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Formulation and Evaluation of Chitosan-Based Polymeric Biodegradable Mucoadhesive Buccal Delivery for Locally Acting Drugs: *In Vitro*, *Ex Vivo* and *In Vivo* Volunteers Characterization

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***SUMMARY*. Pain treatment by means of a local anesthetic and at the same time, controlling the microbial flora with an antiseptic agent can be considered as an option for delivering through mucoadhesive buccal dosage form. The objective of the current study was to develop a chitosan (CHI) based targeted polymeric buccal mucoadhesive fabrication for simultaneous release of lignocaine hydrochloride (LGN) and tibezonium iodide (TBN). Mixed ingredients were compacted via direction compression using chitosan (CHI) with sodium alginate (SA) and hypromellose (HPMC). Outcomes shown that the weight variation and friability were according to the USP specifications and were unaffected by changing amounts of the polymers in the formulations. Maximum swellability was observed in PT8, containing 7.5% of SA and CHI. Surface pH was also in accordance to the normal physiological pH range. Maximum mucoadhesive strength and time values were observed in the formulation PT4 containing 7.5% concentration of CHI and HPMC each, which were 5.76 h and 9.37 g, respectively. Same was the case for PT4 in terms of maximum simultaneous *in vitro* release for both drugs. The release of TBN and LGN was best fitted to Hixon Crowell release model. Formulation PT4 promised optimum results in terms of maximum release with optimum mucoadhesive values till to 4 h.**

RESUMEN. El tratamiento del dolor por medio de un anestésico local y al mismo tiempo, el control de la flora microbiana con un agente antiséptico puede considerarse como una opción para su administración a través de una forma de dosificación bucal mucoadhesiva. El objetivo del presente estudio fue desarrollar una fabricación mucoadhesiva bucal polimérica dirigida basada en quitosano (CHI) para la liberación simultánea de clorhidrato de lignocaína (LGN) y yoduro de tibezonio (TBN). Los ingredientes mezclados se compactaron mediante compresión direccional usando quitosano (CHI) con alginato de sodio (SA) e hipromelosa (HPMC). Los resultados mostraron que la variación de peso y la friabilidad estaban de acuerdo con las especificaciones de la USP y no se vieron afectadas por las cantidades cambiantes de los polímeros en las formulaciones. Se observó una máxima hinchabilidad en PT8, que contenía un 7,5% de SA y CHI. El pH de la superficie también estuvo de acuerdo con el rango de pH fisiológico normal. Se observaron valores máximos de fuerza mucoadhesiva y tiempo en la formulación PT4 que contenía una concentración del 7,5% de CHI y HPMC cada una, que fueron 5,76 hy 9,37 g, respectivamente. Lo mismo sucedió con PT4 en términos de liberación *in vitro* simultánea máxima de ambos fármacos. El lanzamiento de TBN y LGN se ajustó mejor al modelo de lanzamiento de Hixon Crowell. La formulación PT4 prometía resultados óptimos en términos de liberación máxima con valores mucoadhesivos óptimos hasta las 4 h.

INTRODUCTION

Various opportunities for mucoadhesive buccal drug delivery like films [1], gels [2], tablets [3], sprays [4] and particulate dosage form are present for mucoadhesive drug delivery system to deliver the local or systemic release of the medicament in the buccal region. Contrary to the conventional buccal tablets, the mucoadhesive tablets are, however, static in its position in the buccal cavity [5]. In other words, the mucoadhesive buccal tablets adhere to the mucosal surface in the suitable regional spot in the mouth and continue to deliver the drug over the period of time [6]. This route has been extensively stud-

KEY WORDS: anesthetic lozenges, antiseptic buccal tablet, lignocaine HCl, mucoadhesive buccal tablet, mucoadhesive strength tester, tibezonium iodide.

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ies over last two decades [7,8] at *in vitro* [9], *ex vivo* and *in vivo* level [10] in order to characterize the dosage form. It is one of such dosage forms that has gained the potential to commercialize the dosage form [11] based on the assessment of the dosage form. It is because the mucoadhesive buccal tablet first converts to gelatinous form after imbibing the fluid medium so that it is converted to some form of hydrogel [12]. Then release of the drug can be controlled or sustained depending upon the strategy involved. It also offers numerous advantages such as bypassing first pass effect and significant absorption if it is dependent upon the vasculature below the tongue. The dosage form can also be removed in case of any emergency [13]. If the ghost structure is present at the end of complete drug release, then pharmaceutical excipients can simply be expelled or spit out conclusively. For treating local remedies of buccal mucosa, mucoadhesive dosage form mainstay important landmark for the delivery of drugs locally, in spite of loading heavy doses systemically. It is because the drug delivery staying at the site of action and releasing the dug for local action beneficial to reduce the systemic site action. The dose of the drug in this scenario can be reduced [2].

Lignocaine hydrochloride (LGN) is a local anesthetic substance that is delivered to the buccal cavity and other body sites for local action [15]. It is generally delivered as a buccal gels or pastes for temporary loss of sensation, or in other words the periodic relief from the pain perception [16,17]. It is water soluble and is part of the dental procedures as well to induce local anesthesia. It is well tolerated by the patients generally and is effective since it is part of certain soothing oral cavity gels as well. In the current study, it was designed to use LGN as a locally releasing agent for non-sensation of the pain receptors [18]. It was designed to combine it with the tibezonium iodide (TIB) which is a locally acting antiseptic agent [19]. It is commercialized [20] in different regions of the world under different trade names. In Pakistan, it is marketed as Maxius® lozenge with a therapeutic chewable buccal tablet unit dose of 5 mg [21]. For the delivery of both drugs, chitosan (CHI) was based for the release of drugs because it has been used for mucoadhesive drug delivery [22], biodegradable [23], biocompatible [24] and possesses antimicrobial properties [25] which are relatively desirable in this case. It was planned to combine CHI with sodium alginate (SA) and hydroxypropyl methyl cellulose (HPMC). Both polymers are mucoadhesive [26,27], swellable and has been extensively reviewed in the literature. HPMC also possesses the sustainability in releasing the drug over time [12].

So, aim of the current study was to develop a mucoadhesive drug delivery system for the simultaneous release of a local antiseptic as well as local anesthetic agent for local ailments like sore throat infection, bronchitis, pain associated with oral sores, stomatitis or such related. It was accomplished with the aid of biodegradable semisynthetic mucoadhesive polymers for the local release of tibezonium iodide and lignocaine hydrochloride. The objectives of the study were to evaluate the formulated dosage form for *in vitro*, *ex vivo* and *in vivo* characters in healthy volunteers and results were concluded.

MATERIALS AND METHODS

Tibezonium iodide (TBN) was procured as a gift sample from Pacific Pharmaceuticals Limited (Lahore, Pakistan). Low molecular weight chitosan (CHI) polymer was purchased from Sigma-Aldrich®. Whereas lignocaine hydrochloride (LGN), sodium alginate (SA), hydroxypropyl methyl cellulose grade K15 (HP) and polyvinyl pyrollidone k30 (PVP) were attained from Hoover Pharmaceuticals, Pakistan on generous base.

Fabrication technique

As referred (Table 1), all the drugs, polymers and added excipients were accurately weighed and mixed with the help of spatula manually for 2 min. The designated weight of the tablet was set at 200 mg. Sucralose was added in the formulation as sweetening agent while magnesium stearate was used for powder lubrication. The method used for the compaction of powder into tablets by was direct compression method [28]. For this purpose, a force of 1.8 ton was applied on the pre-compressed mixed powder for 10 s using Okeda Chem. Co. Ltd. using 8 mm flat-faced punch.

Characterization of buccal mucoadhesive compressed dosage form

Directly compressed tablets were evaluated for the following physical and physicochemical evaluation tests.

Physical characterization

Following physical tests were evaluated to characterize the physical properties of compressed tablets.

General appearance

Formulations were investigated for the general appearance of the surface of the tablet. The surface smoothness was analyzed for this purpose and intra variation of the tablets for each batch was noted, if present.

Weight variation

To observe variation in the weight of the tablet in each formulation, twenty tablets were chosen randomly and weighed individually. Then average weight for each formulation were calculated to estimate the extent of deviation in the respective formulation code.

Thickness

From each coded formulation, ten tablets were chosen randomly and were estimated for thickness measurement. Digital Vernier caliper was used to calculate thickness results were presented as a mean of standard deviation.

Diameter

For diameter, same procedure was employed for the determination of thickness and results were expressed similar to thickness.

Hardness

Digital hardness tester MT-2020 was used to examine the hardness of tablet. For this purpose, ten tablets were analyzed for hardness.

Friability

Friability was calculated by weighing thirty three tablets and put inside in the Roche friabilator. Rotation was set at 25 rpm for 4 min. After the time interval, the tablets were removed, dedusted and reweighed. Then percentage particle loss due to friability for each formulation was evaluated [3] using Eq. [**1**] and expressed as a percentile loss.

$$\text{Percent loss} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100 \quad [1]$$

Physicochemical characterization

The compressed dosage form was evaluated on physicochemical grounds for surface pH, swelling index, matrix erosion for the swelled tablet at 6 h, *ex vivo* mucoadhesive strength, *ex vivo* mucoadhesive time, *in vivo* residence time, *in vitro* release for both drugs, *in vivo* drugs study on healthy human volunteers, *in vitro* release kinetics.

Surface pH

Measurement of the pH on the surface of mucoadhesive buccal formulations were carried out by placing the respective tablet form each batch in a petri dish containing buffer adjusted to pH 6.8 with the help of orthophosphoric acid. At the end of 2 h, surface pH was determined by touching the proximal surface of the tablet with the tip of the electrode of digital pH meter. The value was recorded when stabilized. The experiment was repeated thrice [12].

Swelling index (SI)

To measure the swelling index of the prepared mucoadhesive formulations, dry tablet from each formulation code were weighed and placed in a petri dish containing 10 mL of phosphate buffer adjusted to pH 6.8. Weight gained by the tablet after water sorption over time was calculated by reweighing the tablet on specified time intervals on a sensitive digital weighing balance. The weight gained by the tablet was considered as the swelling index of the relevant tablet at a specified interval point using the Eq. [**2**] [3]. The values of swelling index were expressed as the standard deviation of the average value of three tablets taken from same formulation code.

$$\text{Swelling index} = \frac{\text{swollen weight at time (t)} - \text{dry weight}}{\text{dry weight}} \times 100 \quad [2]$$

For each time interval 't', the swollen weight was estimated and the results were calculated.

Matrix erosion analysis (ME)

The swollen tablets after 6 h from the swelling index experimentation were used to assess ME. It was accomplished by placing the tablet in a dry heat oven up to 24 h at 60 °C until constant weight was achieved (W3). The extent of moisture loss (W1), given in Eq. [**3**] was estimated [29] and presented as a mean of three observations.

$$ME = \frac{W1 - W3}{W1} \times 100 \quad [3]$$

Where W1 is the initial dry weight of the tablet.

***Ex vivo* mucoadhesive strength**

All prepared mucoadhesive buccal formulation were investigated for its mucoadhesive strength using an improved physical balance having a pan in one arm rest of the balance and a moveable glass slides attached to the other side of the limb as reported [30]. In between the glass slides freshly slaughtered rabbit buccal mucosa was attached in such a way that the tablet was sandwiched between the mucosa at-

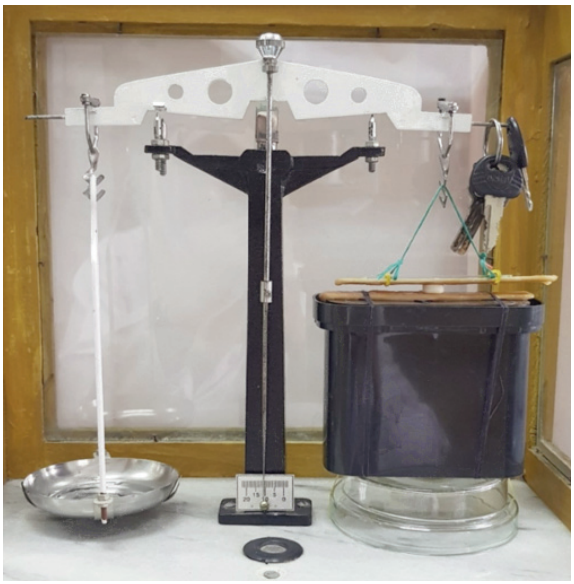

Figure 1. Depiction of the reconstructed mucoadhesive strength tester for the measurement of mucoadhesive strength as reported 30 in the literature.

tached on the glass slides as shown in Fig. 1. Weight was added in the form of water drops on the left side of the unmodified pan. The measurement was started when whole of the system was static and no tension was present on the threads till around 2 g. Experiment was initiated by adding water in the empty pan slowly at a constant rate with plunger without needle at a rate of approximately 1 mL per 10 s until the tablet was detached. The weight at which the mucoadhesive dosage form detached from either side of the mucosa, slide, was considered as the respective *ex vivo* mucoadhesive strength of the respective buccal formulation. The experiment was repeated thrice and presented in the Results section as a mean of standard deviation.

Ex vivo mucoadhesive time

Mucoadhesive time of a buccal formulation was estimated by isolating the buccal mucosa of freshly sacrificed rabbit which was then attached and fixed onto the glass slide with an adhesive material. For that an Ethical Approval no. IREC-2019- 125A was obtained from the Institutional Review Board of The University of Lahore. A tablet was attached from one side after wetting with around 300 µL PBS, pH 6.8 and pressed gently pressed for 20 s for peaceful portal on to the mucosal membrane for the start of the estimation of mucoadhesive time. The glass slide was inserted at an inclined angle into the beaker containing 800 mL of PBS adjusted to pH 6.8. The whole system was put on the hot plate and the solution in the beaker containing slide was maintained at 37.5 °C throughout the experiment. The whole setup was agitated with the help of a magnetic stirrer at 100 rpm. When the tablet was detached or disintegrated from the mucous membrane was considered as mucoadhesive time for the related formulation [12]. The average was expressed for the performance of experiment in thrice.

In vitro drugs release study

The *in vitro* drugs release for TBN and LGN were quantified using the USP type II (ERWEKA DT-700) paddle apparatus with a speed of paddles revolving at 50 rpm [12]. The dissolution media was set based on the solubility and sinking conditions of both drugs and was composed of 900 mL of sodium lauryl sulphate (SLS) in weight to volume ratio of 0.25%. Temperature of the system was maintained at 37.5 ± 0.5 °C during the experiment. The pH of the dissolution fluid was adjusted to 6.8 to mimic the buccal cavity dissolution conditions. For quantitative assessment of both drugs, 5 mL of samples was removed from the dissolution apparatus at time intervals of 0.5, 1, 2, 4, and 6 h while same amount of fresh volume was refilled to justify sink conditions. The samples were filtered and was placed in the auto sampler vial of the HPLC machine for drugs analysis.

There was no method reported for the simultaneous evaluation of drugs in the literature for which a new HPLC instrumental conditions were devised that has been briefed in the Result section.

In vitro TBN and LGN release kinetics

The kinetics of drug release from its dosage form was studied on the optimized dosage form. To accomplish, DD Solver® (Microsoft® Excel Add-in function) was applied [31] to assess the kinetics of TBN and LGN release from the desired dosage form. The *in vitro* release kinetic models that were applied on the optimum compressed formulation included zero order, first order, Korsmeyer-Peppas, Higuchi and Hixon Crowell models. The best fit model was selected to understand the mechanism of drugs release [30].

In vivo residence time

The capacity of the tablet to reside in the mouth of human body was performed in

healthy volunteers who were willing to participate in the study. The prepared mucoadhesive buccal tablet was compressed without the addition of drugs (TIB and LGN) in the dosage form. An ethical approval was obtained from the University board for the conductance of experiment in healthy volunteers and was performed according to the protocols of Helsinki. The volunteers were aged between 20-28 years and were informed not to eat during the experiment while volunteers consumed water during the experiment. The tablet was applied on the frontal part of the buccal mucosa in the mouth. But the tablet was wetted with 200 µL of distilled water and was pressed gently to its portal site for settlement. The time at which the tablet either particle break off due to disintegration or detached from the mucosal surface was considered as the *in vivo* residence time of the tablet [32].

Fourier Transform Infrared (FTIR) analysis

Fourier transform-infrared (FTIR) spectra were measured using Bruker Alpha™ (software operated by OPUS) Platinum-ATR in transmission mode in the range of 4000-600 cm^{-1}. Infrared spectral analysis of the drugs, polymers and optimized formulation were carried out in order to study the presence of normal functional groups as well as detecting any new or unusual peak, if any [31].

Differential Scanning Calorimetry (DSC) analysis

It was performed on the samples of TBN, LGN, CHI, HPMC, SA, and the optimized formulation. For this, around 8 mg of the sample, to be analyzed, was sealed inside aluminum cup covered with the lid and placed inside the DSC TL Q2000TM machine. The scanning temperature range was set between 40 to 250 °C at an incremental rate of 20 °C/min while the inert gas was purged with a volumetric rate of 50 mL/min.

RESULTS AND DISCUSSION

For the preparation of mucoadhesive buccal dosage form, chitosan (CHI) was based in all the formulations. It was because chitosan possesses antimicrobial properties as well in addition to its pharmaceutical role. However, the properties of CHI well expressed in film and gel dosage form. So, initially the properties of CHI at concentration of less than 10% was designed to evaluate along with sodium alginate (SA) and hypromellose (HPMC) in such a way that each of the SA and HPMC were combined with CHI in two levels of concentration levels in the current study *i.e.* 5% and 7.5% in all possible combinations as shown in Table 1. So, eight possible formulations were prepared and coded from PT1-PT8. The formulations were tested for all the methods except *in vitro* release kinetics which was only applied on the release rate of the optimized formulation for both drugs. The dose of tibezonium iodide (TIB) and lignocaine hydrochloride (LGN) in the current study was set to be 5 mg [21] and 20 mg [12], respectively. Initially, mannitol was added in the formulation as diluent agent which posed compression problems and poor friability was observed. Then polyvinyl pyrollidone (PVP) k30 was included as a binder and lactose was added in place of mannitol along with to improve the physical characteristics in order to make up the filling powder amount for a tablet of 200 mg. Similarly, sucralose and magnesium stearate were added in the formulation recipe as sweetener and lubricant, respectively, in fixed amount as listed (Table 1).

Ingredients	PT1	PT2	PT3	PT4	PT5	PT6	PT7	PT8
CHI	5	7.5	5	7.5	5	7.5	5	7.5
HP	5	5	7.5	7.5	-	-	-	-
SA	-	-	-	-	5	5	7.5	7.5
TBN	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
LGN	10	10	10	10	10	10	10	10
PVP	5	5	5	5	5	5	5	5
sweetener	5	5	5	5	5	5	5	5
lubricant	4	4	4	4	4	4	4	4
diluent	68.5	66	66	63.5	68.5	63.5	68.5	63.5

Table 1. Tabulated representation of percentile composition of different mucoadhesive buccal formulations (PT-PT8) prepared in the current study.

Physical characterization

The tablets were evaluated for physical appearance for its surface as well as the extent of smoothness. Results revealed absence of change in color in all the formulations. There was neither any abrasion in the corners, cracks, spots nor pitted marks on the surface of the tablets. The color of surface of the tablet was nearly off white. The diameter of the punched tablets was in the range of 8.13 to 8.18 mm while maximum deviation in the mean diameter value was observed with the formulation, PT8, containing highest concentration of SA and CHI. The deviation was not more than 5% and was considered insignificant. The thickness of formulations in the current study was found to be in the range of 2.60-2.71 mm for the formulations PT5 and PT8, respectively. Least deviation was observed with the formulations PT2 and PT7 which was 0.05. The preset weight of the tablet was 200 mg. This weight falls in the deviation limit of ± 7.5% according to United States Pharmacopeia (USP) [33]. The weight variation test is significant in sense that if it is outside the compendial deviation limits, it means that there is significant variation in the amount of the active moiety present in the unit dosage form. The weight variation of all the formulations were within the Pharmacopeial standards and no tablet was outside the compendial limit while least deviation was observed with the formulation PT6. However, the average weight of all the formulations was ranged between187.56-201.66 mg. After performing trials on the hardness of the tablet, the hardness value was finalized at 5-7 Kg/cm^2. Results showed that hardness of all the formulations was also in such range. Friability parameter is an important determinant of the physical resistance possessed by the formulation to mechanical shock during locomotion. The friability of all the tablets was also in the official limit of USP, which is less than 1%. However, maximum and minimum friability were shown by formulations PT7 and PT5, which were 0.697 and 0.386, respectively. All the values of physical tests have been detailed (Table 2).

Physicochemical characterization

The physicochemical properties of the formulations are important since it is the major determinant of extent of irritation, swellability, mucoadhesion, and movement of the drugs out of the dosage form. Results of physicochemical evaluation are as follows.

Surface pH

Surface pH is an important parameter since the surface of the mucoadhesive formulation is directly in touch with the buccal mucosal membrane. Highly acidic or basic pH may cause pathological irritation. So, the value near to normal range is theoretically more acceptable. The normal pH of saliva is around 6.7 and this value varies in the normal range limit of 6.2-7.6 [34].

Results of salivary pH of mucoadhesive formulations have been listed (Table 3). The calculated lower and upper range of pH was 6.23-7.12, respectively for PT4 and PT2. These values occur in between the aforesaid normal salivary pH. So, it can be conferred that the found pH of the mucoadhesive formulation was mimicking the physiological pH [35].

***Ex vivo* mucoadhesive strength**

Mucoadhesive strength provides an estimation that how much force is present for adhesion as well as to detach the dosage form from the mucosal surface. The *ex vivo* mucoadhesion

Code	Diameter Mean (mm) ± SD	Thickness (mm) Mean± SD	Weight variation Mean (mg) ± SD	Hardness (kg/cm^2)	Friability (%)
PT1	8.14 ± 0.04	2.61 ± 0.21	187.56 ± 1.81	5.68	0.503
PT2	8.16 ± 0.05	2.62 ± 0.05	195.12 ± 1.33	5.21	0.532
PT3	8.13 ± 0.03	2.61 ± 0.20	201.66 ± 3.71	5.54	0.479
PT4	8.16 ± 0.03	2.61 ± 0.13	191.30 ± 1.29	5.97	0.634
PT5	8.16 ± 0.05	2.60 ± 0.09	195.15 ± 1.34	5.91	0.697
PT6	8.18 ± 0.02	2.63 ± 0.20	196.81 ± 0.54	7.02	0.485
PT7	8.18 ± 0.02	2.61 ± 0.05	190.20 ± 1.61	6.96	0.386
PT8	8.17 ± 0.06	2.71 ± 0.20	200.19 ± 0.37	6.37	0.605

Table 2. Response of the mucoadhesive buccal formulations prepared in the study in terms of physical characterization of the prepared dosage form.

Code	Surface pH	*Ex vivo* mucoadhesive time (h) ± SD	*Ex vivo* mucoadhesive strength (g) ± SD	*In vivo* residence time (h) ± SD	ME (%)
PT1	7.02	3.28 ± 2.89	6.83 ± 2.48	0.26 ± 2.10	67.76
PT2	7.12	3.01 ± 3.12	6.19 ± 0.73	0.31 ± 0.98	70.19
PT3	6.72	4.50 ± 2.84	7.56 ± 1.98	1.25 ± 2.83	58.31
PT4	6.23	5.76 ± 4.91	9.37 ± 3.73	2.91 ± 1.18	36.24
PT5	6.45	1.68 ± 2.21	6.74 ± 4.8	0.48 ± 3.09	69.44
PT6	6.98	0.99 ± 2.70	6.08 ± 3.67	0.35 ± 1.80	71.02
PT7	7.01	1.36 ± 0.81	5.96 ± 2.14	0.52 ± 2.24	78.68
PT8	6.39	2.06 ± 3.91	6.79 ± 4.24	0.58 ± 2.69	54.5

Table 3. Response of the mucoadhesive tablets towards mucoadhesive and physicochemical characterization for the formulations (PT1-PT8) prepared in the study.

testing was performed on a modified balance (Fig. 1) in the laboratory under ambient conditions. Buccal mucosa of rabbit was freshly obtained, isolated and adhered on to the surface of the glass slides. Results revealed that there was a general increasing trend for the mucoadhesion force with respect to the increasing concentration of HPMC [12], if we consider look into the formulations PT2-PT4. CHI has been used in mucoadhesive dosage form since it is biocompatible and biodegradable, but possesses comparatively less mucoadhesion properties [22]. CHI requires some conditions to swell and this property was linked to the poor mucoadhesion results of the polymer. In order to achieve more satisfactory results for mucoadhesive strength it is recommended to add some higher concentration of the polymers to adjust the desirability of the ingredients. For formulations containing SA, generally, lower values of force were calculated compared with HPMC containing formulations. The higher amounts of SA *i.e.* 7.5% used in the formulations PT7 and PT8 did not have significant effect on the values of mucoadhesion and the values were low with respect to HPMC containing formulations in amounts of 7.5%, respectively. Overall, lowest value was observed PT7 containing SA and CHI in amounts of 7.5% and 5%, respectively. While highest force was observed with the formulation PT4, which was 9.73 g, containing both CHI and HPMC in amounts of 7.5% of the drug tablet weight as depicted (Table 3).

Ex vivo mucoadhesive time

Mucoadhesive time is important since it approximates the time required for the release of the drug into the regional mucosa. It was the formulations and as the concentration of HPMC was increased from 5% to 7.5%, the values of time were incremented from ≈ 3 h to more than 4 h. The highest mucoadhesive force was achieved when HPMC and CHI were both delivered in the concentrations of 7.5% in PT4 and the value was 5.76 h. Similar to performed under *ex vivo* conditions and conducted after obtaining ethical approval for experimenting in animals. The mucoadhesive time for all the formulations was ranged from 0.58-5.76 h under conditions stated in the methodology section. The mucoadhesion was linked with HPMC concentration in such results, the values of SA containing formulations were less compared with HPMC containing formulations. The values of time for SA containing formulations was ranged between 0.99-2.06 h, respectively, although time values were slightly increased as the concentration was increased from 5 to 7.5%, both for CHI and SA (PT5-PT8). It could be due to the swelling properties of the SA in addition to the disintegrant action in the range of 2.5-10% 3. If the concentration of both SA is increased above this range, then there can be a possibility for higher values of time.

In vivo residence time

The *in vivo* residence time was observed for all the formulations not containing the active moieties in the mucoadhesive formulations prepared in the current study. The safety of the polymeric components was presented to the committee for the approval of conducting experiment of *in vivo* residence time in volunteers. Results of the *in vivo* residence time have been tabulated (Table 3), which showed that the *in vivo* time for all the formulations were within 2 h and the tablet steadily vanished from the point of administration into buccal mucosa. The

time of formulations containing 7.5% HPMC (PT3 and PT4) was able to stay in the mucosa for more than 1 h, while rest of the formulations did not able to survive. It refers that in order to show the *in vivo* performance, there should be some higher concentration of HPMC required since HPMC is an established sustained release agent [36]. On the other hand, SA containing formulations could not be able to survive for more than 1 h and the disintegration of swollen tablet as particulate gel shed off at the point of administration.

Swelling index (SI)

Swellability index is the estimation of the formulation to absorb water over time. It is necessary since as the water influx the dosage form, the drug will be able to leak out of the mucoadhesive drug delivery. There was a general swelling trend of prepared formulations over time and it gradually decreased till the 6 h. As depicted, the values of SI were generally higher for SA (PT1-PT4) as compared with HPMC containing formulations (PT1-PT4). It was due to the fact that SA has generally been accept as well modest swellable agent as compared with HPMC [37] and as the concentration of SA was increased, the SI was also increased as depicted in Fig. 2. Highest swelling index was observed with PT8, containing 7% concentration each for SA and CHI. It was also observed in the SA containing formulation (PT1-PT4) that a modest decrease in the swelled tablet contents was observed at the end of 6 h which was not observed with HPMC containing formulations. Although, there was little impact of CHI on swellability behavior, but further studies are required using the amounts of the polymeric combination versus each polymer alone.

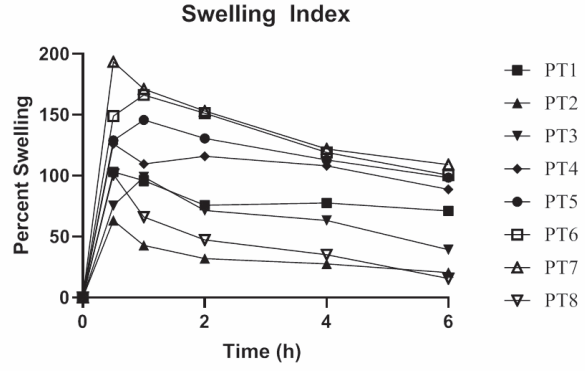

Figure 2. Graphic representation showing the swelling index of mucoadhesive buccal formulations compressed in the current study.

Matrix erosion (ME)

Matrix erosion was assessed to estimate the disrupted matrix of the swollen tablets at the 6 h [29]. It also explained that under stressed conditions of temperature in an unsaturated oven, the ability of the hydrogel/ swollen dosage form to lose its swellability. Results (Table 3) showed that the values lie in the range of 36.24-78.68%. The values were since lower concentration of the polymers used could be a possible justification for the major hydrated loss of the dosage forms. The least value was observed with the formulation containing highest amounts of HPMC and CHI added in the formulation. On the other hand, SA containing formulations were also subjected to comparatively higher values of ME. It could be a possibility that formulations capable of swelling more have higher values of ME.

HPLC instrumental conditions

Since, no method was present in the literature to date in the knowledge of authors for the simultaneous determination of TBN and LGN using high performance liquid chromatography (HPLC), a simple binary mixture mobile phase solution was devised for the estimation of both drugs. The regression value (r2) found for TBN and LGN were 0.9995 and 0.992 using C_8 column maintained at 35 °C, at a flow rate of 1 mL/min, detected at 242 nm. The mobile phase comprised of acetonitrile and 0.02M monobasic potassium phosphate previously adjusted to pH value of 4.5 with phosphoric acid, in a ratio of 70:30, volumetrically. The retention of TBN and LGN in the column were 4.2 and 2.3 min, correspondingly. The validation testing according to ICH guidelines for drugs confirmed that the calculated values of accuracy and precision for TBN were 100.01% ± 1.72 and 100.03 ± 1.61, respectively. For LGN, it was found to be 99.15% ± 0.65 and 99.05% ± 0.48, respectively. The robustness values of flow rate, changing pH and temperature for both TBN and LGN were less

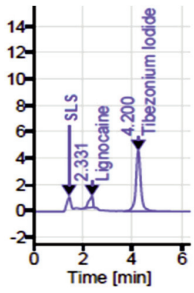

Figure 3. Diagram showing the chromatographic peaks of TBN and LGN using the devised HPLC instrumental conditions, at intervals of 4.2 and 2.3 min, correspondingly.

than 2%. The values for the linearity range of TBN and LGN were found to be 0.07-10.08 and 0.14-20.16 µg/mL, respectively. Similarly, the limit of detection (LOD) and limit of quantification (LOQ) parameters for TBN were calculated to be 12 and 37 ng/mL, respectively. For LGN, the values of LOD and LOQ were 67 and 22 ng/mL respectively (Fig. 3). The detailed version of the HPLC method development is under review as another study by the authors.

In vitro drugs release

For the *in vitro* release of both drugs, the preset criterial of release up to 6 h was defined or if the quantitative value was more than 95%, the sampling was stopped for the respective formulation. Samples of elutes of dissolution medium were removed for quantitative determination of TBN and LGN in accordance with the devised HPLC methods. After filtration, 10 µL of the analytical volume was withdrawn by the Agilent® 1260 infinity equipped with auto sampler for analysis and results can be observed in Figs. 4 and 5 for both drugs.

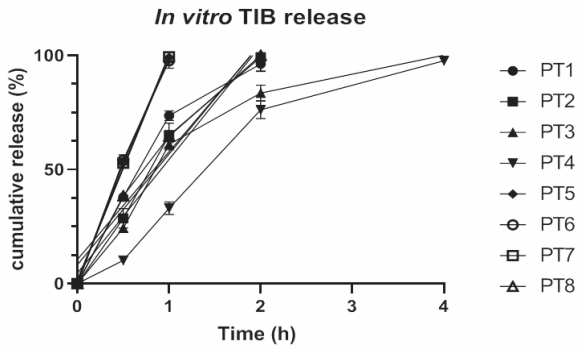

Figure 4. Graphical illustration of *in vitro* TIB release behavior of mucoadhesive buccal formulations prepared in the study.

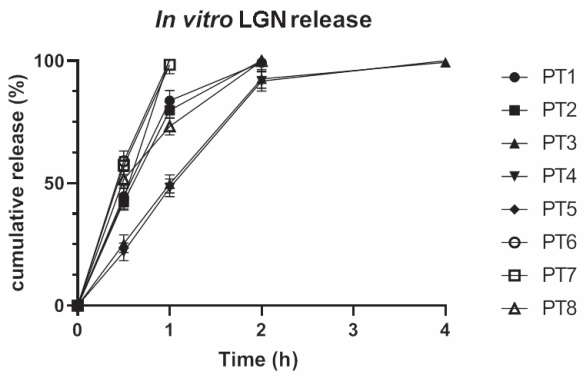

Figure 5. Graphical illustration of *in vitro* LGN release behavior of mucoadhesive buccal formulations prepared in the study.

Results showed that all SA containing formulations, except PT8, released LGN before 2 h as shown in Fig. 5. While the formulations containing 7.5% of HPMC were able to sustain the release of the LGN till the 4 h. While with 5% concentration of HPMC, complete release of LGN was achieved at 2 h. So, this value suggests that if the formulation is desired to release drug for a longer period of time, then the amount of HPMC is required to be increased for sustain action. HPMC has a defined profile of sustain release [38]. In powdered form, CHI has a poor impact on sustaining release as compared with HPMC [22]. Almost same profile was observed for TBN that release of the drug was unable to be sustained with SA containing formulations as compared with HPMC as shown in Fig. 4. It is suggested to use higher concentrations of the used polymers if further sustainability is required.

On the basis of release profile, formulations PT3 and PT4 were selected as the optimum formulations for releasing both drugs to extent of sustainability, out of the prepared formulations. But, PT3 and PT4 were further compared from each other in terms of mucoadhesive properties and swelling perspective in order to select the optimum dosage form based on maximum output response of the evaluation parameters. The formulation PT4 was superior to PT3, since it possessed better mucoadhesive strength and time. For that, it was selected for *in vitro* release kinetic evaluation, being the optimized dosage form. CHI has shown poor release and mucoadhesive properties in solid dosage form [22] because of slow and poor hydration compared to HPMC.

In vitro LGN and TIB release kinetics

For understanding the mechanism of *in vitro* drugs release kinetics, DD Solver® software was employed. Various release models, listed in Table 4, were applied on the optimized drug

Kinetic model	TBN		LGN	
	r^2	n	r^2	n
Zero order	0.9096	-	0.7628	-
First order	0.9375	-	0.9569	-
Higuchi	0.8844	-	0.9230	-
Korsmeyer-Peppas	0.9447	0.759	0.9276	0.560
Hixon Crowell	0.9630	-	0.9785	-

Table 4. *In vitro* release kinetics of the optimized formulation (PT4) using DD solver®. Note that the values are based on best fit model.

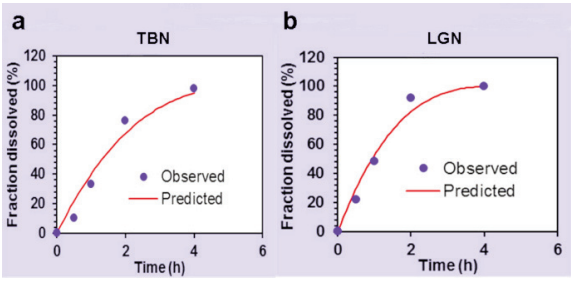

Figure 6. Graph showing best fit kinetic release model for optimized formulation (PT4) for **a**) TBN and **b**) LGN.

formulation (PT4). The model constant having the maximum value was considered to be the best fit model. Result showed that TBN and LGN followed Hixon Crowell mode of release as depicted in Fig. 6, since the values of coefficient of release model was maximum for both drugs, which were 0.9630 and 0.9785, respectively.

Hixon Crowell explain the mathematical modeling for the release of the drugs when the erosion rate of the matrix is high that the release is based on changing surface erosion. This release of LGN from the polymeric swollen matrix in the dissolution medium was dependent upon eroding matrix layer of the constantly changing tablet surface [39].

FTIR spectral analysis

As depicted in Fig. 7, the spectrum of CHI is expressing peaks at 3335 and 3253 cm^{-1} correspondingly the N-H and O-H type stretching inside the molecule of the polymer. The absorption band approximately at 2912 cm^{-1} is the characteristic trait of asymmetric or symmetric C-H stretching and is found typically in carrageenan [40] and glucan [41]. The small sharp peaks around 1654 cm^{-1} [42] corresponded the presence of C=O stretching of amide as well as medium stretching of the C-N in the third amide group at 1197 cm^{-1} [43]. The identified absorption band around 1578 cm^{-1} was correlated with the bending of the N-H as the primary amine. The response of the peak around 1029 and 1061 cm^{-1} depicted the presence of C-O stretching. Similar bands have been reported in the literature [42]. The bending vibration of the methyl and methylene groups were depicted around 1440 and 1375 cm^{-1}. For HPMC, the peak corresponding to O-H vibration based on bond stretching was marked around 3445 cm^{-1} as well as the C-H stretching approximately at 2894 cm^{-1} respectively. Then the vibration of asymmetric carbon due to bending was marked at 1374 cm^{-1} while a strong peak in the region of approximately 1051 cm^{-1} was seen which corresponds to the C-O-C stretching vibration. The absorption spectra of LGN was also characterized by the existence of N-H bond stretching at 3449 and 3384 cm^{-1} showing the presence of amines [44]. The amide group with C=O in LGN was depicted by the absorption band at 1654 cm^{-1}. The C=N in the chemical structure of LGN was expressed in the absorption spectrum as medium intensity peak at 1670 cm^{-1} while the stretch vibration of C-H bond was depicted by peak at 2995 cm^{-1}. In the absorption spectrum of TBN, the sharp peak at the wavenumber of 1580 cm^{-1} confirmed the presence of C=N in the chemical structure [45]. The presence of the cyclic structures of benzene was confirmed by the sharp peak in absorption spectrum approximately at 1438 cm^{-1}. The absorption peak corresponding to the value of 2972 cm^{-1} depicted the presence of C-H bond stretching. The FTIR region of the optimized formulation (PT4) was performed to evaluate the presence of the new

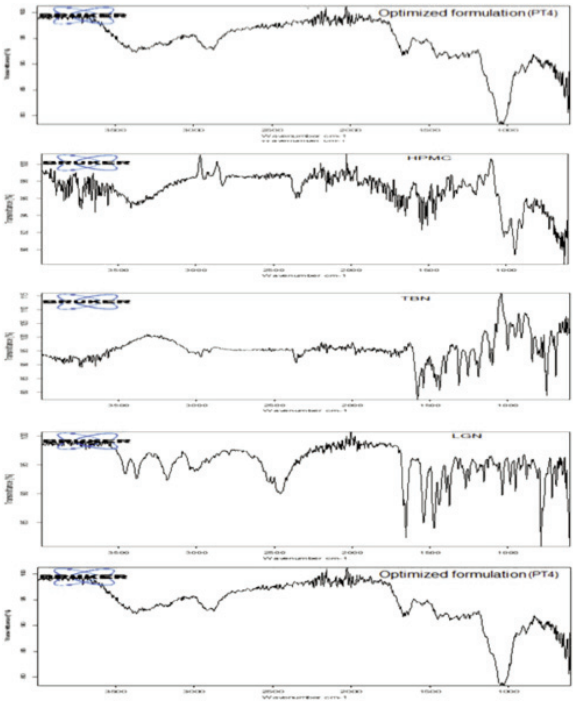

Figure 7. Diagram depicting the FTIR spectral analysis of the chitosan (CHI), hydroxypropyl methylcellulose (HPMC), tibezonium iodide (TBN), lignocaine hydrochloride (LGN) and the optimized formulation (PT4).

peak. While the FTIR absorption region of the individual polymers as well as the drugs were studied to identify each and then it could be compared from the FTIR analysis of individual components added in the formulation. Fig. 7 reveals that the FTIR of the optimized formulation (PT4) contained the peaks from the individual components *e.g.* the C-O-C stretching of the HPMC in the optimized formulation was depicted by strong absorption band at 1051 cm^{-1}.

DSC analysis

The DSC thermogram of pure drug powder, polymers as well as its optimized formulation (PT4) have been depicted in the Fig. 8. As seen, sharp endothermic peak of the lignocaine hydrochloride was obtained at 83.27 °C, which reflected the melting point of the drug. The powder started melting at 75.82 °C. While for TBN, the sharp endotherm of the crystalline drug was seen around 161.4 °C while the powder started melting around 152 °C. Similarly, the polymeric endotherms of HPMC [46] as well as the CHI [47] corresponded to the values reported. If the thermogram of the optimized formulation is observed, it is evident no additional peak was observed and single deep value in the range where the thermal temperature of the ingredients was observed. Therefore, it is evident that the mixture had no unusual peaks.

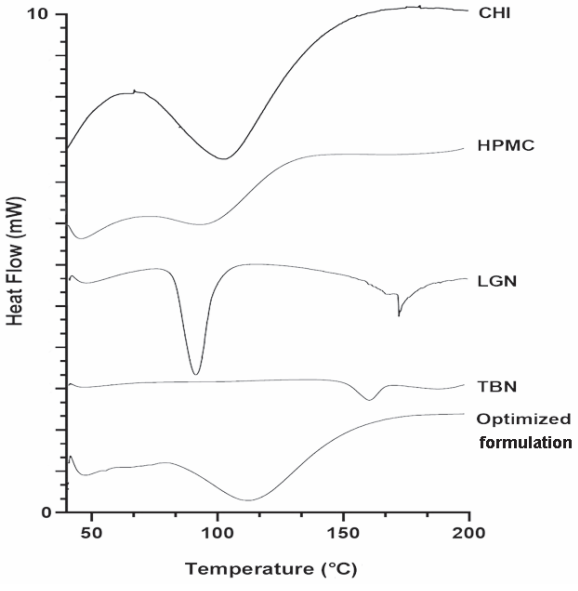

Figure 8. Diagram depicting the graphical illustration of results of differential scanning calorimetry (DSC) for pure drug powder, polymers as well as the optimized formulation (PT4).

CONCLUSION

The weight variation and friability of the prepared mucoadhesive buccal dosage form was in accordance with the United States Pharmacopeial standards and almost unaffected by the changes in the polymers for different mucoadhesive formulations. But the physicochemical characters were affected by changing concentrations of the polymers. *In vitro* drugs release, *ex vivo* mucoadhesive strength and time were increased with the increasing concentration of HPMC, whereas SA affected the swelling property of the dosage form. The polymers HPMC and CHI in the concentration of 7.5% was able to sustain the release of both drugs up to 4 h. The *in vivo* volunteer residence time was also associated with the concentration of HPMC. For higher sustainability of drugs or modified mucoadhesion as well as swelling, the quantities of the polymers may be increased for such response.

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Author's contribution. SH performed experimentation, data analysis and writing. RMS designed the project and critically review the manuscript. MAS helped in review, analysis and interpretation of results. RS aided in experimentation and data analysis. JI assisted in interpreting results. SHM also aided in the experimentation.

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