Development And Evaluation Of Polymeric Chitosan Composites Mucoadhesive Films Containing Benzocaine For Treatment Of Oral Disease

Vivek M Thorat[1]*, Satish Kumar Sarankar[2]

[1]*Research Scholar at Faculty of Pharmacy Mansarovar Global University Sheore.
Email: vivekthorat1992@gmail.com
[2]Professor and Principal at Faculty of Pharmacy Mansarover Global University, Sheore.
Email: satish_sarnkar@yahoo.co.in

***Corresponding Author:** Vivek M Thorat
*Research Scholar at Faculty of Pharmacy Mansarovar Global University Sheore.
Email: vivekthorat1992@gmail.com

Abstract:
A buccal mucoadhesive Benzocaine local delivery system was the focus of the current study's optimisation and evaluation efforts. To determine how much chitosan and HPMC was used, a full 3^2 factorial design was used to assess the effects on buccal film properties. The results demonstrated that PVP concentration had a large synergistic effect on film swelling whereas chitosan just minimally and insignificantly slowed film swelling. Moreover, chitosan's agonistic influence on mechanical film characteristics was the only one that was notable. Additionally, HPMC dramatically increased the Higuchi diffusion slope of the drug release from buccal films, whereas chitosan blocked this effect. For buccal films, the optimised recipe demonstrated sufficient mechanical characteristics. This study showed that the improved Benzocaine mucoadhesive buccal film is a superior alternative dose form and can help patients avoid the unintended side effects of the medication.

Keywords: Benzocaine, Chitosan, HPMC, Factorial design, Buccal film, etc.

Introduction

The buccal route is gaining popularity as an alternative to other conventional means of systemic medication administration due to its many benefits. It is generally recognised that therapeutic chemicals absorbed via the oral mucosa allow a direct entry of the medication into the systemic circulation, bypassing the first pass hepatic metabolism and gastrointestinal drug degradation that are linked to oral administration. The oral cavity is conveniently accessible for self-medication, which leads to patient acceptance and safety because the device can be quickly applied and even removed from the application site, preventing drug ingestion anytime desired [1].

There are two basic restrictions on the amount of medication that can be absorbed by the oral mucosa after delivery via solutions or traditional buccal dose forms: i) An important portion of the medication may not be available for absorption due to involuntary swallowing of the dosage form itself or a portion of it, and ii) they do not permit speaking, eating, or drinking. Because of this, the duration of their administration is limited, making it impossible for such formulations to be used for controlled drug release [2].

As a result of its antibacterial, haemostatic, and wound-healing capabilities, the biodegradable polysaccharide chitosan is used in a variety of biomedical applications [3]. It has positive results in reducing the loss of tooth surface caused by abrasion and erosion [4]. Electrostatic forces help chitosan molecules, which have a strongly positive zeta potential due to the presence of a cationic amino group, adhere to surfaces with a strong negative zeta potential, like tooth enamel [5]. In addition, it was shown that chitosan works very well as a mucoadhesive polymer when it is hydrated or swollen. This feature is thought to be caused by the polymer's cationic composition, which aids in the formation of ionic interactions between the negatively charged mucin in the mucus membranes and the amino groups in chitosan [6]. Chitosan is also a linear polymer with extremely flexible polymeric chains. To promote strong mucoadhesion, these chains physically entangle with mucous membranes [7]. Furthermore, chitosan's capacity to stop enamel demineralization and erosion as well as its antibacterial and wound-healing properties suggest its potential use as a mucoadhesive application [8]. Several disorders connected to pain can be treated with benzocaine. It may be used for local anaesthetic of the mucous membranes of the mouth and pharynx (sore throat, cold sores, canker sores, toothache, sore gums, denture discomfort) [9].

In order to avoid the unfavourable systemic side effects, the current study set out to develop, optimise, and evaluate a buccal mucoadhesive Benzocaine local delivery method. In addition to the films' physicochemical properties, a full 3^2 factorial design was used to assess how the concentration of chitosan and HPMC, which were independent factors,

affected buccal film characteristics such as film extension at break load, film swelling, in vitro bioadhesion, and Higuchi diffusion slope. The designed and tested in vitro and in vivo buccal film recipe was optimised.

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

Benzocaine was received as a sample gift from DCI Pharmaceuticals Ltd., India.
Benzocaine was received as a sample gift from DCI Pharmaceuticals Ltd., 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 Benzocaine buccal films [10-18]

A statistical tool (Stat graphics Plus, version 5) was used to optimise the formulations of buccal films that include benzocaine. The design consisted of two variables, three levels, and 3^2 full factorials. In the three levels shown in the accompanying table, the concentrations of chitosan (X_1) and HPMC (X_2) were employed as independent factors.

Table 1: Variables in 3^2 full factorial design of Benzocaine buccal film formulations

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

In order to assess the influence of the independent parameters on the film extension at break load (mm; Y1), film swelling (%; Y2), in vitro bio adhesion capacity (%; Y3), and Higuchi diffusion slope (%/t 0.5; Y4), statistical models with main, quadratic, and interaction modes were created. In the table below, the composition of the buccal films F1–F9 produced in accordance with the experimental design is shown.

Table 2: Composition of Benzocaine buccal film formulations

Ingredients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Benzocaine (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 chitosan (a bioadhesive polymer) and HPMC (a film-forming polymer) were dispersed in 21 ml of an aqueous solution containing 1.5% acetic acid in order to make buccal films. With the use of a magnetic stirrer, a clear viscous solution was created by slowly stirring the polymeric dispersion. The amount of benzocaine in the formula was dissolved in 10 ml of aqueous solution containing 1.5% acetic acid, 1.25 ml propylene glycol (plasticizer), 5 ml of 100% ethanol, and 4-5 drops of triethanolamine. This allowed the benzocaine to dissolve and produce a transparent film. After gently combining the polymeric solution and drug solution, the drug solution was added. For the purpose of removing air bubbles, the dispersion was left overnight. A 40 °C oven was used to dry the films for 12 hours after they were cast into Teflon plates with a diameter of 6.3 cm. Cut into 1 × 1 cm pieces, wrapped in aluminium foil, and kept in a desiccator for further analysis, the dried buccal films were then examined.

Benzocaine content
A slice of 1 cm^2 was dispersed in 100 mL of methanol to determine the drug concentration of the produced buccal films. After being sonicated for 15 minutes, the dispersion was filtered, and the absorbance at a wavelength of no greater than 380 nm was determined spectrophotometrically.

Film thickness
With the use of 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 identical patches, one from each of the centre and two other points on each film, were taken.

Swelling study
The surface of 1% (w/v) agar gel plates made in simulated saliva and incubated at 37 0.5 °C was covered with patches of buccal films that were each 1 1 cm, weighed separately (W1), and allowed to swell independently. The samples were taken out of the petri plate after 7 hours, and any extra surface water was carefully blotted off with blotting paper. Then, using the formula in the following equation, the swelled film was reweighed (W2), and the swelling percentage was determined:

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

W1 denotes the film's initial weight, and W2 denotes its final weight after seven hours. The test was run three more times for each observation.

Mechanical properties
Using an Instron universal testing equipment with a 5 kg load cell at room temperature, the mechanical characteristics of several produced films were investigated. At first, average values were collected after measuring the thickness of the rectangular filmstrips (1 × 2 cm). In between the machine's two grip fixtures, the films were then held. In order to determine the force and elongation when the films broke, the top grasp was pulled back at a rate of 20 mm/min. Tensile strength (TS) and extension at break load calculations were made for the resulting profile analysis utilising specialised software.

In-vitro bio adhesion
A modified version of a traditional tensile testing experiment was used to evaluate the bioadhesive qualities of the prepared films. Two equals, specially made cylindrical metallic supports each had a circular surface that measured 1.7 cm in diameter, and the testing apparatus used was an Instron universal testing machine with a 5 kg load cell. One metal support was entirely covered with a patch of each film (n = 3), which was then fastened to the machine's top grip fixture using cyanoacrylate adhesive. The second metal support, which was joined to the bottom grasp fixture, was covered in adhesive, and the exterior side of a chicken pouch was adhered to it. Following the sacrifice of the chicken, we bought chicken pouches from a nearby abattoir. Before 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 (chicken pouch and film) had been in contact for 3 minutes with an initial force of 1.5 N while being moistened with simulated saliva. In order to determine the bioadhesive force of the films, the force was measured as a function of displacement up until the break point. This study used specialised software to gather and compute data for each film, which was conducted at room temperature and a relative humidity of 50%.

In vitro drug release
A modified version of the approach described by El-Kamel et al. was used to study the drug release from the films. Buccal Film (1 × 1 cm), which is equal to 2 mg of Benzocaine film, was placed in 250 ml conical flasks with 100 ml of simulated saliva as the dissolution medium. The dissolving agent was stored in a sink-like environment. At a speed of 50 rpm, the flasks were shaken in a thermostatic horizontal shaker with the temperature set at 37 ± 0.5 °C. Fresh medium was substituted for the 2 ml samples at predefined intervals of 0.25, 0.5, 1, 2, 3, 4, 5, 6 and 7 h. Using a UV-visible spectrophotometer set to a maximum wavelength of 380 nm, the samples were filtered and examined. The experiment was run three times, and the outcomes were assessed and presented as mean and standard deviation (SD). To determine the pattern of drug release from the various films, the in vitro release data were fitted into zero order-, first order-, Higuchi, and Peppas models. By incorporating the in vitro findings into the Peppas model, the drug release pathway 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 Benzocaine buccal film formula
Utilising multiple response optimisation, the composition of the Benzocaine buccal film formula is optimised 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 (%/t 0.5).

Thermal analysis
In order to determine the degree of homogeneity and crystallinity of benzocaine using a calorimeter (DSC-60, Shimadzu, Japan), differential scanning calorimetry (DSC) studies were conducted for optimised medicated and non-medicated buccal films compared to the individual film constituents (chitosan and HPMC). A temperature range of 25–250 °C was used with samples that weighed 3-5 mg, and they were heated at a rate of 10 °C/min. A 30 ml/min flow of nitrogen gas was employed to purge the system. A TA 50I PC system running Shimadzu software was used to record 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 gauge the degree of drug crystallinity. The target was Cu K radiation operating at 40 KV and 40 mA (k = 1.5418). The continuous scan mode with a 2° range of 4° to 60° was used to carry out the diffraction measurements.

In vivo bio adhesion residence time
After educating the six healthy female volunteers on the formulation's ingredients and obtaining their written informed consent, the adhesion strength of Benzocaine mucoadhesive films was evaluated on six healthy volunteers between the ages of 18 and 40. The institutional ethics committee gave its consent to this work. The films were to be pressed for 60 seconds against the buccal mucosa by the volunteers. The participants were instructed to note the residence time, which is the period of time during which the film completely erodes or separates from the buccal mucus membrane, and to keep an eye out for any fragmentation, irritation, foul taste, dry mouth, or increase in salivary flow.

Statistical analysis
Using Graph Pad Prism statistical software and the Tukey Kramer multiple comparisons, one-way analysis of variance (ANOVA) was used to study the statistical data. Analyses also included the student t-test. Every variation that met the criteria for statistical significance ($p < 0.05$) was expected. The mean minus standard deviation of each number is determined.

Result and Discussion
API characterization

Table 3: Organoleptic properties of Benzocaine

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

Identification of pure drug
a) Melting Point

Table 4: Melting point of Benzocaine

Sr. No.	Obtained range (ºC)	Mean value (ºC)	Reference value
1.	91	90℃	88-90℃
2.	90		
3.	89		

Melting point of Benzocaine was found to be 90℃, which is in range as given in literature (88-90˚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.

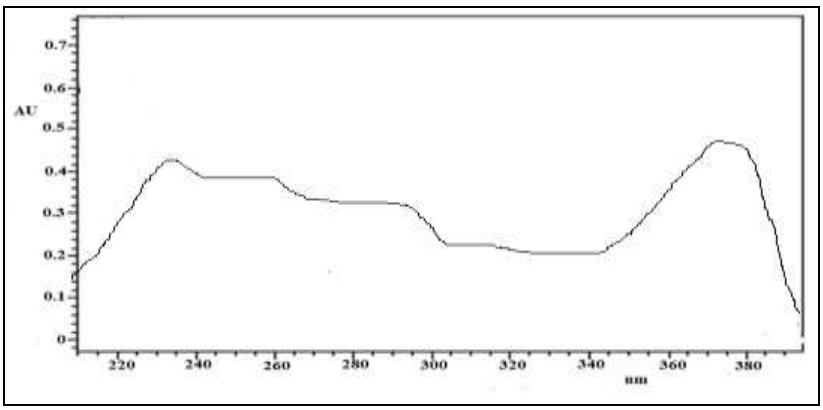

Fig No 1: UV Spectrum of Benzocaine

An absorption maximum was found to be at 380 nm. Hence 380 nm was selected as λ max for further studies

Calibration curve of Benzocaine 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 380 nm. The linearity was found between the concentration range of 10-35 mcg/ml for UV spectroscopy**.**

Table 5: Different concentration & absorbance of Benzocaine

Sr. No.	Concentration (µg/ml)	Absorbance
1	0	0
2	10	0.176
3	15	0.257
4	20	0.335
5	25	0.423
6	30	0.502
7	35	0.578

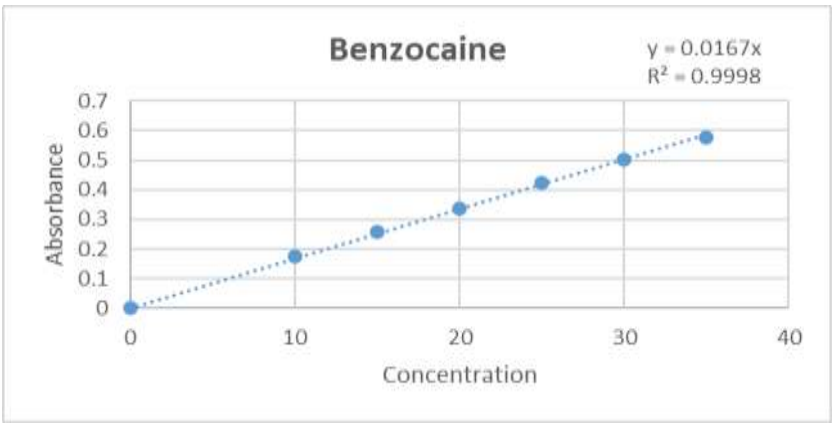

Fig. no 2 Calibration curve of Benzocaine in Methanol

Table 6: Parameters found in calibration curve

Sr. No.	Parameter	Finding
1	Wavelength detection	380 nm
2	Regression equation	y = 0.0167x – 0.0041
3	Correlation coefficient	R^2 = 0.9992

Solubility study

Table 7: Solubility study of Benzocaine

Sr. no.	Different buffers	Solubility
1	Water	0.347
2	0.1 N HCl (pH 1.2)	0.248
3	Acetate buffer (pH 4)	0.294
4	Phosphate buffer solution (pH 6.8)	0.316
5	Phosphate buffer solution (pH 7.4)	0.275

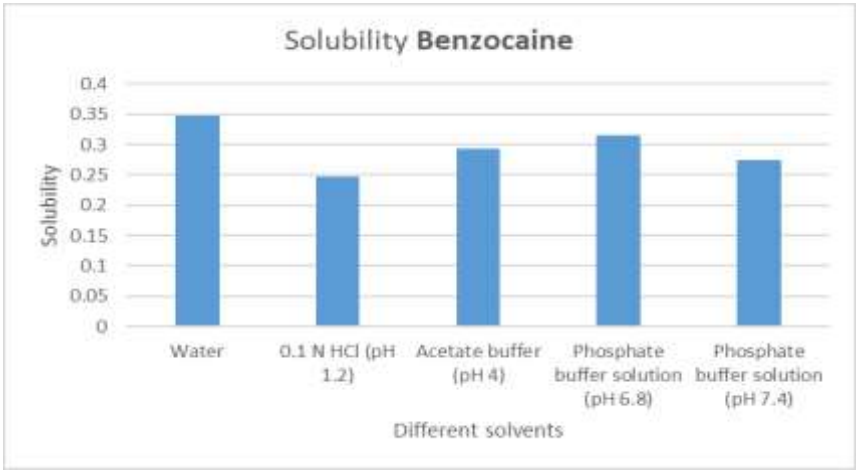

Fig. no. 3 Solubility study of Benzocaine in water and different buffer

Drug and excipient interaction study

A. Fourier Transformation Infrared Spectroscopy (FTIR)

FTIR spectrum of Benzocaine 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 Benzocaine was of pure quality.

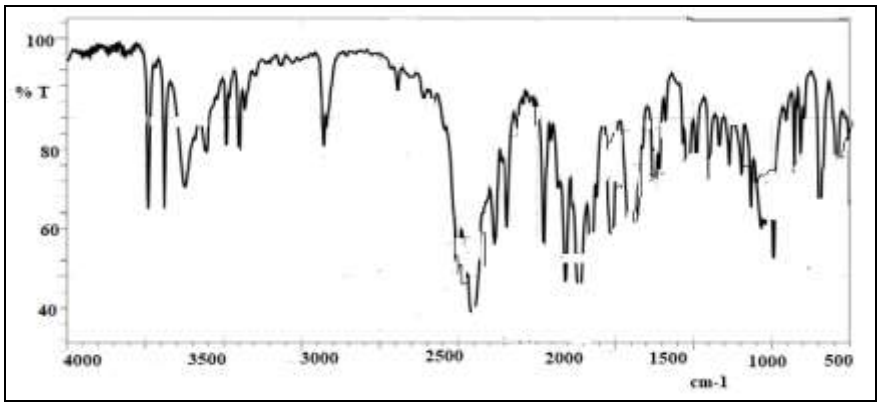

Fig. no. 4 FTIR spectra of Benzocaine

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

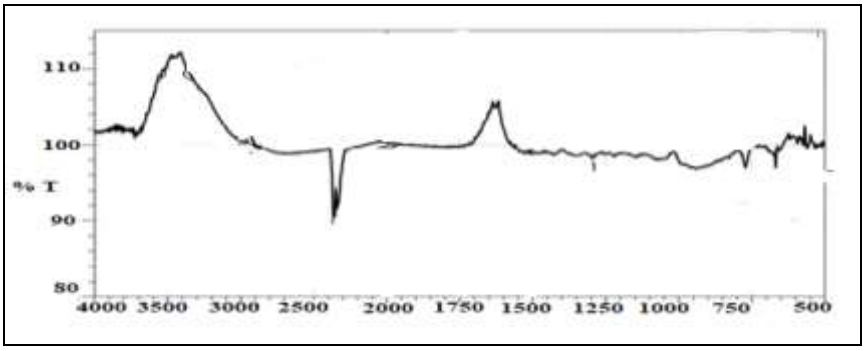

Fig. no. 5 FTIR spectra of Chitosan

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.

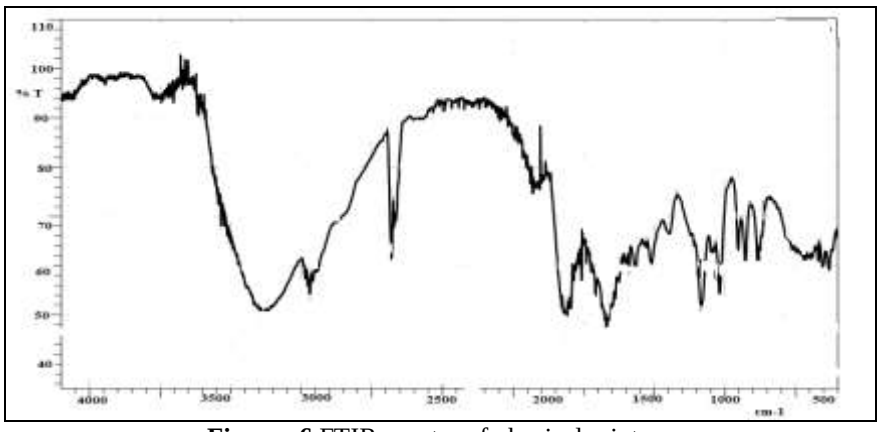

Fig. no. 6 FTIR spectra of physical mixture

After interpretation of FT-IR Spectrum of Chitosan and its physical mixture with drug Benzocaine, it was concluded that all the characteristic peaks corresponding to the functional group present in molecular structure of Benzocaine 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 Benzocaine and Chitosan was studied by using DSC as shown in figure respectively. The Benzocaine shows an endothermic peak at approximately 95℃ and it corresponds to its melting point (fig.). Chitosan shows a sharp endothermic peak at 91.12℃ corresponds to its melting point (fig.).

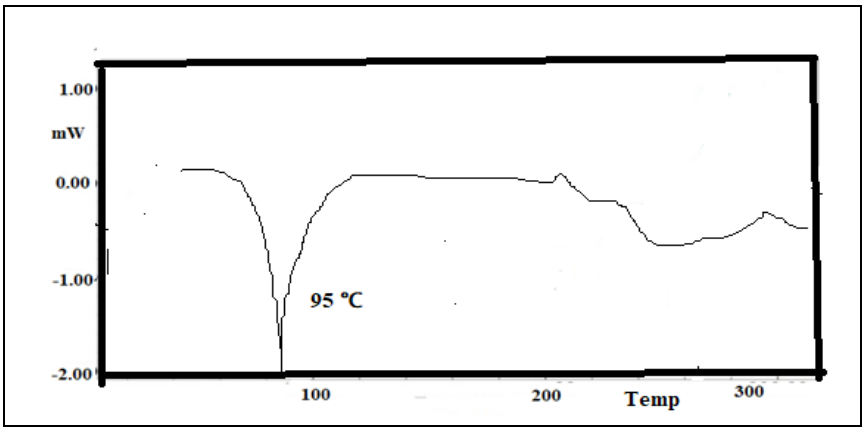

Fig. no. 7 DSC thermogram of Benzocaine

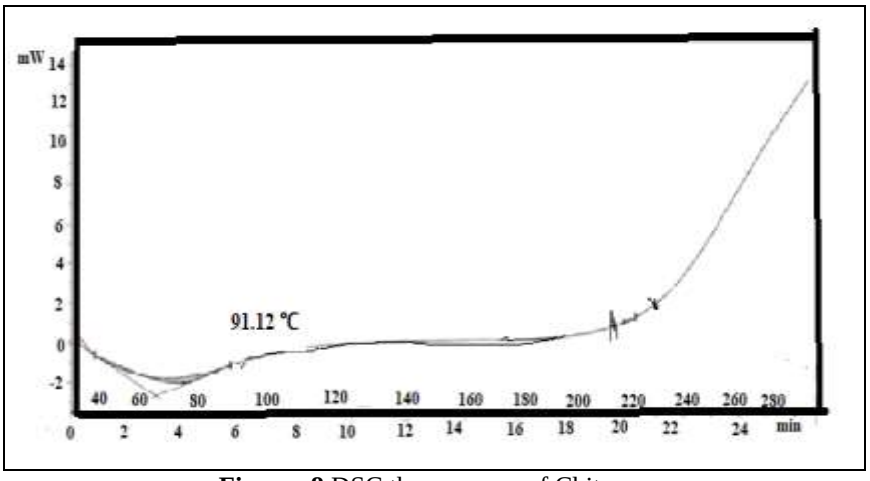

Fig. no. 8 DSC thermogram of Chitosan

Benzocaine content

According to the accompanying table, all formulations had a measured Benzocaine content in the produced chitosan film of 1.90 to 2.13 mg (96.8-108% of the theoretical drug load):

Table 8: Properties of different Benzocaine buccal film formulations

Formula	Benzocaine content (mg)	Film thickness (mm)	Tensile stress at break (MPa)	Extension at break load (mm)	Film swelling (%)	In-vitro bio adhesion (N)
F1	1.96 ± 0.10	0.79 ± 0.11	0.89 ± 0.75	2.59 ± 0.64	257.93 ± 0.35	5.933 ± 0.58
F2	1.90 ± 0.14	0.65 ± 0.03	0.06 ± 0.04	3.19 ± 0.03	187.53 ± 0.24	5.423 ± 0.11
F3	2.05 ± 0.12	1.05 ± 0.17	0.35 ± 0.27	2.64 ± 0.29	246.60 ± 0.12	14.96 ± 0.70
F4	1.92 ± 0.15	0.77 ± 0.12	0.39 ± 0.22	3.01 ± 0.24	264.53 ± 0.59	17.29 ± 2.61
F5	2.00 ± 0.11	0.64 ± 0.10	0.06 ± 0.03	1.96 ± 0.08	94.28 ± 0.31	41.84 ± 10.6
F6	2.13 ± 0.09	0.67 ± 0.09	0.20 ± 0.07	2.07 ± 0.443	224.16 ± 0.03	6.778 ± 0.03
F7	2.11 ± 0.16	0.59 ± 0.07	0.51 ± 0.04	2.16 ± 0.55	199.85 ± 0.10	12.74 ± 2.49
F8	1.91 ± 0.12	0.67 ± 0.07	0.13 ± 0.04	2.54 ± 0.29	219.15 ± 0.15	65.63 ± 2.16
F9	2.02 ± 0.13	0.83 ± 0.03	0.23 ± 0.07	4.15 ± 0.30	302.22 ± 0.07	12.59 ± 3.89

Film thickness

The table above contains a calculation of the average thicknesses of all Benzocaine films. As seen, these values vary between 0.59 ± 0.07 millimetres and 1.05 ± 0.17 millimetres, which is consistent with the recommended thickness for optimum buccal films (50 to 1000 millimetres) to prevent any application-related discomfort. Since the thickness measurements are made using various regions of each film, these figures also demonstrate that the manufactured films have uniformity in their thicknesses, which reflects dose accuracy. Notably, the thickness of the formulation with the highest percentages of chitosan and HPMC (F3) differed significantly ($p< 0.05$) from the thickness of the other formulations under investigation.

Swelling study

The important characteristic of polymer swelling behaviour that influences the mucoadhesion of polymeric films is that it is required for the film to first encounter the buccal mucosa. Additionally, polymeric films' level of hydration and swelling have a significant impact on how well drugs dissolve from them. We found in this investigation that the type of polymer and its concentrations in the casting solution had an impact on the swelling percentage. The following table shows the synergistic effect that HPMC concentration has on film swelling, which is statistically significant ($p = 0.041$).

Table 9: Analysis of variance for the effect of chitosan (X1) and HPMC (X2) on the responses of Benzocaine buccal film formulations

Extension at break load (mm)	Source	Sum of squares	F-Ratio	P-Value
Y1	A: Chitosan	1.75	12.68	0.050
	B: HPMC	1.07	6.47	0.094
	AA	0.09	0.41	0.583
	AB	0.05	0.15	0.753
	BB	0.44	2.54	0.213
Film swelling (%)	A: Chitosan	3466.92	2.153	0.251
Y2	B: HPMC	22032.7	15.36	0.042
	AA	1999.92	1.103	0.374
	AB	375.103	0.243	0.658
	BB	285.113	0.183	0.701
In-vitro bio adhesion (N)	A: Chitosan	69.893	0.12	0.788
Y3	B: HPMC	51.493	0.09	0.819
	AA	317.99	0.45	0.566
	AB	260.42	0.37	0.602
	BB	187.05	0.28	0.658
Higuchi diffusion slope (% /t 0.5)	A: Chitosan	4885.74	232.32	0.004
Y4	B: HPMC	306.033	18.163	0.033
	AA	861.913	45.983	0.006
	AB	860.903	45.933	0.006
	BB	18.1603	0.7903	0.451

Chitosan, however, only had a minimal impact on film swelling, just marginally delaying it. Additionally, a statistical programme (Stat graphics Plus, version 5) was used to assess both the quadratic and interaction effects. The computer programme examines the individual effects (chitosan concentration and HPMC concentration), their quadratic effects (chitosan concentration and HPMC concentration), and the interactive impact (chitosan-HPMC). Insignificant is also how the studied polymers' quadratic and interaction effects on film swelling are affected. When the concentration of the HPMC polymer was raised from 1% to 5%, the swelling values rose, as shown by the reaction surface plot in Fig. 9A. When 0.5% chitosan was used, the swelling percentages were in the following order: F9 > F8 > F2. Comparable results were also achieved when 1% and 1.5% of chitosan were used. The buccal films' surface wettability and swelling appear to be improved by the inclusion of the hydrophilic polymer HPMC. Chitosan's swelling percentage decreased from 187.53 ± 0.24 percent to 94.28 ± 0.31 percent for F2 and F5, respectively, when its concentration was

increased from 0.5% to 1.5% (w/v) using 1% w/v HPMC, whereas an effect from increasing the concentration from 0.5% to 1% (w/v) was not statistically significant ($p > 0.05$). The swelling values reduced with increasing chitosan concentrations for F1 (1% chitosan) and F6 (1.5% chitosan), respectively, from 257.93 ± 0.35% to 224.16 ± 0.03% while using 3% HPMC. Additionally, the rank order of swelling was 302.22 ± 0.07% (F9) > 264.53 ± 0.59 %, (F4) > 246.60 ± 0.12%, (F3) using 5% HPMC. In F9, which had the highest concentration of HPMC (5%) and the lowest concentration of chitosan (0.5%) during the experiment, the highest swelling value (302.22 ± 0.07) was recorded. Aside from that, F5 displayed the lowest swelling value (94.28 ± 0.31%).

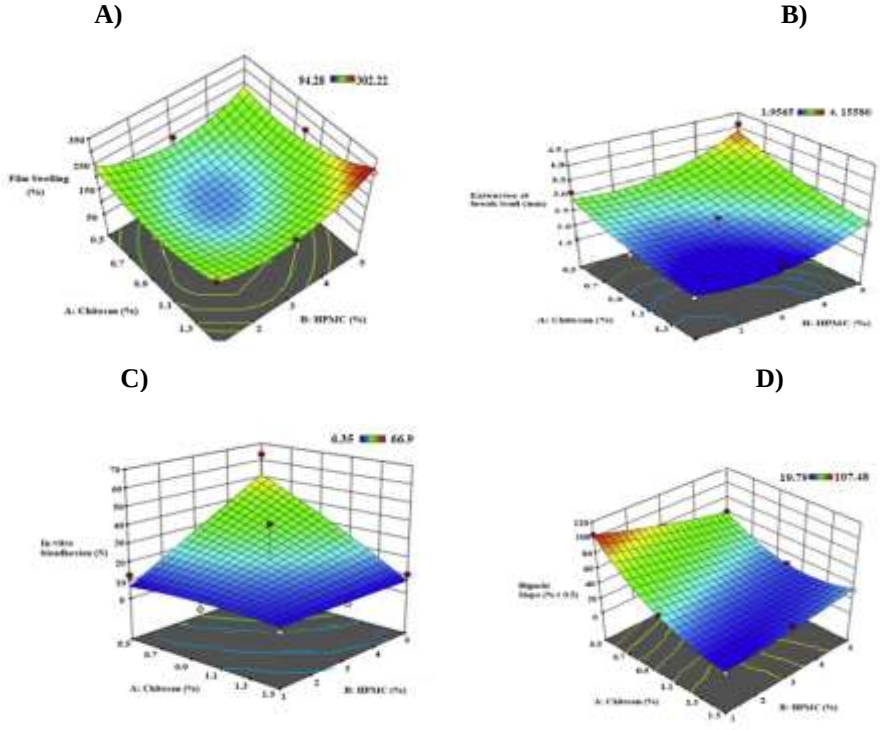

Fig.9: Response surface plots for the effect of independent variable on a) film swelling percentage, b) extension at break load, c) in-vitro bioadhesion, and d) Higuchi release slope

Mechanical properties

In order to assess the physical integrity of buccal films, it is crucial to measure their mechanical properties. The results of this study, which looked at the mechanical characteristics of several Benzocaine films, including their TS and extension at break stress, are shown in the table above. The TS is a measure of a film's strength that represents the highest stress per unit of cross-sectional area that it can withstand before breaking. While the extension at break load, which is defined as the percentage of the change in sample length relative to its initial length, measures the ability of the film to extend before breaking and provides information about the elasticity of the film. The ANOVA study of the impact of independent variables on the mechanical characteristics of buccal film is shown in the table above (extension at break load is shown as Y1 in millimetres). The response surface plot in Fig. 9B shows that Chitosan had the only significant agonistic effect on film mechanical characteristics ($p = 0.050$), whereas HPMC had a negligible but unimportant effect ($p = 0.10$).

The produced films' TS values ranged from 0.06 ± 0.004 MPa to 0.89 ± 0.75 MPa, and their extension at break loads varied from 1.96 ± 0.08 mm to 4.15 ± 0.30 mm. When chitosan content was increased from 0.5% to 1.5% in films containing 1 % HPMC, it was discovered that the extension at break load value for F2 and F5 decreased dramatically from 3.19 ± 0.03 mm to 1.96 ± 0.08 mm. However, increasing the chitosan concentration from 0.5% to 1% greatly enhanced the films' TS, raising it for F3 and F7 from 0.35 ± 0.27 MPa to 0.51 ± 0.4 MPa, respectively. Significantly lowering the TS values as demonstrated for F5 and F7 was achieved by increasing chitosan from 1% to 1.5%. This demonstrates the value of employing a factorial design study to optimise the mechanical properties of films. As evidenced by its extension at break load value (4.15 ± 0.30 mm), Film F9, which has the highest HPMC content (5%) and the lowest chitosan concentration (0.5%), is the formula with the strongest resistance. Given that HPMC is notorious for being rigid and brittle and is frequently added to films to enhance their mechanical properties, particularly membrane flexibility and resistance, this may be attributable to the high concentration of HPMC.

In-vitro bioadhesion

Mucoadhesive polymers are widely known for their ability to create strong adherence to mucus surfaces. Several distinct mechanistic theories of mucoadhesion have been suggested as potential causes for this feature. The mechanical, adsorption, diffusion, and electrical theories are a few of them. To evaluate the bioadhesive power of various films, the peak detachment force, which is most usually evaluated, was employed in this case. In the accompanying table, the measured peak detachment force for several produced films is shown. Researchers

discovered that increasing the amount of chitosan in films containing 1% HPMC from 0.5% (F2) to 1.5% (F5) raised the films' bioadhesion property from 5.423 ± 0.11 N to 41.84 ± 10.6 N, however the effect is negligible (p = 0.80) (Fig. 9C).

The well-known mucoadhesive qualities of chitosan are what cause this outcome to be anticipated. Furthermore, chitosan was increased from 0.5% (F9) to 1.5% (F3) in films containing 5% HPMC, however there was no discernible difference in the bioadhesive capabilities of the films. This may be because the high concentration of HPMC may have interfered with the true mucoadhesive function of the chitosan. On the other hand, there was no discernible difference in bioadhesion when the concentration of chitosan was maintained at 1% while HPMC was increased from 1% to 5%. These findings demonstrate the critical influence of polymer type and concentration on the bioadhesive characteristics of produced films.

In-vitro drug release

The independent variables clearly affected the in vitro drug release from buccal films, as indicated in the above table and Fig. 9D.

The Higuchi diffusion slope of drug release from buccal films was significantly enhanced by the HPMC concentration (p = 0.04) while negatively impacted by chitosan (p = 0.001). Additionally, there were significant impacts (p = 0.008) on the drug release rate from the quadratic effect of chitosan concentration (X12) and the interactive effect of chitosan with HPMC (X1X2). These findings demonstrate the relationship between medication release rate and the type and quantity of the polymers used. This observation may be explained by HPMC's hydrophilic polymer properties, which, as was already indicated, allow it to bind to water molecules, increasing the produced films' percentage swelling. The drug's rate of breakdown from the matrix was subsequently accelerated as a result. In addition, whereas chitosan naturally exhibits hydrophilicity, it also exhibits some hydrophobicity due to the high level of deacetylation (99%). Here, the formulations' high chitosan concentrations led to an increase in viscosity as well as a decrease in the pace at which the matrix hydrates or allows solvent molecules to enter the system. As a result, the rate at which the medication was released from the matrix was reduced.

The release of Benzocaine increased with an increase in HPMC concentration from 1% to 5%, and for F2, F8, and F9, respectively, the release was finished after 1, 2, and 3 hours. Additionally, formula F5 showed the slowest drug release of 52.82 ± 6.65% after 7 h of all film formulations (Fig. 10), despite having the greatest chitosan and lowest concentration of HPMC.

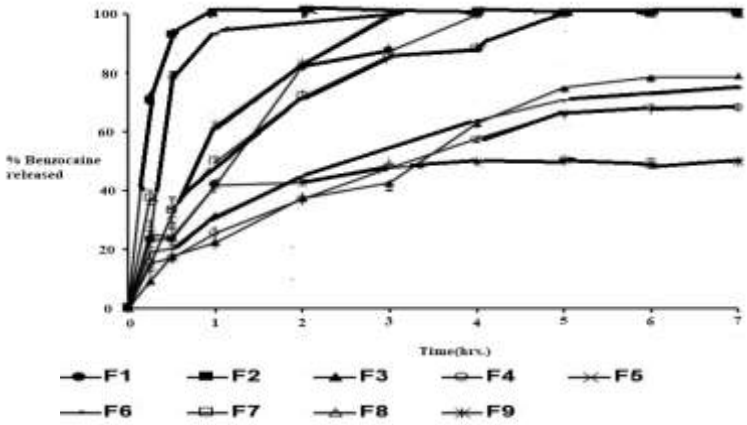

Fig. 10. In-vitro release profiles of Benzocaine from different buccal film formulations

These findings highlight the relationship between the swelling measurements and the pace at which Benzocaine is released from the manufactured buccal films. The in vitro release data were adjusted using the Higuchi-diffusion, first order, and zero-order kinetic models. The kinetic data are summarised in the following table.

Table 10: Kinetic modelling of Benzocaine 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	0.989	9.613	0.990	−0.076	0.963	30.99	0.993	0.584
F2	0.868	91.30	N/A	N/A	0.963	107.48	0.963	0.263
F3	0.963	13.54	0.983	−0.106	0.963	34.95	0.983	0.663
F4	0.993	15.91	0.993	−0.090	0.963	29.76	0.983	0.524
F5	0.763	5.113	0.813	−0.036	0.903	18.79	0.944	0.245
F6	0.983	9.973	0.993	−0.088	0.963	31.41	0.984	0.475
F7	0.963	18.94	0.993	−0.223	0.963	46.14	0.965	0.383
F8	0.853	48.71	0.743	−0.053	0.933	80.43	0.874	0.667
F9	0.992	34.75	0.973	−0.063	0.963	64.06	0.994	0.785

Based on the correlation coefficient value, the preferred release mechanism was determined. According to the findings, benzocaine release from buccal films followed the Higuchi diffusion model, based on the maximum value of the correlation coefficient of the plotted percentage drug release versus square root of time. Furthermore, it was discovered that the calculated values of n (derived from the Korsmeyer-Peppas model) were both lower and higher than 0.48, but that all values were lower than 1, indicating non-Fickian or anomalous drug release (coupled diffusion/polymer relaxation).

Optimization of Benzocaine buccal film

A promising method that is still quite new to pharmacy practise, formulation optimisation has shown it to be an efficient use of factorial design. The factorial design technique was used in this study to forecast the values of the different characterisation parameters and optimise the planned buccal film by calculating its best potential composition. The projected values for the characterisation parameters were then compared to the experienced ones in order to validate the outcomes of applying the optimised independent variables (% of chitosan and HPMC in the formulations). As a result, the optimised buccal film formula and the responses predicted using the factorial design technique are shown in the following table.

Table 11: Composition and predicted response values of the optimized buccal film formula

Factors (X)	**Optimized**				**Observed values**
Chitosan (X1) %	0.52				
HPMC (X2) %	4.38				
Responses	**Goal**	**Lower Limit**	**Upper Limit**	**Optimized**	
Extension at break load (mm)	maximize	1.943	4.1303	3.4603	3.484 ± 0.483
Film swelling (%)	maximize	93.28	300.22	273.30	252.97 ± 0.42
In vitro bio adhesion (N)	maximizes	5.323	64.603	32.153	37.22 ± 6.103
Higuchi slope (%/t 0.5)	maximize	18.79	105.48	69.263	68.92 ± 3.663

0.52 % chitosan and 4.38% HPMC were combined to create this mixture. Comparing the measured and projected bioadhesion results 37.22 ± 6.103 N, respectively—was the first step in the validation procedure. Additionally, whereas the projected value for film swelling was 273.30 %, the optimised Benzocaine film formula revealed a value of 252.97 ± 0.42%. Additionally, the mechanical characteristics of the optimised film formula were 3.51 ± 0.51 mm, which was comparable to the forecasted value of 3.48 mm for extension at break load. The optimised formula demonstrated good mechanical properties, and the thicknesses, TS, and extension at break load values were suitable for this application. In addition, the experimental Higuchi release slope was lower than predicted at 68.92 ± 3.66 %/t 0.5. These results corroborated the formulation optimisation results obtained by using the optimised independent variables and demonstrated the potency of the factorial design technique.

Thermal analysis

Using the optimised buccal film recipe, DSC was used to identify how Benzocaine interacted with the film polymeric composition. The polymer peak was seen at 92 °C and the drug endothermic peak was seen at 96 °C in the case of the optimised film.

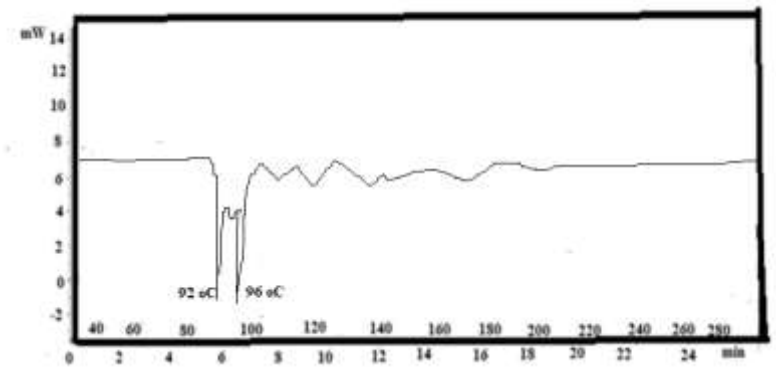

Fig. no. 11 Optimized Benzocaine loaded buccal film

X-ray powder diffraction (XRPD)

The optimised buccal film was subjected to XRPD to identify crystalline changes in the Benzocaine. The XRPD spectrum of benzocaine indicates that it is crystalline due to the presence of several diffraction peaks at 2 theta diffraction angles of 13, 15.7, 16.6, 23.6, 27.3, and 30.1. Conversely, diffraction peaks could be seen for chitosan at 9.35 and 20.34 or HPMC at 19.65, 22.45 and 23.68. However, the XRPD spectra of the Benzocaine buccal film showed that the drug's diffraction peaks at 21.45 and 25.67.

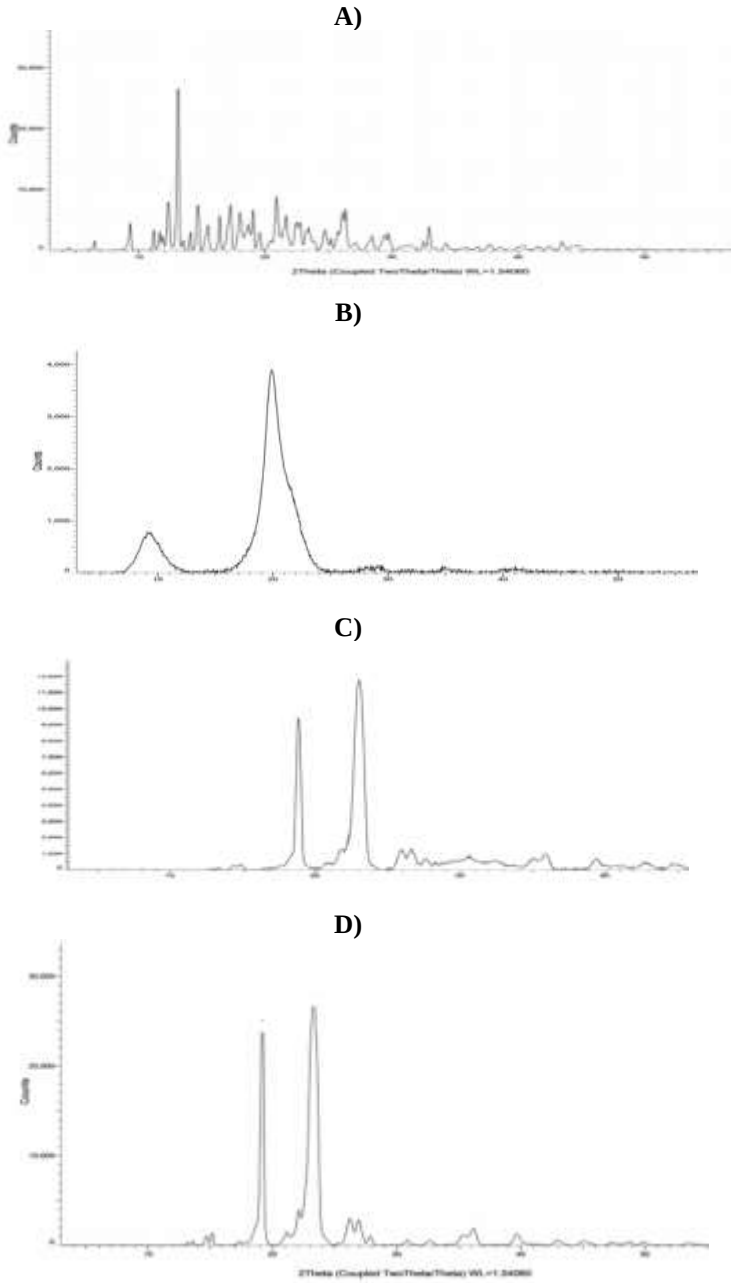

Fig. no. 12 X-ray powder diffraction pattern of Benzocaine (A), chitosan (B), HPMC (C), and optimized Benzocaine loaded buccal film (D)

In-vivo bioadhesion residence time

The optimised Benzocaine film was tested for in-vivo bioadhesion residence duration on six healthy human volunteers, and the results showed minimal irritation or excessive salivation, while the taste was mildly bitter. According to the findings, the optimised film had an in-vivo bioadhesion residence duration of 1.28 ± 0.20 h before being separated from the buccal mucosa and eroding in all six human participants. The over-hydration of the polymer, which causes disentanglement at the polymer-mucus interface and a rapid fall in mucoadhesive strength, could be the cause of this decrease in the bioadhesion residence time. Additionally, it could be brought on by more extreme circumstances than those that often affect in-vivo trials, like mouth movement during speech and swallowing.

Conclusion

This study develops a mucoadhesive Benzocaine local delivery method that is optimised for treating oral disease. This film had good mechanical and bioadhesion qualities and was simple for patients to apply. The medication was delivered in the buccal cavity in a controlled manner over a period of 6 hours in a concentration that is higher than the drug's IC50, even though the film only contains a little amount of the drug compared to the oral dose. As a result, the improved Benzocaine mucoadhesive buccal film may be regarded as an ideal substitute dosage form for oral medication in the treatment of oral disease and may offer a way to counteract the unintended side effects of drugs

taken orally. The fact that the distribution system does not require expert application or removal is another factor that is anticipated to promote patient compliance.

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