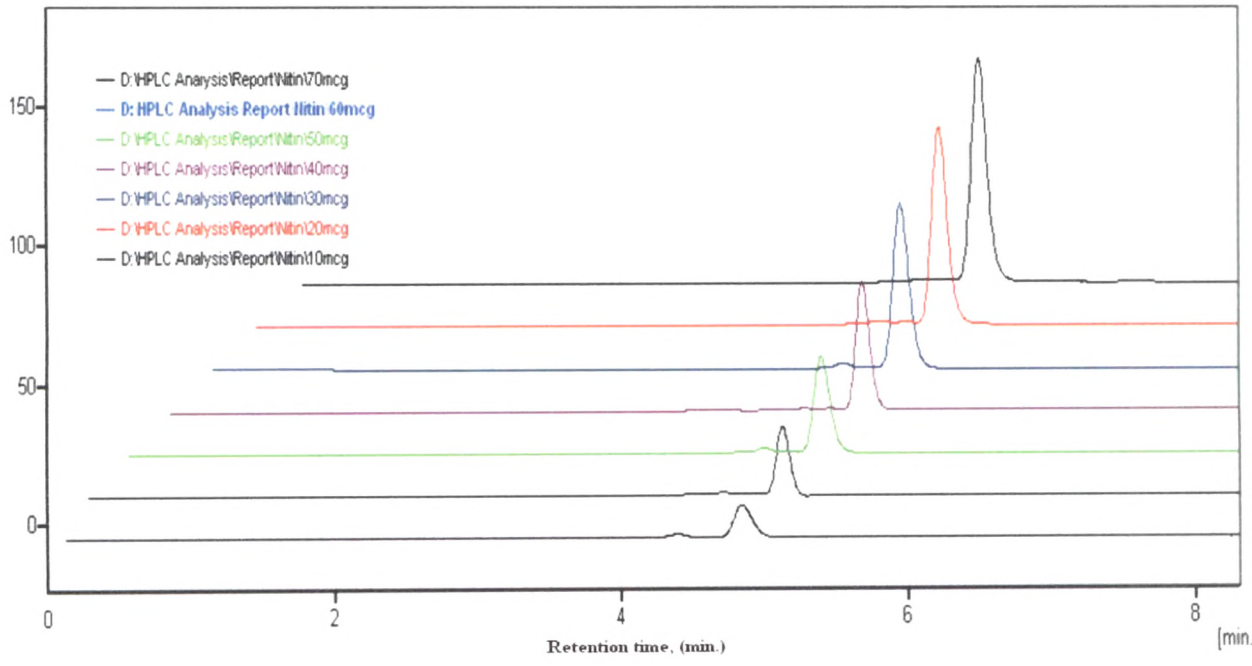

Fig. 6.1b Overlay HPLC chromatograms of CL

The specificity of method was confirmed from **Fig.** 6.2 as excipients did not showed any interference peak at retention time corresponds to CL peak

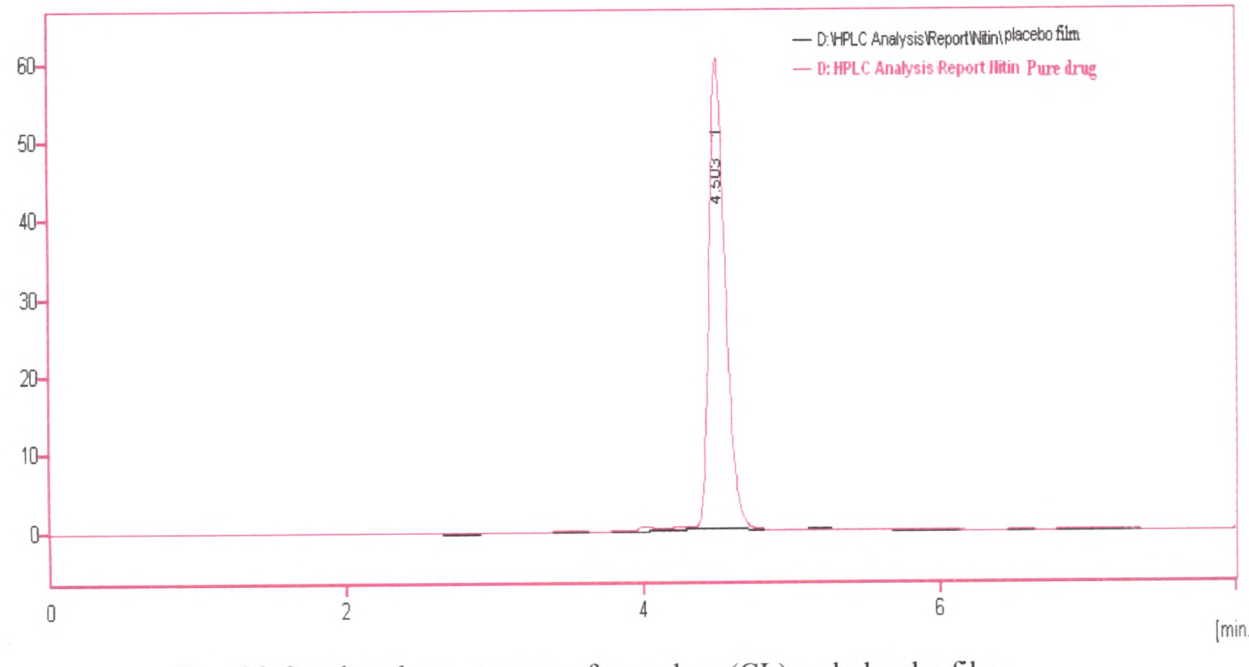

Fig. 6.2 Overlay chromatogram of pure drug (CL) and placebo film

Table 6.6 Spectral and statistical data for estimation of CL by proposed HPLC Method

Parameter	Value
Absorption maxima, λ_{max} (nm)	205
Linearity range (µg/ml)	10-70
Coefficient of determination (r^2)	0.9992
Slope	8.759
Intercept	5.454
LOD (µg/ml)	2.36
LOQ (µg/ml)	7.88

Accuracy of the proposed method was established by recovery experiments. Recovery study was performed by the standard addition of pure drug (80%, 100% & 120% of the label claim) to pharmaceutical formulation and mixture was analyzed by the proposed method. The results obtained from recovery studies are shown in **Table** 6.7. The results are in good agreement with the label claim which shows the accuracy of method.

Table 6.7 Accuracy of the proposed HPLC method by recovery studies

Level	Replicate	Amount			Recovery	
		Added (ppm)	Found (ppm)	Recovery %	Mean	% RSD
Sample solution	1	0.00	97.72	97.72	98.1	1.42
	2	0.00	99.68	99.68		
	3	0.00	96.98	96.98		
Level- 1 (80% addition of label claimed)	1	177.72	176.38	99.25	99.3	0.43
	2	179.68	179.24	99.76		
	3	176.98	175.06	98.92		
Level- 2 (100%addition of label claimed)	1	197.72	195.76	99.01	99.1	0.23
	2	199.68	198.43	99.37		
	3	196.98	194.93	98.96		
Level- 3 (120% addition of label claimed)	1	217.72	217.12	99.73	99.2	0.67
	2	219.68	215.81	98.24		
	3	216.98	216.29	99.68		

Precision of the proposed method were assessed by performing three replicate analyses of the standard solutions. Three different lowest, intermediate, and highest

concentrations of the calibration curve were prepared and analyzed to determine intra-day and inter-day variability. The within and between-day precision were calculated in terms of %RSD. The results and the mean values were shown in **Table** 6.8 demonstrating good precision.

Table 6.8 Interday and intraday precision of the proposed HPLC method

Nominal concentration (µg/ml)	Mean Area	S.D.	Precision (%RSD)
Inter day			
20	164.57	2.23	1.35
50	432.25	4.11	0.95
70	609.6	7.38	1.21
Intraday			
20	166.14	2.78	1.67
50	431.74	4.87	1.13
70	601.12	7.69	1.28

6.4.2 Selection of Film Forming Polymer and Plasticizer

A selection of excipient is one of the most important steps towards optimization of desired formulation. As the desired product must dissolve in vaginal fluid, only water soluble polymers and plasticizers were explored for preparation of CL films. The physical characteristics of CL films prepared with various polymers and plasticizers are mentioned in **Table** 6.9. Film containing glycerol as plasticizers could not form film. On the other hand, films containing PEG 400 as plasticizer were easy to remove from casting surface, but brittle in nature. Film containing d- sorbitol as plasticizer also forms brittle film. Film prepared with sodium alginate/guar gum could not peel from casting surface. While film containing HPMC or HPC were found easy to peel, but brittle in nature. 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.

Another water-soluble polymer such as HEC, guar gum, sodium alginate and xanthan gum was investigated along with HPC to improve physical characteristics of CL film. Film obtained with HPMC and HPC possessed good peelability, but were brittle in nature and had spots on the surface.

Table 6.9 Physical characteristics of CL films prepared with various polymers and plasticizers

Film	Polymers	Plasticizer	Composition (CL:polymer: polymer:plasticizer)	Physical Characteristic of Film
F_1	HPMC	d-sorbitol	1:0:1.0:0.0:0.5	Brittle and hard to peel
F_2	HPMC	Glycerol	1:0:1.0:0.0:0.5	Film does not form
F_3	HPMC	PEG 400	1:0:1.0:0.0:0.5	Easy to peel, Brittle
F_4	HPC	d-sorbitol	1:0:1.0:0.0:0.5	Brittle, hard to peel
F_5	HPC	Glycerol	1:0:1.0:0.0:0.5	Film does not form
F_6	HPC	PEG 400	1:0:1.0:0.0:0.5	Brittle film
F_7	HPC: HPMC	PEG 400	1:0:1.0:0.5:0.5	Easy to peel, but brittle film formed
F_8	**HPC: Xanthan gum**	**PEG 400**	**1:0:1.0:0.5:0.5**	**Homogeneous, soft and easy to peel from casting surface**
F_9	HPC: HEC	PEG 400	1:0:1.0:0.5:0.5	Film form, but more soft and difficult to peel, Air bubble entrapped in film
F_{10}	HPC: guar gum	PEG 400	1 :0:1.0:0.5:0.5	Not easy to peel from casting surface
F_{11}	HPC: sodium alginate	PEG 400	1:0:1.0:0.5:0.5	Difficult to peel
F_{12}	Guar gum	d-sorbitol	1:0:1.0:0.0:0.5	Film does not form
F_{13}	Guar gum	PEG 400	1:0:1.0:0.0:0.5	Film does not form
F_{14}	Sodium alginate	d-sorbitol	1:0:1.0:0.0:0.5	Film does not form
F_{15}	Sodium alginate	PEG 400	1:0:1.0:0.0:0.5	Film does not form

Use of sodium alginate along with HPC did not improve the physical characteristics of the film. In addition to HEC and guar gum, high viscosity of aqueous solutions causes difficult to remove entrapped air bubbles. Non homogeneous surface of films obtained with HEC/guar gum and HPC was posing the problems, such as unequal distribution of drug in film. This problem was overcome by using xanthan gum along with HPC as film forming

polymer. Aqueous solution of CL films containing HPC and xanthan gum was less viscous and uniformly spread on casting surface. Physical characteristics and other mechanical properties of CL film containing HPC, xanthan gum and PEG 400 was acceptable and selected for further optimizations of the formulation.

6.4.3 Drug Excipients Compatibility

Drug-excipients compatibility studies are conducted with the objective of selecting a reasonable composition for vaginal bioadhesive film. Any kind of incompatibility between CL and film forming polymer affects its performance to a significant extent. DSC thermogram of pure CL did not showed any sharp endotherms that confirm combustions of the compound instead of melting at higher temperature. Therefore, it was difficult to study compatibility between CL and film forming polymer by DSC. Hence, an isothermal testing method was carried out for drug excipient compatibility study.

For isothermal stress testing, the samples kept under stress condition (50°C) were analyzed after three weeks for assay of CL by HPLC. No significant difference was observed between control sample which was stored at refrigerator condition and test sample (kept under stress condition (50°C)). No any additional peak was found in chrmatogram of CL film which stored under stress condition (50°C). **Fig.** 6.3 confirmed that the compatibility between CL and excipeints are to be used for preparation of film.

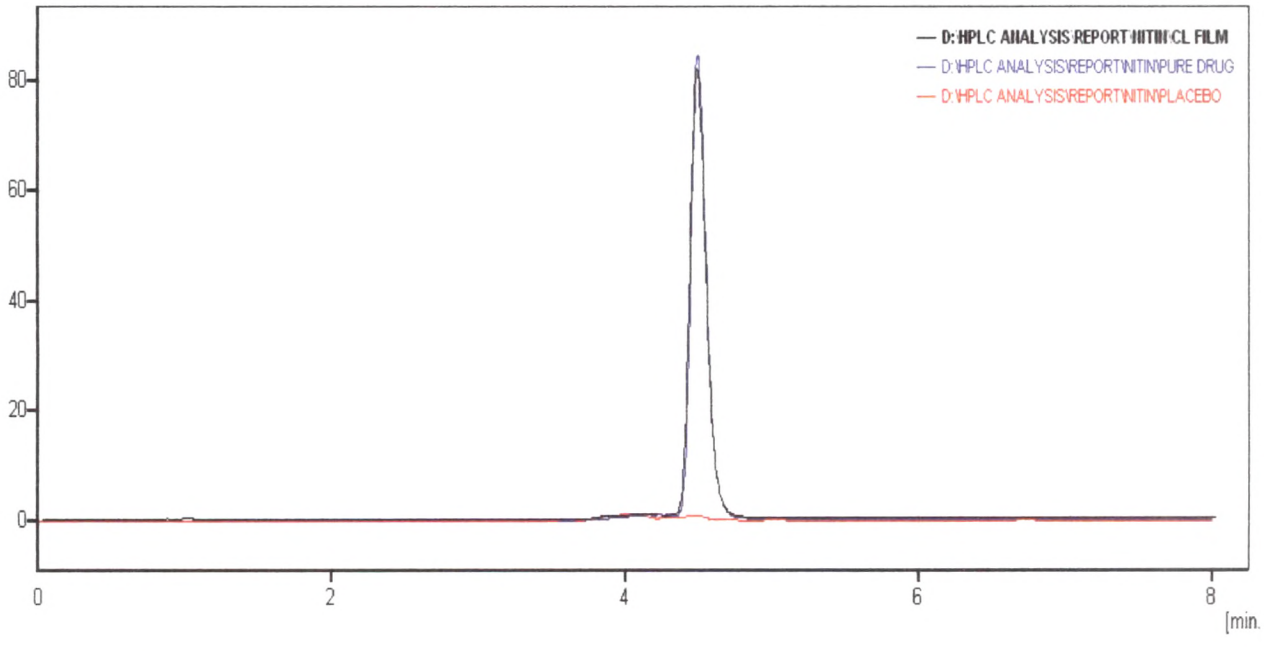

Fig. 6.3 Overlay chromatogram of pure drug, CL film and their placebo for drug-excipients compatibility study.

6.4.4 Experimental Design and Study of Response Variable

Overall results of preliminary experiments carried out in the laboratory revealed that independent variables X_1 play significant role in film formation while X_2 was important for retaining desired physicodynamic properties and for its retention behaviors over vaginal mucosa. Hence, three formulation variables such as % EB, TS and Y_{8h} were selected for systematic optimization studies. Results of experiments carried out as per 3^2 full factorial designs are shown in **Table** 6.10.

Desirability function was utilized to find out optimum level of HPC and xanthan gum out of nine batches. Desirability function was calculated for TS, %EB and Y_{8h}. Batch F_{86} showed highest overall desirability of 0.99. Therefore, this batch was considered to be optimized batch and values of independent variables were considered to be optimum values for bioadhesive vaginal film formulation (see **Table** 6.11). Excipients used in films are GRAS listed and approved for vaginal use.

Table 6.10 Experimental results 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	TS (N/mm^2)	% EB	Y_{8hr}	
F_{81}	-1	-1	4.49	79.02	0.00	0.00
F_{82}	-1	0	3.86	86.04	0.00	0.00
F_{83}	-1	1	3.37	94.96	6.18	0.00
F_{84}	0	-1	4.73	75.61	0.00	0.00
F_{85}	0	0	4.18	82.17	9.47	0.61
F_{86}	**0**	**1**	**4.03**	**91.35**	**13.48**	**0.99**
F_{87}	1	-1	4.61	72.21	0.00	0.00
F_{88}	1	0	4.21	77.47	9.05	0.48
F_{89}	1	1	4.15	86.42	13.65	0.90

Table 6.11 Optimized composition of film formulation containing CL.

Formulation variable	Optimum value (mg per film of 2.5 cm ×2.5 cm area)
Clindamycin phosphate	100
HPC	75
Xanthan gum	80
PEG 400	50

The prediction profiles were obtained for measured responses using JMP 5.1, statistical discovery software. The relationship between independent variables and dependent response value of CL film can be further explained by using prediction profile as shown in **Fig** 6.4. Among tested variables, xanthan gum concentration seems to be the most prominent factor in determining response value of film. An interesting observation of these profiles was improved TS, swelling index and bioadhesive strength as concentration of HPC in film increased. The high concentration of xanthan gum could decrease TS, but enhance % EB, bioadhesive strength and swelling index of vaginal film.

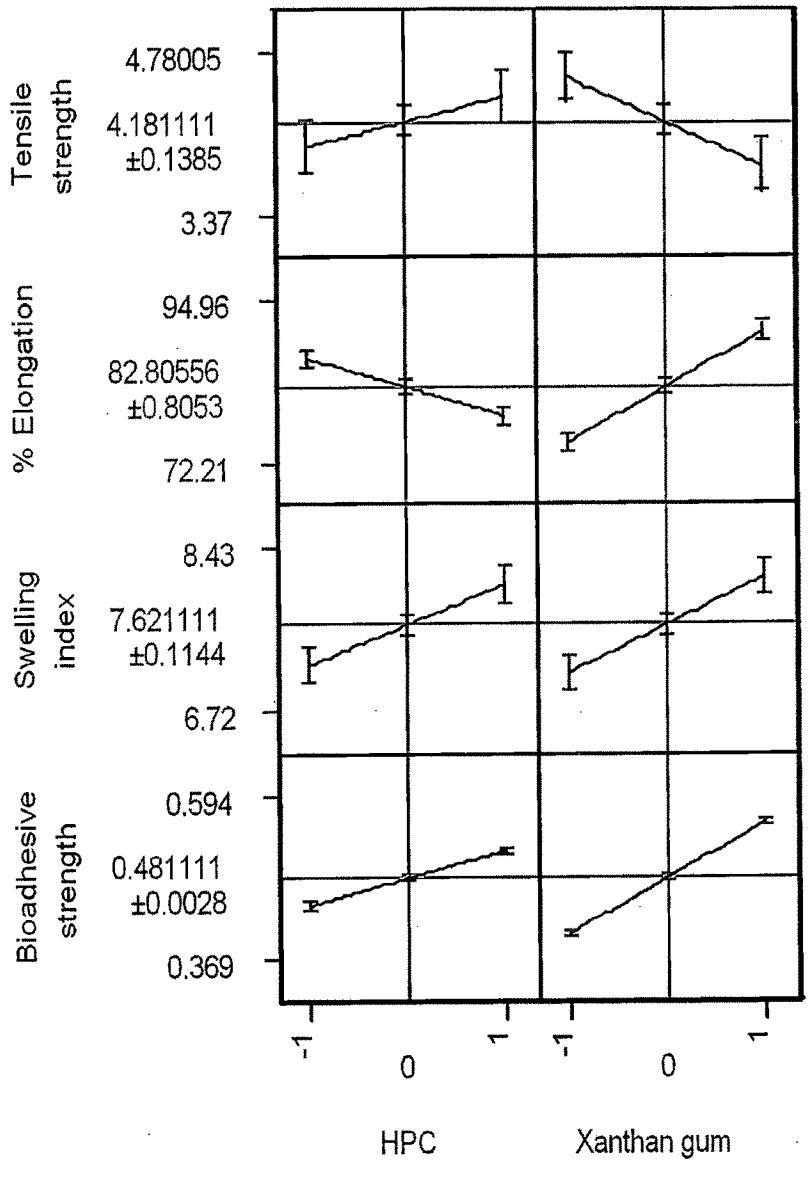

Fig. 6.4. Predictions profile for all the dependent variables against independent variables

Contour plots were obtained for the measured responses based on the model using sigma plot software. The relationship between the independent variables and the responses can be further explained by using these contour plots. **Fig.** 6.5 shows the contour plots for TS and % EB as a function X_1 (amount of HPC) and X_2 (amount of xanthan gum). The contour lines indicate that the addition of a higher amount of HPC resulted a higher TS and lower % EB, while the addition of 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.

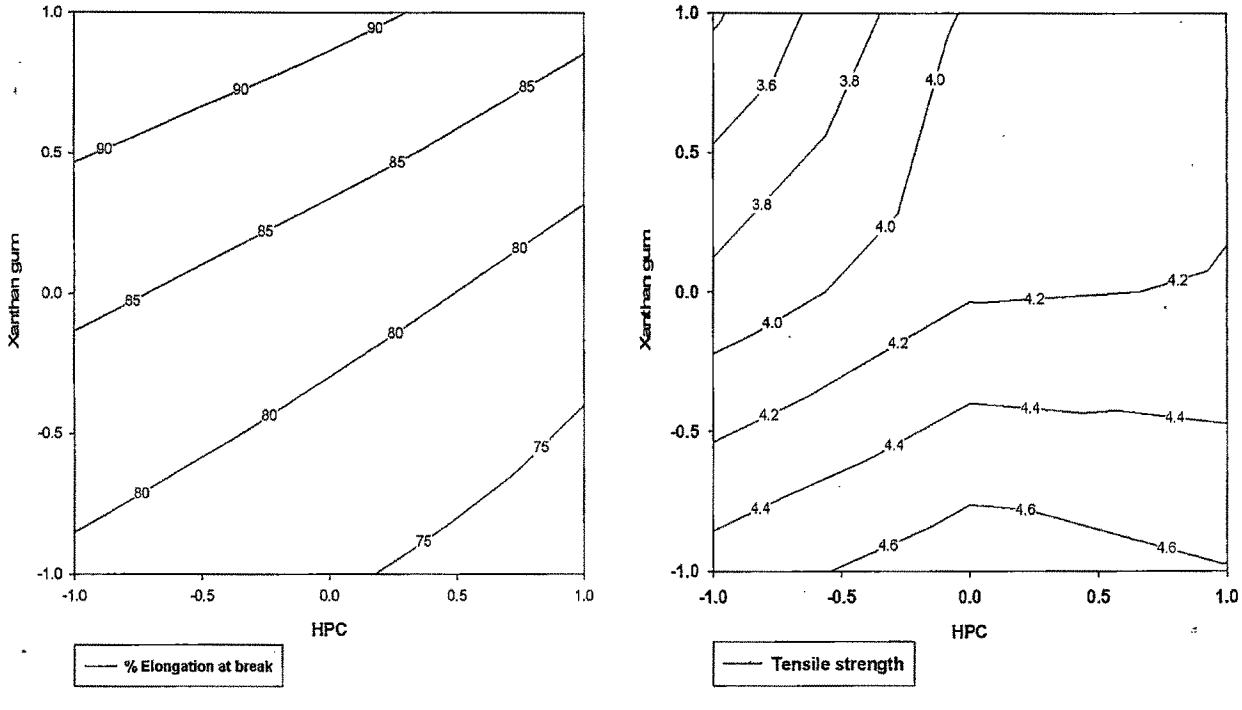

Fig. 6.5 Counter plot showing effect of formulation variables on response variables.

6.4.5 Mechanical properties

The mechanical properties such as TS and %EB gives an indication of the strength and elasticity of the film. It was dependent on the ratio of polymers and their characteristics. Percentage EB and TS of all the design films was found in range of 72.21–91.35 % and 3.37 and 4.73N/mm^2 respectively, suggesting their "soft" and "tough" nature. A soft and weak polymer is characterized by a low TS and %EB; a hard and brittle polymer is defined by a moderate TS and low %EB; a soft and tough polymer is characterized by a moderate TS and high %EB; whereas a hard and tough polymer is characterized by a high TS and %EB

(Rowe R.C., 1983 & Aulton M.E., 1981). Hence, it is suggested that a suitable vaginal film should have a relatively moderate TS and high %EB. The results of mechanical properties are enumerated in **Table** 6.10 suggest that TS of film was improved as increase in HPC concentration, but % EB was found to be decreased. On the other hand, film obtained with high concentration of xanthan gum had less TS with more % EB. An interesting finding of this study was decrease in TS and increase in % EB of film as a function of xanthan gum. This may be due to weakening intermolecular interactions between polymer chains. CL films obtained with HPC and xanthan gum was found tougher and flexible than those obtained without xanthan gum.

6.4.6 Physical Characterization of Film

A newly developed CL films are odorless, flexible, very thin (0.29 ± 0.02mm, Mean ± SD, n = 5), and possess smooth surface. These features of films may reduce chance of mechanical injury during administration and improving patient compliance. It contain 8.40 ± 0.50 % w/w moisture content. The small amount of moisture content in films may help them to remain stable and prevent from being completely dry. Films of three different batches of F_{86} (optimized batch) were found to have similar aesthetic, mechanical and other physicodynamic properties. This suggests that films with desired properties can be prepared consistently and reproducible.

All the performance parameters of F_{86} film have been given in **Table** 6.12. 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 ± SD, n = 5) percentage of added amount of CL per unit of film (2.5 cm ×2.5 cm). Viscosity of formulations after administration in vaginal cavity governs the spreading and retention properties of the formulations which is essential to achieve desired efficacy. The viscosity of polymeric aqueous solution of film was found significantly different (P value <0.0001) in SVF and water. Higher viscosity of polymeric aqueous solution of film in SVF may improve retention of film in vaginal cavity. 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. It may help in successful eradication of unwanted microbial growth from vaginal cavity. The pH of film was found to be slightly acidic (4.39 ± 0.04) in SVF and alkaline (7.18 ± 0.06) in water reveals that vaginal pH as well as micro flora may remain unaffected after administration of BF.

Table 6.12 Performance parameter of bioadhesive (F_{86}) films

Parameter	Optimum values
Size (sq. cm.)	2.5 x 2.5
Weight[a] (mg)	349 ± 5.11
Thickness[a] (mm)	0.29 ± 0.02
Moisture content[a] (%w/w)	8.40 ± 0.50
Tensile strength[b] (N/mm^2)	4.03 ± 0.15
% elongation at break[b]	91.35 ± 1.52
pH of dispersion[c]	
In water	7.18 ± 0.06
In SVF	4.39 ± 0.04
Viscosity (cp) of dispersion[d]	
In water	30.88 ± 1.40
In SVF	126.14 ± 1.42
Bioadhesive strength[e] (N)	0.560 ± 0.02
Retention on isolated sheep vaginal mucosa	13.48% retained amount of drug on vaginal mucosa after 8hr

[a] Mean ± S.D., n = 3

[b] Determined by texture analyzer, Mean ± S.D., n = 5

[c] pH of dispersion (2.5 × 2.5 cm^2 film dissolve in 10ml each of water and SVF at 30°C), Mean ± S.D., n = 3

[d] Viscosity of dispersion measured by Brookfield Viscometer (2.5 ×2.5 cm^2 film dissolved in 10ml each of water and SVF at 29°C), Mean ± S.D., n = 3

[e] Bioadhesive strength determined by texture analyzer, Mean ± S.D., n = 5

6.4.7 Swelling Capability of Films

Films were found to be rapidly swollen within 6 min and thereafter slowly reached to plateau. The effect of various compositions on the swelling index of the films is shown in **Fig** 6.6. Maximum swelling was seen with film containing high concentration of HPC and xanthan gum. The result shows that swelling index increased as the concentration of xanthan gum and HPC increased. Increment in swelling capability of film is more with increase in xanthan gum concentration than HPC concentration. Rapidly swellability of HPC and xanthan gum may prevent the fast erosion of polymeric film.

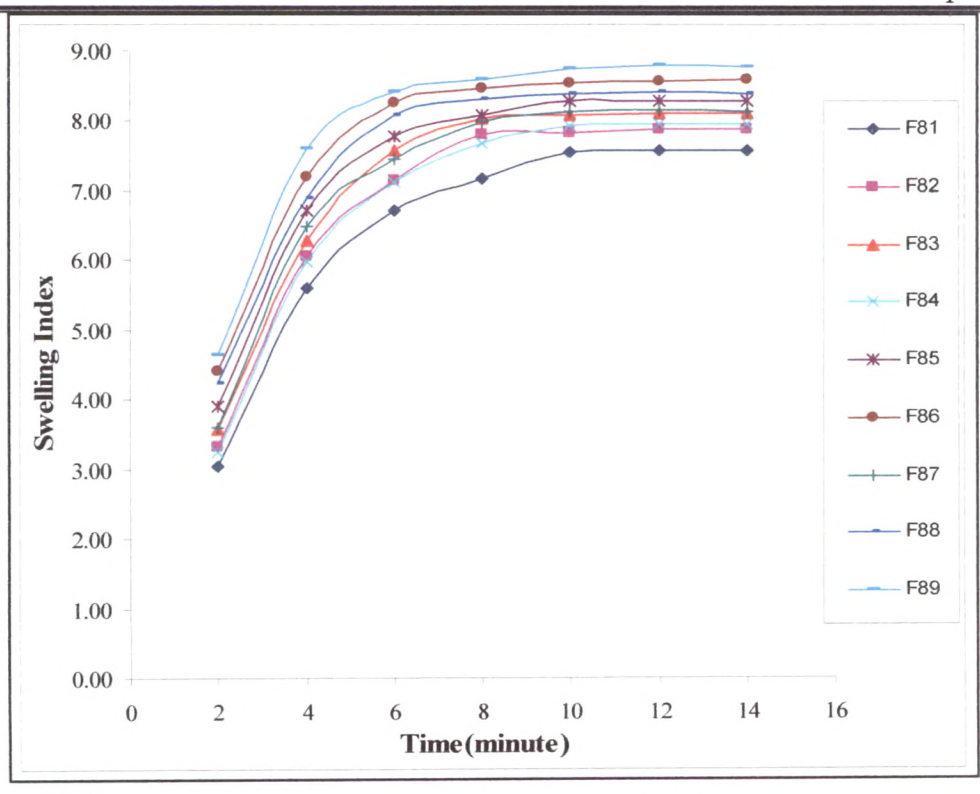

Fig 6.6. Effect of various compositions on the swelling index of the films

The swelling state of polymer was reported to be crucial for its bioadhesive behavior. Adhesion occurs shortly after the beginning of swelling but the bond formed is not very strong.

6.4.8 Bioadhesion Measurement

Bioadhesion is very important aspect for maintaining high drug levels at the site of administration and prevent expulsion of formulation. Maintaining effective drug concentration for longer time in vaginal cavity, one can achieve successful eradication of infectious agent and thereby increase the effectiveness of CL. **Fig.** 6.7 shows bioadhesive strength of film with different polymeric compositions. The bioadhesive strength is the force required to remove polymeric film from the vaginal mucosa, which is found highest with film containing high content of xanthan gum. On the basis of bioadhesive strength and swelling studies, F_{86} comprising xanthan gum and HPC was considered a good candidate for development of novel bioadhesive vaginal delivery system.

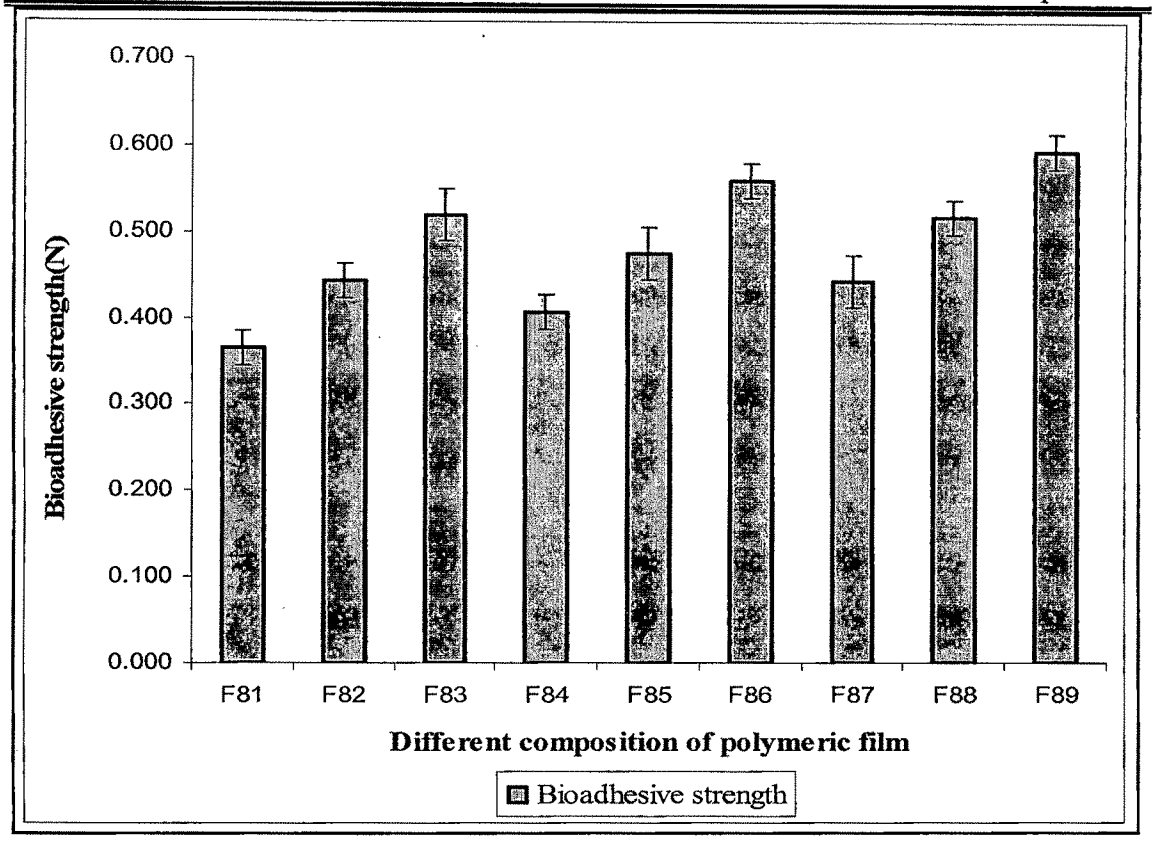

Fig. 6.7 Bioadhesive strength of film with different polymeric compositions.

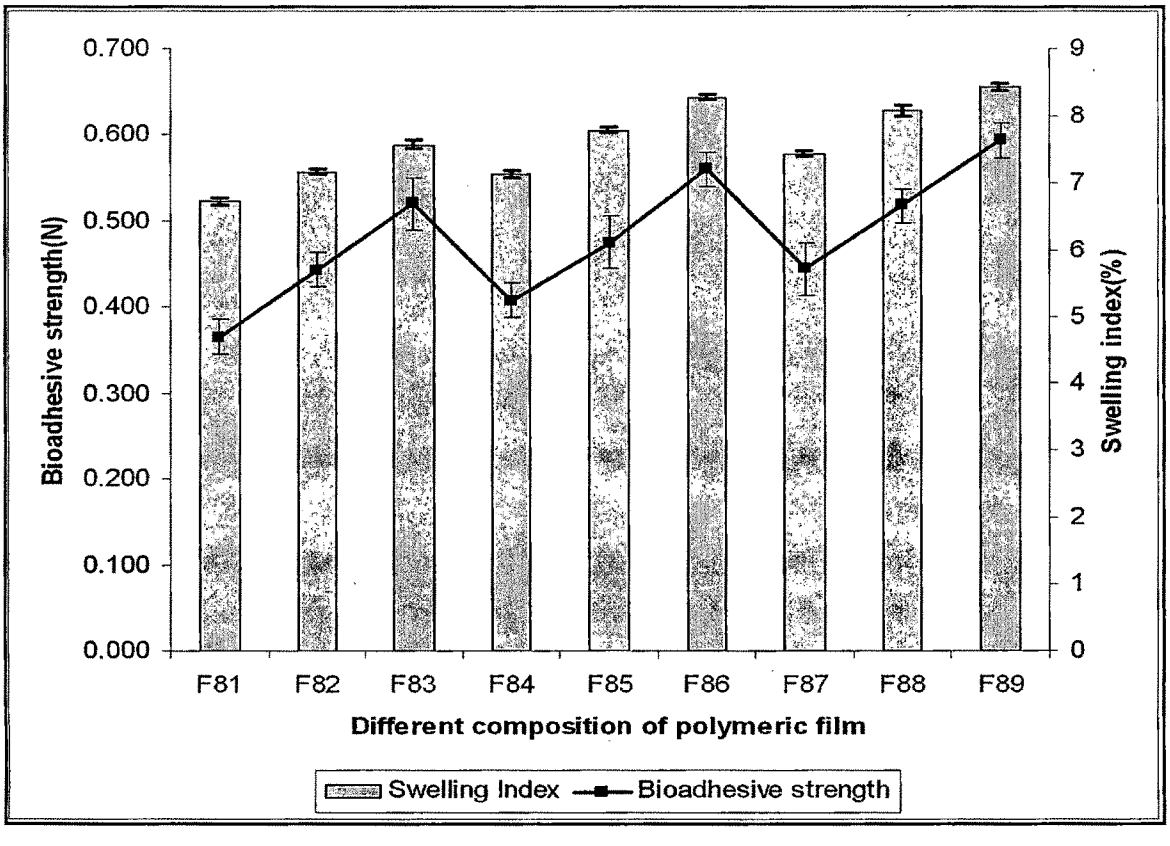

Fig. 6.8 Comparison of bioadhesive strength and swelling capability of polymeric films.

6.4.9 Morphology of film

Morphology of film was characterized by SEM study. Surface image of the film were captured at 150X, 1000X and 5000X (see in **Fig.** 6.9). These images confirms 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.

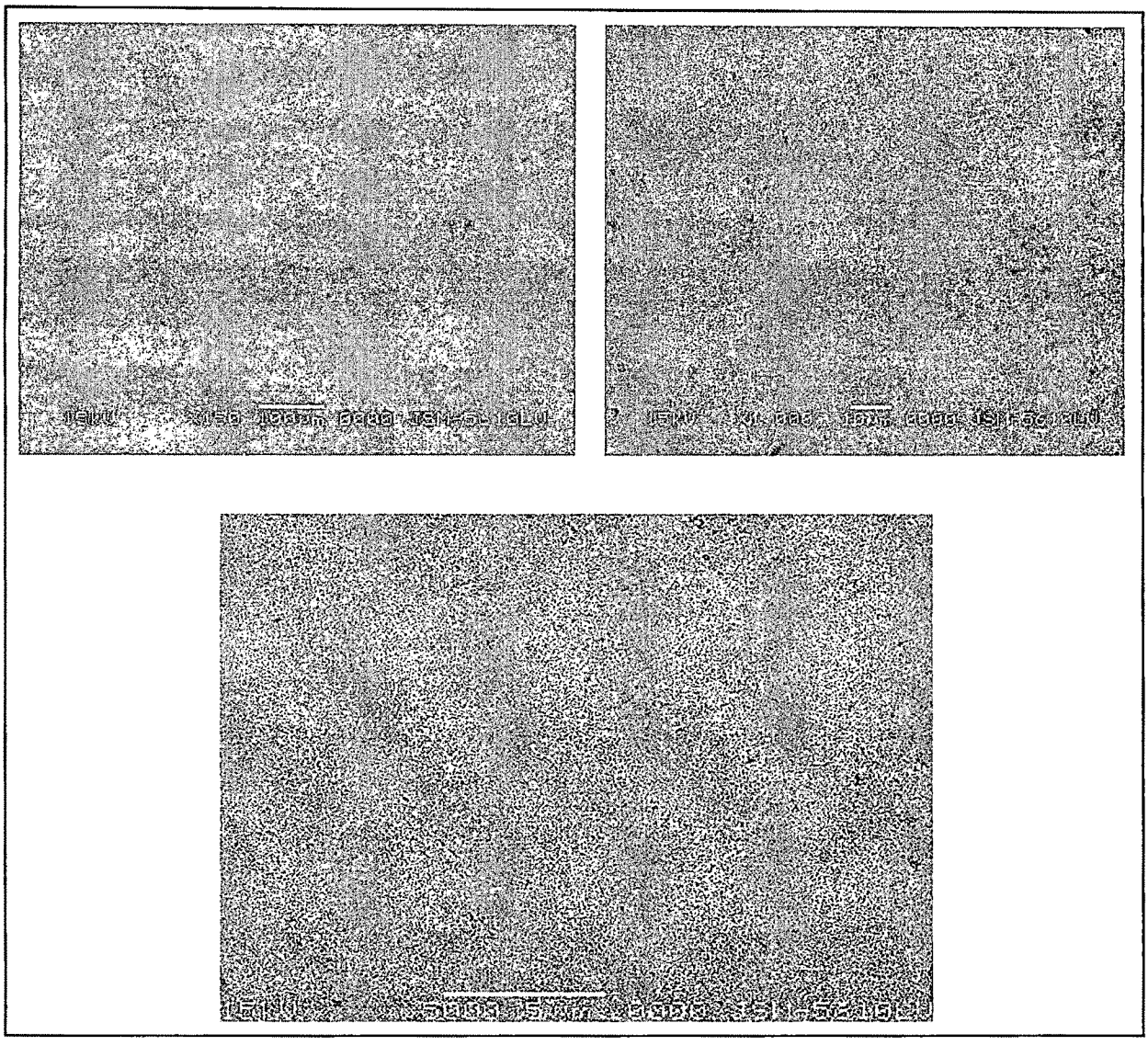

Fig. 6.9 Scanning Electron Micrograph of CL film at 150X, 1000X and 5000X

6.4.10 Retention in simulated vaginal environment

Retention behavior of film was studied using simulated dynamic vaginal system which mimics the physicodynamic conditions of vagina. The film was softened on 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 retained

in vaginal cavity for longer time. Films were eroded as function of time. The remaining percentage of CL on vaginal mucosa at predetermined time interval is mentioned in **Table** 6.13. Time required for entire removal of polymeric film from vaginal mucosa varied with the compositions of film. A film with less polymeric concentration was removed rapidly from vaginal mucosa. As the ratio of HPC to xanthan gum in film increased, the residence time of film increased. This indicates that retention property of polymeric films can be controlled by varying HPC/xanthan gum ratio. The amounts of drug collected into a receiver beaker at predetermined time intervals for each film are shown in **Fig.** 6.10.

Table 6.13 Percentage retention of CL on vaginal mucosa as a function of time.

Time (hr)	Cumulative Percentage Retained of Itraconazole, Mean ± SD (n = 3)								
	F_{81}	F_{82}	F_{83}	F_{84}	F_{85}	F_{86}	F_{87}	F_{88}	F_{89}
1	68.28±0.08	71.02±0.06	72.93±0.09	69.99±0.06	73.32±0.05	76.41±0.06	71.76±0.07	74.85±0.06	76.98±0.05
2	41.05±0.12	44.31±0.11	50.80±0.14	43.34±0.15	44.10±0.08	57.67±0.13	44.54±0.13	50.22±0.11	52.48±0.09
3	25.21±0.21	32.18±0.16	37.32±0.18	29.83±0.24	36.60±0.28	41.98±0.26	34.37±0.22	38.86±0.20	43.02±0.32
4	11.62±0.22	19.65±0.20	27.98±0.35	17.49±0.32	27.91±0.23	32.28±0.32	19.92±0.36	35.31±0.18	36.15±0.27
5	8.15±0.32	10.34±0.37	16.60±0.24	9.51±0.49	19.69±0.31	28.33±0.27	8.53±0.30	27.33±0.26	28.52±0.38
6	-	7.69±0.30	10.59±0.31	-	13.41±0.36	21.29±0.39	5.9±0.51	17.28±0.41	22.85±0.48
7	-	-	6.88±0.42	-	10.85±0.48	15.45±0.34	-	10.72±0.56	16.17±0.42
8	-	-	6.18±0.47	-	9.47±0.42	13.48±0.44	-	9.05±0.49	13.65±0.54

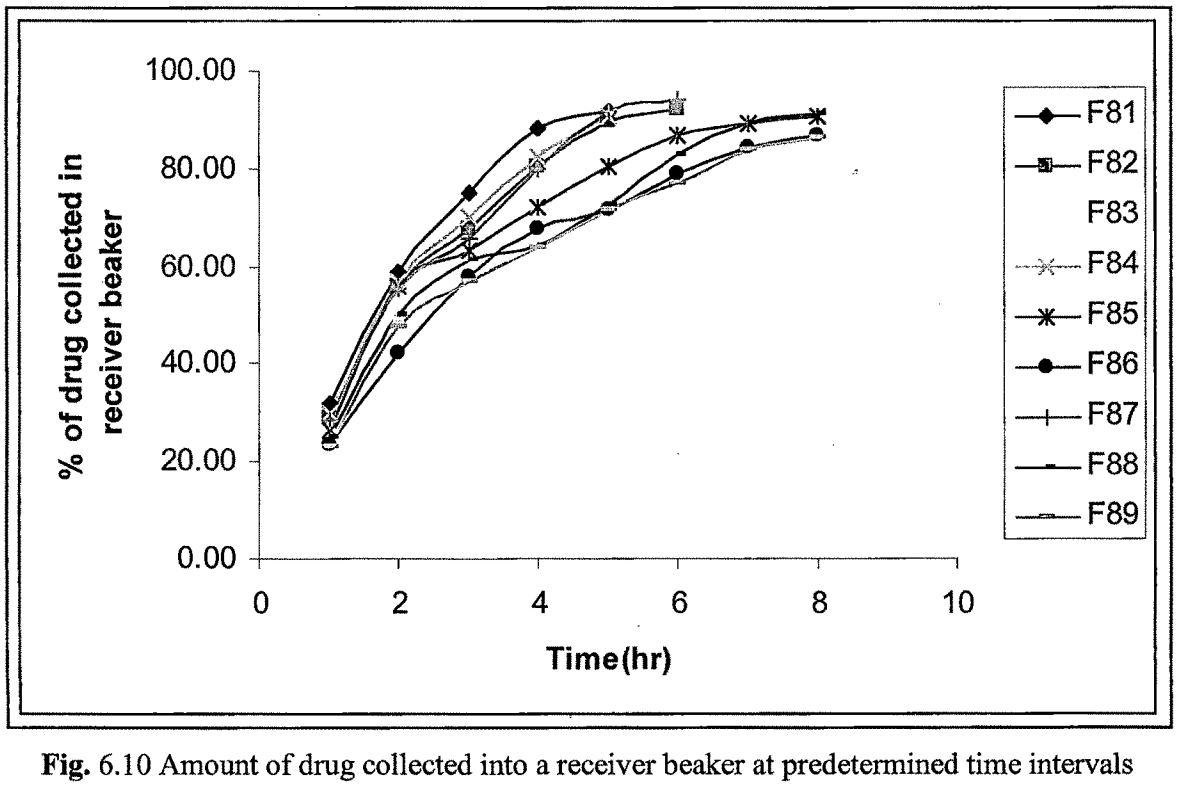

Fig. 6.10 Amount of drug collected into a receiver beaker at predetermined time intervals

6.4.11 Lactobacillus inhibition

Livengood C. H. *et al.* (1990) reported that CL has inhibitory effect against the organisms commonly associated with bacterial vaginosis: namely bacteriodes spicies, Peptococcus spp., Gardnerella vaginalis, Mobiluncus spp. and Mycoplasm hominis, but also negative impact on the growth of lactobacillus spp. The results of lactobacillus inhibition against CL bulk powder, F_{86} film and placebo film are enumerated in **Table** 6.14. CL bulk powder and F_{86} film at a concentration of 10mg per ml showed inhibitory effect on growth of lactobacillus acidophilus after 24h, but after 48h growth resumed and was comparable to that of 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 (Agnew K.J. et al 1995, Flores-Rivera E et al. 1997).

Table 6.14 *In Vitro* Lactobacillus Inhibition Activity

Samples	Zone of inhibition, mm			mean ± SD (n=3)
	I	II	II	
Clindamycin phosphate bulk	7	8	7.5	7.5 ± 0.5
F_{86} film	6.5	6.5	7	6.67 ± 0.29
Placebo film	Nil	Nil	Nil	Nil

6.4.12 Stability studies

The stability studies of the prepared films were done at the conditions stated in the ICH guideline. Stability samples were analyzed at 0, 1, 2, 3 and 6 months time intervals for pH, mechanical properties and assay of CL as per the procedure described in method section. The result of the stability study of CL film is shown in **Table** 6.15. The pH of polymeric dispersion of CL film in SVF was found acidic (4.42± 0.06) suggesting that vaginal pH and microflora remains unaffected. From the stability studies results, it was observed that TS of the film decreased at 75% RH (accelerated storage condition). But, all films exhibited an increase in % EB when stored at accelerated condition (75% RH) as compared to store at intermediate condition (60% RH). The decrease in TS and increase % EB of film may be due to the higher moisture content of the films stored at the higher relative humidity (75% RH).

Overall results of the stability studies indicates that there is no significant change in assay of CL, pH of polymeric dispersion and mechanical properties of CL films which were stored at different stability conditions.

Table 6.15 Results of the stability studies of CL film.

Sample storage condition	**Testing interval (Month)**	**pH of polymeric dispersion** mean ± S.D (n=3)	**Mechanical properties of film**		**Content of Film (%)** mean ± S.D (n=3)
			TS (N/mm^2) mean ± S.D (n=3)	**%EB** mean ± S.D (n=3)	
-	0	4.47 ± 0.07	4.09 ± 0.17	92.19 ± 1.12	98.67 ± 2.42
Intermediate condition (30±2°C and 65±5% RH)	1	4.44 ± 0.03	4.07 ± 0.21	92.62 ± 1.34	98.59 ± 2.61
	2	4.52 ± 0.03	4.11 ± 0.16	91.42 ± 1.11	99.67 ± 2.74
	3	4.34 ± 0.05	4.05 ± 0.11	91.93 ± 1.22	96.83 ± 2.91
	6	4.46 ± 0.03	4.01 ± 0.18	92.31 ± 1.09	96.92 ± 2.59
Accelerated stability conditions (40±2°C and 75±5% RH)	1	4.47 ± 0.06	3.94 ± 0.17	94.47 ± 1.19	98.07 ± 2.49
	2	4.37 ± 0.05	3.90 ± 0.24	95.12 ± 1.07	97.18 ± 2.37
	3	4.49 ± 0.04	3.92 ± 0.28	96.83 ± 1.18	97.54 ± 1.98
	6	4.42 ± 0.06	3.86 ± 0.16	96.78 ± 1.16	96.91 ± 2.87

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