Application of quality by design paradigm in the design of muco-adhesive extended release film for oral ulcer

Manali Prajapat[1,*], Kunal Parmar[2], Shital Bhutani[3], Rajesh Parikh[4], Toral Patel[5]

[1]Student, [2]Drug Inspector, [3]Associate Professor, [4]Professor, [5]Research Scientist, [1]Nirma University, Ahmedabad, Gujarat, [2]Food and drugs control administration, Ahmedabad, [3]Institute of Pharmacy, Nirma University, Ahmedabad, Gujarat, [4]LM College of Pharmacy, Gujarat University, Gujarat, India [5]University of Plymouth, United Kingdom

***Corresponding Author:**
Email: manaliprajapat@gmail.com

Abstract
The present study deals with the formulation of mucoadhesive bilayered buccal film of lidocaine hydrochloride and benzydamine hydrochloride for treatment of oral ulcers (aphthous stomatitis). Eudragit-RLPO and Ethyl cellulose were used as film forming polymers and low viscosity grade hydroxyl propyl cellulose as a mucoadhesive agent. Solvent casting method was adopted for film formation. The film was evaluated for content uniformity, tensile strength, folding endurance, swelling, mucoadhesive strength, ex vivo mucoadhesive time and *in vitro* drug release. The films were characterized for DSC and FTIR. The film showed acceptable film properties and muco-adhesion. The formulation showed predictive drug release, i.e. <30%, 50-65% and >80 in 2, 4 and 6 h respectively. In-vitro drug release study revealed that a combination of Eudragit and Ethyl cellulose was required for better control of the drug release. The DSC and FTIR study confirmed drug-drug and drug–excipient compatibility.

Keywords: Bilayer film, Eudragit-RLPO, Ethyl cellulose, Muco-adhesion, Extended drug release.

Introduction
Amongst the various routes of administration tried so far for novel drug delivery systems, localized delivery to tissues of the oral cavity has been investigated for a number of applications including the treatment of toothaches, periodontal disease, bacterial and fungal infections, aphthous and dental stomatitis and facilitating tooth movement with prostaglandins.[1] In recent years, significant interest has been shown in the development of novel bioadhesive dosage forms for mucosal delivery of drugs. A bioadhesive dosage form necessitates the use of mucoadhesive polymers to adhere to mucosa and withstand salivation, tongue movement and swallowing for significant period of time.[2,3]

Canker sores form on a patient's inner cheek, lips, gums, tongue or soft palate. The condition is medically known as aphthous stomatitis.[1] The buccal mucosa is easily accessible for drug delivery. It allows the patient to interrupt drug administration by simply removing the drug delivery system. The mucoadhesive drug delivery system has the advantage of increased residence time and thus, improves absorption. The bioadhesive polymers are typically hydrophilic macro-molecules containing numerous hydrogen bonding groups. They provide intimate contact between a dosage form and absorbing tissue that may result in high drug flux through the absorbing tissue. In oral cavity, buccal and gingival areas are associated with a smaller flow of saliva as compared to the sublingual region, thus the duration of adhesion of the delivery system would be longer at these sites.[2,4]

Buccal films with backing membrane maintains its position in the mouth for few hours, release drug in a controlled fashion and in unidirectional way towards mucosa and prevents the loss of drug into the oral cavity and increases bioavailability.[5]

A novel method of treating aphthous stomatitis, lesions, sores and blisters comprises of topical mucoadhesive dosage forms containing topically effective antibiotic, antibacterial, anti-infective and antiviral like agents to the affected area.[6]

Lidocaine hydrochloride (a local anaethetic) and benzydamine hydrochloride (a locally acting anti-inflammatory, antipyretic, analgesic and antimicrobial agent) can be used in Combination therapy to achieve better action and patient compliance.[1]

According to the ICH Q8(R2) guidance, quality by design means: "Designing and developing a product and associated manufacturing processes that will be used during product development to ensure that the product consistently attains a predefined or predictable quality at the end of the manufacturing process."

By increasing process understanding, QbD reduces process risk and variability and can move us toward real time quality assurance (Fig. 1). Design of experiments is an integral part of quality by design and hence it was used in the current study.[7]

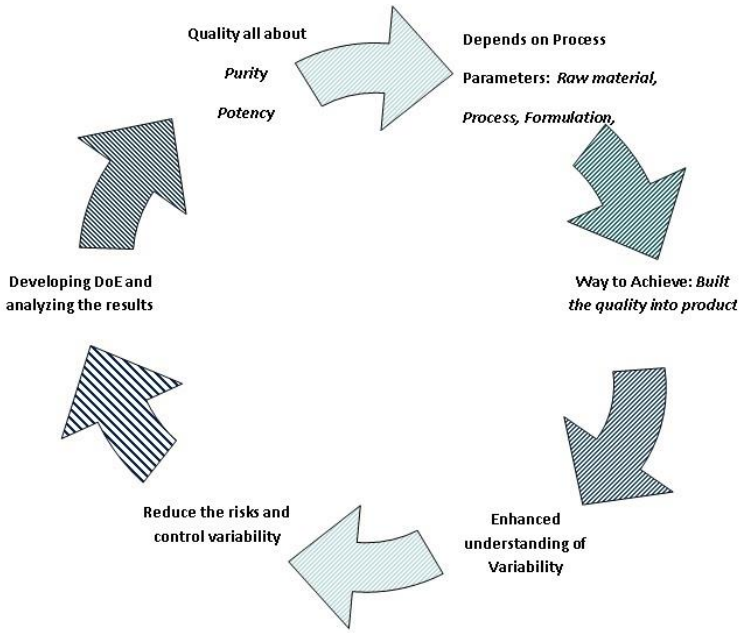

Fig. 1: Steps to minimizing process variability and maximizing product quality

Materials and Method

Materials

Lidocaine hydrochloride BP and Benzydamine hydrochloride BP were received as gift samples from Dishman Pharmaceuticals and Chemicals Ltd, Ahmedabad and Bal Pharma Ltd., Banglore respectively. Polyvinylpyrrolidone K90, Hydroxyl propyl cellulose (low viscosity grade), Eudragit NE 30D and Carbopol 934P were received as gift samples from Zydus Cadila Healthcare Ltd., Ahmedabad. Hydroxy propyl methyl cellulose K4M (HPMC) was a gift sample from Colorcon Asia Pvt. Ltd. Eudragit L100 and Eudragit RLPO were received from Corel Pharma Chem, Ahmedabad and Signet, Mumbai respectively. Ethyl alcohol IP and PEG 400 were purchased from Baroda Chemicals Industries Ltd., Vadodara and Vital Flavors, Mumbai respectively. Glycyrrhizic acid and Benzalkonium chloride IP were purchased from Amsar Pvt. Ltd, Indore and Apex Pharma, Mumbai respectively. Potassium dihydrogen phosphate IP, Sodium hydroxide IP, Ethyl cellulose EP 22 CPs were purchased from Laser Laboratories, Ahmedabad.

Preparation method of bi-layered film

Casting/solvent evaporation technique was selected for the preparation of bilayer film. The backing membrane solution (Ethyl cellulose and Eudragit RLPO) was prepared by dissolving film forming agent in a mixture of Acetone and Isopropyl alcohol (65:35). Polyethylene glycol 400 was used as a plasticizer at a level of 10% by weight of the film former. The solution was poured in petriplate and the solvents were allowed to evaporate at ambient conditions. The polymers of mucoadhesive layer were dissolved in 70% alcohol. Lidocaine hydrochloride (2%) and Benzydamine hydrochloride (0.15%) were subsequently added. Glycyrrhizic acid (4%) and Benzalkonium chloride (0.15%) were dissolved in the solution. Glycyrrhizic acid was added as sweeter. The polymeric solution containing APIs was poured into petriplate containing the dried backing membrane and dried at temp of 40° C for overnight. The dried film was kept in desiccators till further use. The film was cut into 2 × 2 cm (4 cm^2) and evaluated.[7]

Film properties

1. **Content uniformity:** Drug content uniformity was determined by dissolving the patch (2 × 2 cm) by homogenization in 100 ml of an isotonic phosphate buffer (pH 6.8) for 8 h under occasional shaking. Five ml drug solution was further diluted with isotonic phosphate buffer to 20 ml, and the resulting solution was filtered through a 0.45 mm Whatman filter paper. The drug content was determined after appropriate dilution at 263 nm and 307 nm using a UV spectrophotometer (Shimadzu, SPD-10 AVP, Japan) as interference was not observed at λ_{max}. The experiments were carried out in triplicate and average values are reported.[3,8]
2. **Tensile strength:** Universal testing machine (Shimadzu AG100kNG and software - Winsoft tensile and compression testing) was used. The instruments consisted of two jaws: upper jaw (grip II, movable) and lower jaw (grip I, fixed). Film was fixed between the two jaws. Tensile strength was measured and results were recorded.[8]
3. **In vitro drug release:** The USP XXIII rotating paddle method was used to study drug release from the buccal films. The film was cut into a circle with an area of 4 cm^2 and placed at the bottom of the

dissolution vessel. Compendial media is phosphate buffer (pH 6.8, 200 ml 37 ^{0}C) was used as a dissolution medium. The paddle rotation rate was 50 rpm. Ten ml solution was withdrawn at 1, 2, 3, 4, 5 and 6 h and replaced with the fresh dissolution medium. Each sample solution was filtered through 0.45 µm filter medium and analyzed spectrophotometrically at 263 and 307 nm. The experiments were performed in triplicate, and average values are reported.[3,8]

4. **Thickness of film:** The film thickness was measured using micrometer screw gauge (Mitutoyo MMO-25DS) at three different places and the mean value (n=3) was calculated.[9]
5. **Surface pH:** Each patch (2 x 2 cm) was allowed to swell by keeping it in contact with 1 ml of phosphate buffer (pH 6.8) for 2 h at room temperature, and the pH was noted after 1 minute by bringing the electrode in contact with the surface of the patch. The experiments were performed by using digital pH meter (electroequip, model no-pH cal) and average values (n=3) are reported.[8,9]
6. **Folding endurance:** A film strip of 2 x 2 cm was cut and repeatedly folded and unfolded at the same place till it broke. The number of times, the film could be folded at the same place, without breaking was recorded as the value of folding endurance. The experiment was performed in triplicate, and average values are reported.[8,10]
7. **Swelling study:** Buccal films (2 x 2 cm) were weighed (W_1) and separately placed in 2% agar gel plates, incubated at 37±1° C, and examined for any physical changes. At regular one hour time interval, the patches were removed from the plates and surface water was wiped with soft tissue. The swollen patches were then reweighed (W_2) and the swelling index (SI) were calculated using the following formula.[5]

$$SI = (W_2 - W_1) / W_1 \times 100$$

8. **Ex vivo mucoadhesive strength:** A specially fabricated assembly, slightly modified from the method described by Gupta et al (1992), was designed and then the following parameters were calculated from the bioadhesive strength/mucoadhesive strength.[2,5,9,10]:

$$\text{Force of adhesion (N)} = (\text{Bioadhesive strength} \times 9.81) / 1000$$

$$\text{Bond strength (N/ m}^2\text{)} = \text{Force of adhesion / surface area}$$

Ex vivo mucoadhesion time

The ex vivo mucoadhesion time was evaluated after application of the patches onto freshly cut guinea pig buccal mucosa which was previously fixed in the inner side of a glass beaker. Films were wetted with one drop of isotonic phosphate buffer (pH 6.8) and pasted to the buccal mucosa. The beaker was filled with 200 ml of phosphate buffer (pH 6.8). The temperature of the fluid was maintained at 37^0 C. The time required for the patch to detach from the buccal mucosa was recorded as the mucoadhesion time.[3]

9. **Fourier transform infrared spectrophotometer (FTIR) study:** Infrared spectra were recorded on a FTIR (8400S, Shimadzu, Japan). The infrared spectra of lidocaine hydrochloride, benzydamine hydrochloride and of lidocaine hydrochloride plus benzydamine hydrochloride containing optimized film composition were taken.
10. **Differential scanning calorimetry:** Differential scanning calorimetry (DSC) was used to evaluate drug-excipient compatibility studies. Calorimetric analysis was performed using model DSC-7, Perkin Elmer, equipped with a measuring cell DSC 20. The instrument was calibrated with an indium standard. For thermogram acquisition, sample size of 3 to 5 mg was scanned with a heating rate of 5° C/min over a temperature range of 40° C to 150° C. The changes in peak temperature, peak height, peak area as well as occurrence of new peak were selected evaluation parameters.[4]
11. **Stability study:** Stability studies were carried out for selected formulations at room temperature 30^0 C and 75% RH for 45 days. All the films were suitably packed in aluminum foil. The desiccators were used and saturated sodium chloride solution was poured inside the desiccators. The holding plate was placed inside and the desiccators were closed properly. The desiccators were allowed to get saturated for 1-2 h. This gave the humidity chamber of 75% RH. Then the desiccators were reopened and the aluminum foil sealed mucoadhesive bilaminated films were placed inside and the desiccators were closed. At the end of every week, the films were evaluated for different parameters like folding endurance, swelling index, surface pH, mucoadhesive strength, ex vivo mucoadhesion time and *in vitro* drug release.[3]

Results and Discussion

Preparation and evaluation of bi-layered film

Various polymers were screened for the film formation and Ethyl cellulose exhibited best film formation hence it was selected as primary film former. Polyethylene glycol 400 in different ratio was evaluated as plastisizer and 10% level gave good plasticity. Additionally, the films were also prepared using other polymers like Polyvinyl pyrrolidone K-90 (PVP-K90), Carbopol 934P and HPC-L in combination with Ethyl cellulose to increase mucoadhesion as shown in Table 1. HPC-L was selected due to better mucoadhesion in comparison to other mucoadhesives. Two concentrations i.e. 1% and 1.5% of HPC-L showed similar mucoadhesiveness and hence, 1 % of HPC –L was selected for further formulation development. It was aimed to release drug slowly over a period of 6 h but the prepared films were dissolved in time less than 4 h. Hence it was decided to add other release retarding

polymers. The different grades of Eudragi like Eudragit RLPO, Eudragit L-100 and Eudragit NE30D were evaluated as shown in Table 2. The preliminary studies revealed that Eudragit RLPO and Ethyl cellulose exhibited desired film forming property and HPC-L exhibited good mucoadhesive property and hence combination of these three polymers was selected. Ethyl cellulose was used as a backing membrane to release drug in unidirectional way.

All the batches (P1 to P6), showed good film formation and good mucoadhesion but Eudragit RLPO containing films retard the release of both the drugs till 6 h and hence it was selected for further optimization. It was observed after preliminary studies that ethyl cellulose and Eudragit RLPO were critical factors affecting the film formation as well as drug release and hence it was decided to optimize the concentrations of these polymers using central composite design. The amount of ethyl cellulose (X_1) and Eudragit RLPO (X_2) were chosen as independent variables. Drug release at 2 (YL/B_2), 4 (YL/B_4) and 6 ($YL/6_3$) h were dependent variables. The desirability range for the responses Y_1, Y_2 and Y_3 as <30%, 50-65% and >80% respectively. As per central composite design, eleven batches were prepared and evaluated for % release of drug (Table 3 and Fig. 2).

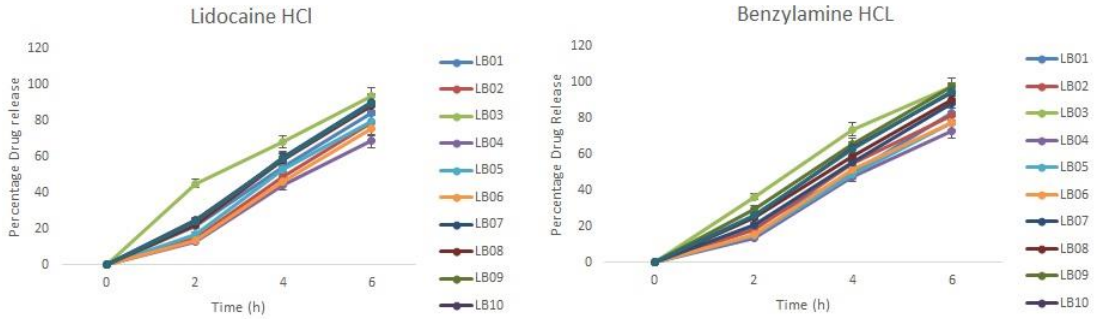

Fig. 2: Lidocaine and benzylamine HCl percentage drug release

Multiple regression analysis was carried out to evolve mathematical models (Table 3)

$YB_1 = 23.13 - 4.40\ X_1 - 3.35\ X_2 + 3.34\ X_1 X_2$ ……..1

$YB_2 = 58.63 - 4.81\ X_1 - 0.97\ X_2 + 2.63\ X_1 X_2 + 0.75\ X_1^2 - 2.26\ X_2^2$……...2

$YB_3 = 88.59 - 5.01\ X_1 + 1.44\ X_2 + 2.36\ X_1 X_2 - 0.26\ X_1^2 - 1.92\ X_2^2$…… ..3

$YL_4 = 30.92 - 2.27\ X_1 - 2.66\ X_2 - 2.32\ X_1 X_2$……..4

$YL_5 = 62.14 - 3.69\ X_1 - 3.25\ X_2 - 2.33\ X_1 X_2 - 5.5\ X_1^2 - 3.47\ X_2^2$…….. 5

$YL_6 = 90.71 - 4.08\ X_1 - 3.85\ X_2 - 3.64\ X_1 X_2 - 6.00\ X_1^2 - 2.50\ X_2^2$…….6

Fig. 3 (a, b and c) show the effect of concentration of ethyl cellulose (X_1) and Eudragit RLPO (X_2) on percentage of drug release in 2, 4 and 6 h respectively.

A check point batch (within design space) was prepared and evaluated. Optimized batch contained 2% ethyl cellulose and 3.89% Eudragit RLPO for backing layer and 2% Lidocaine hydrochloride, 0.15% Benzydamine hydrochloride, 1.85% ethyl cellulose, 3.89% Eudragit RLPO and 1% HPC-L (Table 4). Comparison between the experimental and the predicted values for the check point batch was done. The results showed good relationship between the experimental and predicted values, which confirms the practicability of the model. Hence, it may be concluded that required product characteristics can be obtained by systematic approach to the formulation development study.

Model fitting was done using an in-house program (FORTRAN). Zero-order, first-order, Higuchi, Hixson-Crowell, Korsmeyer-Peppas and Weibull model were tested (Table 5). The best fit model was selected on the basis of least sum of squares of residuals (SSR) and least F-values. It is evident from the data shown in the Korsmeyer and Peppas model best explained the drug release. The overlay plot of responses showed important area of film of the optimized batch is shown in Fig. 3(d).

Folding endurance, surface pH, swelling index, drug content uniformity, mucoadhesive strength, force of adhesion and bond strength were also evaluated (Fig. 4).

Fourier transform infrared spectrophotometer (FTIR)

The infrared spectra of lidocaine hydrochloride, benzydamine hydrochloride and of lidocaine hydrochloride and benydamine hydrochloride (Fig. 5) containing optimized film composition were comparable and the peaks of lidocaine hydrochloride and benzydamine hydrochloride containing optimized film composition are of lower intensity than the pure drug, lidocaine hydrochloride showed strong peak at 1650 cm−1 representing the carbonyl group stretching of the amide group and two sharp bands at the range 1450–1550 cm−1 due to C-N stretching where the one with higher energy was due to the bond with higher inductive effect (O-C-N). Figure also reveals the drug-drug and drug-excipient compatibility.[11]

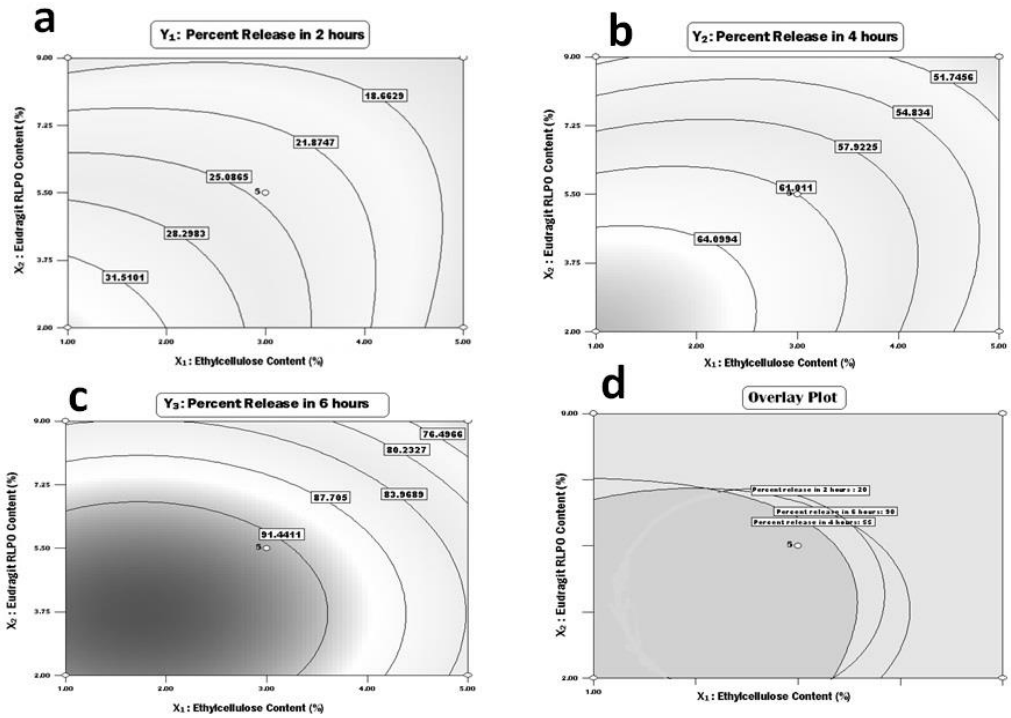

Fig. 3: a) Contour plot for response Y_1, b) Contour plot for response Y_2, c) Contour plot for response Y_3, d) Overlay plot of responses Y_1, Y_2 and Y_3

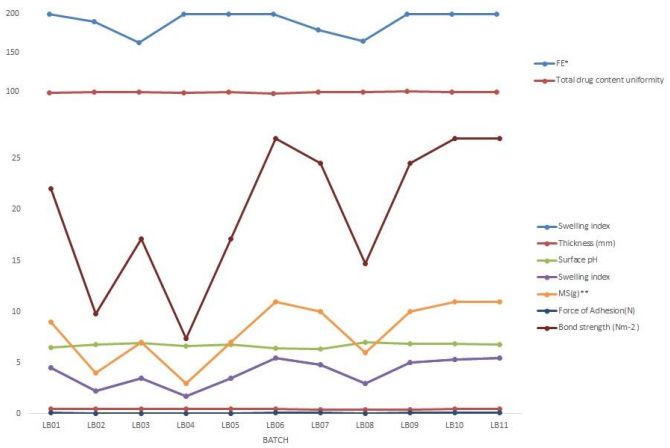

Fig. 4: Evaluated parameter Graph

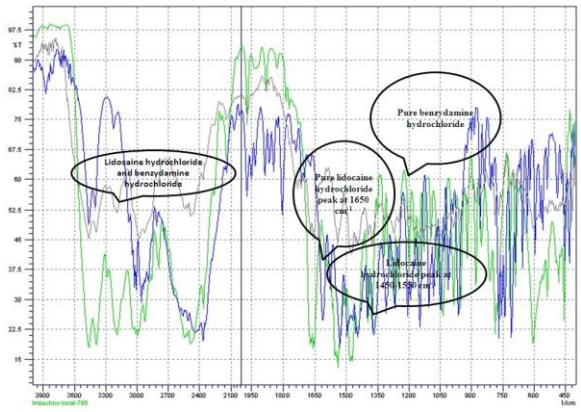

Fig. 5: Spectra in green color shows pure benzydamine hydrochloride drug, blue color shows pure lidocaine hydrochloride drug and black shows lidocaine hydrochloride and benzydamine hydrochloride optimized film composition

Differential scanning calorimetry (DSC)

Lidocaine hydrochloride showed two sharp endothermic peaks that correspond to melting in the range of 84.18 and 244.67 ^{0}C, as shown in Fig. 6(a). Benzydamine hydrochloride showed one sharp endothermic peak that corresponds to melting at 164.44 ^{0}C, as shown in Fig. 6(b). Lidocaine hydrochloride-benzydamine hydrochloride optimized buccal film composition Fig. 6(c) also showed three characteristic peak at 84.38 ^{0}C, 164.76 ^{0} C and 244.99 ^{0} C with decreased intensity showing compatibility between drug and excipient.[12,13]

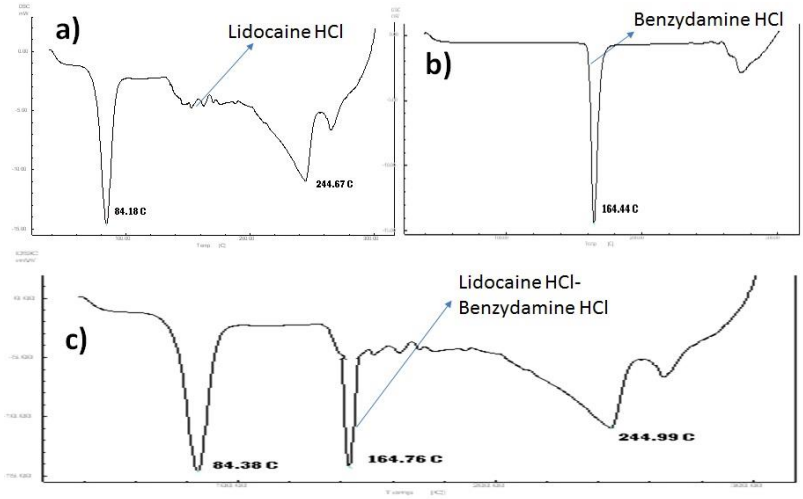

Fig. 6: a) DSC thermogram of pure lidocaine hydrochloride drug; b) DSC thermogram of pure benzydamine hydrochloride drug; c) DSC thermogram of lidocaine hydrochloride and benydamine hydrochloride optimized film composition

Stability study

The stability study was performed to check physical and chemical integrity of the formulation. In the present work stability studies were carried out for selected formulations at room temperature 45±2 °C /75 ±5 %RH for 45 days.[11] Comparison of in-vitro dissolution of buccal film after 45 days is depicted in Table 7 and results of evaluation of buccal film after 45 days show in Table 8. Paired t-test was carried out (Table 9).

Conclusion

The optimized mucoadhesive buccal dosage forms expected to provide clinicians with a new choice of an economical, safe and more bioavailable formulation in the management of oral ulcers. It is a novel method of treating aphthous stomatitis, lesions, sores and blisters affecting the mucous membranes and surrounding areas comprises of topical mucoadhesive dosage forms containing minor amounts of topically effective antibiotic-, antibacterial-, antimicrobial-, anti-infective and antiviral-like agents to the affected area.

Acknowledgement

The authors gratefully acknowledge Dishman Pharmaceutical and Chemicals Ltd, Ahmedabad, Bal Pharma Ltd, Banglore, Zydus Cadila Healthcare Ltd, Ahmedabad, and Colorcon Asia Pvt, Ltd for materials and council of scientific and industrial research (CSIR), India for support.

References

1. El-Kamel AH, Ashri LY, Alsarra I a. Micromatricial metronidazole benzoate film as a local mucoadhesive delivery system for treatment of periodontal diseases. *AAPS PharmSciTech* 2007;8(3):E75. doi:10.1208/pt0803075.
2. P??l S, Nagy S, Boz?? T, Kocsis B, D??vay A. Technological and biopharmaceutical optimization of nystatin release from a multiparticulate based bioadhesive drug delivery system. *Eur J Pharm Sci* 2013;49(2):258-4. doi:10.1016/j.ejps.2013.03.006.
3. Avachat and Ahire. Characterization and evaluation of spray dried coprocessed excipients and their application in solid dosage forms. *Indian J Pharm Sci* 2007;69(1):1-5. doi:10.4103/0250.
4. Sheet HF. Mouth Sores. 2013:1-2.
5. Shanker G, Kumar CK, Gonugunta CSR, Kumar BV, Veerareddy PR. Formulation and evaluation of bioadhesive buccal drug delivery of tizanidine hydrochloride tablets. *AAPS PharmSciTech* 2009;10(2):530-9. doi:10.1208/s12249-009-9241-2.
6. Audu M, Umale AM, Chinnedu IE. Formulation and evaluation the bioadhesive properties of drug delivery system based on PEGylated mucin matrices. *Asian Pac J Trop Med* 2010;3(6):461-4. doi:10.1016/S1995-7645(10)60111-3.
7. Vanhoutte F, Smans G, Janssens L, Vanstockem M. Criticality Management of a Drug Product and its Manufacturing Process. *Pharm Technol* 2008;32(9):66-80. http://search.ebscohost.com/login.aspx?direct=true&db=bth&AN=34383919&site=ehost-live.
8. Komala M, Shanmugapandiyan P, Shalini N, Bindu KH, Sampath KP. Design and In Vitro Evaluation of Buccal Film of Terfenadine. 2012;1(7).
9. Design and characterization of chitosan - containing mucoadhesive buccal patches of propranolol hydrochloride.

PubMed Commons. 2016;57(1):1-2.
10. Patel VM, Prajapati BG PM. Effect of hydrophyllic polymers on buccoadhesiveEudragit patches of propranolol hydrochloride using factorial design. *AAPS Pharm Sci Tech* 2007;8(2):119-26.
11. Aly SAS. Detachment and recovery index: A new parameter measuring powder compressibility. *J Adv Pharm Technol Res* 2010;1(4):1-11. doi:10.4103/0110.
12. Sciencedirect S. 2/25/2016 Carbohydrate polymers as wound management aids. 2016:1-2. doi:10.1016/S0144.
13. Clas S, Dalton C, Hancock B. Differential scanning calorimetry: applications in drug development. *Pharm Sci Technolo Today* 1999;2(8):311-20. doi:http://dx.doi.org/10.1016/S1461-5347(99)00181-9.