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Development and evaluation of a buccal bioadhesive system for smoking cessation therapy

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The objective of the present study was to develop a bilayered buccal bioadhesive film formulation of nicotine hydrogen tartrate for smoking cessation therapy, comprising a bioadhesive drug layer and a backing layer, which releases the drug at a pre-determined rate for a period of 4 h. Formulations were prepared using various bioadhesive polymers and were evaluated for physical parameters like peelability, flexibility, softness, bioadhesive strength, tensile strength, dispersion time and pharmaceutical parameters such as thickness, swelling, content uniformity, water vapour permeability and drug release. Based on these parameters formulation N_2, containing hydroxypropyl methylcellulose and polycarbophil as the bioadhesive polymers, was selected as the optimized formulation. The formulation showed suitable adhesion and an initial burst release of 40% drug in first 15 min followed by a total 80% drug release in a characteristic manner until 4 h; which is the desired time of application. This release pattern is beneficial for patients suffering from emergent cravings. Backing layers of the films were studied by a moisture vapor permeability test and it was observed that the percentage of moisture which permeated through single layered films was much higher than through bilayered films implying that a backing layer would prevent washing out of drug by the saliva.

1. Introduction

Smoking is the single most common cause of morbidity and mortality worldwide (Panchagnula et al. 2000). Annually, over 30% of the approximately 50 million US adults who smoke daily make a serious attempt to quit smoking, but only 2–5% of them are able to quit permanently (Wood 1995; Sweeney et al. 2001; Corelli and Hudmon 2002).

US FDA has approved sustained release bupropion and four nicotine replacement products: nicotine gum, transdermal nicotine patch, nicotine nasal spray, and nicotine vapour inhaler (Wood 1995; Sweeney et al. 2001; Corelli and Hudmon 2002; Rigotti 2002). Nicotine Replacement Therapy (NRT) is the most widely used therapy for smoking cessation and comprises a combination of medications with passive (transdermal patch) and instantaneous nicotine delivery (e.g. gum, nasal spray, inhaler) with the rationale of providing a slow and steady supply of nicotine to achieve constant concentration levels to relieve craving and withdrawal symptoms. There has been ongoing research on various oral NRT products including biphasic buccal adhesive tablets and bioadhesive buccal tablets (Park and Munday 2002; Ikinci et al. 2004, 2006; Park and Munday 2004).

The buccal route of drug administration offers easy accessibility to systemic circulation for drugs with low bioavailability and thus bypasses the first pass effect. However, limitations associated with this route like smaller surface areas available and limited retention capacity of dosage forms leads to inefficient treatment (deVries et al. 1991; Gupta et al. 1992; Smart 1993). Subsequently, bioadhesive formulations can serve a potential solution to the problem with localized delivery, prolonged residence time and reduced dosing frequency (deVries et al. 1991; Hao and Heng 2003; Nafee et al. 2004). Amongst the bioadhesive formulations, films offer various advantages such as low dose, flexibility in shape and size and drug delivery at a predetermined rate for local or systemic effects (Bhaichwal 1985; Bruschi and de Freitas 2005).

The objective of the present study was to develop and evaluate a bilayered buccal bioadhesive film formulation of nicotine hydrogen tartrate consisting of a bioadhesive drug layer and a backing layer for smoking cessation therapy. The formulation was evaluated for various physical and pharmaceutical parameters and accelerated stability studies were conducted on the optimized formulation.

2. Investigations and results

The physical parameters evaluated for placebo films and drug containing films are reported in Tables 1 and 2.

Swelling property plays an important role in the bioadhesive strength and drug release kinetics of the films. The bioadhesive capacity primarily depends on the concentration and swelling behaviour of the polymer in the aqueous environment. Swelling capacity of the polymers was studied using a 1.4% agar gel as discussed in section 4.5.2.

Table 1: Evaluation of placebo films

Batch	Peelability[a]	Softness[a]	Flexibility[a]	Appearance	Thickness[b] (mm)	Dispersion Time[c] (min)
P_1	***	**	**	Transparent	0.18 ± 0.02	18
P_2	***	*	**	Transparent	0.28 ± 0.02	17
P_3	***	*	*	Transparent	0.30 ± 0.05	15
P_4	**	**	**	Transparent	0.14 ± 0.02	15
P_5	***	**	***	Translucent	0.14 ± 0.02	20
P_6	**	*	*	Polymer precipitation	0.38 ± 0.08	35
P_7	**	**	**	Transparent	0.33 ± 0.08	20
P_8	**	***	***	Transparent	0.30 ± 0.05	7.3
P_9	**	***	***	Transparent	0.06 ± 0.01	4.5
P_{10}	***	**	**	Transparent	0.14 ± 0.02	360

[a] * Average, ** Good, *** Excellent
[b] Values expressed as mean ± Standard Deviation (S.D.), n = 5
[c] Dispersion time was determined from time taken for complete dissolution of bioadhesive layer of film in 10 ml of simulated saliva solution maintained at 37 °C

Table 2: Evaluation of buccal bioadhesive films

Batch	Peelability[a]	Appearance	Flexibility[a]	Thickness (mm)[b]	Bioadhesive strength (N)[b]	Tensile strength (N)[b]
N_1	***	Precipitation of drug on surface	***	0.19 ± 0.02	–	–
N_2	***	Translucent	***	0.036 ± 0.05	5.94 ± 0.66	94.08 ± 7.06
N_3	N	Precipitation of drug and polymer	–	–	–	–

[a] * Average, ** Good, ***Excellent, N Non peelable
[b] Values expressed as mean ± Standard Deviation (S.D.), n = 5

A plot of percentage hydration of N_2 films versus time is shown in Fig. 1. The percentage hydration of N_2 films showed a direct relationship with time. All films were intact after completion of swelling studies.

Bioadhesive strength is a major tool to ascertain the bioadhesive capacity of a polymer in bioadhesive buccal drug delivery systems. The average values of bioadhesive strength and tensile strength of N_2 films (n = 5) was found to be 5.94 (±0.66) N and 94.08 (±7.06) N (Table 2). Also, tensile strength properties are extremely important for any film formulation as they ensure the strength of the film, its behaviour during handling, application and use. The results of tensile strength revealed that N_2 film requires a force of 94 N to break the film (Table 2).

The release profile of N_2 films is shown in Fig. 2. Up to 80% of the drug is released in a sustained manner for 4 h. An initial unexpected drug release of 40% in less than 15 min was observed followed by a sustained release until the end of 4 h.

The content uniformity of N_2 films (n = 3) was determined using an in house HPLC method. The mean percentage recovery of drug was 93.18 (±4.46)%.

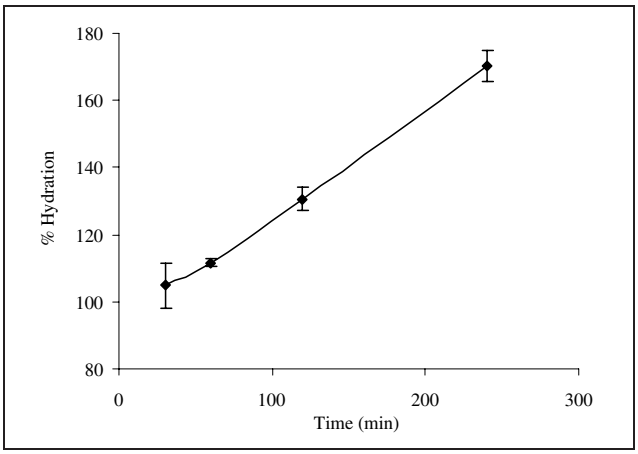

Fig. 1: Swelling behaviour of N2 buccal bioadhesive film (n = 3, mean ± S.D.)

Percentage moisture permeated through N_2 films vs time is shown in Fig. 3.

The thickness of a buccal film should be optimum. It should not be too thick to be felt in the mouth or too thin

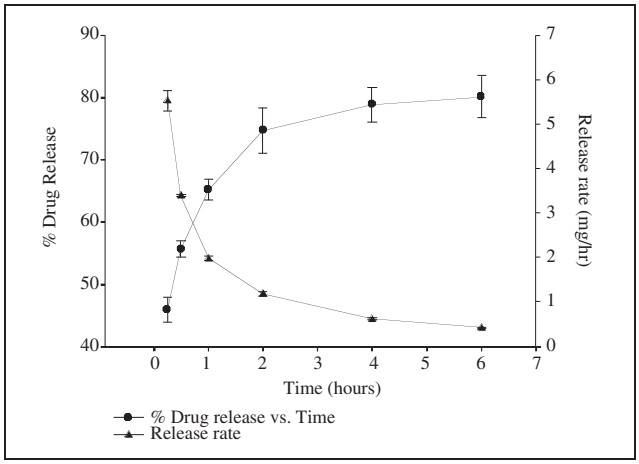

Fig. 2: Percent drug release time and release rate profiles of N2 films [each time point represents mean ± S.D. (n = 3)]

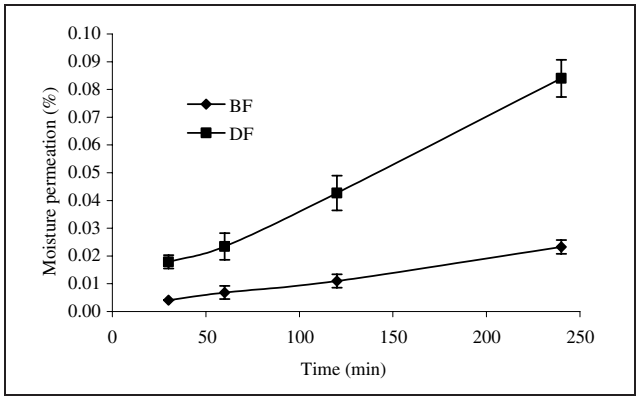

Fig. 3: Percent moisture permeation through BF (Bilayered films) and DF (Drug containing bioadhesive layer without backing layer) of N_2 films [each time point represents mean ± S.D. (n = 3)]

Table 3: Evaluation of backing layer

Batch	Peelability[a]	Softness[a]	Flexibility[a]	Appearance	Thickness[b] (mm)
B_1	N	–	–	–	–
B_2	N	–	–	–	–
B_3	**	**	**	Translucent	0.28 ± 0.05
B_4	***	***	***	Transparent but striations	0.22 ± 0.02
B_5	***	*	**	Transparent but air bubbles	0.23 ± 0.03
B_6	N	–	–	–	–
B_7	**	**	*	Striations on surface	0.23 ± 0.04
B_8	**	**	**	Polymer precipitation	0.21 ± 0.02

[a] * Average, ** Good, *** Excellent, N Not peelable
[b] Values expressed as mean ± Standard Deviation (S.D.), n = 5

Table 4: Stability studies of buccal bioadhesive film N_2

Time points	Thickness (mm)[a]	Weight uniformity[b] (mg)	Bioadhesive strength[c] (N)	Tensile strength[c] (N)
Initial	0.523 ± 0.03	226.9 ± 12.15	5.343 ± 1.408	121.52 ± 13.41
15 days	0.575 ± 0.05	237.3 ± 12.94	6.0 ± 0.59	128.9 ± 17.57

[a] Thickness of stability samples (n = 10) was measured by digital thickness gauge
[b] Values expressed as mean ± Standard Deviation (S.D.), n = 10
[c] Values expressed as mean ± Standard Deviation (S.D.), n = 5

to create problems during handling or dissolve quickly. Also, variation in thickness would lead to non-uniformity in drug content and subsequently alter the drug release and kinetics of drug release (Peh and Wong 1999; Sharma and Hamsa 2001). Average thickness and standard deviation of the films are mentioned in Tables 1, 2 and 3 for placebo films, buccal bioadhesive films and backing layer respectively.

The stability samples of the optimized formulation N_2 were analyzed after 15 days of stressed conditions 40 °C/75% RH and compared to zero time point samples. The results of thickness variation, weight uniformity, bioadhesive strength and tensile strength after 15 days of stressed conditions in stability chambers showed almost the same results as initial samples (Table 4). The percentage drug recovery from N_2 films before and after the stability studies did not show much change as can be seen in Table 4. Content uniformity of initial samples was found to be 111 (±5.3)%, while the 15 days stability sample was 103.3 (±10)%. The release profile and release rate before and after stability analysis showed the same drug release pattern.

3. Discussion

The placebo batches (Table 5) were prepared with different bioadhesive polymers reported in the literature and were evaluated for physical parameters like peelability, softness, flexibility, appearance, thickness, and dispersion time (DT) mentioned in Table 1. The most important criteria for selection of placebo films was dispersion time (DT) and was decided that 4 h would be the desired period of study, so as to retain the formulation until that time. Initially, HPMC was selected as the bioadhesive polymer which has good water absorbing capacity and slow erosion property. Therefore, placebo films were casted with 10% HPMC with different concentrations of glycerol as plasticizer and water as solvent (P_1, P_2 and P_3), the film appeared to be transparent, flexible, and easily peelable but dissolved completely in 18, 17 and 15 min, respectively. Since, the DT obtained was much less, a combination of polymers comprising of water-swellable polymers like Polycarbophil (0.3%) and Carbopol 974 P (0.2%) were chosen along with HPMC (8.5%), since they retard the release of drug in a controlled manner, in the composition P_4. The film was peelable, transparent and highly flexible but DT was obtained as 15 min. So, further concentration of HPMC was reduced (6%) and Polycarbophil was increased (0.5%) with 6% plasticizer and the obtained film (P_5) was easily peelable, translucent, soft in touch, highly flexible and DT of the film was found to be 20 min. Furthermore, in order to increase the DT, a film forming polymer, PVA (5%) was used in combination with HPMC (3.5%), the film (P_6) obtained was peelable, DT increased to 35 min but film was less flexible and showed polymer precipitation. Thus, the ratio of both the polymers was altered (HPMC 2.5% and PVA 6%) to modify the film (P_7) properties, the film obtained was transparent and had good peelability and flexibility, but the DT obtained was 20 min. So, further concentration of PVA was increased (8.5%) and Polycarbophil (0.5%) was added as bioadhesive polymer and the films obtained (P_8) were of good peelability and flexibility but poor DT (7.3 min). Since, these polymeric combinations could not

Table 5: Composition of placebo films prepared using various bioadhesive polymers

Ingredients (%)	P_1	P_2	P_3	P_4	P_5	P_6	P_7	P_8	P_9	P_{10}
HPMC	10	10	10	8.5	6.0	3.5	2.5	–	–	–
PVA	–	–	–	–	–	5.0	6.0	8.5	–	–
Polycarbophil	–	–	–	0.3	0.5	–	–	0.5	–	–
Carbopol 974P NF	–	–	–	0.2	–	–	–	–	–	–
Chitosan Glutamate	–	–	–	–	–	–	–	–	5.0	10
Glycerol	6.0	7.0	9.5	8.5	6.0	8.5	8.5	8.5	0.9	2.0
Water	84	83	80.5	82.5	87.5	83	83	82.5	94.1	88

produce good DT; Chitosan was studied upon, which is reported in the literature to control the release of highly water-soluble drugs up to 6 h. Therefore, film (P_9) was made with 5% of chitosan and glycerin as plasticizer, and was easily peelable, transparent, soft, highly flexible and DT was found to be 4 h but film was sticky. Furthermore, a higher percent of chitosan (P_{10}) was tried upon using 10% polymer, the resultant film was easily peelable, less sticky and DT was found out to be 6 h but film was less flexible. Out of these batches, P_5 and P_9 were selected based on physical evaluation for incorporation of drug and further evaluation.

When drug was incorporated into P_9 placebo batch, precipitation of drug was seen on the surface of the film formulation (N_1). Therefore, P_5 placebo batch was incorporated with drug and films (N_2) obtained were found to be translucent with good peelability and flexibility. Further attempts were made to increase DT by addition of 2% of ethyl cellulose (N_3); the obtained film was non-peelable as the polymer precipitated from the solution. Furthermore, to enhance the DT of the film (N_2), film thickness was increased to double, by keeping the same concentration of polymer and plasticizer but doubling the amount of excipients, obtained film was easily peelable, translucent, highly flexible, and DT was found out to be 3.5 h. So, N_2 was selected as the optimized formulation based on the physical evaluation of drug-containing films (Table 2).

Nicotine is reported to have gastric irritant properties. The rationale of the backing layer is to prevent the release of drug from the bioadhesive layer into the saliva and subsequent avoidance of side effects. So, ethyl cellulose, a water-insoluble polymer was chosen, for the purpose of a backing layer (Rowe et al. 2004). The backing layer was evaluated for parameters like peelability, softness, flexibility, and appearance (Table 3) and optimum composition (B_5) was selected based on these parameters for backing layer of drug-containing bioadhesive layer.

The studies conducted on the optimized formulation exhibited good swelling properties resulting in adequate bioadhesion and desired release profile.

Swelling capacity of a polymer is of significance, since polymer swelling leads to relaxation of the polymer chains and its interpenetration with the mucus layer during bioadhesion. However, excessive swelling may lead to a loss of bioadhesive properties of the film and dislodging of dosage forms from the site of application. Adhesion occurs shortly after the beginning of swelling but the bond formed is not very strong. The adhesion increases with the degree of hydration until a point where overhydration leads to an abrupt drop in adhesive strength due to disentanglement at the polymer/tissue interface (Peh and Wong 1999).

The polymers used in the optimized formulation (N_2) were HPMC (6% w/w) and Polycarbophil (0.5% w/w). The swelling profile of formulation N_2 is shown in Fig. 1. HPMC has been known to have good water absorbing capacity and slow erosion properties, while Polycarbophil is a water-swellable polymer. The direct relationship of percent hydration with respect to time shows good water absorbing and swelling properties of HPMC.

It has been proposed that mucoadhesion occurs in three stages involving the formation of an intimate contact between the mucoadhesive macromolecules and the mucus followed by swelling of the mucoadhesive macromolecules and interpenetration with the mucus macromolecules, finally becoming physically entangled. These molecules interact with each other via secondary, non-covalent bonds such as hydrogen bonds (Duchene et al. 1988; Park and Munday 2002). Textural analysis was employed to measure bioadhesive strength and tensile strength, where the force required to overcome the attractive bonds between the sample (film) and the substrate (cellophane membrane containing 2% w/w mucin gel) was measured as the bioadhesive strength, while, the force required to pull the film until its breaking point as the tensile strength. Table 2 shows the evaluation of drug containing films. Films N_1 containing chitosan glutamate as the bioadhesive polymer exhibited precipitation of drug on the surface of the film, hence, bioadhesive and tensile strength could not be conducted on these films. However, films containing a combination of polymers HPMC and polycarbophil (N_2) demonstrated good bioadhesive strength to sufficiently retain the formulation until the desired period of drug release (4 h). The high bioadhesive strength of these films may be attributed to the combined effect of both the polymers which may have a synergistic effect on the bioadhesive strength (Nafee et al. 2004). The high value of tensile strength reveals good abrasion resistance properties of the films (Peh and Wong 1999; Eouani et al. 2001). In contrast to the N_2 films, these studies could not be conducted upon films containing similar bioadhesive polymers, with addition of insoluble polymer ethyl cellulose (N_3), due to non-formation of films. The bioadhesion data obtained was indicative of good performance as compared to other formulations tested. It is almost impossible to compare the data with other studies due to varying experimental conditions.

HPMC is widely known as the dominant hydrophilic carrier material used for the preparation of oral controlled release drug delivery systems (Siepmann et al. 1999a, b). The transport phenomena involved in the drug release from HPMC matrices are complex because of micro and macro structure of the HPMC exposed to water is strongly time-dependent. Hence, the swelling patterns become essential for the drug release kinetics from these matrices.

Numerous studies have been reported investigating the swelling and drug release kinetics from HPMC matrices (Gao and Meury 1996; Gao et al. 1996; Siepmann et al. 1999a, b; Kavanagh and Corrigan 2004). The proposed mechanism illustrates a series of phenomena resulting in drug release. Initially, polymer swelling occurs upon water imbibition. This initial swelling depends on two factors, the exposed surface area of the film and diffusivity of water into the polymer. Polymer swelling leads to disentanglement and relaxation of the polymer molecules with volume expansion due to increased macromolecular mobilities. The disentanglement of polymer chains facilitates water diffusion into the system resulting in increased water content and drug diffusivity. The drug diffusivity depends on the diffusion coefficient of drug within the system which is dependent on the molecular size of the diffusing species (Siepmann et al. 1999b). It has already been reported that the drug diffusivity from HPMC matrices is independent of external stirring conditions and polymer weight (Reynolds et al. 1998). Polymer swelling is further followed by polymer dissolution. Thus, the drug release from HPMC matrices mainly follows two mechanisms, diffusion through the swollen layer and release by matrix erosion of the swollen gel layer.

In our studies, the initial burst and non-linear release observed indicates surface effect which occurs as a result of drug release from the surface and peripheral boundaries of the film. There is also further blockade of pores due to hydration and swelling which leads to resistance to sol-

vent influx and, thus, the sustained release pattern afterwards. Thus, as can be summarized, initially drug release from HPMC occurs as a burst release followed by diffusion until outer gel layer reaches its critical disentanglement concentration and additional drug release due to polymer erosion. It has been reported that the swelling capacity of Polycarbophil is enhanced by the hydrophilicity of HPMC when these polymers are used in combination (Nafee et al. 2004). Hence, it can be proposed that the same phenomena would have contributed to the increased swelling capacity of the formulation. Furthermore, it has been previously established that the rate of polymer swelling and dissolution and thus drug release rate are dependent on total drug loading and viscosity grade of HPMC with a direct relationship with drug loading and inverse with viscosity grade (Siepmann et al. 1999a). Consequently, it can be postulated that these two factors also play a significant role in the release mechanism.

Water vapour permeability studies conducted for drug-containing films without backing layer and bilayered films showed that percentage moisture permeated through drug-containing layers was much higher compared to bilayered films, which implies that backing layer prevents the permeation of moisture through it. Hence, backing layer would prevent the washing out of drug through saliva.

The stability studies conducted on the formulations showed similar results ($p > 0.05$) indicating stability of the formulation until the end of the studies.

In conclusion**,** Nicotine hydrogen tartrate was successfully formulated as bilayered buccal bioadhesive film using polymeric excipients with backing layer to prevent washing out of drug due to saliva. N_2 was selected as the optimized buccal bioadhesive formulation based on the parameters like peelability, softness, flexibility, thickness and dispersion time. The formulation showed 80% release of nicotine in a characteristic manner after 4 h, which is the desired time of application. It showed an initial release of 40% drug in first 15 min, and followed by sustained release. This release pattern is very beneficial for subjects suffering from emergent cravings, since the initial burst release would relieve from acute cravings. Moisture vapour permeability showed that percentage moisture permeated through drug-containing films was much higher than bilayered films. Hence, it implied that the backing layer would prevent the washing out of drug through saliva. Accelerated stability studies showed the formulation to be stable.

4. Experimental

4.1. Materials

Nicotine hydrogen tartrate (NHT) was purchased from Sigma Chemical Co., USA. Polycarbophil (Noveon™ AA1) was purchased from B. F. Goodrich, USA. Hydroxypropyl methylcellulose K4 M Premium (HPMC) was obtained from Dow Chemicals, USA. Poly Vinyl alcohol (PVA) was obtained from Sigma Chemical Co., USA; Carbopol 974P NF from B. F. Goodrich, USA and Ethocel STD 10 premium (EC), used for preparing a backing layer, was obtained from Dow Chemicals, USA. Glycerol and Dibutyl phthalate (DBP) were used as plasticizers and were obtained from Sisco Research Lab., India and Acros Organics, USA. Dichloromethane (HPLC grade) and Acetone (AR) were used for preparing the backing layer and were obtained from E. Merck Ltd., India and Qualigens Fine Chem., India, respectively.

4.2. Rationale for using nicotine hydrogen tartrate (NHT)

NHT, a crystalline powder, was used in the study for the formulation of bilayered films by solution casting method. This approach is not easily possible using pure basic nicotine which is a highly unstable liquid alkaloid. Also, it is difficult to incorporate the dose of nicotine base (2 mg) compared to nicotine hydrogen tartrate (8.6 mg), which is easy to handle in terms of content uniformity. NHT is advantageous over nicotine base as it is extremely stable and at salivary pH, converts to a readily absorbable unprotonated nicotine form (Park and Munday 2002).

Table 6: Composition of drug containing buccal bioadhesive films

Ingredients	Formulations		
	N_1	N_2	N_3
NHT (mg)	8.6	8.6	8.6
HPMC	–	6	6
Polycarbophil	–	0.5	0.5
Chitosan Glutamate	5	–	–
Ethyl cellulose	–	–	2
Glycerol	0.9	6	6
Water	94.1	85.5	87.5

Table 7: Composition of backing layer of buccal bioadhesive films

Ingredients	B_1	B_2	B_3	B_4	B_5	B_6	B_7	B_8
Ethyl cellulose	10	8	8	8	8	10	15	8
Acetone	70	87	82	–	–	–	–	–
DCM	–	–	–	87	–	–	–	–
DCM/Acetone (7 : 3)	–	–	–	–	87	85	80	–
DCM/Acetone (9 : 1)	–	–	–	–	–	–	–	87
DBP	20	5	10	5	5	5	5	5

4.3. Preparation and optimization of placebo buccal bioadhesive films

Initially, placebo films were prepared by a solution casting method (Perioli et al. 2004) using the bioadhesive polymers. The polymers were weighed separately and were added to the required quantity of solvent with continuous stirring until a complete solution was obtained. Plasticizer was added into the solution and was stirred for 15 min and the solution was sonicated and kept in the refrigerator overnight or centrifuged for 15 min to remove off the entrapped air bubbles. The films were then prepared by casting the solution onto a glass plate and the solvent was allowed to evaporate. The placebo films prepared are listed in Table 5.

4.4. Preparation of bilayered buccal bioadhesive films

Bilayered buccal bioadhesive films were prepared in order to avoid the loss of drug in the saliva. A backing layer made up of water-insoluble polymer served as a barrier to avoid drug loss in saliva, while the bioadhesive layer consisted of NHT containing bioadhesive polymer. A bioadhesive layer gets attached to the buccal mucosa and releases the drug in a controlled manner unidirectionally, thus avoiding its loss in the saliva.

The compositions of the drug containing a bioadhesive layer and a backing layer are shown in Tables 6 and 7, respectively. The backing layer was prepared by dissolving water-insoluble polymer in the solvent; DBP (plasticizer) was added and stirred for 15 min. Then, the solution (30 g) was poured onto a glass plate ($12.6 \times 12.8\ cm^2$) and allowed to dry at room temperature for 3 h. This backing layer was used as a base for casting a drug-containing film. Drug was added to the polymeric solution of bioadhesive polymers and the films were prepared by the process mentioned for placebo films.

4.5. Evaluation of buccal bioadhesive films

4.5.1. Physical parameters

Peelability determines the ease with which a film can be removed or recovered from the plate successfully. It was done manually by peeling the film out of the plate without being torn off from any end. Softness of the film was judged by folding film once lengthwise and again widthwise. The edge of the film after folding should be soft to finger touch. Flexibility is an important property of films as a film should be sufficiently flexible such that processing and handling becomes easy. The film was held between hands and stretched. A good film is such that it should not be torn off with the applied pressure and should come back to its original position when the pressure is released.

4.5.2. Swelling studies

The swelling behaviour of the bioadhesive film formulation was studied for a period of 4 h (intended time of dosage form application) using an agar plate method. A 1.4% w/w agar gel was prepared by dissolving agar

into boiling water, cooled and poured onto a petri dish. The gel was allowed to air dry and maintained at 37 °C. Twelve accurately weighed bioadhesive bilayered films (2×2 cm^2) were placed on agar gel by dividing them into four groups each with three films. The plates were incubated at 37 °C and were carefully removed after a specified time (30, 60, 120, 240 min) and placed on tissue paper for 30 s to remove excess water and the films were weighed. The percentage water absorbed was calculated at each point using the formula:

$$\% \text{ Water absorbed} = \frac{\text{Final Weight} - \text{Initial Weight}}{\text{Initial weight}} \times 100$$

4.5.3. Bioadhesive performance and tensile strength

The bioadhesive strength of film was determined by a method based on the tensile strength measurement. The force required to separate the film from a cellophane membrane was recorded using a commercial texture analyzer with a 5 kg load cell (TA-XT2i, Stable Micro Systems, UK) (Remunan-Lopez et al. 1998; Peh and Wong 1999; Wong et al. 1999; Eouani et al. 2001). A wetted cellophane membrane was placed in the cavity of the mucoadhesive rig (tissue/membrane holder) and 200 µL of the 2% w/w mucin gel was added into the cavity of the tissue holder. A film was cut and attached to 10 mm cylindrical metallic probe using a synthetic cyanoacrylate adhesive. Contact between mucin layer and film was established by lowering the probe at a rate of 0.3 mm/s. After a preload of 0.5 N for 300 s, the film was pulled apart with a constant extension rate of 0.1 mm/s up to a distance of 15 mm. A force versus time diagram was recorded. The bioadhesive force was recorded as the maximum force required for breaking the adhesion between membrane and film.
However, tensile strength of films was evaluated on the commercial texture analyzer using a 50 kg load cell. The films were cut into area 2×2 cm^2 and were placed between two clamps of the assembly positioned at a distance of 5 mm. During measurement, the strips were pulled by the top clamp at a rate of 1 mm/s to a distance of 50 mm before returning to the starting point. The force and area were measured when the film ($n = 5$) broke.

4.5.4. In vitro release of drugs

A USP XXIV type II dissolution apparatus (rotating paddle) was modified for *in vitro* dissolution studies of buccal bioadhesive films (BBF). The paddles of smaller size were fabricated. Dissolution was carried out using 250 ml tall form beakers which were placed in dissolution vessels containing 700 ml of distilled water. Phosphate buffer I.P (100 ml) was added as the dissolution medium into the inner beakers. Bilayered films were affixed on small glass plates using double adhesive tape, with the drug layer facing towards the dissolution medium and the edges of film were sealed by high vacuum grease and dissolution was carried out at 37 ± 0.5 °C at 50 rpm. Samples (5 ml) were withdrawn at intervals of 15, 30, 60, 120, and 240 min. The samples were filtered through 0.45 µm nylon filters and analyzed using in house HPLC method (Tambwekar et al. 2003).

4.5.5. Content uniformity

An individual film was cut and transferred into 100 ml volumetric flask and methanol (HPLC grade, 50 ml) was added into the volumetric flask and sonicated for 30 min. The volume was adjusted to 100 ml with 10 mM phosphate buffer (pH 6.8) and the solution was filtered through 0.45 µm nylon filter and analyzed with an in house chromatographic method in triplicate (Tambwekar et al. 2003).

4.5.6. Water vapour permeability

Preweighed films were pasted onto the mouth of glass vials filled with anhydrous calcium chloride (3 g) of same size and type (30 ± 0.5 ml) by means of cyanoacrylate adhesive. Films were exposed to 9.07 cm^2 surface area of the glass vial. The initial weight of the individual films, vials, and desiccant added in each vial was recorded and kept in a desiccator maintained at $75 \pm 3\%$ RH using a saturated solution of sodium chloride at a temperature of 25 ± 3 °C. The final weight of the container containing desiccant and film was recorded at time intervals of 30, 60, 120, 240 min. The plot of percentage moisture permeated vs time was plotted (Khan et al. 2000; USP24/NF18 2000). The following equation was employed to calculate the rate of moisture permeability:

$$\text{Rate of moisture permeability (mg/min/ml)} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight} \times \text{T} \times \text{V}} \times 100$$

where V is the volume (ml) of the container, and T is the time point at which the sample was withdrawn.

4.5.7. Thickness

Thickness of the film was measured at 5 points, four at the corners and one at the centre of the film using a thickness gauge, and average thickness was calculated.

4.5.8. Accelerated stability studies

The final optimized formulation (N_2) was put on accelerated stability studies in alu-alu packaging material at 40 °C/75% RH for 3 months and samples were analyzed for 15 days for thickness variation, weight uniformity, content uniformity, dissolution studies, tensile strength *and in vitro* bioadhesive strength measurement.

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References

Bhaichwal MR (1985) Advances in drug delivery systems. Bombay, M.S.R foundation.
Bruschi ML, de Freitas O (2005) Oral bioadhesive drug delivery systems. Drug Dev Ind Pharm 31: 293–310.
Corelli RL, Hudmon KS (2002) Medications for smoking cessation West J. Med. 176: 131–135.
deVries ME, Bodde HE (1991) Developments in buccal drug delivery. Crit Rev Therap Drug Carrier Syst 8: 271–303.
Duchene D, Touchard F et al. (1988) Pharmaceutical and medical aspects of bioadhesive systems for drug administration. Drug Dev Ind Pharm 14: 283–318.
Eouani C, Piccerelle P (2001) *In-vitro* comparative study of buccal mucoadhesive performance of different polymeric films.Eur J Pharm Biopharm 52: 45–55.
Gao P, Meury RH (1996) Swelling of hydroxypropyl methylcellulose matrix tablets. 1. Characterization of swelling using a novel optical imaging method. J Pharm Sci 85: 725–731.
Gao P, Skoug JW (1996) Swelling of hydroxypropyl methylcellulose matrix tablets. 2. Mechanistic study of the influence of formulation variables on matrix performance and drug release. J Pharm Sci 85: 732–740.
Gupta A, Garg S (1992) Mucoadhesive drug delivery systems: A review. Indian Drugs 29: 586–593.
Hao J, Heng PW (2003) Buccal delivery systems Drug Dev Ind Pharm 29: 821–832.
Ikinci G, Senel S (2004) Development of a buccal bioadhesive nicotine tablet formulation for smoking cessation Int J Pharm 277: 173–178.
Ikinci G, Senel S (2006) Development and *in vitro/in vivo* evaluations of bioadhesive buccal tablets for nicotine replacement therapy Pharmazie 61: 203–207.
Kavanagh N, Corrigan OI (2004) Swelling and erosion properties of hydroxypropylmethylcellulose (Hypromellose) matrices – influence of agitation rate and dissolution medium composition. Int J Pharm 279: 141–152.
Khan TA, Peh KK (2000) Mechanical, bioadhesive strength and biological evaluation of chitosan films for wound dressing. J Pharm Pharmaceut Sci 3: 303–311.
Nafee NA, Ismail FA (2004) Mucoadhesive delivery systems. I. Evaluation of mucoadhesive polymers for buccal tablet formulation. Drug Dev Ind Pharm 30: 985–993.
Panchagnula R, Jain AK (2000) Nicotine transdermal systems: pharmaceutical and clinical aspects. Methods Find Exp Clin Pharmacol 22: 299–308.
Park CR, Munday DL (2002) Development and evaluation of a biphasic buccal adhesive tablet for nicotine replacement therapy. Int J Pharm 237: 215–226.
Park CR, Munday DL (2004) Evaluation of selected polysaccharide excipients in buccoadhesive tablets for sustained release of nicotine. Drug Dev Ind Pharm 30: 609–617.
Peh KK, Wong CF (1999) Polymeric films as vehicle for buccal delivery: swelling, mechanical, and bioadhesive properties. J Pharm Pharm Sci 2: 53–61.
Perioli L, Ambrogi V (2004) Development of mucoadhesive patches for buccal administration of ibuprofen. J Control Release 99: 73–82.
Remunan-Lopez C, Portero A (1998) Design and evaluation of chitosan/ethylcellulose mucoadhesive bilayered devices for buccal drug delivery. J. Control Release 55: 143–152.
Reynolds TD, Gehrke SH (1998) Polymer erosion and drug release characterization of hydroxypropyl methylcellulose matrices. J Pharm Sci 87: 1115–1123.
Rigotti NA (2002) Treatment of tobacco use and dependence. N Eng J Med 346: 506–512.
Rowe RC, Sheskey PJ (2004) Handbook of pharmaceutical excipients, Pharmaceutical Press.
Sharma P, Hamsa V (2001) Formulation and evaluation of buccal mucoadhesive patches of terbutaline sulphate. STP Pharm Sci. 11: 275–281.
Siepmann J, Kranz H (1999a) HPMC-matrices for controlled drug delivery: a new model combining diffusion, swelling, and dissolution mechanisms and predicting the release kinetics. Pharm Res 16: 1748–1756.
Siepmann J, Podual K (1999b) A new model describing the swelling and drug release kinetics from hydroxypropyl methylcellulose tablets. J Pharm Sci 88: 65–72.

Smart JD (1993) Drug delivery using buccal adhesive systems. Adv. Drug Deliv Rev 11: 253–270.
Sweeney CT, Fart RV (2001) Combination nicotine replacement therapy for smoking cessation, rationale, efficacy and tolerability. CNS Drugs 15: 453–467.
Tambwekar, KR, Kakariya RB (2003) A validated reverse phase HPLC method for analysis of nicotine from bulk and formulations. J Pharm Biomed Anal 32: 441–450.
USP24/NF18 (2000) United States Pharmacoepia Convention. Rockville, MD: 1937–1939.
Wong CF, Yuen KH (1999) An in vitro method for buccal adhesion studies: importance of instrument variables. Int J Pharm 180: 47–57.
Wood AJJ (1995) Nicotine medications for smoking cessation. N Eng J Med 338: 1196–1203.