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Preparation and Evaluation of Buccal Mucoadhesive Patch of Betamethasone Sodium Phosphate for the Treatment of Oral Submucous Fibrosis

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ABSTRACT

The aim of the present study was to develop the mucoadhesive buccal film of Betamethasone sodium phosphate (BSP) by solvent casting method using Hydroxy propyl methyl cellulose (HPMC) E5 LV and carbopol (CP) 940P as polymer, Polyethylene glycol (PEG) 1000 as plasticizer. All the formulations were examined for film thickness, weight variation, drug content, percentage moisture loss, percentage moisture absorption, surface pH, folding endurance, tensile strength, in vitro and in vivo residence time and in vitro release. The all prepared buccal patches were transparent, smooth, consistent and flexible. The percentage moisture loss and percentage moisture absorption of optimised formulation (F3) were found to be 6.59 ± 0.54 and 5.74 ± 0.21 respectively. The surface pH of all formulation showed to be neutral. In vitro and in vivo residence time of all patches showed above 30 minutes. The formulation F3 showed optimum tensile strength (7.72 ± 0.41 kg/mm^2) which indicates less probability of rupture. In vitro drug release of optimised formulation (F3) was found to be 88.59 ± 2.74 at the end of 30 min. Stability studies were performed for optimised formulation (F3) and showed no appreciable change in physical structure and in drug content. The optimized formulation was given for Clinical study at Manipal College of Dental Sciences, Mangalore.

Key words: Betamethasone sodium phosphate (BSP), mucoadhesive buccal film, solvent casting, Hydroxy propyl methyl cellulose (HPMC) E5 LV.

INTRODUCTION

Oral submucous fibrosis is a chronic debilitating disease of the oral cavity characterized by inflammation and progressive fibrosis of the submucosal tissues (lamina propria and deeper

connective tissues). Oral submucous fibrosis results in marked rigidity and an eventual inability to open the mouth. The buccal mucosa is the most commonly involved site, but any part of the oral cavity can be involved, even the pharynx. Worldwide, estimates of oral submucous fibrosis indicate that 2.5 million people are affected, with most cases concentrated on the Indian subcontinent, especially southern India. The rate varies from 0.2-2.3% in males and 1.2-4.57% in females in Indian community. [1] Oral submucous fibrosis also has a significant mortality rate because it can transform into oral cancer, particularly squamous cell carcinoma (Reported cases of 7.6% worldwide) [2-4]. Different classes of drugs such as corticosteroids, extravasations antidotes, interferon, antioxidant, and vasodilator are given to reduce morbidity and to prevent complications which appear due to submucous fibrosis [1].

Betamethasone sodium phosphate is synthetic glucocorticoid that depresses formation, release, and activity of endogenous mediators of inflammation, so act as anti inflammatory agent. It has several side effects but still it is being frequently used in the treatment of submucous fibrosis. The conventional treatment with injections was found to be hazardous, whereas the conservative treatment with buccal patches and gel were found to be safe [5]. Also the parenteral formulation is invasive, causes pain and decreased patient compliance. Retentive buccal mucoadhesive formulations may prove to be a viable alternative to the conventional medications as they can be readily attached to the buccal cavity, retained for a longer period of time and removed at any time [6-13]. Earlier also attempts have been made to formulate various mucoadhesive devices including tablets, films, patches, disks, strips, ointments, and gels. 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 [14]. Hence present study was aimed to formulate the buccal patch of Betamethasone sodium phosphate to overcome the side effects of the injection and also ensure satisfactory level of drug release in the oral cavity for a period of treatment.

EXPERIMENTAL SECTION

Betamethasone Sodium Phosphate was a gift sample Anuh Pharma Ltd**. (**Mumbai, India). Hydroxypropylmethylcellulose (E5 LV) (HPMC) and chloroform were obtained from Loba chemie Pvt. Ltd. (Mumbai, India) and carbopol 940P was obtained from S.D. fine chemicals, (Mumbai, India). Dibutyl phthalate and Ethanol were obtained from Merck specialities Private Limited, (Mumbai, India). Polyethylene glycol 1000 was obtained from Koch-light laboratories Ltd., (England). Aspartame was obtained from HiMedia laboratories Pvt. Ltd. (Mumbai, India). All other chemicals used were of analytical grade and procured from S.D. Fine Chemicals (Mumbai, India)

Preparation of mucoadhesive patches

The films of respective composition were devised using Hydroxy propyl methyl cellulose (HPMC) E5 LV and carbopol (CP) 940P as polymer [15], Polyethylene glycol (PEG) 1000 as plasticizer, aspartame and peppermint oil as sweetening and flavouring agents along with drug and solvent. The solvent system used was 50:50 ratio of ethanol and chloroform. The polymers, PEG 1000 and aspartame were weighed accurately and dissolved in solvent mixture to obtain a viscous solution. The drug was then dispersed uniformly in the viscous solution with continuous mixing on magnetic stirrer. In order to avoid entrapment of the air bubble inside the film, the entire drug-polymer-solvent system was subjected to vacuum treatment with the aid of vacuum

desiccator. Then the solution was poured into moulds lined with aluminum foil for casting and dried for a period of 24 h. Placebo films without the drug were also prepared as mentioned above. After drying medicated patches of 2×2 cm^2 area were cut using a sterile stainless steel borer, each film containing 2.0 mg of drug. The cut patches were used for further studies [16]. The composition of different patches is given in [Table 1].

Table 1: Composition of buccal mucoadhesive patch

Formulations	HPMC E5 LV mg (%w/v)	Carbopol 940P Mg (%w/w)	PEG 1000 mg (%w/w)	Aspartame mg	Peppermint oil ml	Drug mg	Solvent ml
F1	18.75(4)	0.42(1.5)	16.87(60)	0.28	0.002	2	Q.S.
F2	23.47(5)	0.42(1.5)	16.87(60)	0.28	0.002	2	Q.S.
F3	28.12(6)	0.42(1.5)	16.87(60)	0.28	0.002	2	Q.S.
F4	37.50(8)	0.42(1.5)	16.87(60)	0.28	0.002	2	Q.S.
F5	46.87(10)	0.42(1.5)	16.87(60)	0.28	0.002	2	Q.S.
F6	28.12(6)	0.42(1.5)	5.62(20)	0.28	0.002	2	Q.S.
F7	28.12(6)	0.42(1.5)	11.25(40)	0.28	0.002	2	Q.S.

Composition for 2×2 cm^2 film, Value in the bracket indicates concentration with respect to HPMC E5 in %, Q.S.- 0. 12ml, HPMC = Hydroxypropyl methyl cellulose, PEG = Polyethylene glycol

Drug excipient compatibility studies

Infrared spectroscopy was studied using a Shimadzu FTIR 8300 Spectrophotometer and the spectrum was recorded in the region of 2000 to 400 cm^{-1}. The process consisted of dispersing a sample (drug, drug-polymer mixture and patch) in KBr (200-400 mg) and compressing into discs by applying a pressure of 5 tons for 5 min in a hydraulic press. The pellet was placed in the light path and the spectrum was obtained. The spectra obtained for drug, physical mixture of drug with polymer and patch was compared [17].

Physicochemical characterization of buccal mucoadhesive patches

Weight variation

Weight variation test was carried out using digital balance (Mettler Toledo), by weighing three films containing a specific amount of drug from each formulation. The standard deviations (SD) were calculated from individual weight of the film [16-18].

Film thickness

Thickness of films was evaluated by using a puncture test and texture analyzer (Instron® 3366-2716015, Germany). Ten readings were taken and the mean thickness was calculated. The standard deviations (SD) were calculated from individual data value.

Content uniformity of patches

To make sure uniform distribution of BSP in film, a content uniformity test was performed. The film was added to 100 ml of sorensons phosphate buffer (SPB) pH 6.4 contained in a 250 ml beaker was placed on temperature controlled magnetic stirrer maintained at 37 °C. The medium was stirred at 300 rpm with a Teflon coated magnetic bead for 3 h. Then the solution was filtered through 0.45 µm membrane filter and the filtrate was examined for the drug content at 242 nm using UV-Spectrophotometer [16-17].

Surface pH study

The surface pH of the patch was determined in order to investigate the possibility of any side effects (*in-vivo)*. A combined glass electrode was used for this purpose. The patches were allowed to swell by keeping it in contact with 1 ml of distilled water (pH 6.5 ± 0.2) for 15 minutes at room temperature, and pH was noted down by bringing the electrode in contact with the surface of the patch and allowing it to equilibrate for 1 minute [18, 23-24].

Percentage moisture absorption

The percentage moisture absorption test was carried out to ensure physical stability or integrity of buccal films. Buccal films were weighed and placed in a desiccator containing 100 ml of saturated solution of aluminum chloride and 75 ± 5% RH was maintained. After three days the buccal films were taken out and reweighed. The percentage moisture absorption was calculated using this formula [17, 19, 22].

$$\% \text{ Moisture absorption} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

Percentage moisture loss

The percentage moisture loss was carried out to evaluate integrity of the film in dry conditions. Buccal films were weighed and kept in a desiccator containing anhydrous calcium chloride. After three days, the patches were taken out and reweighed. The percentage moisture loss was calculated using the formula [17, 19, 22].

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

Tensile strength

Area of the films and maximum load which film can tolerate were measured using a puncture test and texture analyzer (Instron® 3366-2716015, Germany) (n = 3). Film specimens were mounted on a film holder. The puncture probe was driven through the film at a speed of 0.1 mm/s. Force vs. displacement curves were recorded with a 50 N load cell. Load versus displacement curves were recorded until rupture of the film and used to determine the tensile strength of films and backing membrane [17, 24].

$$\text{Tensile strength} = \frac{\text{Maximum force}}{\text{Area}}$$

Folding endurance

A small strip of film was cut evenly and separately folded at the same place until it broke. The number of times the film could be folded at the same place without breaking gives the folding endurance [17, 19, 22-24].

***In vitro* residence time**

The *in-vitro* residence time was determined using a locally modified USP disintegration apparatus (Disintegration tester, Electrolab, Mumbai, India). The disintegration medium was composed of 900 ml of SPB pH 6.4 maintained at temperature 37 ± 2 °C. A segment of pig

buccal mucosa, 3 cm long, was glued to the surface of a glass slab, vertically attached to the apparatus. The mucoadhesive film with backing membrane was hydrated from film surface using 15 µl SPB pH 6.4 and then the hydrated surface were brought into contact with the mucosal membrane. The glass slab was vertically fixed to the apparatus and allowed to move up and down so that the film was completely immersed in the buffer solution at the lowest point and was out at the highest point. The time necessary for complete erosion or detachment of the film from the mucosal surface was recorded (mean of triplicate determinations) [16, 23].

***In vivo* residence time**

In vivo residence time of placebo buccal patch was carried out in healthy human volunteers as subjects (aged 22–30 years, n=4). BSP have some side effect like Hypertension, oedema, increased susceptibility to all kinds of infection, spontaneous fractures, nitrogen depletion etc., so to avoid all these side effect placebo buccal patches were used for *in vivo* residence time study. The experiment was carried out with drug free films. Prior to the test, the volunteers were educated with the procedure and purpose of test. They were asked to rinse their mouth with distilled water before a piece of the drug free patch with water impermeable backing membrane was placed on their buccal mucosa. The bioadhesive film was placed on the buccal mucosa between the cheek and gingiva in the region of the upper canine and gently pressed onto the mucosa for about 30 sec. The film and the inner upper lip were carefully moistened with saliva to prevent film from sticking to the lip. The subjects were not allowed to eat or drink during the study (1 h). They were asked to monitor the ease with which the system was retained on the mucosa and note any tendency to detachment. The adhesion time was indicated by either complete erosion of the film or failure of the adhesive bond. Any complaints and bad feelings were also recorded. The study was repeated after two days on same volunteers [16, 20-21].

***In vitro* release study**

As there was no official method prescribed for *in vitro* drug release studies of buccal patch, a simple in-house laboratory assembly was utilized simulating the conditions of oral cavity. The backing membrane with mucoadhesive patches (2×2 cm^2 equivalent to 2.0 mg BSP) were carefully pressed on to a glass slide with a few drop of the adhesive and left for a minute for the adherence of backing membrane onto the slide. The slide with the adhered mucoadhesive dosage form was then placed into a 100 ml beaker containing 80 ml of SPB pH 6.4, which was pre heated to 37 ± 0.5 ºC. Then the beaker was kept on the temperature controlled magnetic stirrer maintained at temperature at 37 ± 0.5 º C and the medium was stirred at 50 rpm with the help of small teflon coated magnetic bead. The beaker was kept covered throughout the study to preclude evaporation of the medium. Five ml of sample were collected at various time intervals of 5, 10, 15, 30, 45 and 60 min and replaced by the same volume of the buffer. These samples were filtered through 0.45 µm membrane filter and the filtrate was used for estimation of drug concentration by using a UV spectrophotometer at a wavelength of 242 nm. Three patches of each formulation were tested.

Stability studies

The optimized films of betamethasone sodium phosphate with backing membrane were placed in an amber coloured bottle with aluminium cap as a closure. It was tightly sealed and kept in the incubator maintained at 40 ± 2 °C and 75 ± 5% RH. The stability studies were carried out for a

period of 3 months. Samples were collected at 0, 15, 30, 45, 60 and 90 days and observed for appearance and drug content of the films was investigated in triplicate [16, 23].

RESULTS AND DISCUSSION

Buccal patches of BSP were prepared by solvent casting method. The prepared buccal patches were transparent, smooth, uniform and flexible.

IR spectroscopy

The major IR peaks (wave number, cm^{-1}) of pure drug, drug and HPMC 5LV mixture and optimized formulation F3 are given below;

Pure BSP: 1722.19, 1660.77, 1620.26, 1095.6, 985.66, 895.36; drug+ HPMC 5LV: 1720.70, 1666.56, 1616.41, 1097.60, 974.8, 882.92; Optimized Formulation F3: 1727.56, 1657.91, 1629.90, 1085.96, 977.94, 888.71. The result showed that the principle IR peak of pure drug, its physical mixture with HPMC 5LV and optimized formulation F3 were almost similar, signifying no interaction between drug and polymer during formation of patch [25-26].

Weight variation, film thickness and content uniformity of patches

The results of weight variation, film thickness and content uniformity are represented in Table 2. The weights and thickness of different formulations were ranged between 37.42 ± 0.19 mg to 67.55 ± 0.55 mg and 60 ± 2.12 µm to 116 ± 2.46 µm, because of different concentration of polymer and plasticizer. All the formulations exhibited fairly uniform drug content ranging from 90.65 ± 0.57% to 97.95 ± 0.43%, Formulation procedures involving fewer processing steps, no major drug loss was observed during the preparation of the films.

Table 2: Weight variation, Thickness, %Drug content and surface pH of developed buccal mucoadhesive patch

Formulation	Weight variation (mg)	Thickness (µm)	% Drug content	Surface pH
F1	38.55 ± 0.31	60 ± 2.12	91.60 ± 0.51	7.23 ± 0.11
F2	44.05 ± 0.27	66 ± 2.61	97.95 ± 0.43	6.87 ± 0.08
F3	48.83 ± 0.15	86 ± 2.94	94.71 ±1.29	6.65 ± 0.06
F4	56.57 ± 0.21	94 ± 3.47	90.65 ± 0.81	6.54 ± 0.11
F5	67.55 ± 0.35	116 ± 2.46	95.45 ± 1.4	6.50 ± 0.08
F6	37.42 ± 0.19	61 ± 2.23	93.23 ± 0.85	7.14 ± 0.09
F7	41.86 ± 0.37	68 ± 3.47	92.08 ± 0.92	7.05 ± 0.06

Surface pH, percentage moisture loss and moisture absorption

As an acidic or alkaline pH may cause irritation to the buccal mucosa, an attempt was made to keep the surface pH as close to neutral as possible. The surface pH of formulations was found to be in the range of 6.50 ± 0.08 to 7.23 ± 0.11, as shown in [Table 2]. The surface pH for all the formulations was well within range of neutral pH and has not cause irritation and ultimately achieves patient compliance. The percentage moisture loss was found to be between 4.10 ± 0.32 to 9.44 ± 0.65 and percentage moisture absorption was found to be 3.9 ± 0.11 to 6.98 ± 0.43, as shown in [Table 3]. The result revealed that the moisture absorption and loss was found to increase with increasing concentration of hydrophilic polymers as well as increase the concentration of hydrophilic plasticizer. The optimum moisture content in the formulations helps

the film to remain stable, non brittle and free from complete drying. Optimum values of moisture absorption in F3 formulation indicate less chance of microbial contamination and maintain integrity through the films shelf life [19, 22].

Tensile strength and folding endurance

The tensile strength measures the ability of a patch to withstand rupture. As the concentration of hydrophilic polymer HPMC E5 and CP 940p was increased there is increase in tensile strength, as shown in [Table 3]. Polymers contain large number of chain of molecules and between these chains, homopolar bond and other types of bonds are possible. These bonds are either strong or feeble depending on the nature of polymer. According to the bonds formed force required to break the bonds and rupture the patch will differ [21]. The mean value of tensile strength of patch containing different concentrations of HPMC E5 varies between 4.06 ± 1.71 to 11.30 ± 0.48 kg/mm^2 (F1 to F5). As the concentration of plasticizer PEG 1000 was increased (20 to 60%) there is decrease in tensile strength, as shown in formulation F6, F7 and F3. The mean value of tensile strength of patch containing different concentration of plasticizer was found to be 11.31 ± 1.31, 9.20 ± 0.68 and 7.72 ± 0.41 kg/mm^2 for formulation F6, F7 and F3 respectively. Presence of plasticizer in the formulation helps in imparting strength to the films by lubrication effect of the plasticizer and reduction of the cohesive force between chain molecules of polymer. As a result tensile strength of the films will be reduced [27]. The formulation F3 showed optimum tensile strength which indicates less probability of rupture. The values for folding endurance varied from 25 ± 3.87 to180 ± 4.27, as shown in [Table 3]. The value depends on hydrophilic polymer as well as plasticizer concentrations used. Folding endurance test results indicated that the patches would not break and would maintain their integrity with general skin folding when applied.

***In vitro* and *in vivo* residence time**

In vitro and i*n vivo* residence time studies showed that all patches adhered immediately to the buccal mucosa and showed residence times above 30 minutes. HPMC E5 LV is a non-ionic polymer having unique gelling characteristics, which in turn are responsible for its adhesive properties, in addition to its high mechanical strength, tack, and high elasticity. The chains of HPMC E5 LV exhibit strong bioadhesive behavior either because of hydrogen bonding due to hydroxyl groups or because of significant chain penetration or both. From *in vivo* study it was found that no patches produce unwanted taste, irritation, or pain. None of the formulations were detached from the oral mucosa over the study period, which indicated that the bioadhesion values of all formulations were satisfactory to retain the film on the buccal mucosa [16].

Table 3: Tensile strength, Folding endurance, % Moisture absorption and Moisture loss of developed formulations of betamethasone sodium phosphate

Formulation	% moisture absorption	% moisture loss	Folding endurance	Tensile strength kg/mm^2
F1	3.9 ± 0.11	5.53 ± 0.24	25 ± 3.87	4.06 ± 1.71
F2	5.23 ± 0.32	5.96 ± 0.41	63 ± 2.83	5.48 ± 0.97
F3	5.74 ± 0.21	6.59 ± 0.54	130 ± 3.58	7.72 ± 0.41
F4	6.11 ± 0.33	7.33 ± 0.30	180 ± 4.37	8.55 ± 1.55
F5	6.98 ± 0.43	9.44 ± 0.65	85 ± 4.12	11.30 ± 0.48
F6	4.54 ± 0.54	4.10 ± 0.32	58 ± 2.01	11.31 ± 1.31
F7	6.23 ± 0.42	5.34 ± 0.44	110 ± 4.02	9.20 ± 0.68

***In vitro* drug release studies**

The release profile of formulation F1 to F5 which contain different concentration of HPMC E5 LV is illustrated in [Figure 1]. The cumulative percent drug release from the formulations F1, F2, F3, F4 and F5 was found to be 97.42 ± 3.77, 94.55 ± 2.4, , 88.59 ± 2.74, 68.96 ± 3.42 and 57.89 ± 2.42 at the end of 30 minutes. It was found that increase in the concentration of HPMC E5 LV significantly decreased the drug release. The slow drug release mechanism for higher polymer concentration can be explained by reduction in permeability due to change in the morphology of the polymer. Increased polymer concentration may have provided the matrix with higher tortuosity and poor water porosity for diffusion of drug. Moreover, higher polymer concentration would have resulted in viscous environment of the system inhibiting movement of water into the matrix for easy diffusion of the drug into the surroundings [21]. *In vitro* release of drug also depends on nature of plasticizer. As the concentration of hydrophilic plasticizer was increased the release of drug was also found to be increased, as shown in [Figure 2]. It may be due to quick absorption of water by formation of large number of hydrogen bonds and helped in faster diffusion of drug from system. From *in vitro* drug release study, it was found that F3 showed maximum release (88.59 ± 2.74) at the end of 30 min which was the prerequisite for the achievement of therapeutic action. However formulations F1 and F2 containing lower concentration of HPMC E5 LV showed more release compared to F3 at the end of 30 min, but tensile strength was lesser than F3.

Stability studies

Optimized formulation did not show any physical changes during the study period and also exhibit excellent drug content over the storage period, as shown in [Table 4].

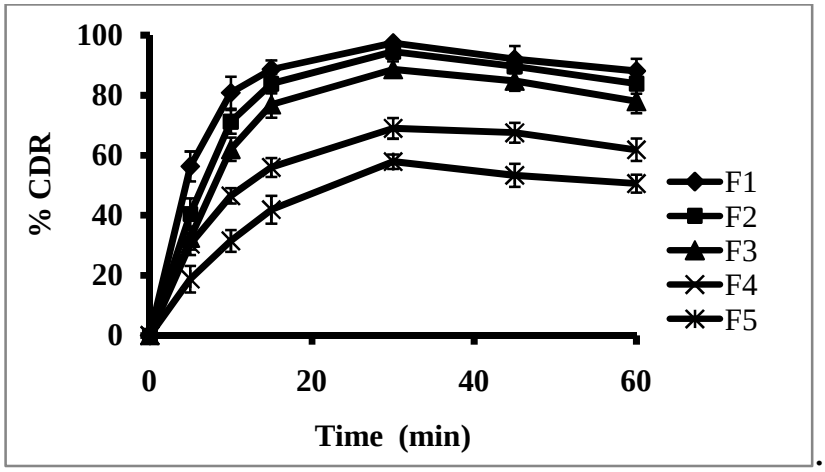

Figure 1: *In vitro* release studies of betamethasone sodium phosphate from F1 to F5 formulations containing 3%, 4%, 6%, 8% and 10% HPMC E 5LV respectively in SPB pH 6.4

Table 4: Stability study of betamethasone sodium phosphate patches at 40 ± 2 °C and 75 ± 5% RH

Time (days)	0	15	30	45	60	90
% drug remaining	94.71	94.12	93.43	93.02	92.67	91.89

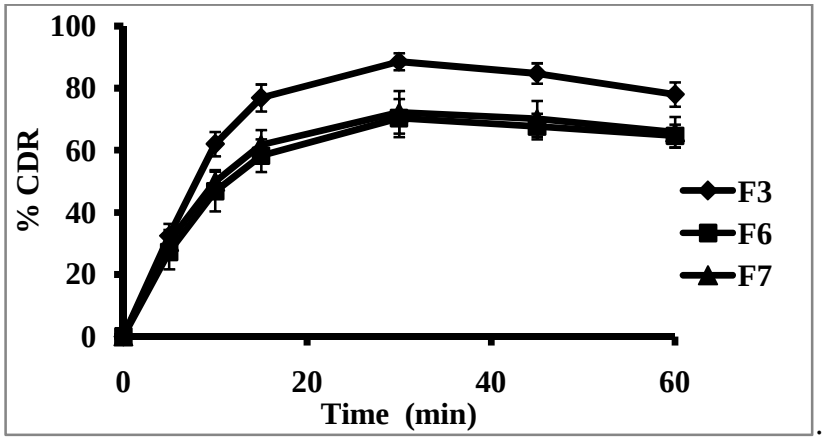

Figure 2: *In vitro* release studies of betamethasone sodium phosphate from F3, F6 and F7 formulation containing 60%, 20% and 40% plasticizer respectively in SPB pH 6.4

CONCLUSION

The most important advantage of the mucoadhesive buccal films is that it contains a lower drug dose, adequate for therapeutic effect as it is placed directly on the site of the inflammation, when compared to conventional administration. Moreover, this mucoadhesive buccal patch is very contented because it is non-irritant and self administration is possible. Clinical study of optimized formulation was performed by Manipal College of Dental Sciences, Mangalore and the result was found to be positive.

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