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Design and Development of Mucoahesive Vaginal Drug Delivery System of Raloxifene Hydrochloride

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Abstract : Objective : Raloxifene hydrochloride is a selective estrogen receptor modulator with a very poor oral absolute bioavailability (2%) due to high hepatic first-pass metabolism. Mucoadhesive vaginal tablets of Raloxifene hydrochloride can bypass high hepatic first pass metabolism and also improve its solubility and dissolution behaviour.
Methods : Inclusion complex of drug with β-cyclodextrin was prepared by kneading method. Composition of the mucoadhesive tablet was optimized using 3^2 full factorial design where amount of sodium CMC (X_1) and amount of Polycarbophil (X_2) were taken as independent variables. Drug release at 6 hour (Q_6), mucoadhesive strength and swelling index were considered as dependent variables. The formulations of design batches were characterized for weight variation, hardness, thickness, friability, drug content, swelling index, *ex-vivo* mucoadhesive strength, surface pH, drug release at 6 hrs, *ex-vivo* residence time, drug release data modelling. Optimized batch was subjected to *ex-vivo* permeation study and short term stability study.
Results : The optimized formulation (F5) comprises 20 mg of sodium CMC and 15 mg of polycarbophil had shown mucoadhesive strength (0.343N), swelling index (36.04%) and % drug release at 6 hours (95.90%).ex-vivo permeation was found to be 47.93% at 6 hr. Results of drug release data modelling suggested zero order drug release kinetics (R2=0.9983) with case II transport release mechanism (n=0.9513) for optimised batch.
Conclusion : Raloxifene hydrochloride mucoadhesive tablet is a promising approach for the effective treatment of disease as it provides control drug release and bypasses the hepatic first pass metabolism.
Key words : osteoporosis, factorial design, Contour plot, β-cyclodextrin, phase solubility, Job's plot.

1. Intrdoduction

Raloxifene hydrochloride is a second-generation selective estrogen-receptor modulators (SERM) endorsed by the Food and drug administration in 1997 for the treatment of osteoporosis. It is commercially available under the trade name of Evista (Eli Lilly, Indianapolis, IN) in 60-mg dose tablets. Oral bioavailability of this drug is only 2% which limits its oral administration. It is Biopharmaceutical Classification System (BCS) class II drug having low solubility and high permeability and it also undergoes extensive first pass metabolism which leads to poor bioavailability [1]. Various strategies have been reported to improve the solubility and

bioavailability of raloxifene hydrochloride, such as lipid-based delivery systems, inclusion complexes, and co-grinding [2-4].

The vaginal epithelium is permeable to a wide range of drugs, like hormones, antimycotics, peptides and proteins [5]. The vagina provides a promising site for local effect as well as systemic drug delivery because of its large surface area, rich blood supply, and avoidance of the first-pass effect, relatively high permeability for many drugs and self-insertion [6, 7]. In addition, a prolonged contact of a delivery system with the vaginal mucosa may be achieved more easily than at other absorption sites like rectum or intestinal mucosa [8].

Conventional vaginal delivery systems include solutions, suspensions, gels, foams and tablets. Vaginal creams and gels provide lubrication, but tend to be messy and cluttered, and are easily washed off if they are water soluble and easily dispersive. Suspensions and solutions tend to spread widely in the vaginal cavity. Vaginal Foams are provided excessive lubrication and leakage from the vagina. Thus vaginal tablets are to be useful and most convenient dosage form as ease for application and portability [9].

The term "Mucoadhesion" describes materials that bind to biological substrates, such as mucosal members. Adhesion of bioadhesive drug delivery devices to the mucosal tissue offers the possibility of creating an intimate and prolonged contact at the site of administration. This prolonged residence time can result in enhanced absorption and in combination with a controlled release of drug also improved patient compliance by reducing the frequency of administration [10, 11].

Mucoadhesive vaginal drug delivery system (MVDDS) has been used for the treatment of local diseases affecting the vagina like candidiasis, sexually transmitted disease, vaginal dryness, and also can be successfully deliver drugs to systemic circulation via vaginal mucosa for treatment of various diseases like migraine and osteoporosis [12]. It can offer other numerous advantages as (i) avoiding hepatic first-pass metabolism, (ii) use of small doses, in comparison to oral administration, (iii) side effect minimization, (iv) provides intimate contact between a dosage form and the vaginal mucosa, which may result in high concentration in a local area and hence high drug flux through the vaginal mucosa and (v) easy removal [13]. The efficacy of MVDDS is affected by the biological environment and the properties of the polymer and the drug[8]. Acrylic acid polymers (Carbomer or polycarbophil) and cellulose derivatives (hydroxyethylcellulose, hydroxypropylcellulose or hydroxypropyl methylcellulose) have been widely used polymers in MVDDS [14-17]. Degim *et al* successfully developed vaginal drug delivery system of insulin using chitosan gel to achieve extended release [18]. Thiolated polymers are also one of the successfully used approaches for mucosal drug delivery systems [19, 20]. Beta-cyclodextrin, citric acid, Tween 80 and Polaxamers are also added in vaginal formulation to increase drug solubility [21].

The present research work includes improvement of solubility of Raloxifene hydrochloride by complexation with cyclodextrin and development of mucoadhesive vaginal tablets of the complex using 3^2 full factorial design.

2. Materials and Methods

2.1 Materials

Raloxifene hydrochloride (R-HCl) was gifted by Aarti drugs limited, Mumbai. B cyclodextrin, Polycarbophil, Sodium carboxy methyl cellulose, Polyox, Xanthan gum, HPMC K4M, and Microcrystalline cellulose were purchased from Lyka pharmaceuticals(Ankleshwar), Lubrizol (Belgium), Lobachemie (Mumbai), Molychem (Mumbai); respectively. Magnesium stearate and talc were purchased from Ases chemical works, Jodhpur. All reagents were used of analytical grade and were used as received.

2.2. Methods

2.2.1 Phase solubility study

An excess amount of Raloxifene hydrochloride (R-HCl) was placed in a 25 ml glass flask containing different concentrations of β- cyclodextrin (β-CD) (0-10 mM). Flasks were covered with cellophane membrane to avoid solvent loss and then shaken (50 agitations/min) in incubator shaker for 24 hr at 37 ± 0.5ºC. Supernatant was withdrawn and filtered through 42 µm Whatman Filter Paper. The filtrates were analyzed

using a UV Visible spectrophotometer (UV 1800, Schimadzu, Japan) at 287 nm after suitable dilution against blank prepared of the same concentration of β-CD in water to cancel any interference of β-CD [22-25]. Phase solubility diagram was plotted (Figure 1) and apparent solubility constant (K_C) was calculated using equation 1.

$$K_c = \frac{Slope}{S_0(1 - slope)} \quad (Equation\ 1)$$

Where, S_0=Solubility of the drug in the absence of β-CD.

2.2.2 Job's plot (continuous variation method)

It was used to demonstrate the stoichiometry of R-HCl: β-CD inclusion complexes. It involves preparing an equimolar solution of drug and cyclodextrin derivative and mixing them in different proportion so that keeping the final concentration constant. Absorbance of the complex was plotted against the mole fractions of these two components (Figure 2) [26]. The maximum on the plot corresponds to the stoichiometry of the inclusion complex formation.

2.2.3 Differential scanning calorimetry (DSC) study

DSC analysis was performed to check drug excipient compatibility using Shimadzu DSC 60 (Schimadzu, Japan) using 10 mg sample. Sample was heated in aluminium pan at a rate of 10ºC/min in the temperature range of 30 to 300ºC under nitrogen flow of 40 mL/min. An empty aluminium pan was used as a reference [27].

2.2.4 Preparation and evaluation of inclusion complex of R-HCl with β-CD using kneading method

A solid complex of R-HCl with β-CD in 1:1 equimolar ratio was prepared by kneading method. Cyclodextrin was triturated in a mortar with purified water to obtain a paste and then drug was incorporated. The resulting mixture was triturated for 1 hr in mortar followed by drying in an oven at 45°C. The dried mass was pulverized; passed through a 60-mess sieve and evaluated for drug content, saturated solubility study, DSC study and FTIR study [28, 29].

2.2.5 Saturated solubility studies

Saturated solubility of R-HCL complex in water and phosphate buffer pH 7.4 was determined by adding excess amounts of inclusion complex to water and phosphate buffer pH 7.4 at 37 ± 0.5°C; respectively [30]. Saturated solubility of pure drug in water was also determined for reference point. The solutions were equilibrated under continuous agitation for 24 h and filtered through Whatman filter paper to obtain a clear solution. The absorbance of the samples was measured by UV spectrophotometer at 287 nm and the concentrations in µg/mL were determined.

2.2.6 Formulation of R-HCl mucoadhesive vaginal tablets (preliminary study)

2.2.6.1 Preliminary studies

2.2.6.1.1 Formulation of R-HCl mucoadhesive vaginal tablets (Batches P_1 to P_8)

Preliminary batches P_1 to P_8 were prepared using 35 mg Carbopol 934, Thiolated chitosan, Sodium CMC, Polyox, Polycabophil, Sodium alginate, HPMC K4M, Xanthan gum; respectively. Each batch contained 35 mg of polymer, drug complex (196.2 mg), MCC (65.8mg), Talc (1 mg) and Magnesium stearate (2 mg). Tablets were prepared by direct compression method and polymers were selected for further study by evaluating tablets for mucoadhesive strength and swelling index.

2.2.6.1.2 Formulation of R-HCl mucoadhesive vaginal tablets (Batches P_9 to P_{18})

Compositions of batches P_9 to P_{18} are mentioned in Table 1**.** Tablets were prepared by direct compression method and evaluated for drug release at 6 hour (Q_6), mucoadhesive strength and swelling index.

Table 1 Composition of raloxifene hydrochloride mucoadhesive vaginal tablets (Preliminary batches P_9 to P_{18})

Ingredients	F9	F10	F11	F12	F13	F14	F15	F16	F17	F18
Raloxifene+ β-cyclodextrin complex	196.2	196.2	196.2	196.2	196.2	196.2	196.2	196.2	196.2	196.2
Polycarbophil	-	-	-	25	15	10	15	20	25	15
Sodium Carboxy Methyl Cellulose	25	25	25	-	-	25	25	25	25	15
Polyox 301	25	15	10	-	-	-	-	-	-	-
Hydroxy Propyl Methyl cellulose (HPMC) K4M	-	-	-	25	25	-	-	-	-	-
Microcrystalline cellulose	49	59	64	49	59	64	59	64	49	69
Magnesium Stearate	2	2	2	2	2	2	2	2	2	2
Talc	1	1	1	1	1	1	1	1	1	1

2.2.6.2 Formulation of R-HCl mucoadhesive vaginal tablets prepared using 3^2 full factorial design

Formulation optimization was done using 3^2 full factorial design [31]. In this design, two factors namely amount of sodium CMC (X_1) and amount of Polycarbophil (X_2) were evaluated, each at three levels (-1, 0 and +1). -1, 0 and +1 level for factor X_1 represents 15, 20 and 25 mg, respectively whereas, -1, 0 and +1 level for factor X_2 represents 10, 15 and 20 mg, respectively. Experimental trials were carried out for all nine possible combinations (Table 2). Drug release at 6 hour (Q_6), mucoadhesive strength and swelling index were selected as dependent variables. As shown in equation (2), a statistical model incorporating interactive and polynomial terms was used to evaluate the responses.

$$Y = b_0 + b_1X_1 + b_2X_2 + b_{12}X_1X_2 + b_{11}{X_1}^2 + b_{22}{X_2}^2 \quad \text{(Equation 2)}$$

Where, Y are the dependent variables, namely, Drug release at 6 hour (Q_6) (Y_1), mucoadhesive strength (Y_2), and swelling index (Y_3); b_0 is the arithmetic mean response of the 9 runs; and b_1 and b_2 are the estimated coefficients for the factors X_1 and X_2, respectively. The main effects (X_1 and X_2) represent the average result of changing one factor at a time from its low to high value. b_{12} is the coefficient of interaction and b_{11} and b_{22} are coefficients of quadratic terms. The interaction term (X_1X_2) shows how the response changes when two factors are simultaneously changed. The polynomial terms (X_{12} and X_{22}) are included to investigate nonlinearity.

Table 2 Compositions of R-HCl mucoadhesive vaginal tablets prepared using 3^2 full factorial design (Batches F_1 to F_9)

Ingredients	F_1	F_2	F_3	F_4	F_5	F_6	F_7	F_8	F_9
Raloxifene+ β- cyclodextrin complex	196.2	196.2	196.2	196.2	196.2	196.2	196.2	196.2	196.2
Sodium Carboxy Methyl Cellulose	15	20	25	15	20	25	15	20	25
Polycarbophil	10	10	10	15	15	15	20	20	20
Microcrystalline cellulose	76	71	66	71	66	61	66	61	56
Magnesium stearate	2	2	2	2	2	2	2	2	2
Talc	1	1	1	1	1	1	1	1	1

2.2.6.3 Evaluation of tablets

2.2.6.3.1 Pre-compression parameters

Tablets were prepared by direct compression method hence micromeritics studies (angle of repose, bulk density, tapped density, % compressibility) of powder blend were evaluated prior to manufacturing of compression coated tablets [32].

2.2.6.3.2 Post compression parameters

Tablets were characterized for weight variation, thickness, hardness, friability, *In vitro* swelling rate, *Ex-vivo m*ucoadhesion study, surface pH, *In vitro* dissolution study, *Ex-vivo* residence time. Hardness, thickness and friability were determined by Monsanto hardness tester, digital Vernier callipers and friabilator respectively [31, 33].

2.2.6.3.3 *In vitro* swelling rate

The weight of medicated tablets was determined and denoted as W_1. Each tablet was placed separately in a petridish with a wire mesh to avoiding direct contact with the petridish which contained a 5 mL phosphate buffer pH 7.4. Tablets were removed at different time intervals, wiped with filter paper and reweighed (W_2) it after deducting a weight of cut piece of sieve. The swelling index was calculated using equation 2 and plotted as a function of time [34].

$$Swelling\ index = \frac{(W_2 - W_1)}{W_1} \quad (Equation\ 2)$$

2.2.6.3.4 *Ex-vivo* Mucoadhesion study

The *ex-vivo* mucoadhesive Strength was performed after application of the vaginal tablet on freshly cut goat buccal mucosa [35]. The fresh goat buccal mucosa was tied on the glass slide, and a mucoadhesive core side of each tablet was wetted with phosphate buffer pH 7.4 and adhered to the sheep buccal mucosa by applying a light force with a fingertip for 30 seconds. The modified physical balance was adjusted by keeping glass beaker on another side. Water was added gradually by burette and observes the weight of water needed to detach the tablet from goat buccal mucosa was recorded to measure the mucoadhesive strength in grams [36]. Force of adhesion (N) was calculated using following equation 3.

$$\text{Force of adhesion (N)} = \frac{Mucoadhesive\ strength}{100} X\ 9.81 \quad (Equation\ 3)$$

2.2.6.3.5 Surface pH:

Surface pH of tablet was determined by adding 2 ml of distilled water to tablet placed in a beaker which was allowed to swell at room temperature for 2 hours. pH measurement was done by contacting the electrode with the tablet surface for 2 min [37].

2.2.6.3.6 *In vitro* dissolution study

Dissolution was performed in 900 ml pH 7.4 phosphate buffer using USP dissolution apparatus II equipped with a paddle operating at the speed of 50 rpm. Temperature of dissolution medium was maintained at 37 ±0.5 ºC. Five ml sample was withdrawn at regular time interval from the dissolution medium and replaced with fresh medium to maintain the sink conditions. The amount of drug released was measured at suitable time intervals using UV spectrophotometer (UV 1800, Schimadzu, Japan). The test was performed in triplicate.

2.2.6.3.7 Drug release data modeling

Several equations are reported in the literature to identify the mechanism of drug release from the dosage from. Drug release data of optimized batch of R-HCl mucoadhesive vaginal tablets was evaluated according to the following equations:

Zero order release model (Equation 4) [38]:

$$Q_t = Q_0 + K_0 t \quad (Equation\ 4)$$

First order release model (Equation 5) [39]:

$$lnQ_t = lnQ_0 + K_1 t \quad (Equation\ 5)$$

Higuchi model (Equation 6) [40-42]:

$$Q = K_H t^{1/2} \quad (Equation\ 6)$$

Hixson-Crowell model (Equation 7) [43]:

$$K_s t = Q_0^{1/3} - Q_t^{1/3} \quad (Equation\ 7)$$

Korsmeyer Peppas model (Equation 4) [44]:

$$\frac{Q_t}{Q_\infty} = K_K t^n$$

Where Qt is the amount of drug dissolved in time t, Q_0 is the initial amount of drug, K_0 is the zero-order release constant, K_H is the Higuchi rate constant, K_K is a release constant, and n is the release exponent that characterizes the mechanism of drug release. For cylindrical matrix tablets, n = 0.45 indicates drug release mechanism by fickian diffusion, and if 0.45 < n <0.89, then it is non-Fickian or anomalous diffusion [45].

2.2.6.3.8 Ex-vivo residence time

The tablet was applied on the goat buccal mucosa which was fixed on the glass slide with cyanoacrylate glue [5, 46]. The slide was tied to the disintegration apparatus and suspended in the beaker filled with 500 mL simulated vaginal pH 7.4. The slide was allowed to reciprocate in the medium until the tablet got detached or eroded from the mucosa. The test was performed in triplicate. Time for the detachment of the tablet was recorded as in vitro residence time.

2.2.6.3.8 *Ex vivo* Permeation study

Diffusion study was carried out to evaluate the permeability of drug across the goat buccal mucosal membrane using Franz diffusion cell. Goat buccal mucosa was obtained from a local slaughterhouse and was used within 2 h of slaughter. The tissue was stored in ice cold water, solution upon collection. The epithelium was separated from underlying connective tissues with surgical scissors and placed in between donor and receiver chambers of the diffusion cells for permeation studies. Receptor compartment contained 20 mL of pH 7.4 phosphate buffer, while donor compartment was filled with 3 mL simulated vaginal pH of 7.4. The tablet was placed on the mucosal surface in donor compartment, and 2 mL aliquots were removed at suitable intervals from the receptor compartment while the solution is being stirred continuously using magnetic stirrer, replacing it with fresh 2 mL medium each time. The absorbance was measured at 287 nm using UV visible spectrophotometer (UV 1800, Schimadzu, Japan) [47].

2.2.6.4 Short-term stability study

The stability of the optimized tablets was assessed by packing them in aluminum foil, sealed tightly and stored for 30 days at 40 ±2ºC and 75 ±5 % RH. The tablets were analyzed for drug release, mucoadhesive strength and % swelling index after 1 month of withdrawal of sample [48].

3. Results And Discussion

3.1 Phase solubility study

Solubility of R-HCL increased with increasing the amount of β-CD (Figure 1). This behaviour of linear increase in drug solubility with increased carrier concentration was indicative of the A_L type of solubility phase diagram [49]. The linear host-guest correlation with slope of less than 1 suggested the formation of a 1:1complex with β-CD concentration [50]. Stability constant for β-CD was found to be 990.40 M^{-1}. The magnitude of apparent stability constant for several drug/CD complexes, K in M^{-1}, ranges from 0 to 100000 [51].

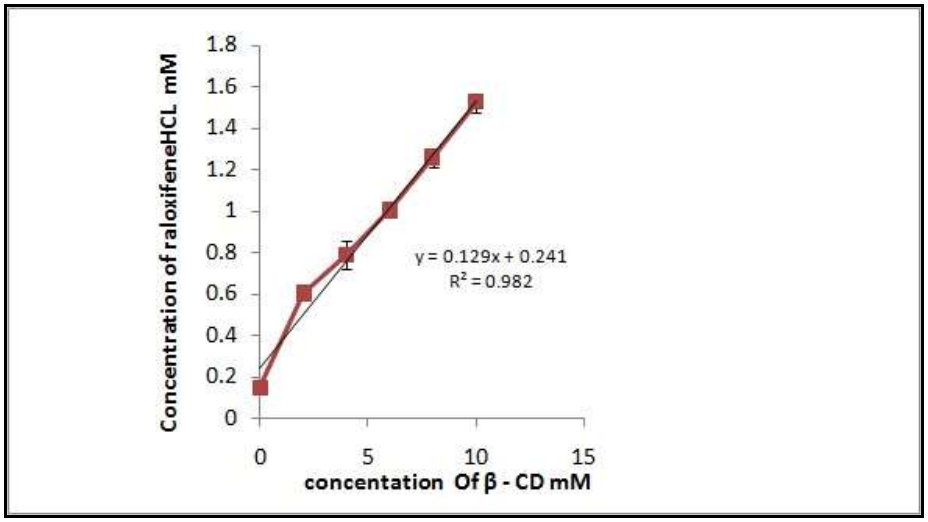
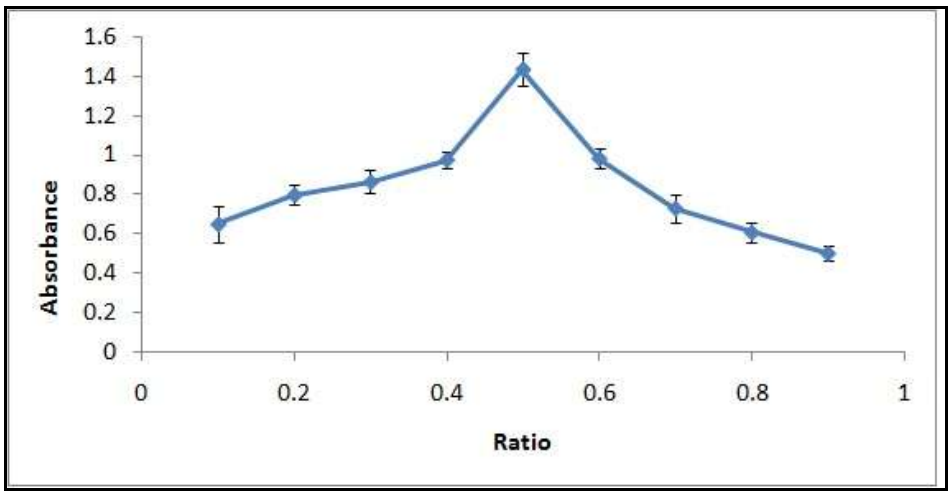


Fig. 1: Plot of phase solubility study

3.2 Job's plot (continuous variation method)

The maximum absorbance variation for R-HCl in β-CD was observed for 0.5 mole fraction, which indicated that the main stoichiometry is 1:1, in agreement with the stoichiometry suggested from the phase-solubility study [52].



3.3 Differential scanning calorimetry (DSC) study

In DSC thermogram, melting endotherm of R-HCl was present at 265.96°C (Figure 3(a)). Figure 4 shows characteristic thermogram for individual excipients and overlay thermogram for drug with a mixture of polymeric material. Peak of drug was preserved in the same temperature region in case of drug-excipient mixtures indicated compatibility of drug with selected excipients [53]. The intensity of peak was reduced in physical mixture due to dilution effect; however it had no incompatibility issue.

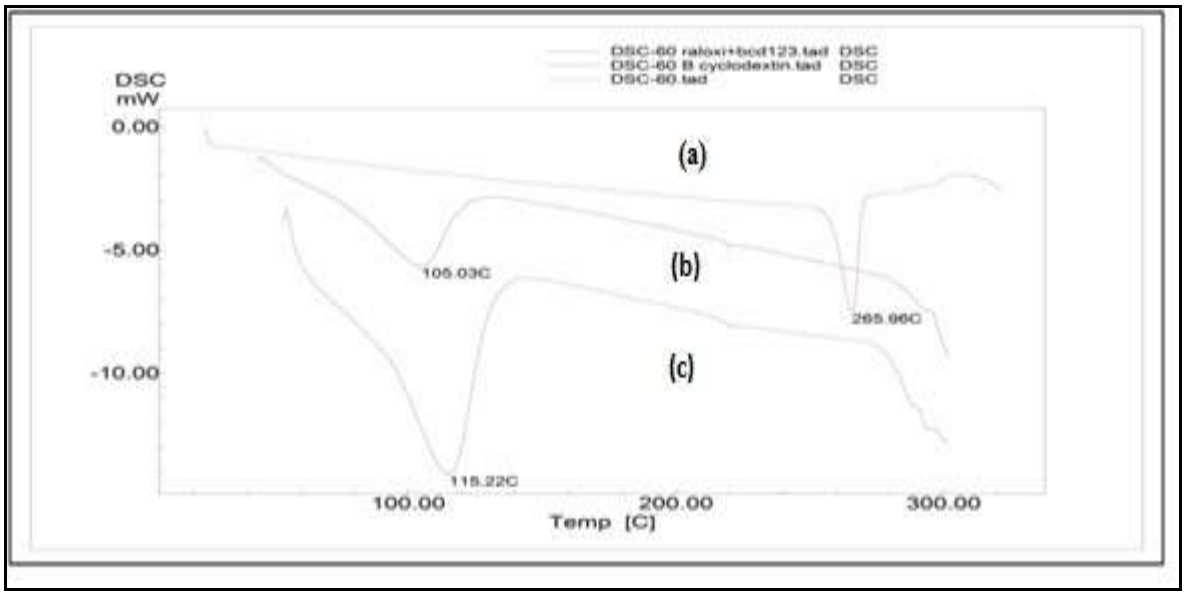


Fig 3: Comparative DSC spectra of drug (a), β-cyclodextrin (b) and inclusion complex(c)

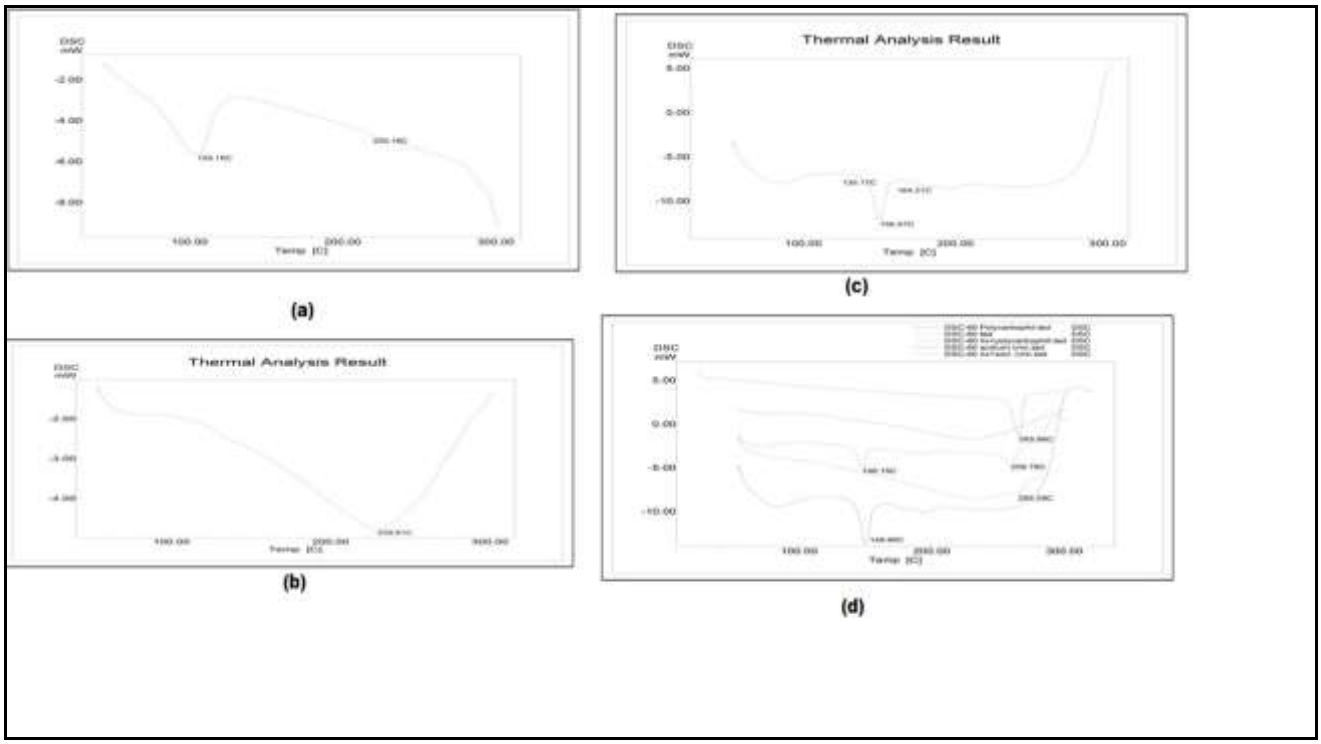


Fig. 4: DSC thermograms of B-CD(a), Polycarbophi(b), Sodium CMC, Overlay spectra of drug-excipient physical mixture

3.4 Evaluation of inclusion complex of R-HCl with β-CD using kneading method

Percentage drug content in inclusion complex was performed in triplicate and it was found to be 96.33± 0.7288%. Solubility of uncomplexed drug in water, complexed drug in phosphate buffer and complexed drug in water was found to be 20.79, 60.32 and 68.54 µg/ml; respectively which indicated enhancement of solubility of

R-HCl due to ß-CD complexation. DSC thermograms of R-HCl exhibited only one endothermic peak corresponding to the melting points at 265.96°C (Figure 3 (a)). The endothermic peak at 105°C in the β-CD was observed due to the evaporation of the absorbed water (Figure 3 (b)). [50].Drug peak was completely disappeared in the inclusion complex prepared by the kneading method (Figure 3(c)) as drug was present within the cavity of the β-CD ring molecule [54].

3.5 Evaluation of R-HCl mucoadhesive vaginal tablets

3.5.1 Preliminary studies

3.5.1.1 Evaluation of R-HCl mucoadhesive vaginal tablets (Batches $P_1$to P_8)

The swelling behavior and mucoadhesive strength are the two main concerns for effective bioadhesive tablet formulations for vaginal delivery [55]. Percentage swelling and mucoadhesive strength of preliminary batches (P_1 to P_8) are in the range of 0.272 to 0.533 and 24.14 to 49.41, respectively. Desired mucoadhesive strength is 0.3 to 0.4 N. Xanthan gum and sodium alginate showed lower mucoadhesive strength than desired value. Lower mucoadhesion leads to less residence time of formulation in the vagina. Thiolated chitosan showed acceptable mucoadhesive strength but it failed to provide acceptable swelling. Tablets with Sodium CMC and HPMC K_4 M exhibited desired mucoadhesive strength but in swelling they form rigid structure which may prolong drug release. Tablet should release more than 90% of drug within 8-10 hours to avoid vaginal irritation [56, 57]. Polycarbophil, polyox and carbopol 934 exhibited the highest mucoadhesive strength among all the selected polymers. However, Carbopol 934 showed gradual and extensive swelling and if the degree of swelling is too great, a slippy mucilage results which can be easily removed from the substrate [56, 57]. Polycarbophil and Polyox were selected as mucoadhesive polymers in further studies.

3.5.1.2 Evaluation of R-HCl mucoadhesive vaginal tablets (Batches P_9 to P_{18})

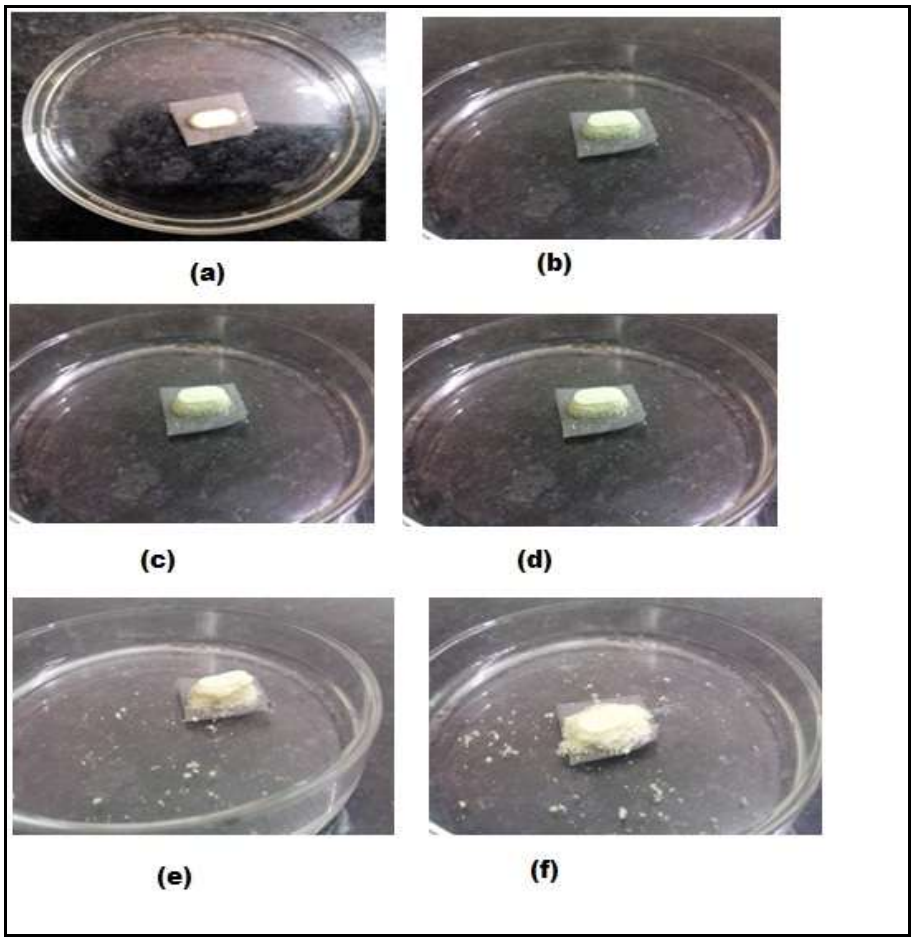


Fig. 5: Swelling behaviour of mucoadhesive vaginal tablet

Formulation P_9 having high mucoadhesive strength than desired value which may lead to local irritation in vagina [58]. Formulation P_{10} exhibited prolonged drug release up to 10 h because higher concentration of sodium CMC swelled rapidly when in contact with water to form a gel, prevented fast disintegration of tablet. Formulation P_{11} having lower mucoadhesive strength which can lead to less residence time of dosage from to vaginal mucosa. Formulation P_{12} containing a polycarbophil as a mucoadhesive polymer and HPMC K_4M as a release retardant polymer but tablet was broken within 5h in swelling study. Formulation P_{13} exhibited 88.81% drug release within 4 hr followed by breaking of tablets within 5h due to presence of erodible polymer, HPMC K_4M. Formulation P_{14} and P_{18} had lower mucoadhesive strength whereas formulation P_{16} and P_{17} had higher mucoadhesive strength than desired value. Formulation P_{15} comprising combination of polycarbophil as a mucoadhesive polymer(15 mg) and sodium CMC as a release retardant polymer (25 mg) in was considered a good candidate due to acceptable criteria of mucoadhesive strength(N), swelling index and drug release. Swelling behavior of this tablet at regular time is shown in figure 5. Fast swellability of sodium CMC prevented the premature disintegration of polycarbophil tablet and polycarbophil prevented the fast erosion of sodium CMC [59, 60].

3.5.2 Evaluation of R-HCl mucoadhesive vaginal tablets prepared using 3^2 full factorial design

3.5.2.1 Pre-compression parameters

The results for bulk density, tapped density, Carr's index, Hausner's ratio and angle of repose of powder blends of batches F_1 to F_9 indicated good flowability and compressibility of the blend [32].

3.5.2.2 Post compression parameters

The prepared tablets showed acceptable pharmaco-technical properties. For batches F_1 to F_9, hardness of tablets were in the range of 4-5 kg/cm^2 and friability was less than 1% w/w indicated sufficient mechanical strength of tablets. All formulations were found to be within IP 2007 [61] limits as per weight variation test. Assay results of all batches were found to be in the Pharmacopoeial limits of 95–105 %.

3.5.2.3 *In vitro* swelling rate

Swelling study helps in analysis of important parameters like drug release mechanism from a matrix system, possibility of water penetration for drug release and lag time for insoluble drug release from matrix system. The swelling Characteristics of a polymer can also contributes to its bioadhesive behavior [62]. Swelling index of each batch (F_1 to F_9) is reported in table 3. Guobin Yi, *et al* prepared hydrogel and suggested that sodium CMC could be potential for preparation of porous and rapid swelling hydrogels [63]. Polycarbophil is an anionic polymer in with Carboxylic group of it binds hydroxyl group of oligosaccharide of mucus glycoprotein so increment in pH enhances swelling. Lower swelling behavior leads to quite deficient adhesion [64].

3.5.2.4 *Ex-vivo* Mucoadhesion study

Tablets of batches F_3 to F_7 showed acceptable mucoadhesive strength (table 3). Highest mucoadhesion was observed in batch F_9. Polycarbophil is insoluble in aqueous media but in the neutral pH conditions, it has a high swelling capacity and the volume can be increased to 100 times, allowing high levels of entanglement within the mucus layer. Comprehensive adhesion and the inherent characteristics of polycarbophil, the bioadhesive effect is produced by the carboxylic acid groups binding to the mucosal surfaces via hydrogen bonding interaction. In the non-swollen state, the macromolecules are tightly coiled, so the volume and viscosity are very small. When dispersed in water, the molecules will hydrate and uncoil to some extent, though the molecular chains don't achieve the greatest degree of expansion, the viscosity of the system could be improved to a greater extent. The performance of polymers will be maximized when they are fully uncoiled and extended, which can be accomplished by neutralization or hydrogen bonding. The hydrogen bonding force makes the viscosity increased significantly [65].

3.5.2.5 Surface pH:

Surface pH of tablets of all the batches F_1 to F_9 was in the range of 7 to 7.4 (table 3) which indicated suitability of R-HCl tablets for vaginal route. Raloxifene approved for prevention and treatment of osteoporosis

and prevention of invasive breast cancer, and ospemifene approved for treatment of dyspareunia from menopausal vaginal atrophy [66]. Vaginal pH is 6.0 to 7.5 in case of menopause [67].

3.5.2.6 *In vitro* dissolution study

Tablets of batch F_1 to F_5 exhibited desired release profile (Figure 6). In The dissolution medium, no disintegration of the tablets was observed during the test period, a fact that may be due to the persistent gel layer surrounding the tablets. It was reported that upon hydration, a mixture of MCC and sodium CMC as present in formulation generates a rheological system showing thixotropic properties over a pH range of 3.5 to 11.0. This behavior results from the formation of a three-dimensional gel structure leading to immobilization of water molecules inside it and slowing drug release in buffer. The network produced would involve hydrogen bonding between the negative oxygen of the carboxyl group of sodium CMC and hydrogen of the hydroxyl group of MCC. Moreover, the combination of MCC and sodium CMC needs little hydration time in such a medium. A higher proportion of sodium CMC, showed a slower release. The excess free sodium CMC content in formulation may form a gel layer surrounding the tablet and hindering dissolution. During storage, some water from the environment is adsorbed on the tablet surface, causing the start of ionization of exposed carboxylate groups of sodium CMC and some interaction with hydroxyl groups of MCC, and production of a gel film around the matrix with subsequent clogging of the surface pores. Consequently, the retardation of drug release in water could be due to decrease of available pores for water penetration34. Polycarbophil is reported to be used in vaginal suppository base to overcome the shortcoming of stranded short time of the site, which was observed in traditional creams, suppositories and vaginal tablets, and it can also improve the hydration of the vaginal tissue. Wang Chengwei [68] *et al* developed the nonoxynol vaginal sustained release gel used PCP, Carbopol 971P and glyceryl behenate, and examined the released results *in vitro*. The consequences showed that the preparation could prolong the contact time, release the effective dose quickly and continue for 24 h of an effective dose, which reduced the drug dose, the toxicity and adverse reactions it caused.

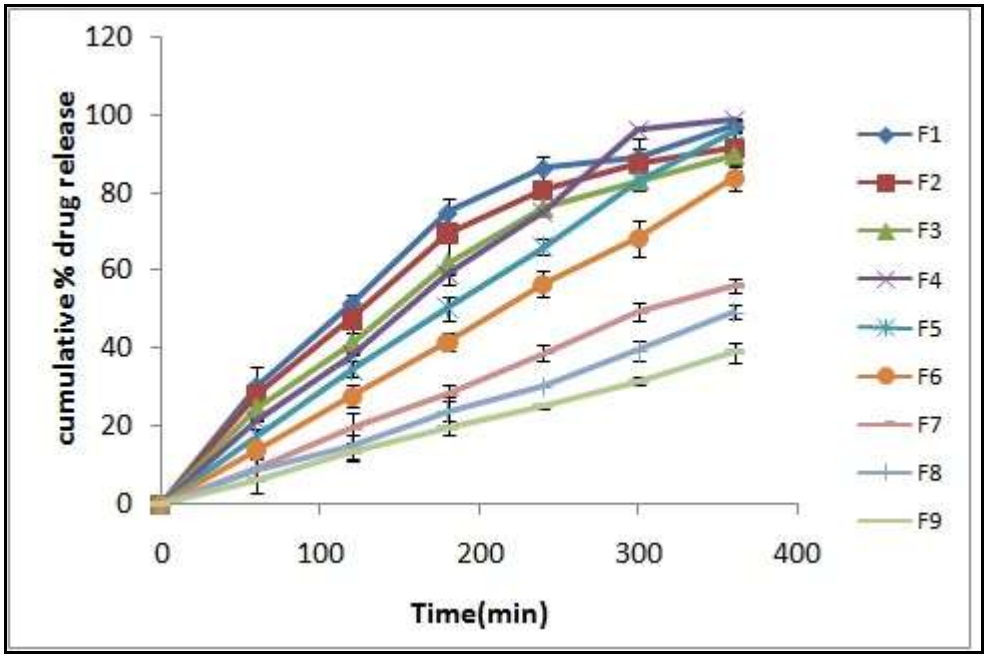


Fig. 6: *In vitro* drug release profile of study design batches F_1 to F_9

3.5.2.7 *Ex-vivo* residence time

The tablet softened inside the vaginal tube after absorbing simulated vaginal fluid and became a swollen structure, helping it to adhere to the vaginal mucosa. Swollen bioadhesive polymers held the solid content of the tablet inside the vagina; at the same time preventing premature leakage. As the concentration of bioadhesive polymer increased, the residence time also increased. This examination reveals the mucoadhesive capacity of polymers used in formulations. Polycarbophil had much more effect on the retention time than sodium CMC and formulation containing higher concentration of polycarbophil showed higher retention time.

Table 3: Results of swelling index, mucoadhesion strength, surface PH and *Ex vivo* residence time of tablets of batches F_1 to F_9

Batch No.	Swelling index	Mucoadhesive strength (N)	Surface pH	*Ex vivo* residence time
F_1	33.73±0.38%	0.267±0.28	7.0±0.2	5.22
F_2	35.23±0.27%	0.294±0.31	7.2±0.3	5.38
F_3	37.73±0.25 %	0.304±0.25	7.4±0.4	5.45
F_4	34.23±0.61 %	0.338±0.61	7.0±0.1	5.55
F_5	36.04±0.13%	0.343±0.19	7.4±0.1	6.10
F_6	38.4±0.34 %	0.362±0.34	7.2±0.2	6.25
F_7	35.02±0.22%	0.389±0.64	7.0±0.2	6.40
F_8	37.17±0.15%	0.412±0.39	7.2±0.1	6.55
F_9	40.64±0.41%	0.43±0.56	7.4±0.2	7.11

3.6 Statistical Data Analysis

3.6.1 Data analysis for Mucoadhesive strength (Y_1)

The observed values of response Y_1 for different batches showed wide variation i.e., values ranged from a minimum of 0.267 to a maximum 0.43 N. There was not much difference between actual and predicted values. The response (Y_1) obtained at three levels of the two independent variables (X_1 and X_2) were subjected to multiple regression to yield a polynomial equation 4 (full model). The coefficients b_1 and b_2 were found to be significant having $p < 0.05$ whereas, the coefficient for b_{11}, b_{22} and b_{12} were insignificant having $p > 0.05$. Reduced model equation was generated by omitting insignificant terms (Equation 5) [69].

$$Y = 0.349 + 0.017X_1 + 0.061X_2 + 0.001X_1X_2 - 0.0013X_1^2 + 0.001X_2^2 \_ (Equation\ 4)$$

$$Y = 0.349 + 0.017X_1 + 0.061_2X_2 \_\_\_\_\_ (Equation\ 5)$$

Positive sign of coefficient indicate a synergistic effect while negative sign indicate an antagonistic effect on the response. In the present study, coefficients b_1 and b_2 possessed positive sign which indicated agonistic effect of both variables X_1 and X_2 on response Y_1. Among the two independent variables, X_2 (amount of Polycarbophil) has prominent effect ($b_2 = 0.061$ and $p = 1.34E\text{-}07$) on mucoadhesive strength after that to some extent X_1 (amount of sodium CMC) affects the results ($b1 = 0.017$ and $p = 0.000229$) Hence increasing the concentration of X_2 polymer in tablet formulation will mainly influence to achieve desired mucoadhesive strength

3.6.2 Data analysis for % swelling (Y_2)

The observed values of response Y_2 for different batches showed wide variation i.e., values ranged from a minimum of 33.73 to a maximum 40.64%. There was not much difference between actual and predicted values. Full model equation for the response (Y_2) obtained at three levels of the two independent variables (X_1 and X_2) is given as equation 6. The coefficients b_1, b_2 and b_{11} were found to be significant having $p < 0.05$ whereas, the coefficient for b_{22} and b_{12} were insignificant having $p > 0.05$. Reduced model equation was generated by omitting insignificant terms (Equation 7).

$$Y = 35.90 + 2.299X_1 + 1.023X_2 + 0.479X_1X_2 + 0.363X_1^2 + 0.001X_2^2 \_ (Equation\ 6)$$

$$Y = 36.47 + 2.299X_1 + 1.023X_2 + 0.405X_1^2 \_\_\_\_\_ (Equation\ 7)$$

Coefficients b_1 and b_2 possessed positive sign which indicated agonistic effect of both variables X_1 and X_2 on response Y_2. Among the two independent variables, X_1 (amount of sodium CMC) has prominent effect ($b_1 = 2.299$ and $p = 4.44E\text{-}05$) on % swelling after that to some extent X_2 (amount of polycarbophil) affects the results ($b_2 = 1.023$ and $p = 0.002017$).

3.6.3 Data analysis for Q_6 (Y_3)

Full model equation and reduced model equation for the response (Y_3) is given as equation 8 and 9, respectively. The high values of the coefficient of determination indicate a good fit i.e. good agreement between the dependent and independent variables.

$$Y = 93.83 - 6.65X_1 - 22.29X_2 - 2.405X_1X_2 - 1.43{X_1}^2 - 22.37{X_2}^2 \_ (Equation\ 8)$$

$$Y = 92.88 - 6.65X_1 - 22.29X_2 - 22.37{X_2}^2 (Equation\ 9)$$

Coefficients b_1 and b_2 possessed negative sign which indicated antagonistic effect of both variables X_1 and X_2 on response Y_3. Among the two independent variables, X_2 (amount of polycarbophil) has prominent effect (b_2 =22.29 and p = 8.5E-06) on Q_6 after that to some extent X_1 (amount of sodium CMC) affects the results (b_1 = 6.65 and p = 0.002688).

3.7 Contour plots and response surface analysis

Two-dimensional contour plots and three-dimensional response surface plots are very useful to study the interaction effects of the factors on the responses. These types of plots are useful in study of the effects of two factors on the response at one time. Six contour plots and response surface plots were generated using software. Representative figure for only response for mucoadhesive strength are given in Figure 7. Non-linear relationship was observed between two factors (X_1 and X_2) with all three responses (Mucoadhesive strength, swelling index and Q_6).

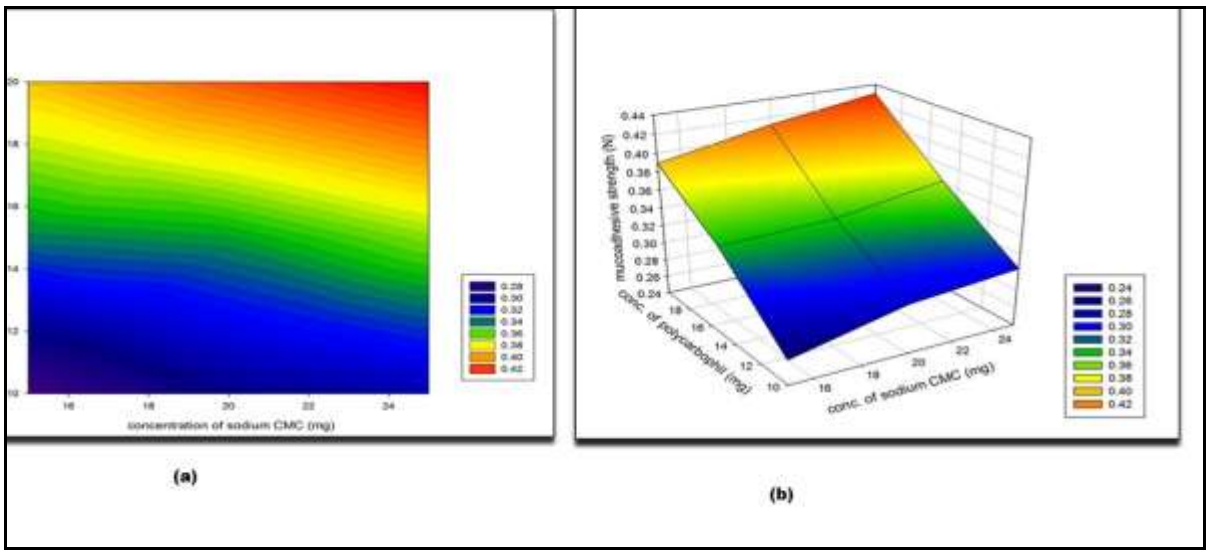


Fig. 7: (a) Contour plot and (b) 3D surface polo for response mucoadhesive strength

3.8 Optimization of formulation

The optimum formulation was selected based on the criteria of attaining the minimum, target and maximum range of the dependent variables in minitab 17 software. Optimized batch was prepared using amount of X_1(sodium CMC) 20 mg and amount of X_2(polycarbophil) 15 mg. Desirability of optimized batch was found to be 0.9880.

3.9 Prediction of Release Mechanism

Batches F_1 to F_9 showed n value between 0.5 to 1, so drug released by non-Fickian transport mechanism (anomalous transport) which is controlled by a combination of diffusion and chain relaxation mechanism and having a correlation coefficient value fitted in zero order drug release profile.

3.10 *Ex- vivo* permeation study

Result of ex-vivo permeation of the optimized formulation is displayed in figure 8. This result can give idea about systemic absorption of the drug. The vagina stands as an important alternative to the oral route for those systemic drugs that are poorly absorbed orally or are rapidly metabolized by the liver. Drug permeation through the vaginal tissue can be estimated by using in vitro, ex vivo and in vivo models. The latter ones, although more realistic, assume ethical and biological limitations due to animal handling. Therefore, in vitro and ex vivo models have been developed to predict drug absorption through the vagina route [70].

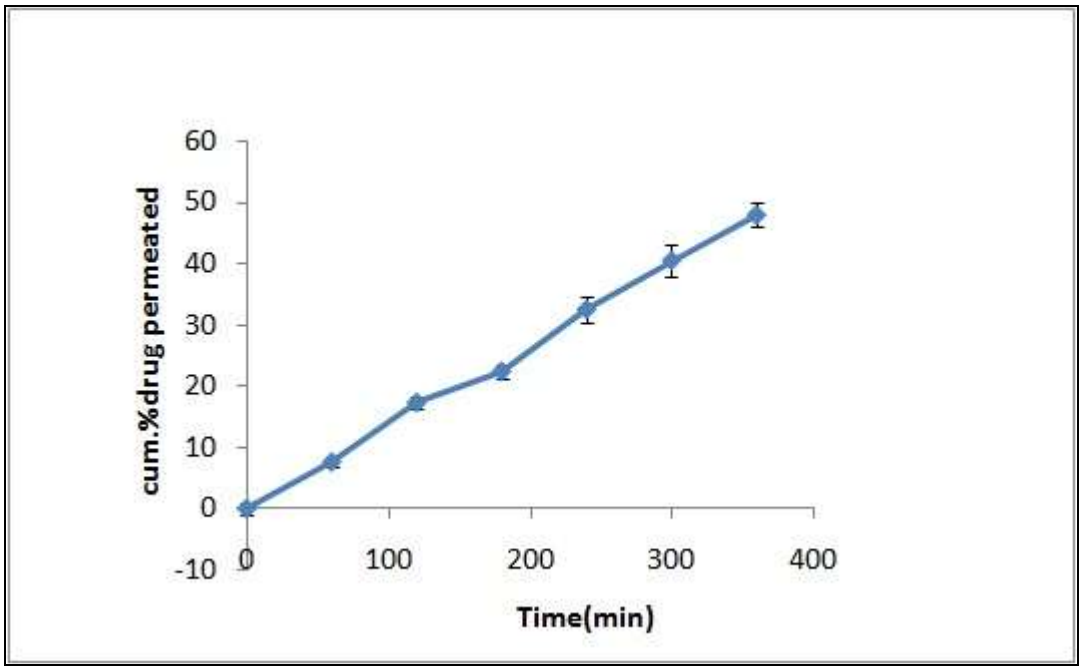


Fig. 8: Result of *ex-vivo* permeation of the optimized formulation

3.11 Validation of design model

To confirm the validity of design, the optimized batch was prepared; three responses were measured and % relative error was calculated which was found to be less than the 5% which was indicated goodness of fit in model. It was found that both factor had statistically significant influence on all dependent variables as $P < 0.05$. Thus both reasons were confirm the validity of design.

3.12 Short-term stability study

The formulation retained the pale yellow appearance. No remarkable change was observed in Surface pH, Mucoadhesive strength, Swelling index and Drug release after 6 hr (%). There was small increase in swelling index, which led to slightly lower the drug release after 6hr, but changes were insignificant. Negligible difference was observed in results obtained during optimization and those after stability study. Thus the formulation retained the good stability at accelerated condition of temperature and humidity.

4. Conclusion

Raloxifene hydrochloride is a selective estrogen receptor modulator with very poor oral absolute bioavailability (2%) due to high hepatic first-pass metabolism. In present research, Raloxifene hydrochloride mucoadhesive vaginal tablets were developed for the effective treatment of disease as it provides control drug release and bypasses the hepatic first pass metabolism. Raloxifene hydrochloride was complexed with β-cyclodextrin to improve the solubility of drug. Amount of sodium carboxymethyl cellulose and amount of polycarbophil showed agonistic effect on mucoadhesive strength and percentage swelling whereas, antagonistic effect on drug release at 6 hr. Based on experimental results, applied statistics and response surface methodology; 20 mg sodium carboxymethyl cellulose and 15 mg polycarbophil was selected as optimized batch to formulate tablets.

5. References

1. Elsheikh MA, Elnaggar YSR, Gohar EY, Abdallah OY. Nanoemulsion liquid preconcentrates for raloxifene hydrochloride: optimization and in vivo appraisal. Int J Nanomedicine., 2012, 7; 3787–802.
2. Jagadish B, Yelchuri R, Bindu K, Tangi H, Maroju S, Rao VU. Enhanced dissolution and bioavailability of raloxifene hydro-chloride by co-grinding with different superdisintegrants. Chem Pharm Bull., 2010, 58; 293–300.
3. Michael FW, Wacher VJ, Ruble KM, Ramsey MG, Edgar KJ, Buchanan NL, *et al*. Pharmacokinetics of raloxifene in male Wistar–Hannover rats: influence of complexation with hydroxybutenyl- beta-cyclodextrin. Int J Pharm., 2008, 346; 25–37.
4. Garg A, Singh S, Rao VU, Bindu K, Balasubramaniam J. Solid state interaction of raloxifene HCl with different hydrophilic carriers during co-grinding and its effect on dissolution rate. Drug Dev Ind Pharm., 2009, 35; 455–70.
5. Valenta C, Kast CE, Harich I, Bernkop-Schnürch A. Development and in vitro evaluation of a mucoadhesive vaginal delivery system for progesterone. J Control Release., 2001, 77; 323–32.
6. Valenta C. The use of mucoadhesive polymers in vaginal delivery. Adv Drug Deliv Rev., 2005, 57; 1692-712.
7. Knuth K, Amiji M, Robinson JR. Hydrogel delivery systems for vaginal and oral applications. Formulation and biological considerations. Adv Drug Deliv Rev., 1993, 11; 137-67.
8. Pereira RRDA, Bruschi ML. Vaginal mucoadhesive drug delivery systems. Drug Dev and Ind Pharm., 2012, 38; 643-52.
9. Karasulu HY, Hilmioğlu S, Metin DY, Güneri T. Efficacy of a new ketoconazole bioadhesive vaginal tablet on Candida albicans. Farmaco., 2004, 59; 163-7.
10. Bagul U, Gujar K, Dhat S, Aphale S, Bhavsar M. In vitro study of mucoadhesive strength of polymers for Mucoadhesive drug delivery systems. Int J Curr Pharm Res., 2009, 1; 42-6.
11. Meneshwari M, Hazari S, Maheshwararao VU , Morsu A, Arun kumar B. A brief review on mucoadhesive microspheres. Int J Res Rev Pharm Appl sci., 2014, 4; 975-86.
12. Bassi P, Kaur G. Innovations in bioadhesive vaginal drug delivery system. Expert Opin Ther Pat., 2012, 22(9); 1019-32.
13. Perioli L, Ambrogia V, Paganoa C, Scuota S, Rossi C. FG90 chitosan as a new polymer for metronidazole mucoadhesive tablets for vaginal administration. Int J Pharm., 2009, 377; 120–27.
14. Krishna SV, Ashok V, Chatterjee A. A review on vaginal drug delivery systems. Int j Bio Pharm allied Sci., 2012, 1; 152-67.
15. Washington N, Washington C, Wilson CG (eds). Vaginal and intrauterine drug delivery, Physiological Pharmaceutics: Barriers to Drug Absorption. London: CRC Press., 2000, 272-81.
16. Madan J, Banode S, Dangi M. Mucosal drug delivery system. Int j Res Ayurveda Pharm., 2010, 1; 63-70.
17. Hussain A, Ahsan F. The vagina as a route for systemic drug delivery.j control rel., 2005, 103; 301-13.
18. Değim Z, Değim T, Acartürk F, Erdoğan D, Özoğul C, Köksal M. Rectal and vaginal administration of insulin-chitosan formulations: An experimental study in rabbits. J Drug Target., 2005, 13; 563-72.
19. Baloglu E, Ay Senyıgıt Z, Karavana SY, Vetter A, Metın DY, Hilmioglu Polat S, Guneri T, Bernkop-Schnurch A. In vitro evaluation of mucoadhesive vaginal tablets of antifungal drugs prepared with thiolated polymer and development of a new dissolution technique for vaginal formulations. Chem Pharm Bull., 2011, 59; 952-8.
20. Sreenivas SA, Pai KV. Thiolated Chitosans: Novel Polymers for Mucoadhesive Drug Delivery – A Review. Trop J Pharm Res., 2008, 7; 1077-88.
21. Kale VV, Ubgade A. Vaginal Mucosa – A Promising Site for Drug Therapy. Brit J Pharm Res., 2013, 3; 983-1000.
22. Kumar O, Rani AP. Effect of Polyvinylpyrrolidone on Complexation and Dissolution Rate of Beta Cyclodextrin and Hydroxypropyl Beta Cyclodextrin Complexes of Piroxicam. Int J Pharm Phytopharmacol Res., 2012, 1; 301-5.
23. Maski N. Studies on the preparation, characterization and solubility of β-cyclodextrin –diacerein inclusion complexes. Int j pharm pharm sci., 2009, 1; 121-35.
24. Yuksel N, Karataş A, Ozkan Y, Savaşer A, Ozkan SA, Baykara T. Enhanced bioavailability of piroxicam using Gelucire 44/14 and Labrasol: in vitro and in vivo evaluation. Eur J Pharm Biopharm., 2003, 56; 453-9.

25. Patel GC, Asodaria KV, Patel HP, Shah DR. Development of Controlled Release Osmotic Pump Tablet of Glipizide Solid Dispersion. Curr Drug Deliv., 2014, 11; 1-11.
26. Ghasadiya RSD. Solubility improvement of cefpodoxime proxetil by inclusion in 2-hp beta cyclodextrin. Int j pharm res biosci., 2012, 2; 71-80.
27. Kerk JSS, Urleb U, Kanalec A, Kofler B, Smid S, Korban J. Compatibility Study Between Acetylcysteine and Some Commonly Used Tablet Excipients. J Pharm Pharmacol., 1992, 44; 515–8.
28. Ghasadiya R, Shah D. Solubility improvement of cefpodoxime proxetil by inclusion in 2-hp beta cyclodextrin. int j pharm res biosci., 2012, 2; 71-80.
29. Patel HM, Suhagia BN, Shah SA, Rathod IS, Parmar VK. Preparation and characterization of etoricoxib-b-cyclodextrin complexes prepared by the kneading method. Acta Pharm., 2007, 57; 351-9.
30. Singh S, Srinivasan K, Gowthamarajan K, Narayan G. Development and validation of discriminatory dissolution procedure for poorly soluble glyburide. Asian J Pharm Sci., 2010, 4; 205-12.
31. Shah R, Patel S, Patel H, Pandey S, Shah S, Shah D. Formulation development of carvedilol compression coated tablet. Pharm Dev Technol., 2013, 18; 906-15.
32. United States Pharmacopoeia 32- National Formulatory 27. United states Pharmacopeial Convention., 2008.
33. Patel P, Barot V, Prajapati B. Preparation and Evaluation of Effervescent Bioadhesive Vaginal Tablet of Itraconazole for Vaginal Candidiasis. Pharmagene., 2013, 1; 1-5.
34. El-Kamel A, Solkar M, Naggar V, Gamal S. Chitosan and Sodium Alginate–Based Bioadhesive Vaginal Tablets. AAPS PharmSci., 2002, 4; E44.
35. Van der BP, Thompson IO, Squier CA. Comparative permeability of human vaginal and buccal mucosa to water. Eur J Oral Sci., 1997, 105; 571-5.
36. Emami JV, Saljoughian N. Development and evaluation of controlled-release buccoadhesive verapamil hydrochloride tablets. Daru., 2008, 16; 60-9.
37. Patel VM, Prajapati BG, Patel HV, Patel KM. Mucoadhesive Bilayer Tablets of Propranolol Hydrochloride. AAPS PharmSciTech., 2007, 8; E77.
38. Gibaldi M, Feldman S. Establishment of sink conditions in dissolution rate determinations. Theoretical considerations and application to nondisintegrating dosage forms. J Pharm Sci., 1967, 56; 1238–42.
39. Wagner JG. Interpretation of percent dissolved-time plots derived from in vitro testing of conventional tablets and capsules. J Pharm Sci., 1969, 58; 1253–57.
40. Higuchi T. Rate of release of medicaments from ointment bases containing drugs in suspension. J Pharm Sci., 1961, 50; 874–75.
41. Higuchi T. Mechanism of sustained-action medication. Theoretical analysis of rate of release of solid drugs dispersed in solid matrices. J Pharm Sci., 1963, 52; 1145–49.
42. Cobby J, Mayersohn M, Walker GC. Influence of shape factors on kinetics of drug release from matrix tablets. II. Experimental. J Pharm Sci., 1974, 63; 732–37.
43. Hixson AW, Crowell JH. Dependence of reaction velocity upon surface and agitation. Ind Eng Chem., 1931, 23; 923–31.
44. Korsmeyer RW, Gurny R, Doelker EM, Buri P, Peppas NA. Mechanism of solute release from porous hydrophilic polymers. Int J Pharm., 1983, 15; 25–35.
45. Verma S, Narang S. Development and in vitro evaluation of floating matrix tablets of anti Retroviral drug. Int J Pharm Pharm Sci., 2011, 3; 208-11.
46. Ghosh A, Sujit Das AS, Ghosh T. Studies on Formulations and Design of Zidovudine Loaded Particulated VaginalBioadhesive Tablet. Int J PharmTech Res., 2011, 3; 82-92.
47. Shidhaye SS, Thakkar PV. Buccal Drug Delivery of Pravastatin Sodium. AAPS PharmSciTech., 2010, 11; 416-24.
48. Bhat SR, Shivakumar HG. Bioadhesive Controlled Release Clotrimazole Vaginal Tablets. Trop J Pharm Res., 2010, 9; 339-46.
49. Aly AM, Qato MK, Ahmad MO. Enhancement of the dissolution rate and bioavailability of glipizide through cyclodextrin inclusion complex. Pharm Technol., 2003, 26; 54-62.
50. George S, Vasudevan D. Studies on the Preparation, Characterization, and Solubility of 2-HP-β-Cyclodextrin-Meclizine HCl Inclusion Complexes. J Young Pharm., 2012, 4; 220-7.
51. Challa R, Ahuja A, Ali J, Khar RK. Cyclodextrins in drug delivery: an updated review. AAPS PharmSciTech., 2005, 6; E329-57.

52. Renny JS, Tomasevich LL, Tallmadge EH, Collum DB. Method of continuous variations: applications of job plots to the study of molecular associations in organometallic chemistry. Angew Chem Int Ed Engl., 2013, 52; 11998-2013.
53. Lachman L, Liberman HA, Kanig JL. The theory and practice of Industrial Pharmacy. Mumbai: varghese publishing house., 1990, 179.
54. Veiga F, Fernandes C, Maincent P. Influence of the preparation method on the physicochemical properties of tolbutamide/cyclodextrin binary system. Drug Dev Ind Pharm., 2001, 27; 523–32.
55. Wang L, Tang X. A novel ketoconazole bioadhesive effervescent tablet for vaginal delivery: Design, in vitro and in vivo evaluation. Int J Pharm., 2008, 350; 181-87.
56. Shaikh R, Singh TRR, Garland MJ, Woolfson AD, Donnelly RF. Mucoadhesive drug delivery systems. J Pharm Bioallied Sci., 2011, 3; 89–100.
57. Patel A, Patel K, Patel J. Development and Evaluation of Mucoadhesive Vaginal Tablet of Sertaconazole for Vaginal Candidiasis Int J PharmTech Res., 2011, 3; 2175-82.
58. Patel A, Patel J. Design, development and in vitro evaluation of sertaconazole mucoadhesive vaginal tablet. Der Pharmacia Lettre., 2012, 4; 418-27.
59. Sinko PJ. Martin's physical pharmacy and pharmaceutical sciences. USA: Lippinkott Williams and Wilkins., 2010.
60. Alam MA, Ahmad FJ, Khan ZI, Khar RK, Ali M. Development and Evaluation of Acid-buffering Bioadhesive Vaginal Tablet for Mixed Vaginal Infections AAPS PharmSciTech., 2007, 8; E 109.
61. Indian Pharmacopeia. Monograph of Ketoprofen. The Indian Pharmacopoeia Commission, Ghaziabad., 2010, 2.
62. García-González N, Kellaway IW, Blanco-Fuente H, Anguiano-Igea S, Delgado-Charro B, Otero-Espinar FJ, Blanco-Méndez J. Design and evaluation of buccoadhesive metoclopramide hydrogels composed of poly (-acrylic acid) cross-linked with sucrose. Int J Pharm., 1993, 100; 65-70.
63. Yi G, HuangAffiliated withFaculty of Chemical Engineering and Light Industry, Guangdong University of Technology Y, Xiong F, Liao B, Yang J, Chen X. Preparation and swelling behaviors of rapid responsive semi-IPN NaCMC/PNIPAm hydrogels. Journal of Wuhan University of Technology-Mater. Sci. Ed., 2011, 26; 1073-78.
64. Morishita, Park K, editors. Biodrug delivery system: Fundamental applications and clinical development. Mariko Volume 194. New York: CRC Press., 2009.
65. Zhaolu Zhu, Yinglei Zhai,Ning Zhang, Donglei Leng, Pingtian Ding. The development of polycarbophil as a bioadhesive material in pharmacy. Asian J Pharm Sci., 2013, 8; 218–27.
66. Pinkerton JV, Thomas S. Use of SERMs for treatment in postmenopausal women. J Steroid Biochem Mol Biol., 2014, 142; 142-54.
67. Caillouette JC1, Sharp CF Jr, Zimmerman GJ, Roy S. Vaginal pH as a marker for bacterial pathogens and menopausal status. Am J Obstet Gynecol., 1997, 176; 1275-7.
68. Wang C, Tang X, Yao J. Preparation and in vitro drug release characterization of nonoxyno-l 9 sustained-release gel for vagina. J Shenyang Pharm Univ., 2004, 21; 245–49.
69. Karen HD, Shah HM, Shah PM, Patel CN. Development and optimization of fast dissolving strip of tizanidine hydrochloride using 32 full factorial design. Int j pharm res biosci., 2014, 3; 880-99.
70. Machado RM, Palmeira-de-Oliveira A, Gaspar C, Martinez-de-Oliveira J, Palmeira-de-Oliveira R. Studies and methodologies on vaginal drug permeation.Adv Drug Deliv Rev., 2015, 92; 14-26.

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