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Design and development of a mucoadhesive buccal film bearing progesterone

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The purpose of this research was to develop and evaluate mucoadhesive films for buccal administration of progesterone using film-forming and mucoadhesive polymers. Buccal films of chitosan bearing progesterone were prepared by solvent casting technique. The films have been evaluated in terms of film weight, thickness, density, surface pH, FT-IR, X-ray diffraction analysis, bioadhesion, swelling properties, *in vitro* drug release and *in vivo* studies. It was found that the film formulations of 2 cm^2 size having weight in the range of 239 ± 0.32 to 290 ± 3.23 mg and film thickness were in the range of 0.49 ± 0.21 to 0.60 ± 0.26 mm. Density of the films was found to be 0.108 to 0.139 g/mL. Drug content was found to be uniform in a range of 9.21 ± 0.051 to 9.67 ± 0.086 mg/cm^2 for formulation F_1 to F_4. Maximum bioadhesion force was recorded for PVP buccal films (formulation F_2) i.e. 0.45 ± 0.53 N as compared to other films. *In vitro* residence time was in range of 1.85 ± 0.08 to 8.94 ± 0.08 h. The drug release studies revealed that formulations follows non-fickian diffusion. *In vivo* residence time data confirmed that none of the polymers detached from the oral mucosa over the study period, which indicated that the bioadhesion values of all polymers were satisfactory to retain the film on the buccal mucosa. These mucoadhesive formulations could offer many advantages in comparison to traditional treatments and their efficacy as an effective contraception is assessed.

1. Introduction

The pharmacological approach to fertility control is mainly by oral administration of steroids although controlled release systems such as Progestasert and Norplant, which deliver progesterone or levonorgestrel from non-biodegradable polymer matrices, have met a reasonable degree of clinical success. Recently, various bioadhesive mucosal dosage forms including adhesive tablets, vaginal gels (Columbia Laboratories Inc.), progesterone troches and recently, films have been developed (Lee et al. 2000; Peh and Wong 1999). Buccal films are superior to tablets or pills for contraceptive delivery since they avoid first pass metabolism. In this study, mucoadhesive films were developed by using the casting method (Wong et al. 1999). For this purpose, film-forming polymer such as polyvinylpyrrolidone (PVP), and a mucoadhesive polymer, as chitosan were used and evaluated for various physicochemical and mechanical properties.

Many researchers have tried to deliver a drug topically through the buccal cavity including antimicrobials (Senel et al. 2000; Jones et al. 2000), topical corticosteroids (Shin et al. 2000), and polypeptides (Langoth et al. 2000). But to our knowledge no one has tried to deliver an antifertility drug through buccal mucosa using natural polysaccharides so far.

There is considerable literature on the use of synthetic biodegradable polymers as carriers for anti-fertility steroids (Han et al. 2000; Vaugelade et al. 2001), but natural polymers such as polysaccharides have received less attention. Chitosan was chosen as drug carrier matrix, since it is known to act as a mucoadhesive biopolymer for mucosal drug delivery systems (Bernkop-Schnürch 2000; Senel et al. 2000). Its favorable biological properties such as non-toxicity, biocompatibility and biodegradability make chitosan a promising candidate for a safe buccal drug delivery system (Senel and Hincal 2001). In particular, chitosan showed penetration enhancement properties towards either monostratified or pluristratified epithelia. In particular, it is able to enhance absorption of drug molecules through intestinal (Artursson et al. 1994) and nasal mucosae (monostratified and endowed with tight junctions) (Hamman et al. 2002), drug permeation across buccal (Okamoto et al. 2001; Rossi et al. 2003a) and vaginal mucosae (pluristratified and lacking tight junctions) (Rossi et al. 2003b). In those epithelia that are rich in tight junctions, the mechanism of penetration enhancement by chitosan is mainly due to a transient widening of the junctions between cells (Dodane et al. 1999), while the mechanism of penetration enhancement across pluristratified epithelia, which lack tight junctions, has still to be clarified (Okamoto et al. 2001; Senel and Hincal 2001).

The present work deals with the preparation and characterization of mucoadhesive polymeric films containing progesterone for buccal delivery. The films were further tested for *in vitro* and *in vivo* behavior.

2. Investigations, results and discussion

The progesterone delivery system was designed and developed using the biodegradable chitosan buccal films. A potential formulation problem was anticipated since chitosan is only soluble in aqueous acidic solutions, whereas progesterone, being a hydrophobic drug, is insoluble under similar conditions. In early stages of formula optimization studies, it was observed that progesterone was not incorporated into the film but crystallized out as needles owing to lack of solubilization in aqueous chitosan solutions. Hence, initial attempts were directed toward dissolving the progesterone in minimum quantity of the methanol and mixed well with the polymer solution.

It was found that the films F_1 to F_4 of 2 cm^2 size were having weight in the range of 239 ± 0.32 to 290 ± 3.23 mg and films thickness was in the range of 0.46 ± 0.21 to 0.65 ± 0.26 mm. Density of the films was in the range of 0.108 to 0.139 g/ml (Table).

The surface pH of the film was found to be in the range of 6.0 to 7.0 for formulations F_1 to F_4. This film pH is closed to the physiological pH of the buccal mucosa. Hence, these films may not cause any irritation to the buccal mucosa after its application (Table).

The buccal films were observed under the scanning electron microscope to see its surface morphology after keeping the films in dissolution media for 0, 30, 60, 90, and 120 min. It was observed that initially the buccal films were intact with regular surface and shiny drug crystals were uniformly distributed on the films as compared to blank chitosan film (Fig. 1a, b). This superficial drug crystals could be one of the reasons for initial faster drug release from the films. On keeping the films in the dissolution medium, the drug starts dissolving and polymer starts eroding that could be clearly seen in Fig. 1c which exhibits formation of cavities giving rise to irregular surface.

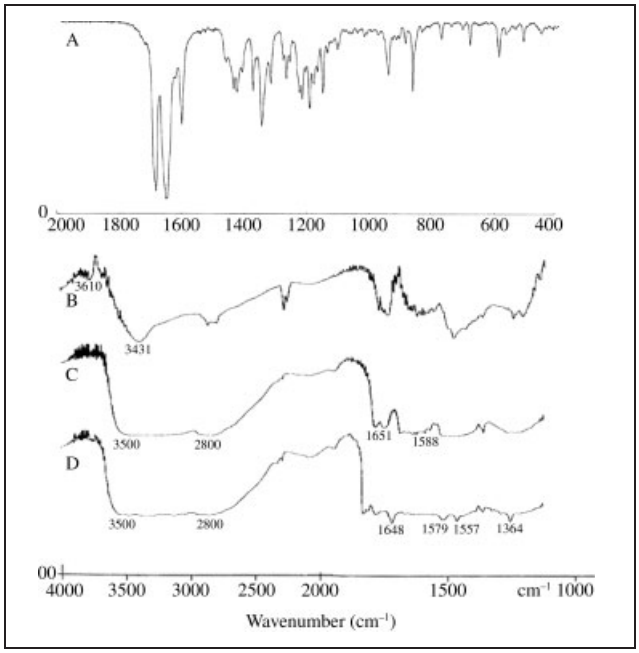

Fig. 2: FT-IR spectra of (A) progesterone, (B) chitosan, (C) chitosan film and (D) progesterone loaded chitosan film (F_2)

Infrared spectra of chitosan powder, chitosan blank film, progesterone chitosan film and progesterone were acquired to draw information on the molecular state of chitosan and progesterone (Fig. 2). Chitosan is an amino glucose characterized by a small proportion of amide groups via an amide linkage with acetic acid. In the IR spectrum, powder chitosan exhibited a broad peak at 3431 cm^{-1}, which is assigned to the N−H and hydrogen bonded O−H stretch vibrational frequencies, while a sharp (shoulder) peak at 3610 cm^{-1} is that of free O−H bond stretch of glucopyranose units. Further, in the C−H stretch region of FTIR spectrum, the higher intensity peak at 2923 cm^{-1} is assigned to the asymmetric and the lower intensity peak at 2857 cm^{-1} is assigned to the symmetric modes of CH_2. In addition, the characteristic band due to CH_2 scissoring, which usually occurs at 1465 cm^{-1} was also present in the

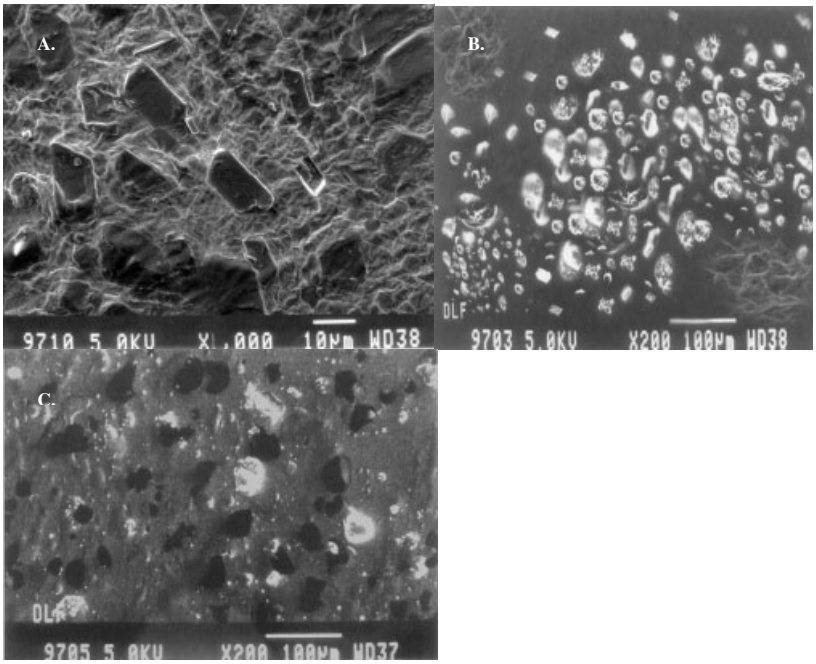

Fig. 1:
SEM photograph of blank chitosan film (A), drug loaded chitosan film after 0 min dissolution C. after 120 min dissolution

sample. Since the grade of chitosan used in the present study was ≥85% deacetylated, an amide bond peak was present in the spectra and the C=O stretch of amide bond was observed at 1661 cm^{-1}. The peaks at 1550 and 1599 cm^{-1} were assigned to strong N−H bending vibrations of secondary amide, which usually occur in the range of 1640 to 1550 cm^{-1} as strong band. In comparison to the chitosan powder, the spectrum was not sharp in film. An overlay of chitosan film and progesterone-chitosan film is shown in Fig. 2. The presence of residual moisture content and glycerol in films resulted in a broad peak from 3500 to 2800 cm^{-1}. The peak at 1651 cm^{-1}, representative of C=O stretch of amide bond in chitosan film, shifted to 1648 cm^{-1} in the presence of progesterone. Further, the peak due to N−H bending vibration, which was observed at 1588 cm^{-1} in chitosan film, was lowered to 1579 cm^{-1} in progesterone-chitosan film; its intensity also decreased. The band due to CH_2 scissoring, which occurred at 1466 cm^{-1}, was broadened due to progesterone in film. However, only two characteristic peaks of progesterone, at 1364 and 1557 cm^{-1}, were observed in progesterone-chitosan film, while other peaks were not discernible due to interference caused by polymers.

X-ray diffraction is a proven tool to study crystal lattice arrangements and yields very useful information on degree of sample crystallinity. X-ray diffraction pattern of progesterone, blank chitosan film, and progesterone loaded chitosan film were obtained and compared, which revealed marked differences in the molecular state of progesterone (Fig. 3). The diffractogram of blank chitosan film has shown two low intensity peaks at 22.1° and 25.3° 2θ with a characteristic broad hump in the range of 7° to 45° 2θ. This halo diffraction pattern (broad hump) is an indication of the predominantly amorphous form of chitosan in films (Fig. 3A). In the case of progesterone, the diffractogram exhibited peaks at the following 2θ values: 10.8°, 11.2°, 12.6°, 13.8°, 15.6°, 18.6° and 19.6° (Fig. 3B). Among these, the peak of highest intensity was located at 17.8° 2θ, and the peaks at 10.6° and 13.6° 2θ were broad. When the diffraction pattern of progesterone in chitosan film was compared with that of progesterone, the pattern differed to a large extent. Several high-angle diffraction peaks were observed in progesterone-chitosan film at the following 2θ values: 9.8°, 10.8°, 11.0°, 11.4°, 13.0°, 14.9°, 16.0°, 17.2°, 19.1°, 24.3°, 26.9° (Fig. 3C). The 17.2° 2θ peak had the highest intensity, and the hump in the baseline occurred from 7° to 45° 2θ, as observed for chitosan film.

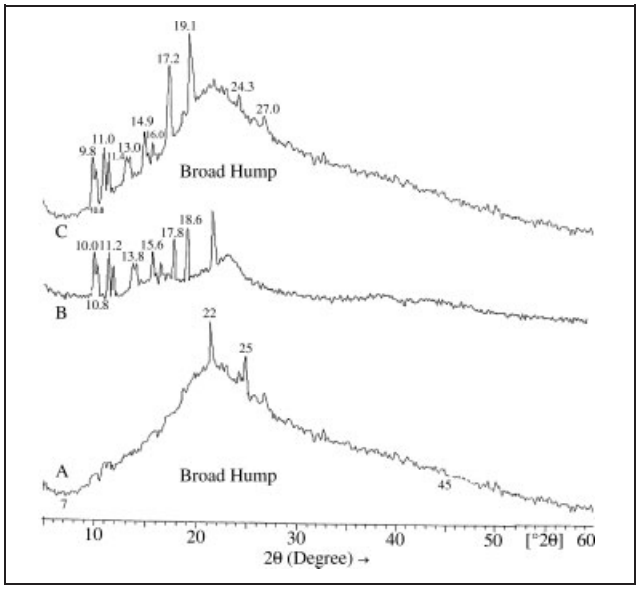

Fig. 3: X-Ray diffraction spectra of (A) chitosan film (B) progesterone and (C) progesterone loaded chitosan film (F_2)

Drug content was found to be uniform in a range of 9.21 ± 0.051 to 9.67 ± 0.086 mg/cm^2 for formulation F_1 to F_4.

Film mucoadhesion time varied from 1.5 to 5.5 h (Table). Formulation F_2 showed the highest adhesion time whereas the films from formulation F_1 showed the lowest mucoadhesion time. This difference may be due to employment of PVP which favors hydration and the outward diffusion of the drug from the film matrix. Another important factor to be considered is the kind of film forming polymer used for the film preparation and the homogeneity of the polymer solution mixtures. In fact, while chitosan polymers are water insoluble, PVP is water soluble and these characteristics influenced miscibility with the mucoadhesive polymer, the uniformity of the film as well as permeability to water. In spite of these differences, *ex vivo* mucoadhesion times were not drastically influenced by the polymer chemical and physical characteristics. These results are concordant with the results of Perioli et al. (2004). They employed HPMC and NaCMC in their buccal formulations and observed that mucoadhesion time in case of HPMC was higher than NaCMC.

Mucoadhesion force values were between 0.15 and 0.45 N (Table). It is observed that films with the highest mucoadhesion force was found to be in the formulation F_2 and then in the formulations F_4 and F_3. The reason behind high mucoadhesion force in F_2 rather than in F_3 and F_4 is that formulation F_2 contains higher percentage of chitosan along with PVP, which acts as co adjuvant by increasing the mucoadhesive properties of chitosan. The comparison of *ex vivo* mucoadhesion times and mucoadhesive forces revealed a good correlation between *ex vivo* data and force values of formulation F_2. This group, besides, having the longest *ex vivo* mucoadhesion times, also showed the highest mucoadhesive forces. Maximum bioadhesion was recorded for PVP buccal films (formulation F_2) i.e., 0.45 ± 0.53 N as compared to other films. Bioadhesion in different formulations was found to be in the order of $F_1 < F_3 < F_4 < F_2$. Our results are concordant with the results of the Nafee et al. (2003) who observed decreased bioadhesion on increasing PVP concentration while studying the mucoadhesive miconazole containing chitosan patches.

According to Henriksen et al. (1996), chitosan is a promising bioadhesive material at neutral or slightly alkaline pH, which is found to be advantageous for adsorption on the mucosal surface. It was suggested that, at this pH, chitosan exhibits numerous amine and hydroxyl groups that may increase the interaction of polymer with the negative mucin. The rheological interaction between chitosan and mucin, and/or hydrophillic additives and mucin produces strong force of attraction between polymer and mucus membrane and in turn influences mucoadhesive property of the films. The cross linking reduces the number of free amino groups for the binding to mucus membrane and it also influences the rheological interaction of polymer and mucin.

Assessment of the swelling behavior was done by measuring radial swelling. In the case of buccal films intended for local therapy, the contact area should be as large as possible, a requirement that must be balanced with patient compliance; excessive increase in patch diameter might cause discomfort and/or dislodgement of the swollen film.

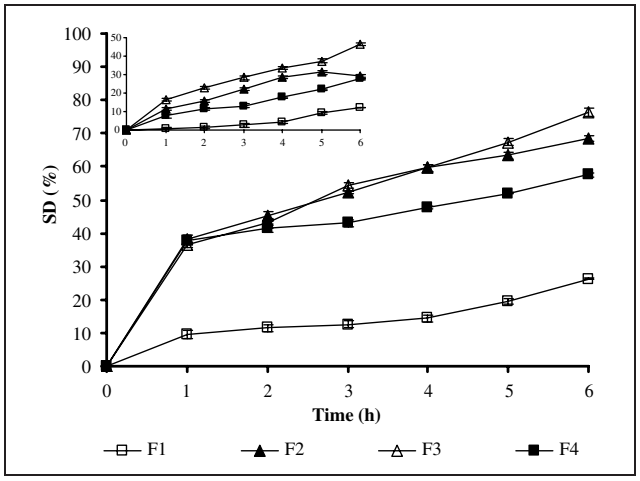

Fig. 4: The radial swelling profiles of mucoadhesive patches containing progesterone. The inset represents the radial swelling of plain patches (SD bars, n = 3)

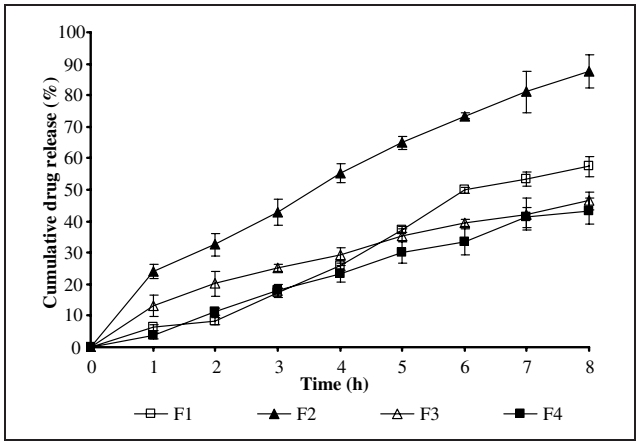

Fig. 5: The drug release from mucoadhesive patches containing progesterone (SD bars, n = 3)

The radial swelling index of different buccal films was determined and it was found that in general, the medicated patches had higher swelling values compared to plain patches (Fig. 4). It is observed that film F_3 is exhibiting maximum percent swelling ($76.36 \pm 0.78\%$) while film F_1 is showing only $26.21 \pm 0.25\%$ swelling. The percent enhancement in percent swelling could be due to the presence of PVP and gelatin in higher concentration i.e., 2 and 3%, respectively in formulation F_3. The water-soluble hydrophilic additive dissolves rapidly introducing porosity. The void volume is thus expected to be occupied by the external solvent diffusing into the film and thereby accelerating the dissolution of the gel (Samuelov et al. 1979). The weak aqueous solubility of the cationic polymer limited the swelling of the patches but the addition of certain amounts of the hydrophilic polymers such as PVP and gelatin increased surface wettability and consequently water penetration within the matrix.

Further, there was a little reduction in swellability ($57.51 \pm 0.53\%$) for film F_4, this could be due to crosslinking of the polymer with glutaraldehyde. Percent radial swelling in different formulations was found to be in the order of $F_1 < F_4 < F_2 < F_3$. Comparing the radial swelling of plain buccal films and those containing progesterone an increase in film swelling by the addition of drug was noted. Undoubtedly, the presence of drug would modify the way water is bound to or taken up by the polymer. Alteration in water distribution within such systems would thus modify the matrix structure (Langoth et al. 2005). The micronized drug particles may exist in between the polymer chains allowing each chain to hydrate freely, which may result in weak hydrogen bonding areas around the progesterone molecules. These areas may increase the strength of the swollen layer followed by an obvious increase in the amount of penetrated water (Artusi et al. 2003).

The drug release from the various prepared buccal films was studied using the USP dissolution apparatus. Chitosan-containing patches produced sustained release in formulation F_2 (Fig. 5). The minimum drug release was observed from the system (F_4) containing 5% w/v PVP where only 3.6% progesterone was released in the first hour and slowly progressed to 43% after 8 h. The subsequent increase in diffusional path length and low attrition may be responsible for the distinct low release profile.

The release profile of the drug from different formulations exhibits that the drug release from these films is following non-fickian diffusion as the value of diffusion release exponent (η) is in the range of 0.7 to 0.8. The value of (η) was calculated from the slope of the log % cumulative drug release vs. log time. The reason for the non-fickian diffusion of the drug from the buccal film could be due to the formation of solvent filled pore in the matrix and erosion of the polymeric matrix at pH 6.8. Therefore, non-fickian diffusion of the drug from these films could be a relative contribution of the polymer erosion as well as their diffusion from solvent filled pores. Hence, a straight line was obtained on plotting a graph between percent cumulative drug release versus $(t)^{0.75}$ and slope of this curve is the drug release rate of a product. The drug release rate is higher ($17.8\ \mu g/h^{0.75}/cm^2$) with the product F_1 and minimum ($10.8\ \mu g/h^{0.75}/cm^2$) with product F_4, as it does not contain anionic polymer i.e., PVP or gelatin. Therefore most probable reason for retardation of drug release is may be due to the charge interaction between drug and polymers (Nafee et al. 2003).

The *in vitro* drug permeation across the buccal mucosal membrane for different buccal formulations was assessed using the diffusion cell and it is observed that film F_2 exhibits maximum drug permeation while formulation F_4 shows minimum drug permeation due to cross linking of chitosan with glutaraldehyde.

Values of the *in vitro* residence time, as shown in the Table, differed from one formulation to the other. Chitosan films (F_1–F_4) remained attached to the membrane during the time of study (10 h) without erosion. However, the addition of PVP and gelatin to films F_3 and F_4 caused dislodgment within 4.3 and 1.8 h, respectively, without erosion. The presence of progesterone, a water insoluble drug, slightly may have affected the residence time of the film.

The *in vitro* residence time of the buccal film on the mucosal membrane was observed and it was noted that formulation F_2 remained on the mucosal membrane longer (8.94 ± 0.08 h) than formulation F_1 (5.05 ± 0.90 h). It could be due to the presence of higher concentration of chitosan polymer (2% in F_2), while the product F_4 exhibited minimum residence (1.85 ± 0.08 h) because the chitosan in this polymeric film was cross linked (Table). The water-soluble hydrophilic additive such as PVP dissolves rapidly introducing porosity. The addition of certain amounts of the hydrophilic polymer PVP increased surface wettability and consequently water penetration within the matrix. Additional shortening in the residence time was observed when a higher percentage of PVP was added to the

patches. The increase in water-soluble content promotes faster dissolution of the patch (Korsmeyer et al. 1983).

No correlation was found between the bioadhesion force and the residence time of the polymers. It seems that highly bioadhesive polymers do not necessarily reside longer on the mucosal surface. Surface charge density and chain flexibility are considered to be prerequisites for bioadhesion, whereas the residence time is primarily dependent on the dissolution rate of the polymer. However, as regards the *in vivo* residence time data (Table), none of the polymers detached from the oral mucosa over the study period, which indicated that the bioadhesion values of all polymers were satisfactory to retain the film on the buccal mucosa.

Comparing the *in vitro* and *in vivo* residence time of the tested buccal films, the higher values were obtained *in vitro* (Table). This may be due to the movements of the mouth when speaking, laughing, swallowing, representing a shearing force promoting faster erosion of the films despite the comparatively larger dissolution medium applied *in vitro* (Bottenberg et al. 1992).

In conclusion mucoadhesive chitosan films were able to deliver the drug at a controlled rate for 8 h. Chitosan-PVP buccal films showed improved uniform and effective progesterone levels *in vitro* and *in vivo*. Moreover, this mucoadhesive buccal film was found to be tolerable and comfortable because of its non-irritant and biocompatible nature. Results indicate that these buccal films are adequate for the systemic delivery of progesterone.

3. Experimental

3.1. Materials

Progesterone was procured from M/s. Sun Pharma, Vadodara, India, Chitosan (purified viscosity grade: 50 cps, molecular weight: 150 KDa, deacetylation degree: 85%) was procured as gift sample from M/s Panacea Biotech as a gift sample. Glutaraldehyde, PVP, Gelatin, Acetic acid was procured from HiMedia, Mumbai, India and other reagents were of analytical grade.

3.2. Method

Buccal films bearing progesterone were prepared by the solvent casting technique employing aluminum foil cups (10 mm in diameter-placed on glass surface) as substrate. Chitosan (1 g) was dissolved in 50 ml of 2% (v/v) acetic acid under constant stirring using a magnetic stirrer for 48 h. The resultant viscous solution was filtered through muslin cloth. The filtrate was left to stand until all air bubbles disappeared. Then, hydrophilic polymers (PVP and gelatin) were dissolved in small volume of distilled water (2 ml) and this solution was added to chitosan solution with stirring. Glutaraldehyde was added drop wise to this solution with constant stirring. Progesterone (1 g) was dissolved in minimum quantity of methanol (5 ml) and mixed well with the above polymer solution. This polymer mixture (20 ml) was poured on the aluminum foil placed on plain glass surface. This whole assembly was then placed in the oven and dried the film at 30 ± 2 °C. After drying, the films were carefully removed from the aluminum foil, checked for any imperfections or air bubbles and cut in to patches 2 cm^2 (2 cm × 1 cm) size. The films were packed in aluminum foil and stored in glass container maintained at room temperature.

Various formulation variables e.g. chitosan concentration (0–3%), gelatin concentration (0–5%), glutaraldehyde concentration (0–0.2%), which could affect the preparation and properties of the films were identified and their concentration was optimized (Table).

3.3. Characterization of buccal films

3.3.1. Film weight

Three buccal films of each formulation were taken, individually weighed and an average weight was determined.

3.3.2. Thickness

Thickness of buccal films was measured using a Screw gauge at different places of randomly selected films. The mean thickness of the buccal films was calculated.

3.3.3. Density

The density of the buccal films was calculated by using formula m/v where m and v are weight and volume of the films of size 2 cm^2. The volume of the film was calculated from its area multiplied by thickness.

3.3.4. Surface pH

Each film (2 cm^2) was left to swell for 2 h on the surface of an agar plate. Then surface pH was measured by mean of a pH paper placed on the surface of the swollen film. The color developed was compared with the standard color scale.

3.3.5. Surface morphology

The films were observed under scanning electron microscope before and after keeping the film in phosphate buffer saline (pH 6.8) for 0, 30, 60, 90 and 120 min. The dried films were coated under argon atmosphere with gold-palladium to achieve a film of 10 nm thicknesses and then observed under a scanning electron microscope.

3.3.6. Fourier transform infrared spectrum analysis

The progesterone loaded chitosan film and blank film was analyzed by FT-IR spectroscopy to confirm loading of the drug in the film. The polymer samples were crushed with KBr to make pellets. Spectra were taken on a FTIR Perkin Elmer (Pyrogon 1000) and scanned between 400–4000 cm^{-1}.

Table: Characteristics of plain and drug loaded buccal films of optimized formulation

Composition/Characteristics	Formulations			
	F1	F2	F3	F4
Progesterone (g)	1	1	1	1
Chitosan (% w/v)	1	2	1	1
Glutaraldehyde (0.2% w/v) ml	0	0	0	2
Polyvinyl pyrrolidone K-30 (% w/v)	0	1	2	5
Gelatin (% w/v)	0	1	3	3
Film thickness (mm)*	0.49 ± 0.21	0.53 ± 0.41	0.54 ± 0.32	0.60 ± 0.26
Density (g/mL)	0.139	0.136	0.110	0.108
Film mass (mg)	257 ± 0.67	290 ± 3.23	239 ± 0.32	283 ± 3.3
Surface pH	7.0	7.0	7.0	6.0
Bioadhesion time (h)	1.5	5.5	3.7	4.9
Bioadhesion force (N)*	0.15 ± 0.23	0.45 ± 0.53	0.24 ± 0.15	0.37 ± 0.89
Residence time (h): *in vitro**	5.05 ± 0.90	8.94 ± 0.08†	4.37 ± 0.43	1.85 ± 0.08
: *in vivo**	3.10 ± 0.20	7.85 ± 0.16†	4.20 ± 0.18	1.90 ± 0.40
Drug content uniformity (mg/cm^2)	9.40 ± 0.06	9.67 ± 0.09	9.33 ± 0.06	9.21 ± 0.05
Drug permeation through buccal mucosa*‡	34.12 ± 1.78	52.23 ± 1.87	42.86 ± 1.23	23.24 ± 0.53

* Mean values ± SE; n = 6 (for patch thickness) and n = 10 (for film mass)
† The patches showed no erosion, disintegration, or detachment during the study
‡ Cumulative percent drug permeated after 7 hrs

3.3.7. X-Ray Diffraction Analysis

The determination of the drug dispersion state in the buccal films was performed by the X-ray diffraction method. The X-ray diffraction patterns were obtained with a powder diffractometer, RU-200 B (M/s Riqaku, Japan). Progesterone-loaded chitosan films (F_2) and blank (without drug) chitosan films have been studied for X-Ray diffraction analysis. The scanning range of 2θ was 10–60°.

3.3.8. Drug content uniformity

Three films (2 cm^2 size each) of each formulation were weighed accurately and transferred into a separate 100 ml volumetric flask, containing 100 ml of PBS (pH 6.8) containing methanol (10%v/v). The contents of the flask were stirred constantly for 24 h using a magnetic stirrer. The solution was filtered and analyzed for drug content at 247 nm with an UV-Spectrophotometer (Cintra 10, Japan). The average of three observations was recorded.

3.3.9. Bioadhesive time and force

The *ex vivo* mucoadhesion time was performed (n = 6) after application of the films on freshly cut porcine buccal mucosa. The porcine buccal mucosa was fixed on the internal side of a beaker with cyanoacrylate glue. Each film was divided in portions of 2 cm^2 and cut, a side of each film was wetted with 50 µl of simulated saliva fluid (SSF) and was pasted to the porcine buccal mucosa by applying a light force with the finger tip for 20 s. The beaker was filled with 800 ml of the simulated saliva fluid and was kept at 37 °C. After 2 min, a 100 rpm stirring rate was applied to simulate the buccal cavity environment and film adhesion was monitored during 8 h (Han et al. 1999).
Ex vivo adhesion strength was assessed by a dynamometer (Eouani et al. 2001) using the above cited porcine buccal mucosa. For mucoadhesive measurements, films were cut in portions of 2 cm^2 and pasted on a support, connected to the dynamometer with cyanoacrylate glue. A piece of buccal mucosa was glued on a support and kept in a vessel placed in a thermostatic bath at 37 °C (±0.2). The free side of the film was wetted with 50 µl of simulated saliva fluid and pasted to the porcine buccal mucosa by applying a light force with the finger tip for 20 s. The vessel was filled with SSF at 37 °C and the measurement was started after 2 min. The maximum adhesive force is the average of three measurements (n = 6) and confidence intervals were also determined at 0.01 significance level.

3.3.10. Swelling

Three films were tested for each formulation. The diameter of the original buccal films was determined. Three films of each formulation were allowed to swell on the surface of agar plate kept in an incubator maintained at 37 °C. Measurement of the diameter of the swollen film was done at one-hour intervals for 6 h. The percent radial swelling (S_D %) was calculated using following equation:

$$S_D(\%) = \frac{[Dt - Do]}{Do} \times 100 \cdots \quad (1)$$

where S_D (%) is the percent swelling obtained by the diameter method, D_t is the diameter of the swollen film after time t, D_o is the initial diameter of film at time zero.

3.3.11. Drug release from buccal films

The USP dissolution apparatus (Type-1) was used throughout the study. A portion of 2 cm^2 (2 cm × 1 cm) of film was used. One film of each formulation was fixed to the central shaft using an acrylate adhesive. The dissolution medium consisted of 900 ml of SSF (2.38 g Na_2HPO_4, 0.19 g KH_2PO_4 and 8.00 g NaCl per liter of distilled water adjusted with phosphoric acid to pH 6.75 and containing 40% v/v PEG-400). The release study was carried out at 37 ± 0.5 °C with a rotation speed of 50 rpm. The release study was performed for 8 h. After every hour, a 3 ml sample was withdrawn from each station and immediately replaced with fresh media. The withdrawn samples were filtered; 2 ml of the filtrate were diluted to 10 ml using SSF (pH 6.75). The samples were analyzed spectrophotometrically at 247 nm.

3.3.12. In vitro drug permeation through buccal mucosa

Buccal tissue (cheek) of pigs weighing 35–70 kg was purchased from a slaughter house (Sagar, India). After removal, the tissue was placed in cold Krebs buffer (pH 7.4) and immediately transported to the laboratory. The buccal mucosa, with a part of sub-mucosa, was carefully separated from fat and muscles using a scalpel. Then, the epithelium was isolated from the underlying tissue. The thickness of samples was about 500 µm. Because of the time dependent viability decline, the buccal epithelium was used within 2 h upon removal.
In vitro drug permeation from different formulations was performed using a Franz diffusion cell (Jain et al. 2005) using porcine buccal mucosa. The SSS (pH 6.75) containing 40% v/v PEG-400 was used as the receptor medium in the diffusion cell. The mucosa was sandwiched between the receptor compartment and donor compartment so that the buccal portion was continuously bathed with the receptor fluid maintained at 37 ± 1 °C by circulating water bath exposed to ambient temperature. The content of the receptor fluid was stirred continuously by a magnetic stirrer. Samples were withdrawn at different time intervals, replaced with the same volume of fresh solution, filtered, and the amount of drug was determined spectrophotometrically at 247 nm (Cintra 10 UV-visible spectrophotometer).

3.3.13. Residence time

The *in vitro* residence time was determined using a laboratory designed apparatus for analysis of *in vitro* residence time. The vessel of test apparatus was filled with 800 ml PBS (pH 6.8) maintained at 37 ± 2 °C. The segment (3 cm) of porcine buccal mucosa was glued (using cyanoacrylate adhesive) to the surface of a glass slab vertically attached to the apparatus. Six mucoadhesive films of each formulation were hydrated from one surface using PBS (pH 6.8) and then the hydrated surface was brought into contact with the mucosal membrane. The glass slab was vertically fixed to the apparatus and allowed to move up and down in such a way that the film was completely immersed in the buffer solution at the lowest point and at the highest point. The time required for complete erosion or detachment of the film from the mucosal surface was recorded. The *in vitro* residence time of different formulations (Table) are 1 compared with plain patch.
Four healthy human volunteers (2 males and 2 females, 25-30 years old) agreed to participate in the *in vivo* test study after signing informed consents. The study was conducted in accordance to the Declaration of Helsinki guidelines. The experiment was carried out with plain films only. The bioadhesive film was placed on the buccal mucosa by applying a light force between the cheek and the gingiva in the region of upper canine and gently pressed onto the mucosa for about 30 s. The patch 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 (5 h). They were asked to monitor the ease with which the system was retained on to the mucosa and note any tendency of detachment. The adhesion time was indicated by either complete erosion of the patch or failure of the adhesive bond. Any complaints and bad feeling were also recorded. Repeated application of the bioadhesive films by same volunteer was allowed after a two-day period.

3.4. Statistical analysis

Data are expressed as the mean ± standard deviation (SD) of the mean. The significance of differences was evaluated by analysis of variance (ANOVA) and differences were considered statistically significant at $P < 0.01$.

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