DEVELOPMENT AND EVALUATION OF POLYMERIC CHITOSAN COMPOSITES MUCOADHESIVE FILMS CONTAINING TRIAMCINOLONE ACETONIDE FOR TREATMENT OF ORAL DISEASE

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Abstract:
The rapid beginning of action, high blood level, avoidance of the first-pass effect, and exposure of the medication to the gastrointestinal tract have lately sparked interest in transmucosal drug delivery (TMD) systems using mucoadhesive polymer. In the presence of chitosan, a brand-new mucoadhesive polymer complex for the TMD system was created using hydroxyl propyl methyl cellulose (HPMC) and chitosan. The film made of the chitosan/HPMC polymer complex contained triamcinolone acetonide (TAA). Without interacting with the polymer complex, TAA was uniformly distributed throughout the chitosan/HPMC polymer complex film. Time, pH, drug loading quantity, and the ratio of chitosan to HPMC all influenced the release behaviour of TAA from the mucoadhesive polymer film. TAA may be released from the chitosan/HPMC polymer complex film, according to the examination of the drug release from the mucoadhesive film.

Keywords: Triamcinolone acetonide, Film, Oral disease, Treatment, Chitosan, HPMC, etc.

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Introduction

For biomedical and medication delivery applications, bioadhesive polymers are crucial. The usage of polymer carriers in these applications should have strong adherence and close contact with the tissue. Hard and soft tissue applications both use bioadhesive polymers [1, 2]. Drug delivery technologies that are mucoadhesive and bioadhesive can also be used. Because mucoadhesive methods extend the period that the drug is in touch with the biological substrate, enhancing drug absorption, they are preferable to conventional drug delivery systems. Greater bioavailability is achieved using mucoadhesive systems that can stick to body locations [3-5]. Currently, release techniques for buccal, nasal, ocular, and vaginal mucoadhesive systems are available [2,6,7]. When compared to other methods, the buccal route appears to have several benefits, including a quick beginning of action, high blood levels, the avoidance of the first-pass effect, and exposure of the drug to the gastrointestinal system [8]. Adhesive tablets [9, 10], gels [11, 12], ointments [13], patches [14, 15], and more recently films [16] have all been created as bioadhesive mucosal dosage forms.

Although polymeric films have been widely used in pharmaceutical tablet coating formulations to protect tablet cores from environmental extremes, improve appearance, cover unpleasant tastes, and regulate drug release, their use for transmucosal drug delivery (TMD) systems has not yet been thoroughly investigated. In terms of comfort and flexibility, buccal film may be preferred to sticky tablets. Additionally, they can avoid the mucosa's relatively brief length of residence for oral gels, which is readily washed away and eliminated by saliva [14,18]. Additionally, the buccal film can shield the wound's surface, which lessens pain and might help to cure oral disorders more successfully.

A (1,4) connected 2-amino-2-deoxy-b-d-glucan called chitosan can be made by N-deacetylating chitin. Natural polysaccharides are advantageous for potential therapeutic usage since they are non-toxic, highly biocompatible, and non-antigenic [19]. Chitosan's ability to act as a mucoadhesive and facilitate macromolecule penetration through the nasal and intestinal barriers has also been demonstrated more recently [20].

Triamcinolone acetonide (TAA) was loaded into the chitosan/HPMC polymer complex film in this work, and the release of TAA from the mucoadhesive polymer film was measured in relation to time, pH, the weight percentage of the loaded medication, and the chitosan/HPMC ratio. TAA is commonly used to treat oral diseases by reducing inflammation [21].

Material and Methods

Formulation Development

Selection of best polymer composite

The concentration of chitosan and blending polymers is important for the formulation of film. As per literature review concentration of all film forming ingredients will be select.

Procurement of drug and polymers

Triamcinolone acetonide was obtained from Ralington pharma LLP India.

Chitosan was purchased from Pure Chem Pvt. Ltd. (Ankleshwar).

HPMC was obtained as a gift sample from Ajanta Pharma, Aurangabad, all other chemicals were purchased from Merck Ltd. (Mumbai).

Pre-formulation studies

Physicochemical behavior of a medicine is understood through pre-formulation studies or preliminary investigations, which produce the supporting data. Modifications are then made in order to design, develop, and test the dosage form. The following tests were run: TLC, UV max, calibration curve, and FTIR excipient compatibility.

Preparation of Triamcinolone acetonide buccal films [22-30]

Using a statistical programme (Stat graphics Plus, version 5) and a full factorial design with two factors, three levels (3^2), triamcinolone acetonide-loaded buccal film formulations were optimised. The concentrations of chitosan (X_1) and HPMC (X_2) were employed in three levels, as shown in the following table, and were regarded as independent factors.

Table: Variables in 3^2 full factorial design of Triamcinolone acetonide buccal film formulations

Independent variable, factor	Low (−1)	Middle (0)	High (1)
X1: Chitosan MMW, %	0.5	1.0	1.5
X2: HPMC, %			
Dependent variable, response	1.0	3.0	5.0
Y1: Extension at break load (mm)			
Y2: Film swelling (%)			
Y3: In vitro bio adhesion (N)			
Y4: Higuchi diffusion slope (%/ t 0.5)			

To assess the impact of the independent parameters on film extension at break load (mm; Y_1), film swelling (% Y_2), in vitro bio adhesion capacity (% Y_3), and Higuchi diffusion slope (%/t 0.5; Y_4), statistical models with main, quadratic, and interaction modes were produced. The following table provides an illustration of the composition of the buccal films F1–F9 produced in accordance with the experimental design.

Table: Composition of Triamcinolone acetonide buccal film formulations

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Triamcinolone acetonide (mg/cm2)	2 mg/cm^2 of the casted film								
Chitosan MMW (%) in casting solution	1.0	0.5	1.5	1.0	1.5	1.5	1.0	0.5	0.5
HPMC (%) in casting solution	3.0	1.0	5.0	5.0	1.0	3.0	1.0	3.0	5.0
Propylene glycol (%) in casting solution	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0

The formula weights of HPMC (a film-forming polymer) and chitosan (a bioadhesive polymer) were distributed in 21 ml of an aqueous solution containing 1.5% acetic acid to create buccal films. A clear viscous solution was created by slowly stirring the polymeric dispersion with a magnetic stirrer. Triamcinolone acetonide, whose formula weight was used, was dissolved in 10 ml of an aqueous solution containing 1.5% acetic acid, 1.25 ml of propylene glycol (a plasticizer), 5 ml of 100% ethanol, and 4-5 drops of triethanolamine. The polymeric solution and medication solution were combined gradually. To allow the air bubbles to be removed, the dispersion was left overnight. Films were cast into Teflon plates with a 6.3 cm diameter and dried for 12 hours in a 40 °C oven. For further analysis, the dried buccal films were divided into 1 × 1 cm pieces, wrapped in aluminium foil, and kept in a desiccator.

Triamcinolone acetonide content

One cm^2 of the produced buccal films was dispersed in 100 mL of methanol to determine their drug content. After being sonicated for 15 minutes, filtered, and measured spectrophotometrically at a wavelength of no greater than 240 nm, the dispersion.

Film thickness

Using a hand-held electronic digital calliper, the thickness of film strips (n = 3) with dimensions of 20 × 10 mm was measured. An average result was then reported. Three patches (in duplicate) from three separate positions on each film, including the middle and two other positions, were taken.

Swelling study

Each 1 × 1 cm patch of buccal film was weighed separately (W1) before being placed on the surface of 1% (w/v) agar gel plates that had been made with simulated saliva and incubated at 37 ± 0.5 °C. The samples were taken out of the petri plate after 7 hours, and any extra surface water was carefully blotted away with blotting paper. The swollen film was then reweighed (W2), and the formula given in the following equation was used to compute the swelling percentage:

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

Where, W1 is the initial weight of the film and W2 is its final weight after 7 h. For each observation, the test was repeated three times.

Mechanical properties

An Instron universal testing equipment with a 5 kg load cell was used to study the mechanical characteristics of various produced films at ambient temperature. The rectangular filmstrips' thicknesses (1 × 2 cm) were first measured, and average results were noted. The films were then secured between the machine's two grip fixtures. When the films broke, the upper grip was pulled back at a rate of 20 mm per minute, and the force and elongation were measured. Tensile strength (TS) and extension at break load calculations were made using specialised software as part of the profile analysis that resulted.

In-vitro bio adhesion

By modifying a traditional tensile testing experiment, the bioadhesive properties of the prepared films were evaluated. Two equals, specially made cylindrical metallic supports with a circular surface measuring 1.7 cm in diameter were used with an Instron universal testing machine. One metal support was entirely covered with a patch of each film (n = 3) using a cyanoacrylate adhesive before being fastened to the machine's upper grip fixture. The second metal support, which was attached to the bottom grasp fixture, had the exterior of a chicken pouch glued to it. After the chicken was sacrificed, we went to a local abattoir and bought chicken pouches. Prior to the in vitro bio adhesion test, they were totally defrosted at room temperature after being frozen

at 10 °C for less than 12 hours in simulated saliva (pH 6.8). The upper support was removed at a rate of 20 mm/min after the two surfaces (film and chicken pouch) had meet an initial force of 1.5 N for 3 min while being moistened with simulated saliva. The peak detachment force was used to evaluate the bioadhesive force of the films. The force was measured as a function of displacement up until the break point. A specialised programme was used to gather and compute data for each film during this experiment, which was conducted at room temperature and a relative humidity of 50%.

In vitro drug release

The reported approach by El-Kamel et al. was modified to study the drug release from the films. Buccal Film (1 ×1 cm), containing 100 ml of simulated saliva as the dissolution media, was placed in 250 ml conical flasks, equivalent to 2 mg of triamcinolone acetonide/film. The dissolving media was maintained in a sink-like environment. The flasks were shaken in a horizontal thermostatic shaker at 50 rpm with the temperature set at 37 ±0.5 °C. 2 ml samples were taken out and replaced with new medium at predefined intervals of 0.25, 0.5, 1, 2, 3, 4, 5, 6 and 7 h. Filtered samples were then examined at a maximum wavelength of 240 nm by a UV-visible spectrophotometer. The experiment was done in triplicate, and the mean and standard deviation (SD) of the data were calculated. The patterns of drug release from the various films were evaluated using the in vitro release data that was fitted into the zero order, first order, Higuchi, and Peppas models. By incorporating the in vitro findings into the Peppas model, the mechanism of drug release was identified:

$$M_t / M_\infty = Kt^n$$

Where, Mt is the amount of drug released in time t; M∞ is the total amount of drug released after infinite time; K is the release rate constant and n is the release exponent.

Optimization of Triamcinolone acetonide buccal film formula

Triamcinolone acetonide buccal film composition is optimised by combining independent variables (that can satisfy the desirability to formulate optimised formula) using multiple response optimisation, which is based on the maximum extension at break load, the least amount of film swelling, the greatest amount of in vitro bio adhesion, and the least amount of Higuchi release slope (% /t0.5).

Thermal analysis

Using a calorimeter (DSC-60, Shimadzu, Japan), differential scanning calorimetry (DSC) studies were conducted for optimised medicated and non-medicated buccal films in comparison to the individual film constituents (chitosan and HPMC) to ascertain the degree of homogeneity and crystallinity of triamcinolone acetonide. Samples weighing 3-5 mg were placed in aluminium pans and covered tightly before being heated at a rate of 10 °C/min between 25 and 250 °C. For purging, nitrogen gas was used at a rate of 30 ml/min. A TA 50I PC system with Shimadzu software programmes was used to log the data.

X-ray powder diffraction (XRPD)

A RIGAKU diffractometer outfitted with a curved graphite crystal monochromator, an automatic divergence slit, and an automatic controller PW/1710 was used to take XRPD scans for medicated buccal films in comparison to the film's constituents and non-medicated films in order to assess the degree of drug crystallinity. Cu K radiation, operating at 40 KV and 40 mA (k = 1.5418), was employed as the target. The measurements of the diffraction were carried out using a continuous scan mode with a 2° range of 4° to 60°.

In vivo bio adhesion residence time

Six healthy female volunteers, ranging in age from 18 to 40, were tested for the ability of triamcinolone acetonide mucoadhesive films to adhere after being told of the formulation's ingredients and completing a written informed permission. The Institutional Ethics Committee gave its approval to this study. The films were to be pressed against the buccal mucosa for 60 seconds by the volunteers. The volunteers were instructed to keep track of the residence time (the period during which the film completely eroded or detached from the buccal mucus membrane) as well as any fragmentation, irritation, foul taste, dry mouth, or increase in salivation.

Statistical analysis

One-way analysis of variance (ANOVA) with Tukey Kramer multiple comparisons was used to study statistical analysis using GraphPad Prism statistical software. For analysis, the student t-test was also used. When $p < 0.05$, all statistically significant differences were expected. Each value is converted to its mean minus standard deviation.

Result and Discussion

Preformulation Study

API characterization

Table: Organoleptic properties of Triamcinolone acetonide

Sr. No.	Name of property	Specification
1.	Colour	White
2.	Odour	Unpleasant
3.	Nature	Crystalline

Identification of pure drug

a) Melting Point

Table: Melting point of Triamcinolone acetonide

Sr. No.	Obtained range (ºC)	Mean value (ºC)	Reference value
1.	293	293℃	292-294℃
2.	295		
3.	291		

Melting point of Triamcinolone acetonide was found to be 293℃, which is in range as given in literature (292-294˚C). Hence the drug can be stated as pure.

b) UV Spectroscopy

Determination of λ max

Accurately weighed 1 mg of drug was transferred to 100 ml of volumetric flask add dissolved in methanol and volume was made up to 100ml and the solution was scanned on UV spectrometer in the range 200-400 nm.

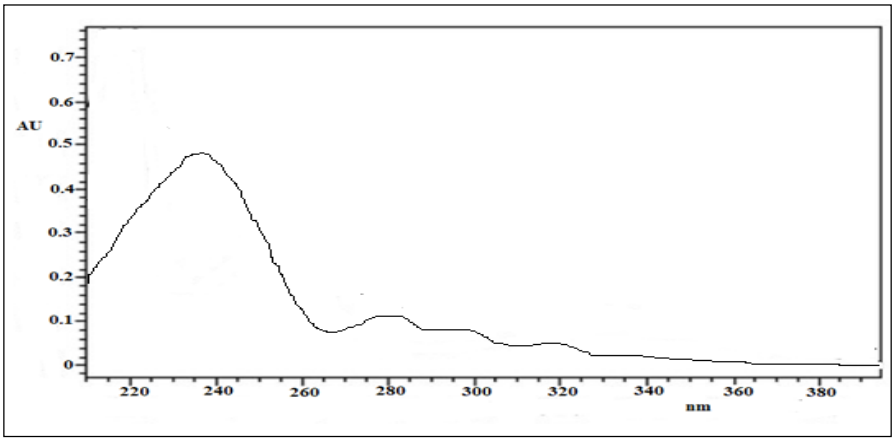


Fig. UV Spectrum of Triamcinolone acetonide

An absorption maximum was found to be at 240 nm. Hence 240 nm was selected as λ max for further studies.

Calibration curve of Triamcinolone acetonide in methanol

The stock solution for the standard drug of 1 mg was prepared using 100 ml of methanol. The maximum absorbance for the drug solution of 10 mcg/ml was found to be at 240 nm. The linearity was found between the concentration range of 10-35 mcg/ml for UV spectroscopy**.**

Table: Different concentration & absorbance of Triamcinolone acetonide

Sr. No.	Concentration (µg/ml)	Absorbance
1	0	0
2	10	0.189
3	15	0.256
4	20	0.378
5	25	0.466
6	30	0.529
7	35	0.611

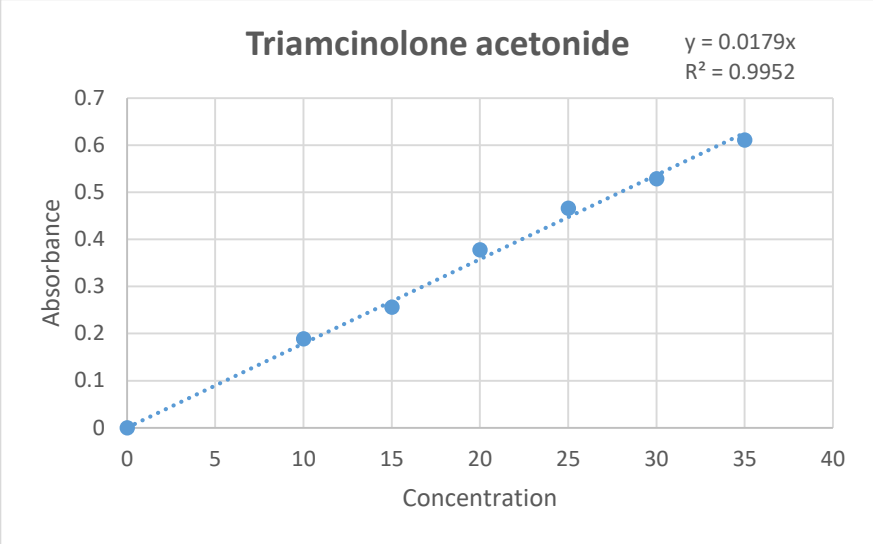


Fig. Calibration curve of Triamcinolone acetonide in Methanol

Table: Parameters found in calibration curve

Sr. No.	Parameter	Finding
1	Wavelength detection	240 nm
2	Regression equation	y = 0.018x – 0.0027
3	Correlation coefficient	R^2 = 0.9951

Solubility study

Triamcinolone acetonide is a BCS class II drug that's why solubility study is mandatory.

Table: Solubility study of Triamcinolone acetonide

Sr.no.	Different buffers	Solubility (mg/ml)
1	Water	1.423
2	0.1 N HCl (pH 1.2)	1.249
3	Acetate buffer (pH 4)	1.614
4	Phosphate buffer solution (pH 6.8)	1.548
5	Phosphate buffer solution (pH 7.4)	1.754

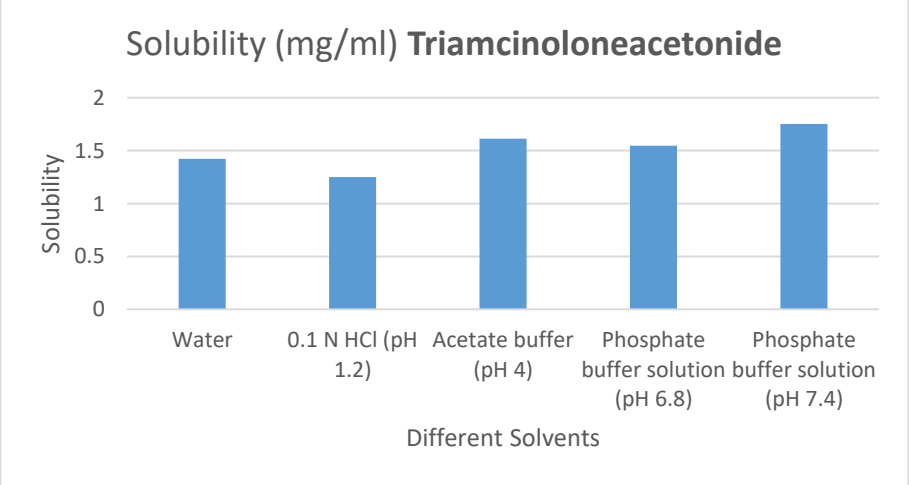


Fig. Solubility study of Triamcinolone acetonide in water and different buffer

Drug and excipient interaction study

A. Fourier Transformation Infrared Spectroscopy (FTIR)

FTIR spectrum of Triamcinolone acetonide was shown in following Fig. revealed that the characteristic peaks representing the presence of functional groups claim by its chemical structure. From this we can consider that the Triamcinolone acetonide was of pure quality.

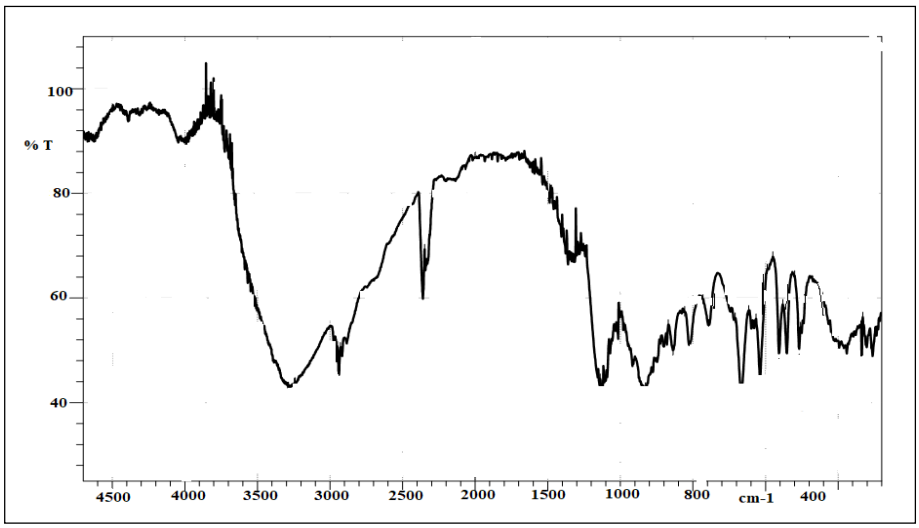


Fig. FTIR spectra of Triamcinolone acetonide

After interpretation of FT-IR Spectrum of Triamcinolone acetonide, it was concluded that all the characteristic peaks corresponding to the functional group present in the molecular structure of Triamcinolone acetonide were found within the reference range and confirming its identity.

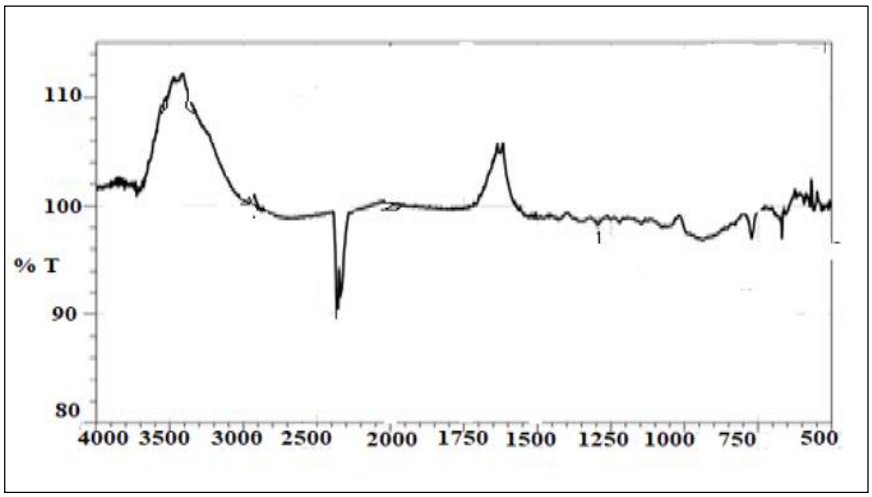


Fig. FTIR spectra of Chitosan

After interpretation of FT-IR Spectrum of Chitosan, it was concluded that all the characteristic peaks corresponding to the functional group present in molecular structure of Chitosan were found within the reference range, confirming its identity.

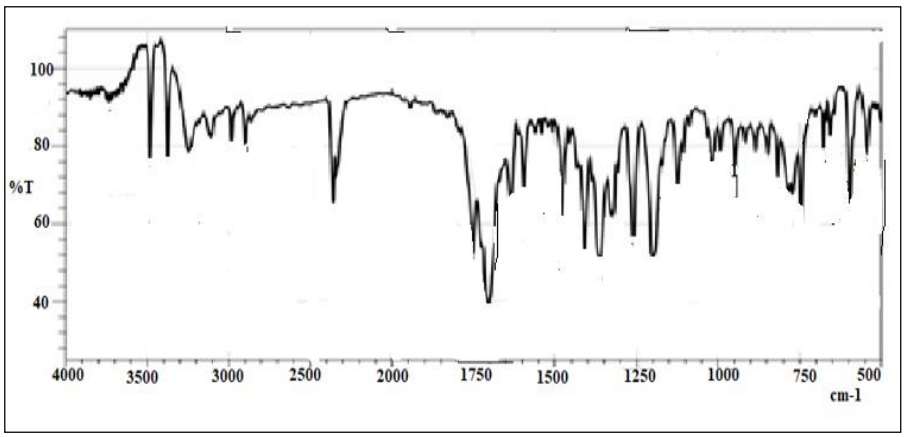


Fig. FTIR spectra of physical mixture

After interpretation of FT-IR Spectrum of Chitosan and its physical mixture with drug Triamcinolone acetonide, it was concluded that all the characteristic peaks corresponding to the functional group present in molecular structure of Triamcinolone acetonide were not found intact within the reference range, confirming its reactivity with chitosan. This interaction further supports the selection of polymer.

B. Differential Scanning Calorimetric analysis (DSC)

The thermal analysis of Triamcinolone acetonide and Chitosan was studied by using DSC as shown in figure respectively. The Triamcinolone acetonide shows an endothermic peak at approximately 290 ℃ and it corresponds to its melting point (fig.). Chitosan shows a sharp endothermic peak at 91.12℃ corresponds to its melting point (fig.).

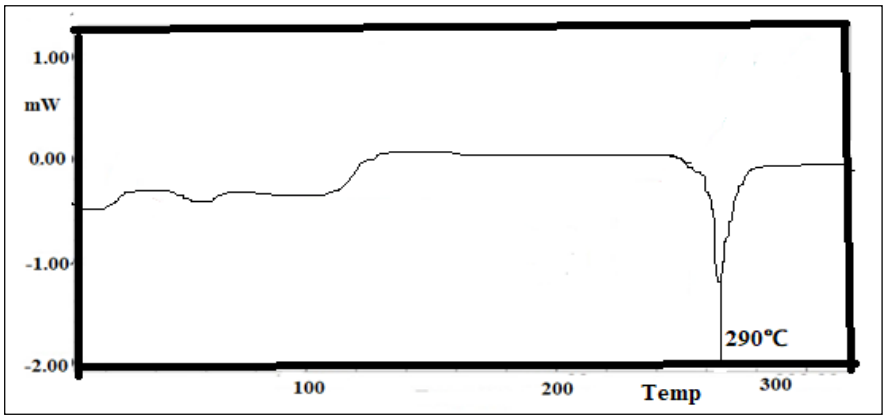


Fig. DSC thermogram of Triamcinolone acetonide

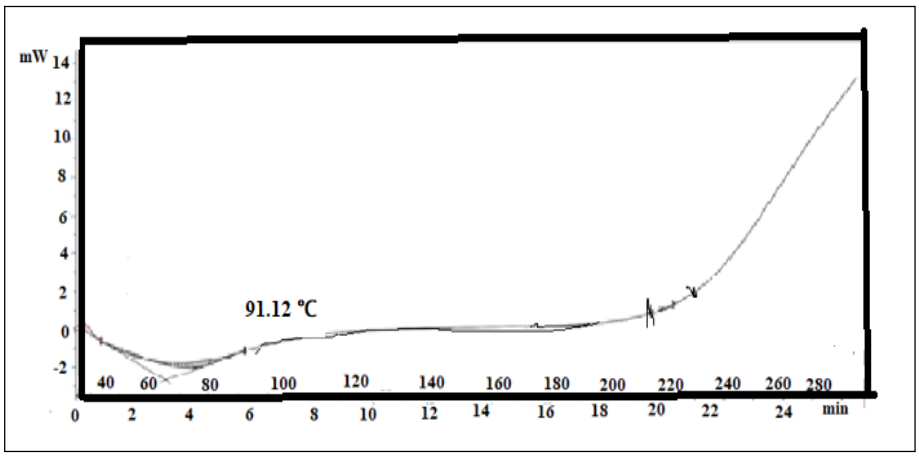


Fig. DSC thermogram of Chitosan

Triamcinolone acetonide content

As indicated in the following table, the measured concentration of triamcinolone acetonide in the produced chitosan film ranged from 1.88 to 2.11 mg (94.6-106% of the theoretical drug load).

Table: Properties of different Triamcinolone acetonide buccal film formulations

Formula	Triamcinolone acetonide content (mg)	Film thickness (mm)	Tensile stress at break (MPa)	Extension at break load (mm)	Film swelling (%)	In-vitro bio adhesion (N)
F1	1.94 ± 0.08	0.77 ± 0.09	0.87 ± 0.73	2.57 ± 0.62	255.91 ± 0.33	5.831 ± 0.56
F2	1.88 ± 0.12	0.63 ± 0.01	0.04 ± 0.02	3.17 ± 0.01	185.51 ± 0.22	5.321 ± 0.09
F3	2.03 ± 0.10	1.03 ± 0.15	0.33 ± 0.25	2.62 ± 0.27	244.58 ± 0.10	12.98 ± 0.68
F4	1.90 ± 0.13	0.75 ± 0.10	0.37 ± 0.20	2.99 ± 0.22	262.51 ± 0.57	15.27 ± 2.59
F5	1.98 ± 0.09	0.63 ± 0.09	0.04 ± 0.01	1.94 ± 0.06	92.251 ± 0.29	39.82 ± 10.8
F6	2.11 ± 0.07	0.65 ± 0.07	0.18 ± 0.05	2.05 ± 0.41	222.14 ± 0.01	6.671 ± 0.01
F7	2.09 ± 0.14	0.57 ± 0.05	0.49 ± 0.02	2.14 ± 0.53	197.83 ± 0.07	10.73 ± 2.47
F8	1.89 ± 0.10	0.65 ± 0.05	0.11 ± 0.02	2.52 ± 0.27	217.13 ± 0.13	63.61 ± 2.14
F9	2.00 ± 0.11	0.80 ± 0.03	0.21 ± 0.05	4.14 ± 0.29	300.20 ± 0.05	10.57 ± 3.87

Film thickness

All Triamcinolone acetonide films' average thicknesses were determined and are shown in the table above. As seen, these values vary from 0.57 ± 0.05 mm to 1.03 ± 0.15 mm, which is consistent with the recommended thickness for optimum buccal films (50-1000 m) to prevent any application-related discomfort. Additionally, given that the thickness measurements are carried out utilising various sections of each film, these values demonstrate that the produced films have uniformity in their thicknesses reflecting dose accuracy. It is important to note that the thickness of the formulation with the highest percentages of chitosan and HPMC (F3) and the other formulations under study differed significantly ($p < 0.05$).

Swelling study

Swelling behaviour of polymers is a crucial characteristic that influences the mucoadhesion of polymeric films, as film swelling is required to begin contact between the film and the buccal mucosa. Additionally, the degree of hydration and swelling in polymeric films has a significant impact on the dissolution of drugs from them. In this investigation, we found that the polymer type and its concentrations in the casting fluid had an impact on the swelling %. We discovered that HPMC concentration has a synergistic effect on film swelling that is statistically significant ($p = 0.040$), as indicated in the accompanying table.

Table: Analysis of variance for the effect of chitosan (X1) and HPMC (X2) on the responses of Triamcinolone acetonide buccal film formulations

Extension at break load (mm) Y1	**Source**	**Sum of squares**	**F-Ratio**	**P-Value**
	A: Chitosan	1.73	10.66	0.048
	B: HPMC	1.05	6.45	0.092
	AA	0.07	0.39	0.581
	AB	0.03	0.13	0.751
	BB	0.42	2.52	0.211
Film swelling (%) Y2	A: Chitosan	3264.90	2.051	0.249
	B: HPMC	20030.5	13.34	0.040
	AA	1798.90	1.101	0.372
	AB	373.101	0.241	0.656
	BB	283.111	0.181	0.699
In-vitro bio adhesion (N) Y3	A: Chitosan	67.891	0.10	0.786
	B: HPMC	49.491	0.07	0.817
	AA	315.97	0.43	0.564
	AB	258.40	0.35	0.600
	BB	185.08	0.26	0.656
Higuchi diffusion slope (% /t 0.5) Y4	A: Chitosan	4683.72	230.30	0.002
	B: HPMC	304.031	16.161	0.031
	AA	859.911	43.981	0.008
	AB	858.901	43.931	0.008
	BB	16.1601	0.7701	0.449

Chitosan, in contrast, had a marginally smaller impact on film swelling. Moreover, a statistical programme (Stat graphics Plus, version 5) was used to assess the quadratic and interaction effects. The programme assesses each effect individually (chitosan concentration and HPMC concentration), quadratically (chitosan concentration and HPMC concentration), and jointly (chitosan-HPMC). The tested polymers' quadratic and interaction effects on film swelling are also negligible. The response surface plot (Fig. 1A) demonstrates that the swelling values rose as the concentration of the HPMC polymer increased from 1% to 5%. Using 0.5% chitosan, the swelling percentages were in the following order: F9 > F8 > F2, and comparable results were achieved using 1% and 1.5% chitosan. The hydrophilic polymer HPMC seems to improve the buccal films' surface wettability and swelling when it is present. In the case of chitosan, the swelling percentage decreased from 185.51 ± 0.22 % to 92.251 ± 0.29 % for F2 and F5, respectively, when the concentration was increased from 0.5% to 1.5% (w/v) using 1% w/v HPMC, whereas a non-significant ($p > 0.05$) effect was obtained when the concentration was raised from 0.5% to 1% (w/v). The similar result was achieved with 3% HPMC; for F1 (1% chitosan) and F6 (1.5% chitosan), the swelling values decreased with increasing chitosan concentrations from 255.91 ± 0.33% to 222.14 ± 0.01%, respectively. Additionally, the rank order of swelling was as

follows: (F9) > 262.51 ± 0.57 %, (F4) > 244.58 ± 0.10 %, (F3) using 5% HPMC. During the experiment, F9 with the lowest concentration of chitosan (0.5%) and the highest concentration of HPMC (5%) had the highest swelling value (300.20 ± 0.05). The lowest swelling value was displayed by F5 (92.251 ± 0.29 %).

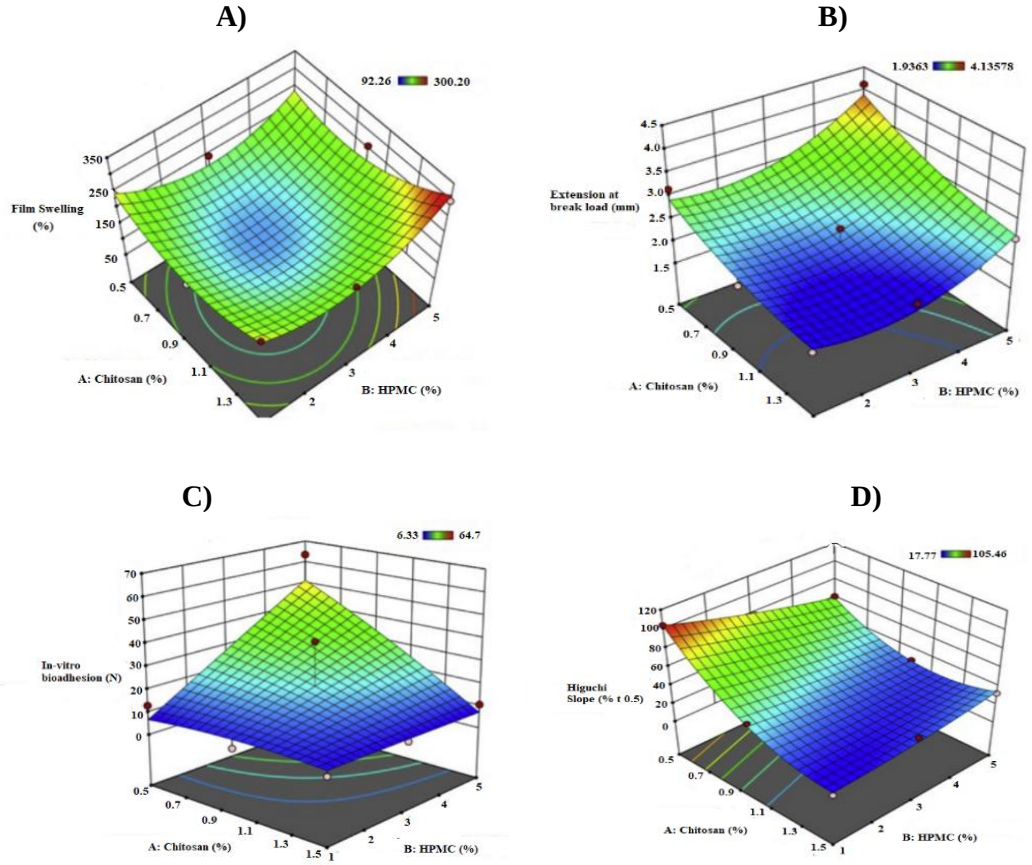


Fig.1: Response surface plots for the effect of independent variable on a) film swelling percentage, b) extension at break load, c) in-vitro bioadhesion, and d) Higuchi release slope

Mechanical properties

It is crucial to measure the mechanical characteristics of buccal films since doing so aids in assessing their structural integrity. Here, the mechanical characteristics of several Triamcinolone Acetonide films were examined. The results are shown in the table above. The TS, which provides information about the film's tensile strength, is the maximum stress per unit of cross-sectional area that the film can withstand before breaking. While the extension at break load, which is defined as the percentage of the change in sample length compared to its initial length, measures the ability of the film to extend before breaking and provides information about the elasticity of the film. The table shows the results of an ANOVA investigation of the impact of independent variables on the mechanical characteristics of the buccal film (expressed as extension at break load in mm; Y1). According to the response surface plot in Fig. 1B, Chitosan had the sole significant agonistic effect on film mechanical characteristics ($p = 0.048$), but HPMC had a small but negligible effect ($p = 0.10$).

The produced films' TS ranged between 0.04 ± 0.02 MPa and 0.87 ± 0.73 MPa, and their extension at break stress varied from 1.94 ± 0.06 mm to 4.14 ± 0.29 mm. The extension at break load value for F2 and F5 decreased dramatically from 3.17 ± 0.01 mm to 1.94 ± 0.06 mm when the chitosan concentration was increased from 0.5% to 1.5% in films containing 1 % HPMC. Contrarily, raising the content of chitosan from 0.5 % to 1 % considerably increased the films' TS from 0.033 ± 0.25 MPa to 0.49 ± 0.02 MPa for F3 and F7, respectively. Chitosan was then increased from 1% to 1.5%, which dramatically decreased the TS values for F5 and F7. This demonstrates the significance of employing a factorial design research to enhance the mechanical properties of films. The formulation of Film F9, which has the highest HPMC concentration (5%) and the lowest chitosan concentration (0.5%), is the most flexible and has the strongest resistance, as shown by the

value of its extension at break load (4.14 ± 0.29 mm). This might be caused by the high concentration of HPMC, which is thought to be hard and brittle and is frequently added to films to enhance their mechanical properties, particularly membrane flexibility and resistance.

In-vitro bioadhesion

The ability of mucoadhesive polymers to effectively adhere to mucus surfaces is well established. It was suggested that a variety of mechanistic hypotheses of mucoadhesion could be combined to explain this feature. Electronic, adsorption, diffusion, and mechanical theory are a few of these theories. Here, the most often examined peak detachment force was employed to evaluate the bioadhesive force of several films. The table above shows the measured peak detachment force for various prepared films. The bioadhesion property of the films improved from 5.321 ± 0.09 N to 39.82 ± 10.8 N when the percentage of chitosan increased from 0.5% (F2) to 1.5% (F5), but the effect is negligible (p = 0.80) (Fig. 1C).

Due to chitosan's well-known mucoadhesive characteristics, this outcome is expected. Additionally, there was no discernible variation in the bioadhesive characteristics of films containing 5% HPMC while increasing the amount of chitosan from 0.5% (F9) to 1.5% (F3), which may have been caused by the high concentration of HPMC, which may have impacted the true mucoadhesive function of chitosan. On the other hand, there was no discernible difference in bioadhesion when HPMC was increased from 1% to 5% while chitosan content was kept at 1%. These findings demonstrate that the bioadhesive capabilities of produced films are significantly influenced by the kind and concentration of polymers used.

In-vitro drug release

As indicated in the above table and Fig. 1D, the independent parameters had a discernible effect on the in vitro drug release from buccal films.

On the Higuchi diffusion slope of drug release from buccal films, the HPMC concentration showed a significant boosting effect (p = 0.04), but chitosan showed an antagonistic effect (p = 0.001). Additionally, the interaction between chitosan and HPMC (X1X2) and the quadratic effect of chitosan concentration (X12) both had a significant impact (p = 0.008) on the drug release rate. These findings demonstrate that the kind and quantity of the polymers used affect the medication release rate. As was already indicated, this result could be explained by HPMC's hydrophilic polymer properties, which enable it to absorb water molecules, increasing the produced films' percentage swelling. In turn, this accelerated the rate at which drugs were released from the matrix. In addition, chitosan exhibits some hydrophobicity while being naturally hydrophilic (it contains 97% deacetylation). Here, the high quantities of chitosan in the formulations led to an increase in viscosity as well as a decrease in the rate of matrix hydration, or the pace at which solvent molecules entered the system. As a result, the drug's rate of breakdown out of the matrix was decreased.

The rate of Triamcinolone acetonide release increased as the concentration of HPMC was raised from 1% to 5%; for F2, F8, and F9, the release was finished after 1, 2, and 3 hours, respectively. Additionally, formula F5, which has the least amount of HPMC and the most chitosan, had the slowest drug release of all the film formulations at 50.80 ± 6.63% after 7 hours (Fig. 2).

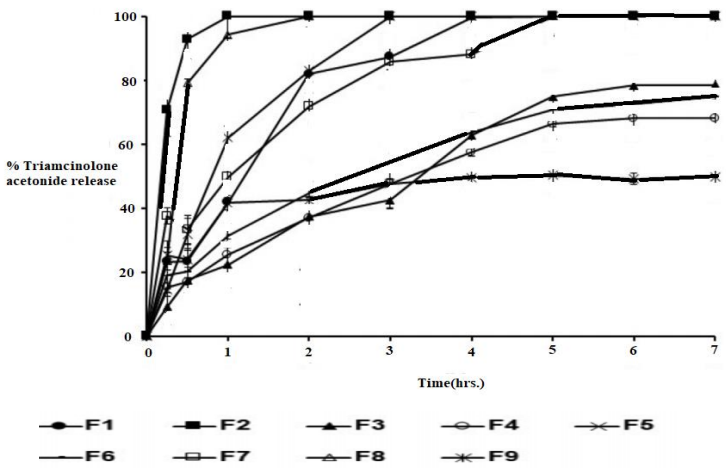


Fig. 2. In-vitro release profiles of Triamcinolone acetonide from different buccal film formulations

These findings highlight the relationship between the swelling values and the Triamcinolone acetonide medication release rate from the manufactured buccal films. The kinetic data are summarised in the following table. The in vitro release data were fitted using the Zero-, first order-, and Higuchi-diffusion kinetic models.

Table: Kinetic modelling of Triamcinolone acetonide release from different buccal film formulations

Formula	Zero order		First order		Higuchi Diffusion model		Peppas model	
	R	slope	r	slope	r	Slope	r	n[a]
F1	0.967	9.611	0.988	−0.074	0.991	28.97	0.991	0.582
F2	0.846	90.28	N/A	N/A	0.961	105.46	0.961	0.261
F3	0.991	12.52	0.981	−0.104	0.991	33.93	0.981	0.661
F4	0.971	14.89	0.991	−0.088	0.991	28.74	0.981	0.521
F5	0.781	5.111	0.811	−0.034	0.901	17.77	0.941	0.241
F6	0.961	9.971	0.991	−0.086	0.991	30.39	0.981	0.471
F7	0.941	17.92	0.991	−0.221	0.991	45.12	0.961	0.381
F8	0.831	47.69	0.731	−0.051	0.931	79.41	0.871	0.661
F9	0.971	33.73	0.961	−0.061	0.991	63.04	0.991	0.781

The correlation coefficient value was used to determine the release method preference. Based on the maximum value of the correlation coefficient of the plotted % drug release versus square root of time, the results showed that triamcinolone acetonide release from buccal films matched the Higuchi diffusion model. Additionally, it was discovered that the calculated values of n (derived from the Korsmeyer-Peppas model) were both lower and higher than 0.46, but that all values were lower than 1, indicating non-Fickian or anomalous drug release (coupled diffusion/polymer relaxation).

Optimization of Triamcinolone acetonide buccal film formula

Although factorial design is a promising method that is still new to pharmacy practise, it has been shown to be successful when applied to formulation optimisation. In this study, the optimum potential composition of the planned buccal film was determined using the factorial design technique, and the values of its various characterisation parameters were predicted. The anticipated characterisation parameter values were then compared to the experienced ones in order to validate the findings of employing the optimised independent variables (% of chitosan and HPMC in the formulations). The optimised buccal film formula is thus displayed in the following table along with responses that were expected using the factorial design technique.

Table: Composition and predicted response values of the optimized buccal film formula

Factors (X)	Optimized			Observed values
Chitosan (X1) %	0.51			
HPMC (X2) %	4.37			
Responses	**Goal**	**Lower Limit**	**Upper Limit**	**Optimized**
Extension at break load (mm)	maximize	1.931	4.1301	3.4501	3.481 ± 0.481
Film swelling (%)	maximize	92.26	299.20	271.28	250.99 ± 0.40
In vitro bio adhesion (N)	maximizes	5.321	63.601	33.151	35.20 ± 6.101
Higuchi slope (%/t 0.5)	maximize	17.77	104.46	68.261	66.90 ± 3.661

4.37 % HPMC and 0.51 % chitosan were combined to create this recipe. Comparing the predicted and observed bioadhesion results 35.20 ± 6.101 N, respectively—was the first step in the validation procedure. Additionally, the anticipated value for the optimised Triamcinolone Acetonide Film Formula was 271.28 %, but the actual value was 250.99 ± 0.40 %. Additionally, the optimised film formula's mechanical characteristics (extension at break stress) were 3.481 ± 0.481 mm, which was comparable to the forecasted value of 3.4501 mm. The improved formula had good mechanical characteristics, and the values for the thicknesses, TS, and extension at break load were suitable for this application. Furthermore, compared to the projected value of 68.261 % /t0.5, the experimental Higuchi release slope was 66.90 ± 3.661 % /t0.5. These results confirmed the efficacy of the factorial design technique in formulation optimisation and supported the effects of applying the optimised independent variables in the optimised formulation.

Thermal analysis

DSC was used to identify how Triamcinolone Acetonide interacted with the film polymeric composition in the optimised buccal film formula. The drug endothermic peak completely vanished in the case of the optimised film, leaving only the polymer peak visible at 104 °C. This could be as a result of the drug's solubility in the melted polymeric matrix. The drug's exothermic breakdown peak, however, was no longer visible in the video that had been optimised.

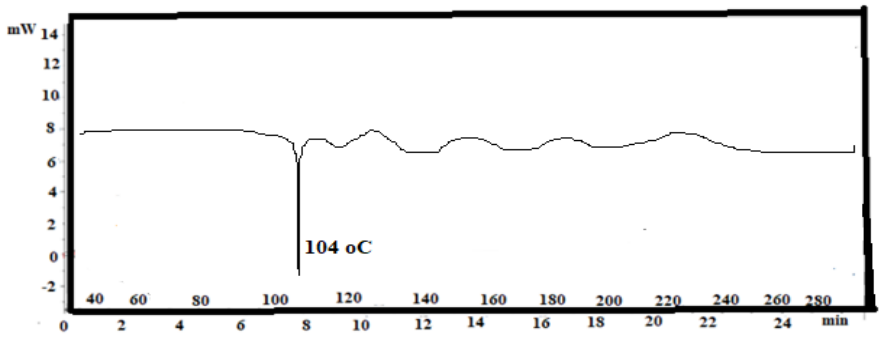
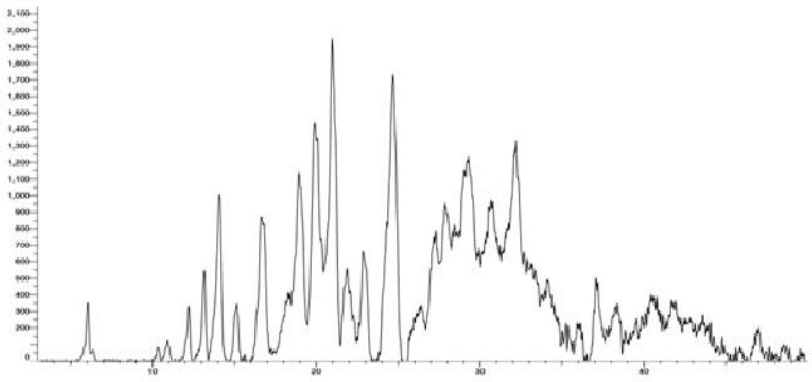


Fig. Optimized Triamcinolone acetonide loaded buccal film

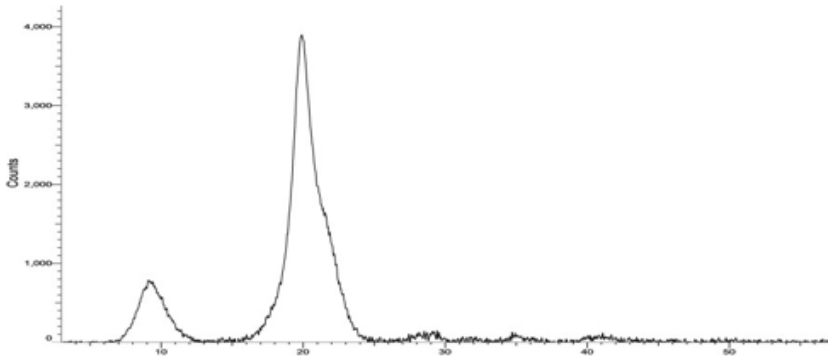
X-ray powder diffraction (XRPD)

Triamcinolone acetonide crystallisation was monitored by XRPD on the optimised buccal film. Triamcinolone acetonide's crystalline nature is defined by the presence of several diffraction peaks at 2 theta diffraction angles of 14.8, 16.8, 18.6, 24.9, 29.4, and 30.8. This is seen in the accompanying image. Conversely, diffraction peaks could be seen for chitosan at 9.35 and 20.34 or HPMC at 19.65, 22.45 and 23.68. Triamcinolone acetonide buccal film's XRPD spectrum, on the other hand, showed a peak at 5.85, 11.43, 15.34, 19.43, 25.34, 28.43.

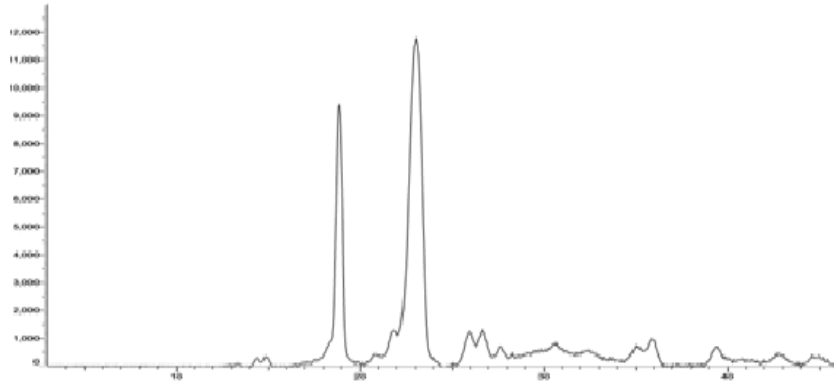
A)



B)



C)



D)

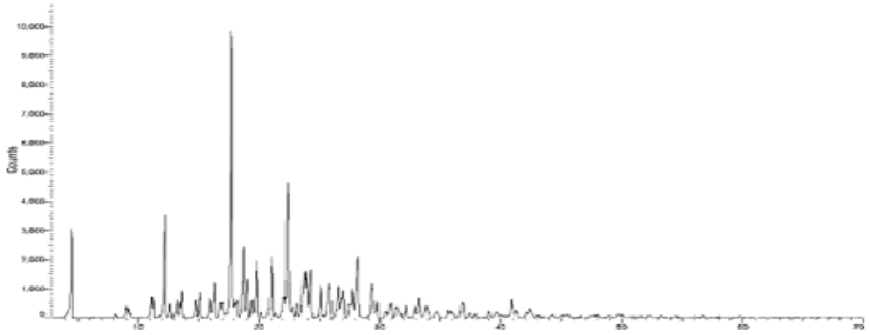


Fig. X-ray powder diffraction pattern of Triamcinolone acetonide (A), chitosan (B), HPMC (C), and optimized Triamcinolone acetonide loaded buccal film (D)

In-vivo bioadhesion residence time

Six healthy human volunteers underwent in-vivo bioadhesion residence time testing with the optimised Triamcinolone acetonide film, and the results showed that there was no discomfort, while the taste was mildly bitter; there was also no irritation or excessive salivation. The findings demonstrated that the optimised film exhibited an in vivo bioadhesion residence duration of 1.26 ± 0.18 h in all six human volunteers before being separated from the buccal mucosa and eroding. The over-hydration of the polymer, which causes disentanglement at the polymer-mucus interface and a rapid fall in the mucoadhesive strength, may be to blame for the decrease in the bioadhesion residence time. Furthermore, it can be caused by more challenging circumstances than those that often affect in vivo research, like mouth movement during speech and swallowing.

Conclusion

Strong adhesive power and limited aqueous solubility were demonstrated by a novel mucoadhesive polymer made via template polymerization of hydroxy propyl methyl cellulose in the presence of chitosan, two crucial characteristics for creating transmucosal drug delivery systems. In the chitosan/HPMC polymer complex film, triamcinolone acetonide (TAA) was loaded. TAA was inertly distributed throughout the chitosan/HPMC polymer complex film. The water quickly dissolved the polymer complex films with a higher chitosan content, hastening the release of TAA from it. The mucoadhesive polymer film's ability to release TAA was also influenced by duration, the pH of the release medium, and the weight percentage of the loaded medication. The chitosan/HPMC polymer complex film may release TAA, according to the analysis of release. The discharge of the TAA and TMD system will likely be controlled by a film made of the HPMC/chitosan polymer combination.

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