INTERNATIONAL JOURNAL OF ADVANCES IN PHARMACY, BIOLOGY AND CHEMISTRY

Research Article

# Development and Statistical Optimization of Mucoadhesive Buccal patches of Indomethacin: *In-vitro* and *Ex-vivo* evaluation

**VA. Deore*, RS Kumar and PS. Gide**

Department of Pharmaceutics, MET's Institute of Pharmacy, Bhujbal Knowledge City, Adgaon, Nashik, Maharashtra-422003, India.

**ABSTACT**

Buccal route offers the advantage of avoiding the first pass metabolism. Indomethacin (IND), an NSAID, in conventional dosage form, is extensively metabolized by the liver. Non-steroidal Anti Inflammatory Drugs (NSAIDs) are known to cause gastrointestinal disorders when given orally. The objective of the present study was to formulate and evaluate buccal patches of Indomethacin. Eudragit RL-100, a hydrophobic polymer was used as the base matrix and Hydroxypropylmethylcellulose (HPMC) as mucoadhesive polymer, were utilised in different ratios to formulate the buccal patches by solvent casting technique. The patches were characterized for various parameters and a $3^2$ full factorial design was employed to study the effect of independent variables. The response of design was analysed using Design Expert® trial version 8.0.7.1; and the tools of the software were used to draw Contour plot and 3D plot. On the basis of the software analysis, formulation S1 with desirability factor of 0.988 was selected as optimized formulation and evaluated for independent parameters. Optimized formulation showed 98.04 ± 0.21% dug release after 6 hrs. The release kinetics of the optimized formulation best fitted the Higuhci, Korsmayer-peppas model. Hence formulation S1 can be considered as a promising formulation for the buccal systemic delivery of Indomethacin.

**Keywords:** Buccal patch, NSAID, Hydroxypropylmethylcellulose, Mucoadhesive polymer, Eudragit RL-100.

## INTRODUCTION

Although oral nonsteroidal anti-inflammatory drugs (NSAIDs) are administered to relieve pain, there have been several reports on the risks of systemic side effects with oral NSAIDs, including gastrointestinal disorders. Thus topical formulations that decrease the risk of side effects and allow the rapid onset of analgesia are an attractive alternative. External formulations that relive pain include films, patches, sprays, ointments and mouthwashes. Film or patch formulations can particularly improve patient Quality Of Life (QOL) because of better localization and drug retention times, as well as protective coverage of the affected site (Tanabe et al., 2008).

The rich vascularization of the oral mucosa and its permeability for many drugs makes this route an attractive alternative to the oral and parenteral routes for systemic drug delivery. Absorption of therapeutic agents from the oral mucosa overcomes premature drug degradation due to enzyme activity and pH of the gastrointestinal tract, avoids active drug loss due to first-pass hepatic metabolism and therapeutic plasma concentration of the drug can be rapidly achieved. (Jian-Hwa, G., Karsten, C.,1999)

Extensive efforts have been recently focused on targeting/ delivering the drugs to a particular region of the body for extended period of time, thus maximizing drug availability and minimizing dose dependent side effects (Kumar et al., 2010). Recent advances in novel buccal drug delivery system aim to enhance safety and efficacy of drug molecule by formulating a convenient dosage form for administration and to achieve better patient compliance. One such approach is formulation of buccal patches containing NSAID for treatment of arthritis, gout etc. Advantages, such as good accessibility, robustness of the epithelium, facile removal of the dosage form in case of need, relatively low enzymatic activity and possible elimination of the dosage form from the buccal area by natural clearance mechanism, satisfactory

patient acceptance and compliance are offered by the buccal route of drug administration. This route also offers the advantage of partly circumventing drug degradation in the GI tract and of avoiding the hepatic first pass metabolism. (Burgalassi et al., 1996).

The drug Indomethacin is Non-steroidal anti-inflammatory drug (NSAID) with anti-inflammatory, analgesic and antipyretic activity. Indomethacin is commonly used as a prescription medication to reduce fever, pain, stiffness, and swelling. Indomethacin is used to treat pain or inflammation caused by many conditions such as arthritis, gout, ankylosing spondylitis, bursitis, or tendinitis (http://www.everydayhealth.com/drugs/indomethacin cited 18/09/2012). The drug can be absorbed through oral mucosa into the systemic circulation. It has a short biological half life and is usually administered in a dose of 50-200 mg 2-3 times a day in order to maintain effective concentrations of drug throughout the day. Indomethacin undergoes first pass metabolism with bioavailability 99 %. Half-life of Indomethacin is 2 to 4.5 hrs. Conventional oral administration has good absorption, but extensively metabolized by the liver. Its pharmacological effect is inhibition of cyclooxygenase (COX), the enzyme responsible for catalyzing the rate-limiting step in prostaglandin synthesis via the arachidonic acid pathway (http://url.ie/fm69 (2011); http://url.ie/fm6c (2012)). Such characteristics make Indomethacin a suitable candidate for controlled drug delivery.

In the present work an attempt was made to control the release of Indomethacin and to optimize the drug plasma concentration using buccal mucosa as a route of delivery. The patch was designed to provide bidirectional drug release, a large contact surface area and good buccal penetration of drug. Various formulation variables and their effect on patch properties were evaluated and compared statistically. The hydrophobic polymer Eudragit RL-100 was used as base matrix and Hydroxypropylmethylcellulose E-15 (HPMC-E15) polymer was used to modify the rate of drug release as well as mucoadhesive polymer.

Buccal patches are highly flexible and thus much more readily tolerated by the patient than tablets. Patches also ensure more accurate dosing of the drug compared to gels and ointments (Nafee et al. 2003). They may be preferred over the mouth dissolve tablets in terms of flexibility, small size, comfort as well as less friable dosage form. In addition, they can also circumvent the sticky feeling in the oral cavity associated with oral gels (Sharma et al. 2007). Also the semisolid and liquid dosage forms intended for buccal drug delivery have problem of residence time at the site of application. This problem can be solved effectively by formulating bioadhesive patches (Kumar et al. 2010).

## MATERIAL AND METHOD

### Materials

Indomethacin was received as a gift sample from Microlab Pvt. Ltd., Bengaluru, India. Eudragit RL-100 was provided by Evonik India Pvt. Ltd. Mumbai, India. HPMC-E15 was obtained from Colorcon Pvt. Ltd., Goa, India. All the solvents and other reagents used were of the best Laboratory reagent (LR) grade.

### Methods

#### Experimental design

A complete $3^2$ randomized full factorial design was used in this study. The amount of HPMC-E15 (X1) and Eudragit-RL100 (X2) were selected as independent variables and the dependent variable was % cumulative drug release. The data obtained was treated using DE software (Design Expert® trial version 8.0.7.1; State-Ease Inc., Minneapolis, MN, USA) and analyzed statistically using analysis of variance (ANOVA).

The levels of these factors were selected on the basis of initial studies and observations. All the other formulation aspects and processing variables were kept invariant throughout the study period. Polynomial models including interaction and quadratic terms were generated for the entire response variables using multiple linear regression analysis (MLRA) approach. The general form of the MLRA model is represented in the Equation

$$Y = b0 + b1X1 + b2X2 + b12X1\,X2$$

Where Y is the dependent variable; b0 is the arithmetic average of all the quantitative outcomes of nine runs. b1, b2, b12 are the estimated coefficients computed from the observed experimental response values of Y and X1 and X2 are the coded levels of the independent variables. The interaction term (X1X2) shows how the response values change when two factors are simultaneously changed.

Table 1 summarizes the translation of the coded levels to the experimental units used in the study and table 2 summarizes the experiment runs used. In this study, factorial design based on the response surface method was adopted to optimize effective factors for the release of the drug from the films.

#### Preparation of Buccal Patches

Buccal patches of indomethacin were formulated by using solvent casting technique. Buccal patches were prepared using HPMC-E15 and Eudragit-RL100 polymers. Polyethylene glycol (PEG) was used as a plasticizer. Dichloromethane: Methanol in 1:1 ratio was used as the solvent system.

Model dose of drug (50 mg pet patch of 4 $cm^2$) was weighed and dissolved in part of the solvent. Required amount of HPMC-E15 was added slowly

in drug solution and it was allowed to stand for complete swelling. In remaining of the solvent Eudragit-RL100 was dissolved and it was added in drug - polymer solution. Polyethylene glycol was added to final solution. The resultant solution was set aside for 2 hrs to remove entrapped air and poured into glass petri plate. The Petri plates were kept on horizontal surface and covered with inverted funnel to allow controlled evaporation of solvent at room temperature till a flexible patch was formed. The formed patches were removed carefully, cut to size, wrapped in aluminium foil and stored in desiccators. Patches with any imperfections, entrapped air, differing in weight or Indomethacin content were excluded from further studies. Table 3 shows Mucoadhesive patch composition (Muchalambe et al. 2010).

**Evaluation**

**Drug polymer interaction (FTIR) study**

FTIR spectroscopy was performed on Fourier transform infrared spectrophotometer (IR Affinity-1, Shimadzu, Japan).

**Thickness and weight of patches**

For each formulation, three randomly selected patches with surface area 4 $cm^2$ were used. Each patch was weighed individually on an analytical balance (Shimadzu, Japan) and average weight calculated. Similarly three patches of each formulation were taken and the patch thickness was measured using a dial caliper (Advance) at three different places and the mean value calculated (Choudhury et al. 2010 and Semalty et al. 2008).

**Surface pH**

Buccal patches were allowed to swell for 2 h on the surface of an agar plate. The surface pH was measured by means of a pH paper placed on the surface of the swollen patch. A mean and of three readings was recorded (Kumar et al. 201l, Nafee et al. 2003).

**Folding endurance test**

The folding endurance of the patches was determined by repeatedly folding and unfolding one patch at the same place till it broke or folded up to 300 times, which is considered satisfactory to reveal good patch properties. The number of times the film could be folded at the same place without breaking gave the value of the folding endurance (Nafee et al. 2003).

**Percentage moisture loss**

The percentage moisture loss was carried out to check integrity of the film at dry conditions. Patches from each batch were weighed and kept in a desiccators containing anhydrous calcium chloride. After three days, the patches were taken out and reweighed. The percentage moisture loss was calculated using the formula (Muchalambe et al. 2010).

$$\%\ Moisture\ loss = \frac{Initial\ weight - Final\ weigth}{Initial\ weigth} \times 100$$

**Swelling study**

Bioadhesion is the phenomenon between two materials, which are held together for extended periods of time by interfacial forces. It is generally referred as bioadhesion when interaction occurs between polymer and epithelial surface; mucoadhesion when occurs with the mucus layer covering a tissue. Generally bioadhesion is deeper than the mucoadhesion (Sudhakar et al. 2006). Swelling behaviour of patches gives an indication about relative water absorption capacities of polymers. On exposure to water or biological fluid, the dry polymer becomes hydrated, swells and forms a gel barrier layer, which retards the diffusion of drug out of the matrix. As the polymer chains become more hydrated and the gel becomes more dilute, the 'disentanglement concentration' may be reached, that is the critical polymer concentration below which the polymer chains disentangle and detach from a gelled matrix. The polymer will then undergo simultaneous swelling, dissolution and diffuse into the bulk medium resulting in erosion of the polymer (Corrigan et al. 2004). However, an excessive water uptake causes a leakage in cohesiveness of dosage forms transforming the formulation into over-hydrated slippery mucilage. This leads to an abrupt drop in adhesive strength due to disentanglement at the polymer tissue interface. The rate and the extent of patch hydration and swelling also affect the patch adhesion and consequently the drug release from the patch (Alanazi et al. 2007). Swelling behaviour of patches gives an indication about relative water absorption capacities of polymers. Buccal patches were weighed individually **(W1)** and placed separately in 2% agar gel plates, incubated at 37 ºC ± 1 ºC, and examined for any physical changes. At regular time intervals of 5 min until 1hr, patches were removed from the gel plates and excess surface water was removed carefully using the filter paper. The swollen patches were then reweighed **(W2)** and swelling index was calculated using the following formula (Patel et al. 2007, Semalthy et al. 2008, Nafee et al. 2003).

$$\%\ Swelling = \frac{W2 - W1}{W1} \times 100$$

**Drug content uniformity**

The drug loaded patch (2 × 2 cm) was allowed to dissolve in 100 mL phosphate buffer (pH 6.8). Solution was filtered, diluted suitably. The amount of Indomethacin in the patch was measured

spectrophotometrically (UV-visible spectrophotometer, JASCO V-630, Japan) at λmax of 266 nm (Verma et al. 2011).

**Mechanical properties of mucoadhesive patches**

**Tensile strength**

The tensile strength of a film/patch is defined as the resistance of the material to a force tending to tear it apart and normally identified as the maximum stress in the stress–strain curve.

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

**Elongation at break**

The elongation at break is a measurement of the maximum deformation the film can undergo before tearing apart and it was determined by formula (Javier et al. 2011 and Kolli et al. 2008),

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

***In vitro* bioadhesion/ mucoadhesion strength**

Mucoadhesion was evaluated using a texture analyzer (CEB Texture Analyzer, Make-Brookfield Engineering Labs, Inc., Model no. Texture Pro CT V1.4 Build 17). Goat buccal mucosa was utilized as the model membrane for mucoadhesive strength determination of various formulations. A patch was carefully attached to a 10-mm cylindrical probe (TA probe) by a double-face tape. The upper platform was moved downward manually near to the mucosa surface and then the polymer sample was brought toward the mucosa at a constant speed of 0.5 mm/s until a predetermined compressive force of 1 N was applied for 60 s. The probe was then removed at 5 mm/s to a distance of 15 mm and maximum detachment force (kg) was determined for each sample. For each new sample, a different mucosa sample was used (Peh and Wong 1999).

***In vitro* residence/ mucoadhesion time**

The *in vitro* adhesion time of patch was evaluated by assessing the time for the patch to detach from goat buccal mucosa in a well stirred beaker filled with 500 mL phosphate buffer pH 6.8 at 37 °C. The mucosal membrane was fixed on the side of the beaker with cyanoacrylate glue. The patch was attached to the membrane by applying light force with finger tip for 60 s. The beaker was then magnetically stirred at an approximate rate of 150 rpm to simulate buccal and saliva movement. The time necessary for complete erosion or detachment of the patches from the mucosal membrane was taken as an indication of the in vitro adhesion time (Basalious et al. 2009 and Jana S et al. 2010).

***In vitro* release study**

The USP apparatus type II (Electrolab-Tablet dissolution tester) rotating paddle method was used to study the drug release from buccal patch with some modifications. The dissolution media consisted of 250 mL of phosphate buffer pH 6.8. The release was performed at 37 ± 0.5 °C, with a rotation speed 50 rpm. The one side of buccal patch was attached to the glass disc with instant adhesive (cyanoacrylate adhesive). The disc was kept at the bottom of the dissolution vessel. Samples (10 mL) were withdrawn at predetermined time intervals and replaced with fresh medium. The samples were filtered through 0.45 µm Whatman filter paper and dilutions were made by withdrawing 1 mL from 10 mL aliquot and assayed UV-spectrophotometrically at 266 nm. (Shidhaye et al. 2008 and Choudhary et al. 2010)

**Residual solvent of patch**

Gas Chromatography (GC) is the most common method employed to determine amounts of residual solvents. The residual concentrations of methanol and dichloromethane were measured by GC (Agilent Technology 7890A GC system with G1888 Network Headspace Sampler) (European medicines agency 2009, Hu and Liu, 1990).

**GC-MS method**

Instrument: Agilent GC-MS equipped with Headspace Analyzer
Column: Agilent HP 5ms, 30 m x 0.25 mm, 0.25 µm
Temperature program: 30 °C for 5 min -----10 °C/min to 120 °C
Injection Port Temp.: 240 °C
Head Space Parameter: 80 °C for 20 min.
Gas flow: 1 mL/min (Helium gas)
Split Ratio: 1:5.

**Determination of zeta potential of patch**

The zeta potential was measured by using a Laser doppler electrophoresis analyzer (Malvern Instrument Ltd., UK). The temperature of the samples was controlled at 25 °C. The dispersions were diluted using deionized water to obtain

appropriate concentrations (count rates >20,000 counts/s) prior to measurement (Pongjanyakul et al. 2009 and Harding et al. 1999).

**Statistical analysis of response by design expert software**

**D**esign Expert® trial version 8.0.7.1; (State-Ease Inc., Minneapolis, MN, USA) was used for the effect of analysis of each variable on the designated response. Contour plots were made for the analysis of each response coefficient for its statistical significance. Qualitative and quantitative contribution of each variable on the response was analysed. The significant polynomial equations generated by design expert were used to validate the statistical design (Bolton, 4th edition). Response surface plots were generated to visualize simultaneous effect of each variable on each response parameter. Possible interactions between X1X2 were also studies.

**Experimental design validation**

The polynomial equations obtained were utilised for validation of experimental design (Bolton). An extra checkpoint formulation S1 was prepared with the predicted value of 97.96% for in vitro drug release (% CDR at 6th hr). Experimental values were determined by formulating and evaluating nine batches and the close resemblance between the predicted and experimental values indicated validity of the generated model. Finally an optimised formulation was selected on the basis of higher in vitro drug release after 6 hr with good desirability factor using software analysis.

**Kinetics of drug release**

The drug release of optimized formulation was fitted to zero order kinetics, first order kinetics, Higuchi model, Hixson-Crowell, Korsmeyer-Peppas model to ascertain the kinetic modeling of drug release and the model with the higher correlation coefficient i.e. higher $R^2$ was considered to be the best fit model (Deshmane et al. 2009, Alanazi et al. 2007).

**Differential scanning calorimetry analysis**

Differential Scanning Calorimetry (DSC) was carried out to study the interaction
between the drug and polymer. Samples (3-5 mg) were sealed in aluminium pans
with lids and heated in a rate of 10 °C/min, using dry nitrogen as carrier gas with a flow rate of 20 mL/min. The heat flow being recorded from 30 to 250 °C. Indium and zinc was used as the standard reference material to calibrate the temperature and energy scales of the DSC (Jade DSC V1.12).

**Effect of temperature and humidity**

Effect of temperature and humidity of optimization formulation was carried out for one month at 40 °C ± 2 °C / 75 % ± 5% maintained in environmental stability chamber (Remi, India). The patches were wrapped in aluminium foil and exposed to the said conditions. Samples were evaluated at 0, 7, 14, 21 and 28 days for the parameters as

a) Appearance
b) Surface pH
c) Folding endurance
d) Drug release (%)

**RESULT AND DISCUSSION**

Buccal patches of Indomethacin were successfully prepared by solvent casting technique using HPMC-E15 as the mucoadhesive polymer, Eudragit RL 100 as the hydrophobic polymer and propylene glycol as platicizer. All the prepared formulations were evaluated for physical characteristics and pharmacotechnical parameters, and are shown to be uniform, transparent, flexible and having smooth appearance without the entrapment air.

**Drug polymer interaction (FTIR) study**

The FTIR spectra of pure drug, Eudragit, HPMC-E15 and Indomethacin patch were shown in (fig. 1). It shows that no incompatibility reactions took place between drug and excipients.

**Evaluation parameters of patches**

Tables 4(a) and (b) summarizes the evaluated parameters of the formulated patches. Patch thickness ranged from 0.29 to 0.43 mm, with a weight range from 110.33 to 210.33 mg. It can be concluded that, as the concentration of Eudragit–RL 100 and HPMC-E15 increases the thickness and weight of the patch also increases. Patches exhibited a surface pH of 6-7 which is within range of physiological salivary pH (5.8-7.4). Hence no mucosal irritation and allergic response is expected. Folding endurance test results indicated that the patches did not show any cracks even after folding 300 times and thus showing good elasticity. Folding endurance was found in the range of 299 to 321 for all patches indicating that the patches will not break and will maintain their integrity with general skin folding when applied. Percentage moisture loss of all patches from each batch was found to be in range of 5.08 to 8.46 %. From moisture loss study it was found that formulation showed maximum amount of moisture loss due to HPMC-E15 as it undergo moisture loss in dry condition. Inspite of the moisture loss, patches were found to maintain their physical stability. The drug content among the patches of all formulations was found to be uniform and ranged from 95.46 to 99.51%.

**Swelling study**

The swelling study was done to calculate degree of swelling of the patches (F1-F9). In the swelling

study it was observed that as time increased, the swelling index was increased, because weight gain by patch was increased proportionally with the rate of hydration up to certain time. The direct relationship was observed between swelling index and amount of HPMC-E15. It was found that the percentage swelling of HPMC-E15 patch was reduced by addition of Eudragit-RL100 (F1 > F2 and F3, F4 > F5 and F6, F7 > F8 and F9). It may be due to poor water solubility of Eudragit that may lead to resistance of the matrix network structure (hydrogen bond) to the movement of water molecule. From the results of the swelling study (table 4a), it can be concluded that patches undergo rapid swelling within 30-40 min. and there after gradually reach a plateau (fig. 2).

**Mechanical properties of patch**

The tensile testing gives an indication of the strength and elasticity of the film, reflected by the parameters, tensile strength (TS) and elongation at break (E/B). From the result of the mechanical properties i.e. TS and E/B (table 4b), it was found that TS increases with increase in polymeric content but E/B values decreased with the increase in polymer content. Maximum TS was exhibited by formulation F9 (16.45 kg $mm^{-2}$) and minimum was exhibited by formulation F1 (14.80 kg $mm^{-2}$). Maximum E/B was seen with F1 (38.39 % $mm^{-2}$) and least was observed for F9 (28.96 kg $mm^{-2}$). F5 was found to have moderate TS and E/B (15.347 kg $mm^{-2}$ and 36.47 % $mm^{-2}$). Addition of eudragit-RL100 in formulations was found to increase in tensile strength. This indicates Eudragit may produce effective cross-linking and strengthen the bonding of polymer chains.

***In vitro* mucoadhesive strength/ bio-adhesive strength**

The *in vitro* mucoadhesive strength (bio-adhesive strength) of polymeric buccal patches was found to be in the range of 6.49 to 8.90 N (table 4b); which indicates that bio-adhesive strength increases with increasing the percentage of polymer.

***In vitro* residence/ mucoadhesion time**

Mucoadhesive time was increased linearly with increasing concentration of HPMC after 5 minutes of contact time with goat buccal mucosa. The increase in mucoadhesivity may be due to the formation of a strong gel that penetrates deeply into the mucin molecules. Time necessary for complete erosion from mucosal membrane was recorded. The *in vitro* residence time in phosphate buffer solution (pH 6.8) varied from 6.10 ± 0.1 hrs to 9.47 ± 0.026 hrs (table 4b).

***In vitro* release study**

From table 5 and 6, it was found that the drug release from the patches varied with respect to the proportion of polymers. Increase in the polymer concentration reduces the diffusion of drug from the matrix. During dissolution, HPMC containing patches swelled forming a gel layer on the exposed film surfaces. The loosely bound polymer molecules in these patches were readily eroded, allowing the easy release of Indomethacin as compared to Eudragit RL-100. After 6 hrs the release was found to be 97.28, 96.07, 95.36, 95.44 and 94.23, 92.93 % in formulation F1, F2, F3, F4, F5 and F6 respectively. In formulation F7, F8 and F9 the release was found to be 89.41, 87.01 % and 85.19 % respectively. In the present study formulations containing more amount of HPMC and Eudragit have shown retarded drug release. Fig. 3 shows graphical presentation of comparative dissolution profile of 9 formulations.

**Residual solvent concentration**

The residual methanol and dichloromethane concentration of samples was determined by GC. The result shows that there is no dichloromethane in the patch but methanol was found to be 500 ppm (fig. 4, 5, 6). The residual limit for dichloromethane is 300 ppm while for methanol it is 3000 ppm (table 7). The residual methanol and dichloromethane concentration of our samples was less than 3000 and 300 ppm, respectively, so they are within the pharmaceutical acceptance limit according to ICH guideline.

**Zeta potential of patch**

The level of mucin adsorption was found to be proportional to the absolute value of positive zeta potential of formulation and negative zeta potential of mucin glycoprotein. Factors leading to a reduction or a reversal of this absolute value (e.g. different pH, or ionic strength of medium used) led to reduction in the amount absorbed. The zeta potential of patch was found to be -0.745 mV which is near to absolute value of positive zeta potential. Therefore good bioadhesion would be expected.

**Experimental design and data analysis**

The aim of present work was to achieve optimized formulations for Indomethacin loaded buccal patches by determining the effects of some important factors (variables) and their interactions during the process of preparation on buccal patches physicochemical properties. Two of the most significant factors had been chosen as the independent variables. In the next step, for determining the low and high levels of each factor, some formulations were made.

According to a $3^2$ factorial design and considering these two variables, 9 experiments had been performed (table 3). Amount of the % drug release in mentioned 9 formulations were obtained. The highest release was related to formulation F1 in which two factors i.e. HPMC-E15 and Eudragit-

RL100 had been used in their low levels. The least response was resulted from F9 in which both factors were used in highest level.

**Analysis of variance and model equations**

The polynomial equation of drug release (coded terms) obtained is as follows;

$$Drug\ release = +93.98 - 4.68 * A - 1.61 * B - 0.33 * A * B - 2.31 * A^2 + 0.33 * B^2$$

Above equation indicates that factor A (HPMC) shows high negative value than the factor B (Eudragit). From this, it can be concluded that by making a minor change in factor A may obtain a significant change in response (i.e. drug release).

Table 8 of ANOVA test for determining the significance of the variables indicates;

The Model F-value 161.99 of implies the model is significant. There is only a 0.08 % chance that a "Model F-Value" this large could occur due to noise. F-test used to check the statistical significance of equation 1 show that the fitted model is strongly significant at 95 % confidence level (P-value < 0.05). In this case A, B, $A^2$ are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. The same tables show the other adequacy measures $R^2$, adjusted $R^2$ and predicted $R^2$. All the adequacy measures are in reasonable agreement whereas they indicate significant relationships. According to table 8 the variables A, B, $A^2$ had significant effects. The "Pred R-Squared" of 0.9593 is in reasonable agreement with the "Adj R-Squared" of 0.9902. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The ratio of 34.842 indicates an adequate signal. This model can be used to navigate the design space. The high values of $R^2$ reveals that model equation represent the system well over the given experimental domain.

From the equation 1 it was concluded that HPMC-E15 (factor A) and Eudragit-RL100 (factor B) having individual effect on the drug release. The main effect of the HPMC-E15 is observed. According to the obtained results, the developed models are statistically accurate and can be used for further analysis.

**Diagnostics case statistics of experimental matrix**

The actual values were obtained from experiments, and the predicted ones were obtained from the model as shown in table 9. The values prove that the predicted data, which were obtained from the empirical model for drug release, are in good agreement with the experimental results due to their low differences. Linear correlation was observed between actual and predicted value as shown in fig. 7.

**Effect of variable A (HPMC) on response**

The graph shows that as the level of HPMC increases, the drug release decreases. From the graph it can be conclude that up to the medium level of HPMC, drug release decreases slowly. As the concentration crosses the medium level of HPMC, drug release decreases rapidly. Therefore minimum concentration of HPMC (i.e. 6-8 %) will lead to maximum drug release. Therefore it was concluded from the graph that the factor A (HPMC) alone might have significant effect on the drug release (fig. 8.)

**Effect of variable B (Eudragit)**

The drug release increases with decrease in the Eudragit concentration. Graph indicates that the range of 100 to 200 mg of Eudragit will have more significant effect on drug release. Therefore it was concluded from the fig. 9 that Eudragit in the formulation might have individual effect on the increase in drug release.

**Effect of combined factors (Interaction of AB)**

There are two lines shown in the fig. 10, the red line represents high level of the variable (A) and the black line referrers to the low level. There is non-significant interaction between HPMC and Eudragit indicate that variables showing individual effect on the drug release.

**Contour plot**

The fig. 11 shows the counter plot of HPMC and Eudragit. It shows that a minimum concentration of HPMC and Eudragit increases the drug release.

**Three dimensional graphical presentations (3D PLOT)**

The 3D response surface plot of the factorial model was drawn to show the effect of the variables on the drug release. Fig. 12 illustrates the effect of the amount of HPMC and Eudragit on the drug release. It is demonstrated that the drug release depends more on the HPMC than the amount of Eudragit. Accordingly, the HPMC has shown a more significant effect on drug release. Meanwhile, this is the most significant factor affecting the drug release in comparison with the other factors i.e. Eudragit.

**Approximation of desired responses (Perturbation)**

Fig. 13 shows the relationship between the drug release on one side and the polymer, HPMC and Eudragit on the other side. The drug release is found to be more responsive to the HPMC than to the Eudragit as found in fig. 8 and 9. In other words, increasing the HPMC would result in a drastic decrease in the drug release more than when increasing the concentration of Eudragit at a certain medium level.

Area highlighted in fig. 13 indicates that the levels of the entire 2 variable considered together can give the optimized response.

**Approches for optimized solution (Desirability approach)**

It provides flexibility and giving importance for each response individually. According to the final results, this program suggested some formulations and also predicted their responses containing a probability factor named "Desirability" that ranged between 0 – 1 that the most presumable answer would be the nearest to 1.

Data analysis showed that lower levels of each variable always would cause in increased drug release in a formulation (fig. 14).

Table 10 includes some of the suggested formulations of DE software and the desirability of each item could be observed. According to software HPMC kept in minimize level (in between 6-8 mg) and Eudragit in the range of 100-200 mg. Out of suggested formulations one solution was selected with high drug release and formulated. The percentage drug release of obtained buccal patch was calculated and listed in table 11.

**Optimized formulations**

From the suggested solutions one formulation (S1) was selected and formulated. For the same formulations drug release was calculated as shown in table 11.

The solution one is selected as optimized formula and it was formulated in triplicates to confirm the optimized formulation. The results obtained are tabulated in table 12.

It can be concluded that application of factorial design demonstrates a useful method for optimization of Indomethacin buccal patches. Furthermore, D.E. 8.0.7.1 analysis of the results described adequately the influence of selected variables (HPMC and Eudragit) at different levels on response under study (% drug release) in this work.

**Determination of best fit model for dissolution kinetics of optimized formulation**

From the results of $R^2$ (table 13 and fig. 15), Zero order, Higuhci, Korsmayer-peppas models were found to be best fit. This indicates that drug diffuse from the patch in sustained manner with erosion of polymer matrix.

**Differential scanning calorimetry**

The DSC thermogram for the Indomethacin and indomethacin loaded buccal patch are shown in fig. 16 and 17. The pure drug Indomethacin gives rise to a sharp endothermic peak that corresponds to melting at approximately 161 °C, indicating its crystalline nature. In DSC of the formulation, sharp endothermic peak of 161 °C was found to be shifted approximately to 240 °C. The DSC results revealed that no interaction between the drug and the used polymers occurred as there was only shift, no change in the melting endothermic peak of drug.

**Effect of temperature and humidity on optimized batch**

From the tabulated results in table 14 it can be concluded that there was no significant physical and chemical changes in the optimized batch after one month. Elasticity of patches was found to be maintained.

**CONCLUSION**

An optimised buccal patch of Indomethacin was successfully developed. The developed buccoadhesive patch exhibited sufficient pharmacotechnical properties and bioadhesive properties. The patch sustained the release of drug for 6hr, showed good folding endurance and surface pH ascertaining its compatibility with the oral mucosa. *Ex-vivo* permeation studies to be carried out to determine the permeability characteristics of Indomethacin. *In-vivo* studies of the optimised patch should be carried out to determine its feasibility in clinical practise.

**Table 1: Amount of variables in $3^2$ factorial design batches**

| Coded Values | Actual values | |
|---|---|---|
| | $X_{1\,(mg)}$ | $X_{2\,(\%)}$ |
| -1 | 6 | 100 |
| 0 | 8 | 200 |
| +1 | 10 | 300 |

**Table 2: A $3^2$ full factorial experimental design layout**

| Formulation Code | Coded Values | |
|---|---|---|
| | $X_1$ | $X_2$ |
| F1 | -1 | -1 |
| F2 | -1 | 0 |
| F3 | -1 | +1 |
| F4 | 0 | -1 |
| F5 | 0 | 0 |
| F6 | 0 | +1 |
| F7 | +1 | -1 |
| F8 | +1 | 0 |
| F9 | +1 | +1 |

**Table 3: Mucoadhesive patch composition**

| Ingredients | Formulations | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 |
| Indomethacin (mg) | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 | 50 |
| HPMC-E15 (%) | 6 | 6 | 6 | 8 | 8 | 8 | 10 | 10 | 10 |
| Eudragit-RL100 (mg) | 100 | 200 | 300 | 100 | 200 | 300 | 100 | 200 | 300 |
| PEG 400 (mL) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| Solvent (mL) | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 | 25 |

**Table 4a: Evaluation parameters of formulated patch**

| Batch code | *Weight (mg) | *Thickness (mm) | Surface pH | *Folding endurance | *Moisture loss (%) | *Swelling index (%) |
|---|---|---|---|---|---|---|
| F1 | 110.33 ± 0.58 | 0.29 ± 0.01 | 6 to 7 | 299 ± 6.24 | 7.88 ± 1.04 | 88.25 ± 1.6 |
| F2 | 130.33 ± 0.57 | 0.26 ± 0.00 | 6 to 7 | 308 ± 3.46 | 6.11 ± 0.96 | 87.74 ± 2.9 |
| F3 | 141.00 ± 0.58 | 0.27 ± 0.01 | 6 to 7 | 310 ± 8.18 | 7.69 ± 0.77 | 86.53 ± 4.0 |
| F4 | 150.33 ± 0.58 | 0.28 ± 0.00 | 6 to 7 | 308 ± 1.52 | 8.46 ± 0.77 | 90.28 ± 1.8 |
| F5 | 162.33 ± 1.00 | 0.33 ± 0.01 | 6 to 7 | 311 ± 3.60 | 6.04 ± 0.95 | 92.90 ± 2.1 |
| F6 | 170.00 ± 1.00 | 0.34 ± 0.00 | 6 to 7 | 315 ± 6.18 | 6.07 ± 0.89 | 94.44 ± 1.3 |
| F7 | 180.33 ± 0.57 | 0.38 ± 0.00 | 6 to 7 | 309 ± 8.08 | 5.08 ± 0.30 | 132.3 ± 5.3 |
| F8 | 190.33 ± 1.52 | 0.43 ± 0.01 | 6 to 7 | 314 ± 6.65 | 5.50 ± 0.50 | 124.9 ± 2.0 |
| F9 | 210.33 ± 0.58 | 0.43 ± 0.019 | 6 to 7 | 321 ± 2.43 | 7.55 ± 0.76 | 123.3 ± 2.9 |

* All values are expressed as mean ± SD, (n= 3)

**Table 4b: Evaluation parameters of formulated patch**

| Batch Code | *Content uniformity (%) | TS ($kg/mm^2$) | EB ($\%mm^{-2}$) | *In vitro* bioadhesion strength (N) | **In vitro* residence time (hrs.) |
|---|---|---|---|---|---|
| F1 | 95.46 ± 0.38 | 14.8085 | 38.39 | 6.59 | 6.10 ± 0.100 |
| F2 | 99.74 ± 0.25 | 14.8557 | 37.99 | 6.49 | 6.21 ± 0.095 |
| F3 | 97.17 ± 0.54 | 14.8924 | 37.48 | 7.50 | 6.27 ± 0.030 |
| F4 | 95.49 ± 0.23 | 15.3470 | 36.47 | 7.65 | 7.27 ± 0.020 |
| F5 | 96.79 ± 0.23 | 15.4112 | 36.01 | 7.67 | 7.31 ± 0.035 |
| F6 | 99.51 ± 0.27 | 15.4629 | 31.70 | 7.83 | 7.36 ± 0.015 |
| F7 | 95.37 ± 0.22 | 16.3891 | 31.40 | 8.28 | 9.28 ± 0.058 |
| F8 | 95.77 ± 0.27 | 16.4161 | 31.37 | 8.66 | 9.39 ± 0.007 |
| F9 | 97.02 ± 0.13 | 16.4526 | 28.96 | 8.90 | 9.47 ± 0.026 |

* All values are expressed as mean ± SD, (n= 3)

**Table 5: Cumulative % drug release from formulation F1 to F4**

| Time (min) | F1 | F2 | F3 | F4 |
|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 |
| 15 | 6.99 ±0.03 | 4.88 ±0.034 | 15.11 ±0.09 | 14.66 ±0.045 |
| 30 | 16.85 ±0.022 | 8.73 ±0.034 | 27.77 ±0.13 | 26.34 ±0.023 |
| 45 | 30.16 ±0.051 | 18.90 ±0.030 | 35.00 ±0.02 | 43.98 ±0.03 |
| 60 | 50.26 ±0.06 | 30.19 ±0.023 | 48.34 ±0.1 | 55.53 ±0.017 |
| 90 | 64.71 ±0.072 | 50.03 ±0.022 | 56.13 ±0.08 | 63.22 ±0.034 |
| 120 | 76.96 ±0.06 | 66.33 ±0.011 | 60.63 ±0.06 | 81.30 ±0.072 |
| 150 | 87.07 ±0.022 | 73.31 ±0.019 | 70.37 ±0.03 | 85.19 ±0.023 |
| 180 | 89.43 ±0.03 | 81.45 ±0.023 | 81.19 ±0.04 | 86.70 ±0.06 |
| 210 | 91.16 ±0.08 | 87.35 ±0.055 | 84.65 ±0.56 | 87.37 ±0.023 |
| 240 | 92.44 ±0.034 | 89.18 ±0.005 | 88.06 ±0.056 | 88.79 ±0.04 |
| 270 | 93.71 ±0.034 | 92.42 ±0.033 | 92.97± 0.05 | 92.97 ±0.056 |
| 300 | 95.12 ±0.75 | 93.93 ±0.77 | 94.26 ±0.16 | 94.26 ±0.04 |
| 330 | 96.60 ±0.27 | 95.05 ±0.73 | 94.60 ±0.13 | 94.35 ±0.23 |
| 360 | 98.28 ±0.18 | 96.07 ±0.023 | 95.36 ±0.6 | 95.44 ±0.16 |

All values are expressed as mean ± SD, (n= 3)

**Table 6: Cumulative % drug release from formulation F5 to F9**

| Time (min) | F5 | F6 | F7 | F8 | F9 |
|---|---|---|---|---|---|
| 0 | 0 | 0 | 0 | 0 | 0 |
| 15 | 16.66 ±0.07 | 2.30 ±0.01 | 1.43 ±0.017 | 1.75 ±0.4 | 1.84 ±0.023 |
| 30 | 20.97 ±0.06 | 18.70 ±0.05 | 11.88 ±0.045 | 14.40 ±0.58 | 7.42 ±0.034 |
| 45 | 35.83 ±0.057 | 31.42 ±0.006 | 22.20 ±0.013 | 27.71 ±0.050 | 19.05 ±0.022 |
| 60 | 47.93 ±0.06 | 46.67 ±0.013 | 32.29 ±0.023 | 43.51 ±0.028 | 35.38 ±0.033 |
| 90 | 54.95 ±0.012 | 57.32 ±0.065 | 51.67 ±0.023 | 55.77 ±0.019 | 47.19 ±0.05 |
| 120 | 60.42 ±0.045 | 68.53 ±0.013 | 65.03 ±0.07 | 61.50 ±0.059 | 59.93 ±0.040 |
| 150 | 74.92 ±0.06 | 76.73 ±0.05 | 77.70 ±0.03 | 71.38 ±0.017 | 71.16 ±0.028 |
| 180 | 85.53 ±0.08 | 82.57 ±0.01 | 81.55 ±0.034 | 73.84 ±0.042 | 73.32 ±0.05 |
| 210 | 89.16 ±0.03 | 83.71 ±0.045 | 85.63 ±0.01 | 75.10 ±0.081 | 74.69 ±0.03 |
| 240 | 90.73 ±0.06 | 87.05 ±0.09 | 85.76 ±0.07 | 78.73 ±0.057 | 76.52±0.033 |
| 270 | 92.31 ±0.04 | 90.13 ±0.065 | 86.92 ±0.08 | 80.95 ±0.19 | 79.26 ±2.5 |
| 300 | 93.93 ±0.03 | 91.45 ±0.045 | 88.29 ±0.6 | 83.07 ±0.14 | 82.70 ±0.7 |
| 330 | 94.16 ±0.017 | 92.78 ±0.2 | 88.80 ±0.05 | 85.16 ±0.12 | 83.59 ±0.99 |
| 360 | 94.23 ±0.05 | 92.93 ±0.35 | 89.41 ±0.5 | 87.01 ±0.06 | 85.19 ±0.33 |

All values are expressed as mean ± SD, (n= 3)

**Table 7: Residual solvent concentration determination**

| Residual solvents | Conc. obtained (ppm) | Acceptance limit (ppm) |
|---|---|---|
| Methanol | 500 | 3000 |
| Dichloromethane | 0 | 300 |

**Table 8: ANOVA test for determining the significance of the variables**

| Source | Sum of Squares | Df | Mean Square | F Value | p-value Prob > F | |
|---|---|---|---|---|---|---|
| Model | 158.47 | 5 | 31.69 | 161.99 | 0.0008 | Significant |
| A-HPMC | 131.60 | 1 | 131.60 | 672.64 | 0.0001 | |
| B-Eudragit | 15.52 | 1 | 15.52 | 79.33 | 0.0030 | |
| AB | 0.42 | 1 | 0.42 | 2.16 | 0.2380 | |
| $A^2$ | 10.70 | 1 | 10.70 | 54.71 | 0.0051 | |
| $B^2$ | 0.22 | 1 | 0.22 | 1.12 | 0.3668 | |
| Residual | 0.59 | 3 | 0.20 | | | |
| Cor Total | 159.05 | 8 | | | | |

| | | | |
|---|---|---|---|
| Std. Dev. | 0.44 | R-Squared | 0.9963 |
| Mean | 92.66 | Adj R-Squared | 0.9902 |
| C.V. % | 0.48 | Pred R-Squared | 0.9593 |
| PRESS | 6.48 | Adeq Precision | 34.842 |

**Table 9: Diagnostic case statistic**

| Standard order | Actual value | Predicted value | Residual |
|---|---|---|---|
| F1 | 98.28 | 97.96 | 0.316111 |
| F2 | 96.07 | 96.35 | -0.47889 |
| F3 | 95.36 | 95.40 | 0.162778 |
| F4 | 95.44 | 96.14 | -0.27889 |
| F5 | 94.23 | 93.98 | 0.027778 |
| F6 | 92.93 | 92.70 | -0.03722 |
| F7 | 89.41 | 89.14 | 0.227778 |
| F8 | 87.01 | 87.20 | -0.19056 |
| F9 | 85.19 | 86.98 | 0.251111 |

**Table 10: Selective formulations that DE.8.0.7.1 predicted out of the specified limit for each variable**

| Number | HPMC-E15 | Eudragit-RL100 | % Release | Desirability |
|---|---|---|---|---|
| S1 | 6.00 | 100.00 | 97.9639 | 0.988 |
| S2 | 6.00 | 103.07 | 97.9045 | 0.986 |
| S3 | 6.04 | 100.00 | 97.968 | 0.979 |
| S4 | 6.00 | 148.06 | 97.105 | 0.954 |
| S5 | 6.00 | 151.98 | 97.0416 | 0.953 |

**Table 11: Predicted percent drug release and related obtained response of suggested solution**

| Solution No. | HPMC-E15 | Eudragit-RL100 | Predicted response | *Obtained response. |
|---|---|---|---|---|
| S1 | 6.00 | 100 | 97.96 | 98.04 ± 0.21 |

* All values are expressed as mean ± SD, (n= 3)

**Table 12: Results of optimized batch**

| Solution | **In vitro* mucoadhesion time (hrs) | *Tensile strength kg.mm$^2$ | *Folding endurance |
|---|---|---|---|
| S1 | 6.09 ± 0.10 | 14.9035 ± 0.9 | 285 ± 1.23 |

* All values are expressed as mean ± SD, (n= 3)

**Table 13: $R^2$ values and slope values for applied values**

| S. No. | Models | $R^2$ values | Slope value |
|---|---|---|---|
| 1 | Zero order | 0.905 | 0.031 |
| 2 | First order | 0.801 | -0.0296 |
| 3 | Higuchi | 0.9127 | 0.7183 |
| 4 | Korsemayer-Peppas | 0.9682 | - 0.0353 |
| 5 | Hixon Crowell | 0.8054 | -0.0004 |

**Table 14: Effect of temperature and humidity on optimized batch (Conditions 40 °C ± 2 °C / 75 % ± 5%)**

| Parameters | Days | | | | |
|---|---|---|---|---|---|
| | 0 | 7 | 14 | 21 | 28 |
| Appearance | Smooth | Smooth | Smooth | Smooth | Smooth |
| Surface pH | 6 to7 | 6 to 7 | 6 to 7 | 6 to 7 | 6 to 7 |
| *Folding endurance | 299 ± 6.24 | 280 ± 1.2 | 275 ± 2.5 | 290 ± 1.3 | 285 ± 0.88 |
| *Drug release (%) | 98.28 ±0.18 | 97.20 ±0.88 | 98.01 ±1.02 | 97.80 ±0.86 | 98.10 ±0.91 |

* All values are expressed as mean ± SD, (n= 3)

a

b

c

d

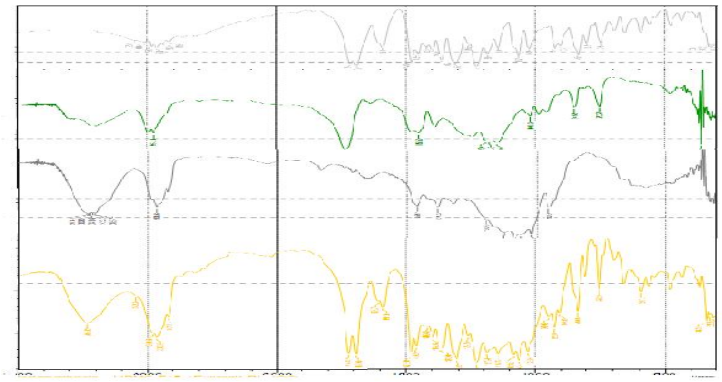

**Fig. 1: FTIR of a) Indomethacin, b) Eudragit-RL100, c) HPMC-E15 and d) Indomethacin buccal patch**

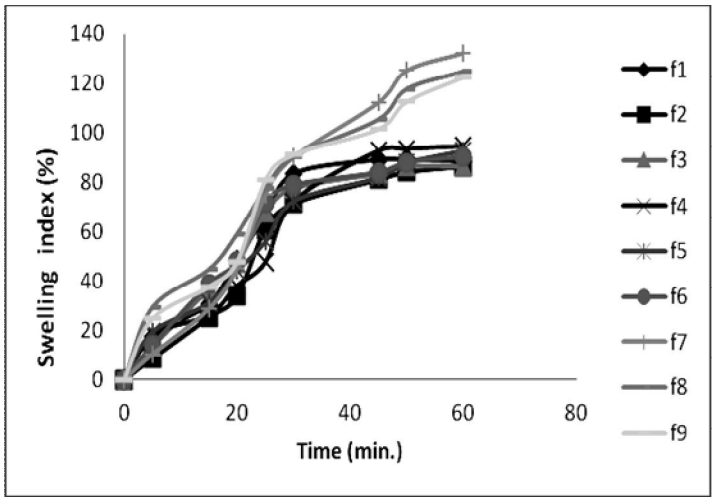


**Fig. 2: Swelling index of formulations F1 to F9**

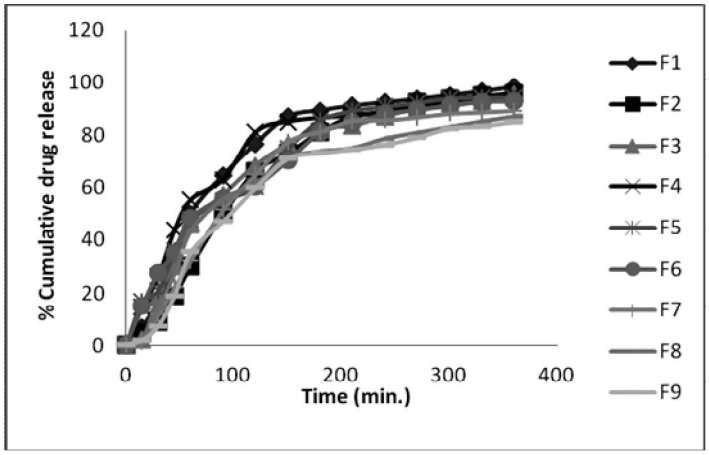


**Fig. 3:Graphical presentation of comparative dissolution profile of 9 formulations**

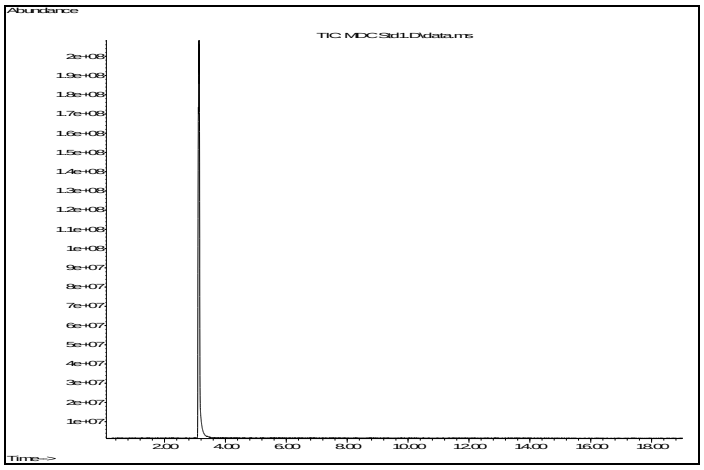


**Fig. 4: Chromatogram of Methanol**

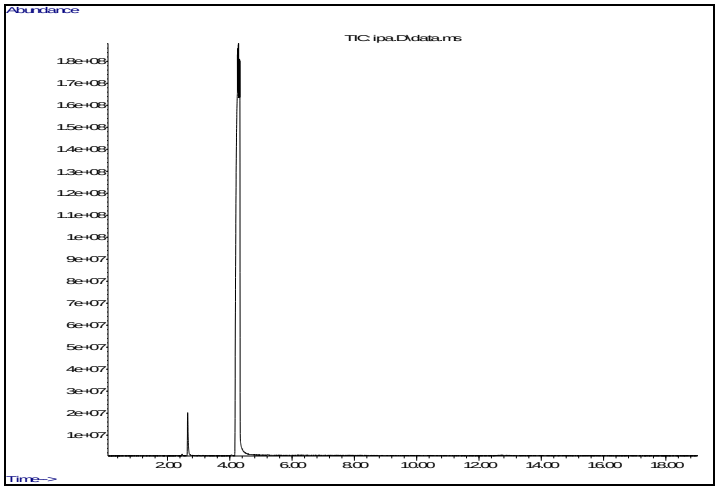


**Fig. 5: Chromatogram of Dichloromethane**

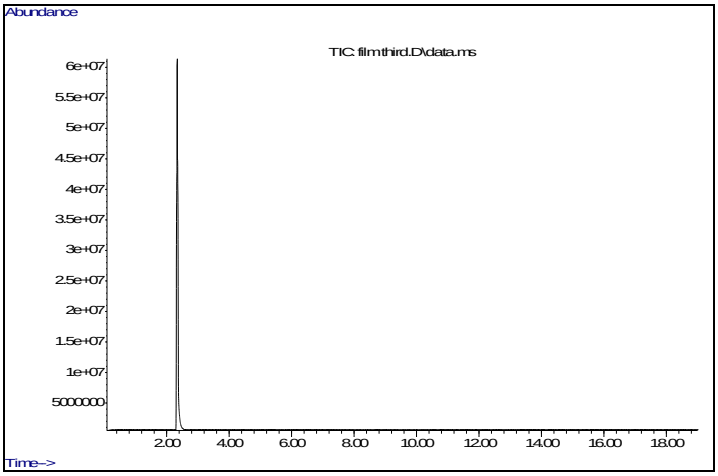


**Fig. 6: Chromatogram of sample**

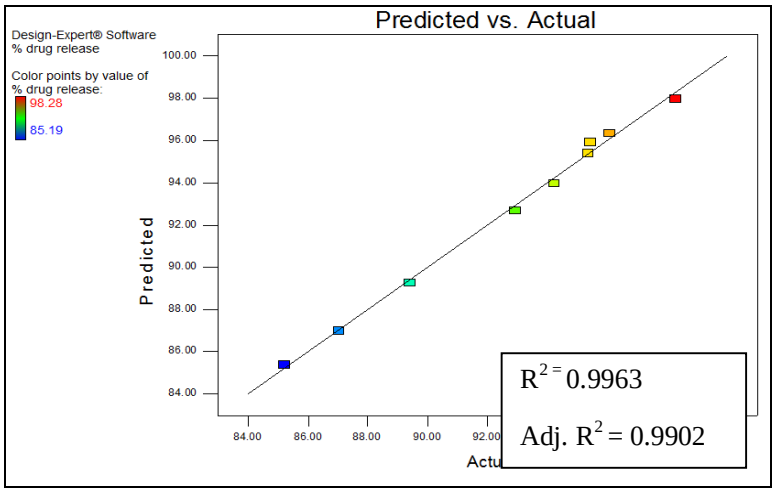


**Fig. 7: Predicted vs. actual values of % drug release**

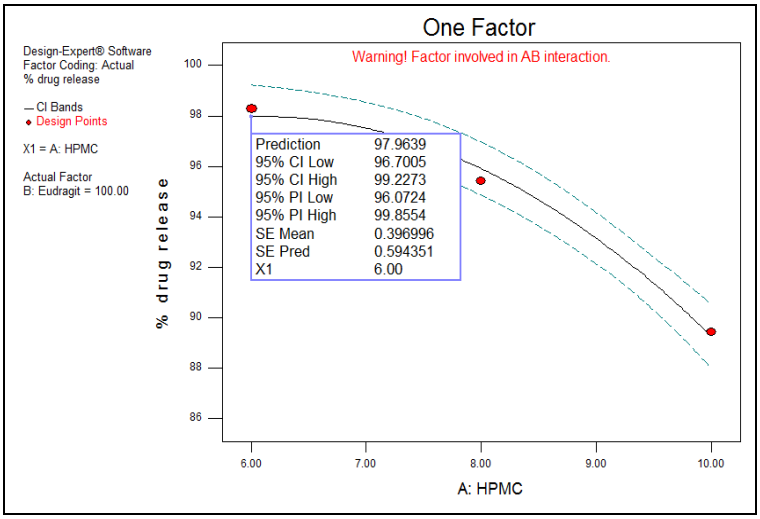


**Fig. 8: Effect of HPMC-E15 on the % drug release**

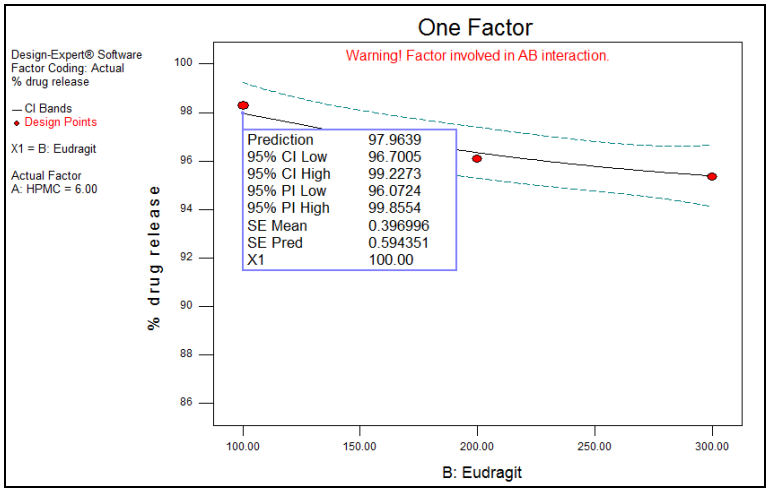


**Fig. 9: Effect of Eudragit-RL100 on the drug release**

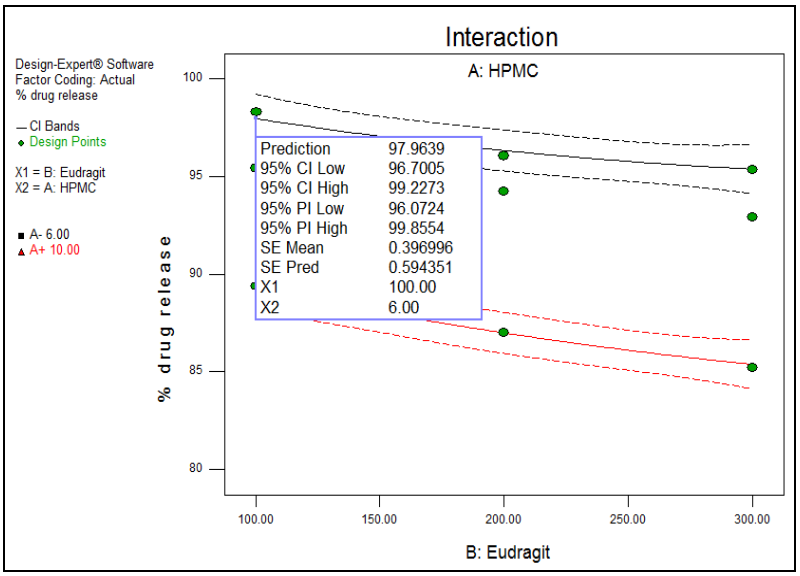


**Fig. 10: The effect of interaction between HPMC and Eudragit on the response**

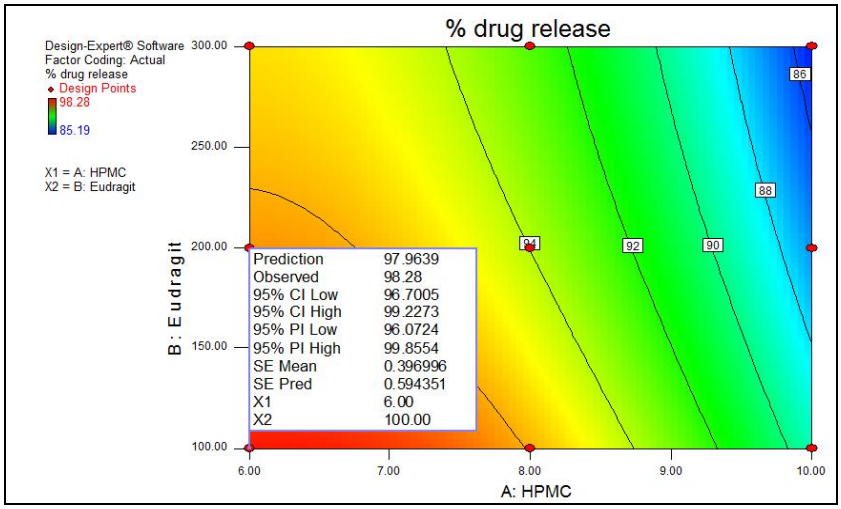


**Fig. 11: Contour plot shows the effect of HPMC and Eudragit on the drug release**

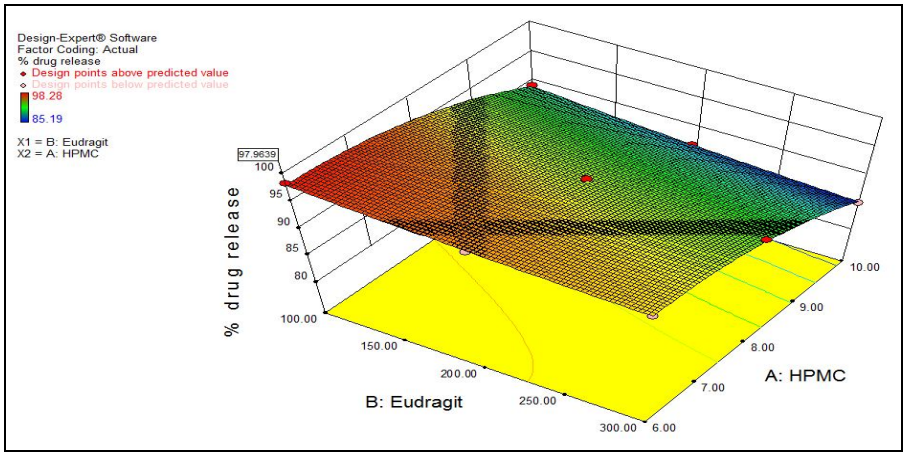


**Fig. 12: Three dimensional view of drug release with respect to HPMC and Eudragit obtained by D.E. 8.0.7.1 related to the given data**

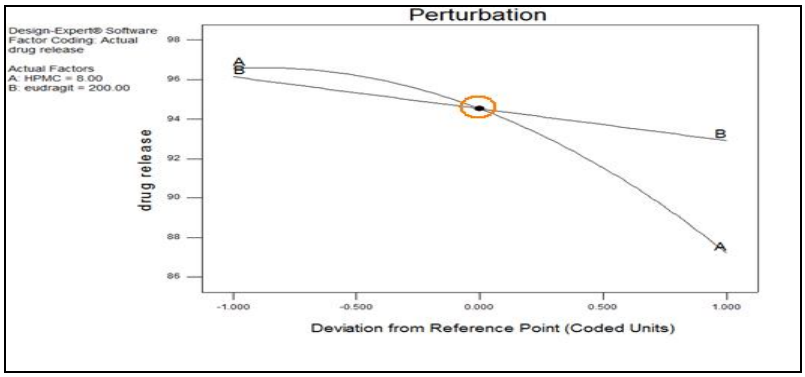


**Fig. 13: Perturbation plot shows the drug release [%] as a function of A: HPMC and B: Eudragit**

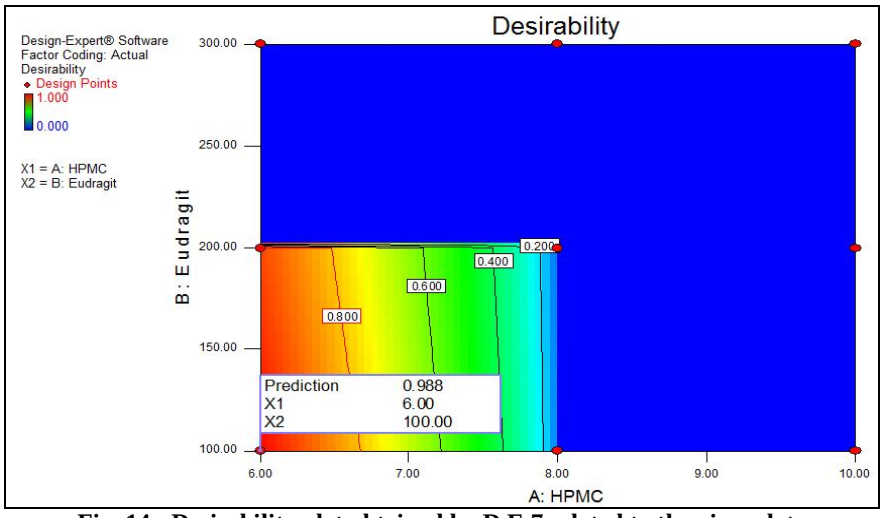


**Fig. 14: Desirability plot obtained by D.E.7 related to the given data**

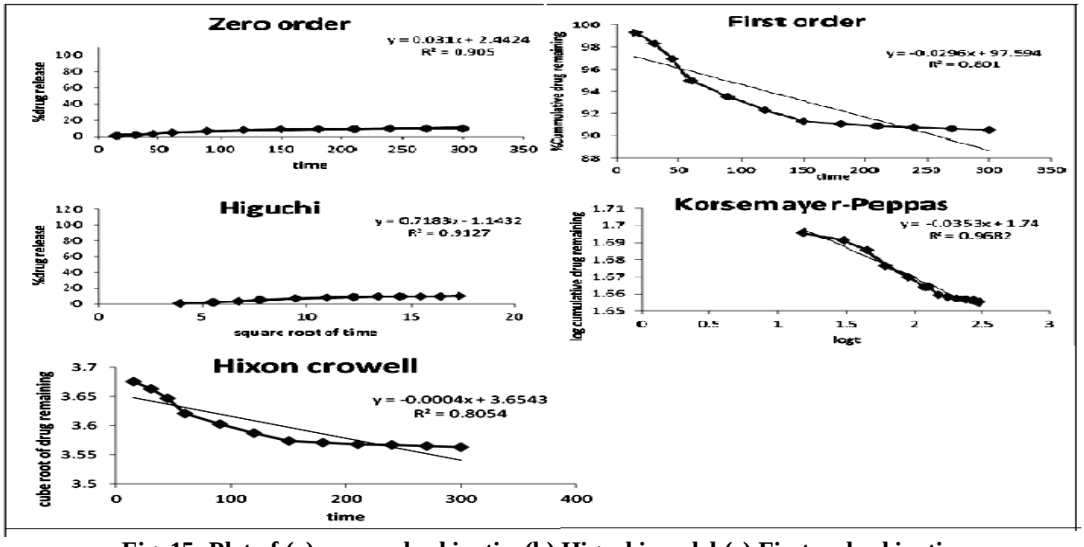


**Fig. 15: Plot of (a) zero order kinetic, (b) Higuchi model (c) First order kinetic (d) Korsmeyer-peppas model (e) Hixon crowell model**

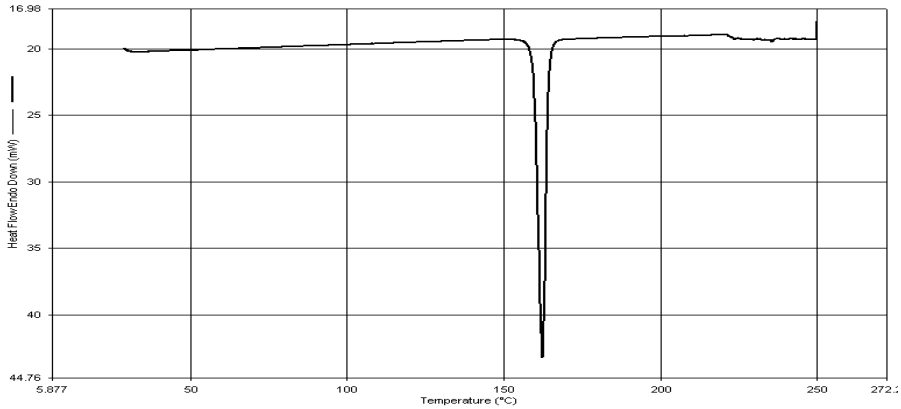


**Fig. 16: DSC of Indomethacin**

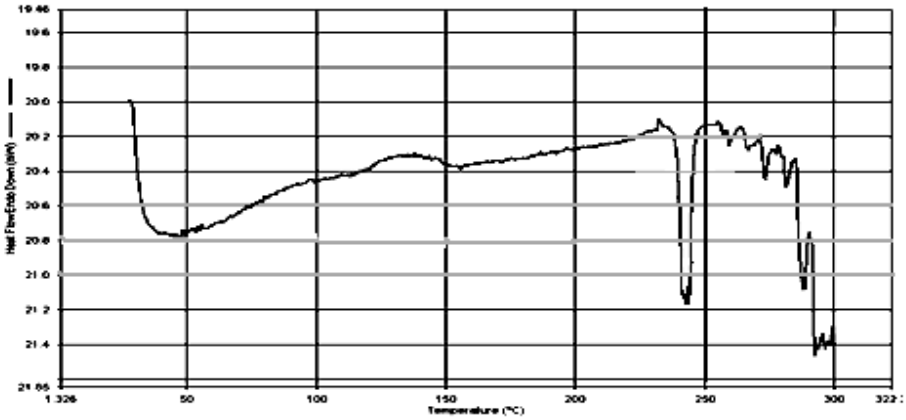


**Fig. 17: DSC of optimized formulation**

**REFERENCES**


1. Tanabe M, Watanabe M, Yanagi M, Nishizawa S, Chigono Y, Matsuda J, Yamaoka K and Inoue K. Controlled indomethacin release from mucoadhesive film in vitro and clinical evaluations. Yakugaku Zasshi. 2008;128(11):1673-1679.
2. Jian-Hwa G and Karsten C. Development of Bioadhesive Buccal Patches. Swarbrick J, Mathiowitz E, editors, Bioadhesive Drug Delivery Systems: Fundamentals, Novel Approaches and Development. Marcel Dekker Inc. 1999; 541-59.
3. Kumar DSD, Reddy KS, Tiwari AK and Dey S. Design and evaluation of buccal patches of Lornoxicam. International Journal of Pharmacy and Bio Sciences. 2010;1(4):587-596.
4. Burgalassi S, Panichi L, Saettone MF, Jacobsen J and Rassing MR. Development and in vitro/in vivo testing of mucoadhesive buccal patches releasing benzydamine and lidocaine. International Journal of Pharmaceutics. 1996;133:1-7.
5. http://www.everydayhealth.com/drugs/indomethacin cited 18/09/2012.Product Information, 2011. [WWW] Indomethacin. Available from: http://url.ie/fm69. [Accessed on: 19/06/2012].
6. Nafee NA, Boraie NA, Ismail FA and Mortada LM. Design and characterization of mucoadhesive buccal patches containing Cetylpyridinium chloride. Acta Pharma. 2003;53:199-212.
7. Sharma R, Parikh RK, Gohel MC and Soniwala MM. Development of taste masked film of valdecoxib for oral use. Indian Journal of Pharmaceutical Sciences. 2007;69(2):320-323.
8. Muchalambe S, Dandagi PM, Yogesh HS, Ravindra R and Gopalakrishna B. Preparation and evaluation of Sparfloxacin dental implants for treatment of Periodontal diseases. International Journal of Pharmaceutical Sciences. 2010;2 (2):606-611.
9. Choudhury A, Das S, Dhangar S, Kapasiya S and Kanangoet A.

Development and characterization buccoadhesive Film of Ciprofloxacin Hydrochloride. International Journal of Pharm. Tech Research. 2010;2 (2):1050-1057.
10. Semalty M, Semalty A, Kumar G and Juyal V.. Development of Mucoadhesive Buccal Films of Glipizide. International Journal of Pharmaceutical Sciences and Nanotechnology. 2008;1(2):184-190.
11. Kumar V, Zakir F, Agarwal G and Choudhary A. Formulation and evaluation of buccal patches of Venlafaxine. International Journal of Pharmacy and Biological Sciences. 2011;1(3):170-182.
12. Sudhakar Y, Kuotsu K and Bandyopadhyay AK. Buccal bioadhesive drug delivery - a promising option for orally less efficient drugs. Journal of Controlled Release. 2006;114 (1):15-40.
13. Corrigan OI and Kavanagh N. Swelling and erosion properties of hydroxypropylmethylcellulose (Hypromellose) matrices—influence of agitation rate and dissolution medium composition. International Journal of Pharmaceutics. 2004;279:141-152.
14. Alanazi FK, Rahman AA, Mahrous GM and Alsarra IA. Formulation and physicochemical characterization of buccoadhesive films containing Ketorolac. J Drug Del Sci. 2007;17(3):183-192.
15. Patel VM, Prajapati BG and Patel MM. Effect of hydrophilic polymers on buccoadhesive Eudragit patches of propranolol hydrochloride using factorial design. AAPS PharmSciTech. 2007;8(2):E1-E8.
16. Verma S, Kaul M, Rawat A and Saini S. An overview on buccal drug delivery system. International Journal of Pharmaceutical Sciences and Research. 2011;2 (6):1303-1321.
17. Javier OM and Jason TM. Manufacture and characterization of mucoadhesive buccal films. European Journal of Pharmaceutics and Biopharmaceutics. 2011; 77:87–199.
18. Kolli CS, Gannu R, Yamsani VV and Yamsani MR. Development of mucoadhesive patches for buccal administration of Prochlorperazine: evaluation of In vitro release and mechanical properties. International Journal of Pharmaceutical Science and Nanotechnology. 2008;1(1):64-70.
19. Peh KK and Wong CY. Polymeric films as vehicle for buccal delivery: swelling, mechanical and bioadhesive properties. J Pharm Pharmaceut Sci. 1999;2(2):53-61.
20. Basalious EB, Yehia SA and Omaima NE. Fluconazole Mucoadhesive Buccal Films: In Vitro/In Vivo Performance. Current Drug Delivery.2009;6:17-27.
21. Jana S, Dibyendu L, Sen KK and Basu SK. Development and evaluation of Epichlorohydrin cross-linked mucoadhesive patches of Tamarind seed polysaccharide for buccal application. International Journal of Pharmaceutical Sciences and Drug Research. 2010;2(3):193-198.
22. Shidhaye SS, Saindane NS, Sutar S and Kadam V. Mucoadhesive bilayered patches for administration of Sumatriptan succinate. AAPS PharmSciTech. 2008;9 (3):909-16.
23. European Medicines Agency. ICH Topic Q3C (R4) Impurities: Guidelines for residual solvents. [WWW] Available from: http://url.ie/fm6h [Accessed on 07/06/2012], 2009.
24. Hu C and Liu Y. Quality Control in Pharmaceutical: Residual Solvents Testing and Analysis. Wide Spectra of Quality Control. Beijing: China. 1990;11:183-210.
25. Pongjanyakul T and Suksri H. Biointerfaces alginate-magnesium aluminum silicate films for buccal delivery of Nicotine. Colloids and Surfaces B: Biointerfaces. 2009;74:103-113.
26. Harding SE, Davis SS, Deacon MP and Fiebrig M. Biopolymer Mucoadhesives. Biotechnology and Genetic Engineering Reviews. 1999;16:41-86.
27. Bolton B and Bon C. Pharmaceutical Statistics, practical and clinical applications, Drug and the pharmaceutical sciences. 4th ed. New York: Marcel Dekker. 2004;265-283, 506-539.
28. Deshmane SV, Channawar MA, Chandewar AV, Joshi UM and Biyani KR. Chitosan based sustained release mucoadhesive buccal patches containing Verapamil HCL. International Journal of Pharmacy and Pharmaceutical Science, 2009;1(1):216-229.