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Chitosan-enhanced mucoadhesive delivery for local action: Salivary pharmacokinetic estimation

Sana Hanif[1], Farhang Hameed Awlqadr[2], Ijaz Ali[3], Saleha Yasir[1], Nariman Shahid[1], Umaira Rehman[4], Rouheena Shakir[5], Syed Hassan Murtaza[1] and Muhammad Ali Syed[6]*

[1]Department of Pharmaceutics, Faculty of Pharmacy, The University of Lahore, Lahore, Pakistan
[2]Food Science and Quality Control, Halabja Technical College, Sulaimani Polytechnic University, Sulaymaniyah - Iraq
[3]Faculty of Pharmaceutical Sciences, Government College University, Faisalabad, Pakistan
[4]Lahore College of Pharmaceutical Sciences, Lahore, Pakistan
[5]Leads College of Pharmacy, Lahore Leads University, Lahore, Pakistan
[6]Department of Pharmaceutical Sciences, Government College University Lahore, Pakistan

Abstract: **Background**: Sore throat ache is a discomforting condition for patients during the disease course while eating, drinking or swallowing. However, improved topical concentration of locally acting drugs can address this issue. **Objectives**: The goal of the current research was to formulate and evaluate the single-dose salivary pharmacokinetics of a chitosan (CT)-based mucoadhesive delivery system containing tibezonium iodide and lignocaine (LIG). **Methods**: Mucoadhesive buccal gels were formulated using a homogenization technique and then subsequently characterized for physical, physicochemical and mucoadhesive properties. The ex vivo mucoadhesive studies were conducted on healthy New Zealand rabbits (aged 12-14 weeks and weight range in between 2.2-2.5 kg). **Results**: Solid-state characterization revealed the absence of any unusual peaks in the physical mixture of the FTIR and DSC, whereas the unchanged physical form of drugs was confirmed through PXRD analysis. Formulations containing sodium alginate (SA) demonstrated greater swelling (15.39% in F4) but could not sustain drug release for up to 3 h at the polymer concentrations studied. Contrarily, poor mucoadhesive strength (MS) and mucoadhesive flow time (FT) were associated in formulations containing SA. Homogenization of CT and HPMC gels, when mixed at respective concentrations of 1.5% and 2% w/v, demonstrated sustained drug release over time, along with improved MS and FT values of 16.34 g and 142.20 min, respectively. Better salivary concentrations (C_{max}) for LIG (5.14 µg/mL) and TIB (4.82 µg/mL) were observed at 2 and 3 h, respectively. **Conclusion**: Our study demonstrated higher C_{max} concentrations of the locally acting drugs with single-dose mucoadhesive delivery in healthy volunteers, designed for sore throat, as a single-dose alternative to their respective conventional lozenges.

Keywords: Buccal gel; Tibezonium iodide; Lignocaine; Local action; Mucoadhesive; Pharmacokinetics

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INTRODUCTION

Buccal gels, without difficulty, are considerably easier to formulate employing a hydrophilic polymer homogenization method. Due to their swelling and mucoadhesive properties, these gels provide good drug delivery and delayed release for local effects (Prezotti *et al.*, 2020). Even though the mucosal surface area is wet and suitable for drug absorption, it is considered to be more efficacious than external human skin. Buccal infections, periodontitis and mucosal inflammation are all pathological diseases that require local delivery of therapeutic medicines. Gels with porous structures and micro dimensional design improve drug loading and have a broad range of applications in drug delivery (Chen *et al.*, 2023). The mucoadhesive properties of the scaffold enable significant adherence of the dosage form to the buccal region, with improved control of drug release. Delivering drug moieties as buccal gels remains effective in an attempt to reduce the dose of the drug for local action. This is because gels are pre-hydrated when inserted into the mucosal cavity and may adhere immediately (Steinberg and Friedman, 2020). This dosage form is preferable, especially when it is intended to cover the wound or affected area, so that a layer may be maintained to prevent pathological symptoms in other healthy tissues within the cavity (Hemmingsen *et al.*, 2021). In pathological conditions, the bacterial infections in the oral cavity are usually associated or may complicate the scenario to cause pharyngitis, allergies, tonsillitis and can complicate surgery at worst. For symptomatic treatment, antiseptics and local anesthetics are suggested. Lozenges, gargles, nebulizers and gels might be included in conventional drug delivery therapy to control sore throat (Hedin *et al.*, 2014). Buccal mucoadhesive administration, on the other hand, differs from traditional dosing in that it allows medications to be released continuously within the buccal cavity over time. The presence of salivary secretions in the buccal area promotes gel swelling and mucoadhesive phenomena. The molecular moieties such as tibezonium iodide (TIB) and lignocaine (LIG) were used as local antiseptics and

*Corresponding author: e-mail: dr.alisyed@gcu.edu.pk

anesthetics, correspondingly, in this investigation. The TIB is an antiseptic that is used to treat infections of the buccal cavity such as pharyngitis, gingivitis, sores and stomatitis (Hanif *et al.,* 2021a). Conversely, LIG is a frequently used local anesthetic that also has a minor analgesic effect. Chitosan (CT) is a biodegradable, non-toxic, long-acting, mucoadhesive, swellable and biocompatible polymer (Liu *et al.,* 2022). As a consequence, the aim of this study was to design a CT-based single-dose sustained mucoadhesive gel for locally acting formulation. In healthy people, the gel was estimated for *in-vitro*, ex vivo as well as *in-vivo* mucoadhesive properties in volunteers. Eventually, the optimized gel formulation was further evaluated for salivary pharmacokinetics, drug stability and solid-state studies.

MATERIALS AND METHODS

Materials

Tibezonium iodide (Recordati®, Italy) was sourced from Pacific Pharmaceuticals Limited (Pakistan) on a kind basis, whilst lignocaine (as hydrochloride), sodium alginate (SA) and hydroxy propyl methyl cellulose k15 (HPMC) were kindly provided by Hoover Pharmaceuticals Pvt. Limited, Pakistan. Low molecular weight chitosan (50 kDa) was purchased from Sigma-Aldrich® (USA). Likewise, dimethyl sulfoxide (DMSO), ortho-phosphoric acid, triethanolamine and/or any other solvent/reagent used in the study belonged to analytical quality. However, reverse osmosis (RO) Millipore® filtered water was used wherever mentioned in the study, unless otherwise stated.

Formulation design

Overall, six formulations (F1-F6) were prepared containing blend of HPMC and SA with CT as presented in table 1. However, the amounts of polymers used in formulating buccal gels were kept low with the aim of keeping the single dose of drugs locally. The polymeric gel concentrations used in the study, for CT and HPMC to formulate buccal gels, were 0.5-1.5 % w/v, while for SA, it was 2-4 % w/v, respectively. Regardless of the concentration, the procedure for the preparation of specific polymer gel was similar (Hanif *et al.,* 2022).

Preparation of buccal gels

Concisely, CT gels were prepared by incorporating the amount to yield 0.5, 1 and 1.5% w/v in 100 mL of 1.0 % (v/v) glacial acetic acid to form gel spontaneously (Soares *et al.,* 2021). It was further homogenized manually for 10 min with spatula to ensure uniformity.

Similarly, the amount of HPMC sufficient to produce 0.5, 1 and 1.5% w/v was taken and polymer was dispersed in distilled water at 70 °C and stirred at 500 rpm using magnetic stirrer for at least 1 h. As the viscosity of the solution increased, it was further cooled to 4°C for almost 6 h (Hanif *et al.,* 2022).

For SA gels, the weighed amount to yield (2, 3 and 4% w/v) was agitated on a magnetic hot plate (HPS-380-1 Infitek, China) for 2 h at 500 rpm and left unobstructed for 1 h. In case of gel composites, equal amount of designated polymer gel concentrations (table 1) was homogenized for 1 h with D-160 propeller homogenizer (D-160) DLAB Scientific Co., Ltd, China. Afterwards, 0.15 and 0.20 mL of glycerol and dimethyl sulfoxide (DMSO) were added to formulation to solubilize the drugs, respectively. It was then tested for evaluation parameters. Then triethanolamine was added to the formulation to maintain the pH of the gels near the pH of the physiological buccal region. Both LIG and TIB were dissolved in DMSO first before being added to the formulation. Optionally, drugs were not added to DMSO for evaluating mucoadhesion of the dosage form in volunteers.

Evaluation of gel formulations

Physical appearance

To assess the morphological appearance, buccal formulations were inspected physically for indication of grittiness, lumps, precipitation, texture and homogeneity (Syed *et al.,* 2022b).

pH

The pH of the surface of each gel was evaluated by touching the electrode of pH meter (PH200, Biobase®, China) to the surface of the gel and the digital reading on the pH meter was noted when it was stable (Prezotti *et al.,* 2020).

Contents uniformity

To evaluate the uniformity of contents of the prepared formulations, weight of the dosage form equivalent to the single dose of LIG and TIB was taken. It was placed in the dissolution medium, 900 mL containing 0.25% w/v sodium lauryl sulphate (SLS), maintained at pH 6.8 with ortho-phosphoric acid with the intention to separate out the polymeric structure and eventually dissolve out the drug contents. For execution, the media was spun at 1000 rpm to destroy the structure of the gels, thereby releasing the drugs entrapped in the gel structure. The setup was maintained at 37.5°C on a stirring hot plate (HPS-380-1 Infitek, China). Afterwards, sample of the dissolution fluid was withdrawn and syringe filtered using parenteral grade Millipore®. The filtrate was auto-injected onto the C_8 column in Agilent® 1260 HPLC machine for the quantitative estimation of drugs (Hanif *et al.,* 2022). The HPLC instrumental conditions are detailed under the estimation of dissolution study.

Swelling (SI) and matrix erosion indices (ME)

To measure SI, approximately 1.5g (w1) of the formulation was transferred to a glass slide.

Table 1: Composition of polymers (w/v %) in mucoadhesive buccal gels (F1-F6).

Code	HPMC	CT	SA
F1	1.5	0.5	-
F2	1	1	-
F3	0.5	1.5	-
F4	-	0.5	4
F5	-	1	3
F6	-	1.5	2

It was placed in a petri dish containing 10 mL of phosphate-buffered saline (PBS) (adjusted to pH 6.8 with orthophosphoric acid), so that the gel formulation was slightly dipped in the PBS to mimic the natural conditions in which gel is administered in the buccal cavity (Hanif *et al.*, 2022). During experiment, petri dish was kept at 37.5 ± 0.5 ˚C using a hot incubator (DLab®, China). The weight (w2) gained by the formulation during intervals of incubation was recorded and increase in the weight due to swelling and imbibition of PBS was calculated according to Equation 1 (Syed *et al.*, 2022a).

$$SI = \frac{w2 - w1}{w1} \times 100 \quad \text{..... (Equation 1)}$$

To estimate the capability of the formulations to erode by losing moisture was estimated using matrix erosion (ME). In short, the swelled formulations were exposed to 60 ˚C for 24 h in a hot incubator or till the dried constant weight (w3) was attained (Muhammad *et al.*, 2022a). Then ME was estimated subsequently (Equation 2).

$$SI = \frac{w1 - w3}{w1} \times 100 \quad \text{..... (Equation 2)}$$

Dissolution study

The United States Pharmacopeia (USP) type II dissolution paddle apparatus (Popular Chemicals®, Pakistan) was used to evaluate the *in-vitro* release of drugs from the gels according to the conditions reported (Hanif *et al.*, 2022). To accomplish, the weight of the dosage form equivalent to the single-dose of drugs was placed in a small petri dish and covered with a 100 mm mesh to avoid the translocation of the gels in the dissolution media and process of dissolution of drugs. This gel-loaded and mesh-covered petri dish was then placed at the bottom of the dissolution vessel already containing 900 mL of 0.25 % w/v SLS, adjusted to pH 6.8 and maintained at 37.5 ± 0.5 °C. The speed of the paddle rotation was adjusted at 50 rpm during the study. At defined intervals or till dissolution of drugs by more than 95%, aliquots of 5 mL of dissolution fluid were removed at 0.5, 1, 2 and 3 h. The deficit volume in the dissolution media was replaced replenished with the fresh volume of medium. The aliquot was filtered through 0.2 µm parenteral grade Millipore® syringe filter and 10 µL of the filtrate was auto-injected for analysis on high performance liquid chromatographic (HPLC) machine.

HPLC analytical conditions

The mobile phase, comprised of binary solution of 0.02M monobasic potassium phosphate adjusted to pH 4.5 with phosphoric acid and acetonitrile in a proportion of 30:70 (v/v), was flowing through C_8 column at a rate of 1 mL/min. The temperature of column was maintained at 35 °C while both drugs were detected at 242 nm using Agilent G1314B/C VWD1A as ultraviolet detector in Agilent® (Canta Clara, USA) HPLC 1260 Infinity machine. The injection volume was set at 10 µL. The OpenLab CDS® software was used for the integration of peaks of drugs.

The standard solution, containing both drugs, was prepared by dissolving 55.56 mg of LIG and 27.78 mg of TBN in 500 mL of 0.25% w/v sodium lauryl sulphate (SLS) solution, adjusted to pH 6.8. For analysis, 10 mL of this standard solution was then diluted to 100 mL with similar SLS solution. Whereas for dissolution samples, 5 mL of aliquot was diluted to 50 mL with 0.25% w/v sodium lauryl sulphate (SLS) solution, adjusted to pH 6.8 (Hanif *et al.*, 2023).

Ex vivo mucoadhesive strength (MS)

Experimental MS of the formulations was executed on freshly excised rabbit's buccal mucosa which was attached on a fixed base as reported in the literature (Syed *et al.*, 2022a). In compliance with the Arrive guidelines, a favorable approval was obtained from the Institutional Research Ethics Committee vide approval number IREC-2019-125A in 03-02-2020 from the Department of Pharmacology, The University of Lahore.

To persue, four Newzealand rabbits were sourced from University and housed under controlled environmental conditions (temperature, humidity and light–dark cycle) prior to tissue collection.

The buccal mucosal tissue was removed from New Zealand white rabbits, with all experimental protocols approved from the ethical committee and conducted in strict accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals ARRIVE guidelines. Animals were fed with standard diet and maintained under controlled conditions. The housing conditions, feeding regimens and pre-experimental acclimatization were consistent with the guidelines.

Briefly, healthy rabbits aged 12-14 weeks and weight range in between 2.2-2.5 kg, were scrutinized. Animals were euthanized via IV administration of sodium pentobarbital (150 mg/kg), the buccal mucosa (cheek pouches) were immediately excised. The procedure was performed by trained personnel. The isolated buccal tissues were rinsed with PBS (pH 6.8) to remove any tissue debris and utilized within an hour post-excision for mucoadhesive testing (Yildiz Pekoz *et al.*, 2016).

To proceed, a modified physical weighing balance was used in which one side of the physical balance was replaced with the thread and it was attached with a movable glass slide containing fixed buccal mucosa of the animal (Fig. 1a). The gel, which MS needs to be evaluated, was placed between the movable and fixed glass slide and the base. When weight was added to the pan, tension in the string increased, thereby achieving the force necessary to detach the formulation from either side of the mucosa (Fig. 1b). This force was considered as MS of the formulation and was executed thrice (Syed *et al.*, 2022b).

Ex vivo mucoadhesive flow time (FT)

The FT was calculated using the technique as reported previously by the researchers (Syed *et. al.*, 2022a). Briefly, freshly sacrificed rabbit's oral cavity mucosa was stick on the concave side of the polyvinyl chloride base using dual sided acrylate adhesive. Then buccal gel, equivalent to the single dose of drugs (approximately 1 g), was gently fixed and allowed to settle for 20 s on the ex vivo mucoadhesive surface. The set up was tilted about an angle of 45° with a constant stream of PBS (set at pH 6.8) at a height of almost 10 cm and with approximately 1 mL/min (Fig. 2). The temperature of PBS was maintained at 37.5°C during the experiment. The time period at which the formulation was displaced from the point of application was known to be the FT of respective formulation (Hanif *et al.*, 2022).

Mucoadhesive study in volunteers (MT)

To be applied on healthy volunteers after obtaining favorable approval from Research Ethics Committee, Department of Pharmacy Practice, The University of Lahore vide approval number REC/DPP/FOP/6A on 20-02-2020 was obtained. However, the mucoadhesion time (MT) was estimated on drug free gel formulations. Before the start of the experiment, a written and verbal consent from the volunteers (20-28 yr) was taken as an inclusion. Wherein, volunteers willing to participate, free of any disease, not having any oral pathology, no restriction of gender, body mass index between 18-30 kg/m^2. The experiment was conducted in accordance with the principles of the Declaration of Helsinki to ensure respect for human subjects, beneficence and justice. Prior to participate, the volunteers provided informed consent after they were fully briefed about the aim and procedure of the study. Confidentiality of data was strictly kept throughout the study. Subjects were allowed to withdraw at during the experiment without penalty, while maintaining the autonomy. Special care was taken to use non-invasive sampling (saliva) to reduce harm and discomfort (Bromley *et al.*, 2015).

Each formulation was evaluated on 5 volunteers (Baus *et al.*, 2019). To brief, dosage form was gently applied onto the slight offset from the center of the lower gingiva (fig. 3). All the protocols of the experiment were followed as explained under "Salivary drug concentration". The condition of gels inside the volunteers was observed and the time at which the gels either eroded or disappeared from the point of application was considered as the MT for a particular formulation code.

Evaluation of optimized formulation

All the forth mentioned tests were applied to all gels. The gel possessing slowest drug release of TIB along with optimal mucoadhesive results was considered as the optimized formulation and following parameters were tested on it.

Fourier transform infrared (FTIR)

The FTIR testing was assessed using Bruker-α® (operated by OPUS) with Platinum-ATR in transmission mode. Samples of the drugs, polymers as well as the physical mixture of polymers and drugs were scanned in the range of 4000-600 cm^{-1}. Briefly, 8 mg of the sample was placed directly onto the lens of the spectroscopic machine and the results were interpreted (Hanif *et al.*, 2022).

Differential scanning calorimetry (DSC)

The DSC analysis was performed on the samples of the polymers, drugs and the physical mixture of each. For thermal analysis, sample of about 8 mg was closed in an aluminum cup, covered with a top and positioned in the DSC (TL Q2000™) heating chamber. Sample was scanned in between 40 - 200°C, incrementing at 20°C/min with a purging rate of 50 mL/min used to combust the inert gas (Syed *et al.*, 2022a).

X-ray powder diffraction (XRPD)

The Rigaku® Miniflex 600 X-ray diffractometer (Japan) was used to study the diffraction pattern of the samples of pure drugs, polymers as well as the physical mixture of each of drugs and polymers. Briefly, the sample was evaluated with a velocity of 5 º/min in the range of 5-45º. Origin® Lab software was used to evaluate the results (Hanif *et al.*, 2021b).

Salivary drug concentration

The salivary concentration of TIB and LIG was performed on the optimized buccal formulation by administering it on the frontal gingiva as depicted in Fig. 3. Satisfactory opinion was acquired prior to conducting the experiment from the Review Board (or Ethics Committee) from the Department of Pharmacology, Faculty of Pharmacy, The University of Lahore (IREC-2019-125A on 03-02-2020).

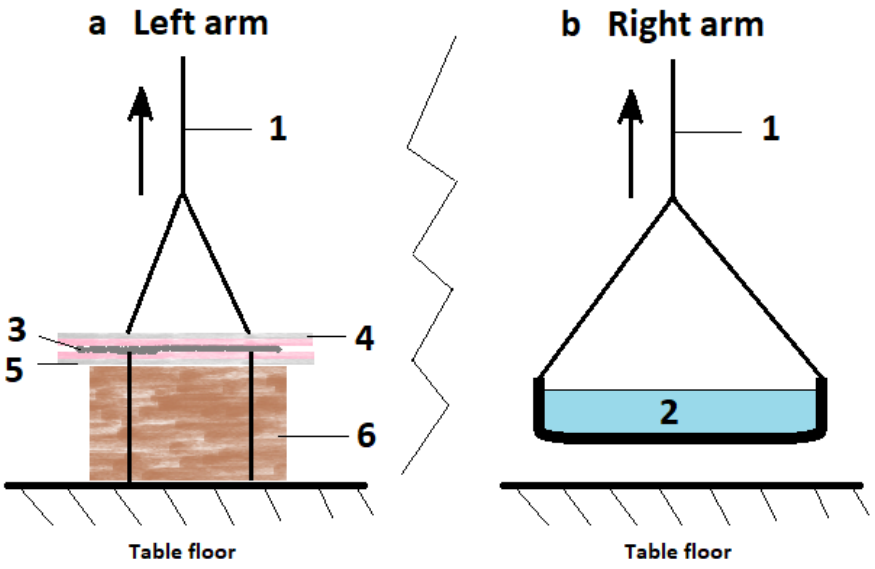

Fig. 1: Modified physical balance for the estimation of MS showing (a) left arm and (b) right arm wherein 1. Thread, 2. water as weight, 3. gel, 4. upper glass-slide, 5. lower glass-slide and 6. base.

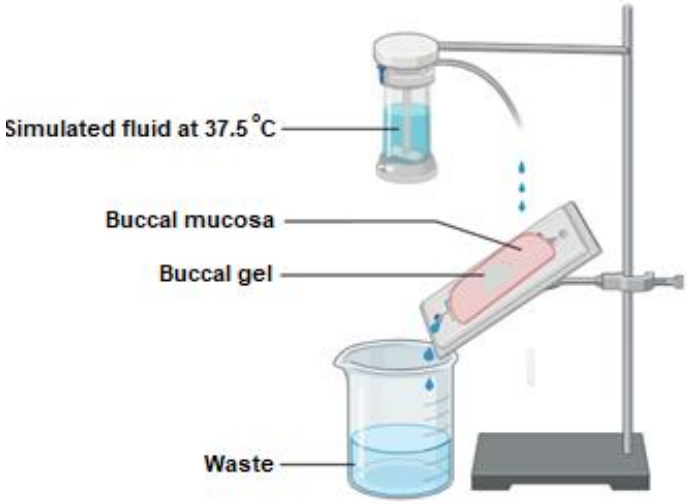

Fig. 2: Experimental visualization for the estimation of FT in buccal gels.

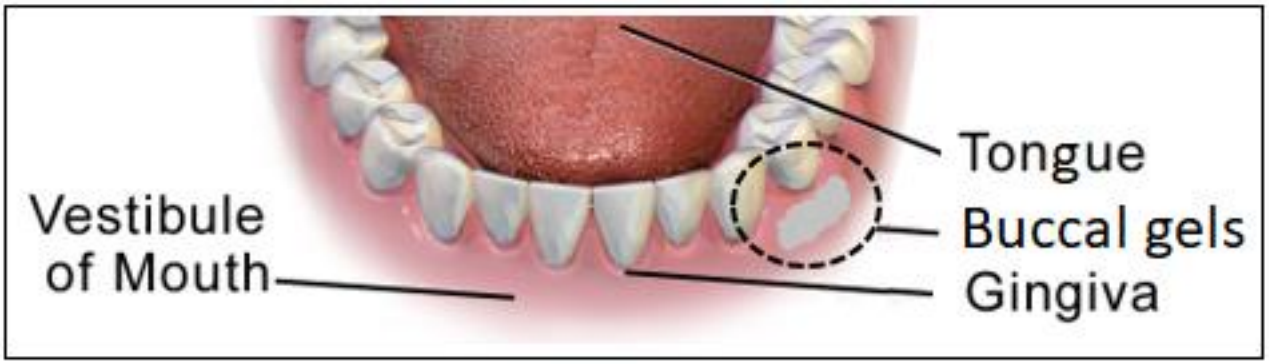

Fig. 3: Anatomical view of the lower jaw with dotted circle depicting point of application of mucoadhesive buccal gels in volunteers.

To brief, eight healthy volunteers, aged 20-25 yr. and agreeing to participate in the study were included. During the experiment, the liquid form of the diet was allowed to be ingested; however, not before 15 minutes of salivary sample collection. The participants were also instructed not to dislodge the gel with voluntary movement of tongue. The salivary sample (100 µL) was removed using micropipette at defined time intervals (0.5, 1 and 3 h). The time points set for the salivary drug estimation study was similar to the time points set in the dissolution study. It was diluted to quantity sufficient, 1 mL of the dissolution fluid discussed earlier and subsequently centrifuged for 15 min at 8000 rpm at 25 °C before parenteral grade Millipore® syringe filtration. For analysis, same procedure was adopted for the prepared sample as explained for dissolution studies using HPLC (Hanif *et al.*, 2022).

Salivary pharmacokinetic estimation

Based on the concentrations of both drugs in saliva, further model independent pharmacokinetic approach was adopted to calculate maximum concentration of drug in saliva (C_{max}), time (t_{max}) to achieve C_{max}, half-life ($t_{1/2}$), elimination rate of drugs from buccal cavity (k_{el}) and area under the curve (AUC) as well as the extrapolated AUC (Villar-Chavero *et al.*, 2020).

In-vitro-salivary drug release kinetics

The mode of drug release from the optimized formulation, when placed in dissolution media as well as the buccal cavity in volunteers, was studied by fitting the data in different kinetic release models. These included zero order, first order, Korsmeyer-Peppas, Hixson-Crowell and Higuchi models. The model depicting the highest values of the coefficient of regression (r^2) was considered as the best fit model for drug release (Pandey *et al.*, 2021).

Marketed product evaluation

As a supplementary evaluation, the salivary drug concentration of the optimized formulation was compared with the marketed formulations containing Tibezonium iodide and lignocaine hydrochloride. Since, no marketed product contains the combination of the either for sore throat. Hence, separate medicine products were tested in order to estimate the concentration of drugs at defined time intervals.

RESULTS

The fabrication of the gels and the concentrations of the polymers were evaluated on a lab scale. They were accomplished on a hit-and-trial method. It was desired, therefore, that formed gels neither have a completely solid appearance nor free-flowing liquid quality. The components of mucoadhesive buccal gels have been discussed.

Physical appearance

The formulations containing CT and SA (F4-F6) appeared as pale, soft and viscous mucoadhesive gels (Hanif *et al.*, 2022). However, it was reduced due to the addition of SA in formulations. Overall, no grittiness, particle sedimentation or precipitation was found in the buccal gels (F1-F6), formulated in the current study.

Surface pH

The pH of gels was found in the range of 6.75-6.84, which displayed that it was considerably close to the salivary pH in the buccal cavity (6.8–7.4). Hence, the pH of the formulations was maintained within the physiological limits (Maslii *et al.*, 2020).

Content uniformity

Uniformity of active agent throughout the gel was measured using the procedure as defined under methodology section and performed in triplicate. All the formulations displayed the values of content uniformity ranging from 95.43-101.57% and 96.84-99.89%, respectively for LIG and TIB (Table 2).

Matrix erosion

The increased trend was observed in gels, including CT and SA, with greater than 99% of the gel matrix erosion after mixing equal amounts of CT (1%) and SA (3%), respectively. When CT was used alone, lower values were observed.

Swelling index (SI)

Overall, an increase in the swellability index was seen in all the formulations, then a fall in SI results was revealed till 6 h, as displayed in Fig. 4. In case of CT based HPMC gels (F1, F2 and F3), a poor response of SI was displayed as compared to CT based SA formulations (F4, F5 and F6) as shown in Fig. 4a. The highest swelling rate was observed with F6, where the plateau was reached at 4h (16%), followed by a steep decline until 6h (Fig. 4b). Whereas, F3 has shown low swelling trend as it contained 1.5% of CT and 0.5% of HPMC, while F6 contained 1.5% CT and 2% of SA. It advocates that SA-containing blends imbibe water to a great level compared to the rest of the formulations. This trend was also reported in previous researches (Pamlényi *et al.*, 2021b).

Dissolution study

Results of the *in-vitro* release of TIB was a little bit slower than LIG due to the poor solubility of TIB in water. The existence of TIB in hydrophilic polymeric mixtures of buccal formulations could not dissolve without the addition of dimethyl sulfoxide, without which the gels might cause a foggy appearance (Hanif *et al.*, 2022). Here, the formulations comprising CT and SA (F4 & F5) displayed a faster release of about 99% within the first hour. Likewise, F6 also displayed a faster release, but slightly less than F4 and F5. This might be due to greater concentration of CT (1.5%) in case of F6 as compared to F4 and F5, having lesser CT concentration of 0.5% and 1.0%, respectively.

Table 2: Outcome of the contents of the drugs found in gels.

Code	Amount (% ± SD)	
	LIG	TIB
F1	96.13 ± 0.32	99.32 ± 0.37
F2	97.89 ± 0.28	98.18 ± 0.21
F3	96.35 ± 0.53	99.35 ± 0.89
F4	95.43 ± 0.38	98.01 ± 0.70
F5	101.57 ± 0.82	99.89 ± 0.86
F6	98.08 ± 1.39	98.84 ± 1.98

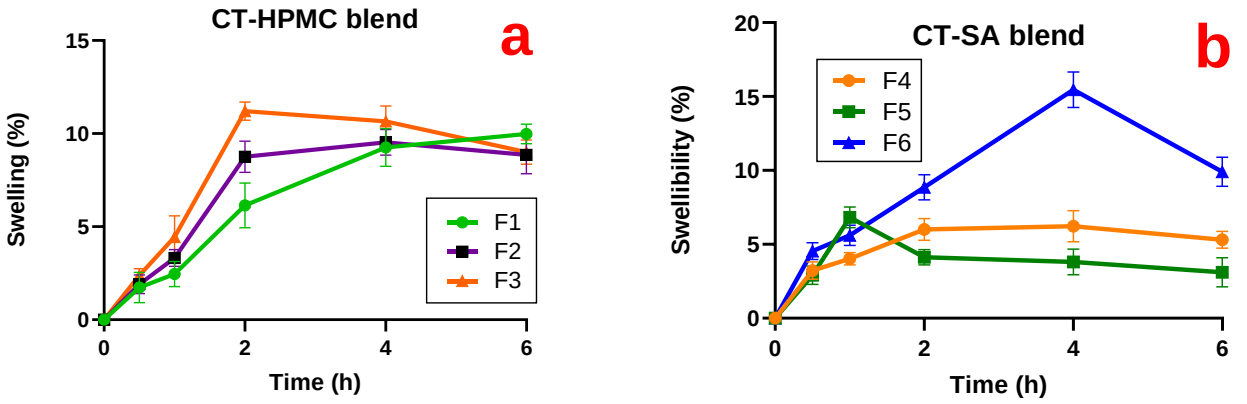

Fig. 4: Swelling indices (SI) of gel formulations. (a) F1–F3; (b) F4–F6. (CT= chitosan, HPMC= hydroxypropylmethyl cellulose and SA= sodium alginate).

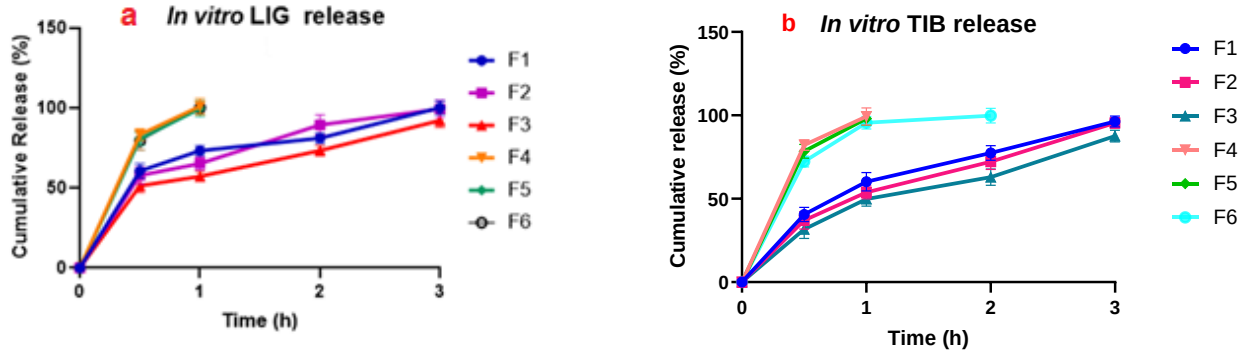

Fig. 5: Response of dissolution studies. (a) lignocaine hydrochloride (LIG); (b) tibezonium iodide (TIB).

The CT exhibits the ability of slow and controlled drug release, enhancing stability and solubility of the drug (Lucio and Martínez-Ohárriz, 2017). While the formulations comprising CT and HMPC (F1, F2 & F3) displayed a sustained release of TIB for up to 3h. Here, F1 displayed a higher drug release of 96.29% while F2 and F3 displayed a cumulative release of 95.45% and 87.59%, respectively, after 3 hours.

Comparatively, the *in-vitro* release of LIG revealed that F4 and F5 release LIG entirely before 1h, as they contain a greater amount of SA than CT. However, F1, F2 and F3 formulations showed a sustained release pattern for drug release up to 3 h (Fig. 5a). LIG is hydrophilic in nature and freely soluble in both water and buffer. Therefore, in combination with a small content of CT in a hydrophilic polymeric gel, LIG demonstrated fast release. Contrarily, LIG release was delayed in the formulations with slightly higher concentration of CT (1.5 %). Formulation F4, which contained 0.5 % CT and 4.0 % of SA, had the fastest early release, of about 100.21 % of the gel. Since SA is a swelling agent as well as a disintegrant, so such rapid release of LIG was related to the 4 % of SA, which resulted in the entire drug release within 1 h (Shivakumara and Demappa, 2019). On the other hand, F3 had the slowest release, with the LIG reaching 94% after 3 h. While F6 exhibited sustained release of LIG till 2h as it contained 2% of SA and 1.5% CT (Fig. 5a). Compared to LIG, the release of TIB was slower which might be attributed to the hydrophobic nature of the drug (Fig. 5b).

Ex vivo mucoadhesive strength (MS)

The Highest attained value for MS was 16.34g, containing 1.5% CT with 0.5% HPMC (F3). Both CT and HPMC have distinct mucoadhesive characteristics. Viscoelastic characteristics of HPMC influence mucoadhesive potentials of CT, eventually synergizing the mucoadhesive strength (Hamed *et al.*, 2018).

Ex vivo mucoadhesive flow time (FT)

A previously reported modified apparatus was used to perform the mucoadhesive flow (Hanif *et al.*, 2022). Experimentally, the buccal gel was applied to the oral mucosa of the animal. In order to assess the time required for total separation of gel components connected to buccal mucosa, a gel containing buccal mucosa was rinsed with a consistent stream of PBS (pH 6.8). It was observed that increased amount of CT associated with the higher values of FT. However, blend of HPMC and CT shown increased values of FT. It could be because HPMC has viscoelastic potentials, further added with a better adhesive agent like CT, produced sustain FT of formulations (Pan *et al.*, 2023).

In the form of a hydrogel, SA acts as a good swelling and also works to disintegrate the substances when in contact with the moisture (Berardi *et al.*, 2021; Pamlényi *et al.*, 2021a). Formulation F4 provided the shortest flow time of 75.28 min. The highest FT values (142.2 min) were seen in F3 comprising CT (0.5%) and HPMC (1.5%).

Residence time (RT)

A greater value of RT (161.1H) was related to the formulation (F3) having polymeric blend of 1.5% CT and 0.5% HPMC (Fig. 6) whereas, lowest value of RT (23.43H) was exhibited in F4, including lowest amount of CT (0.5%) and the highest amount of SA (4%).

Evaluation of the optimized formulation

FTIR spectral analysis

Fig. 7a demonstrates that the CT band around 1578 cm^{-1} represented N-H stretching, while the presence of C-O stretching was accompanied by the peaks around 1029 and 1061 cm^{-1}. In the literature, similar bands have been observed (Shahid *et al.*, 2022). Around 1440 and 1375 cm^{-1} region, the bending vibrations of the CH_3 and methylene groups were observed. The peak related to O-H vibration based on bond stretching was detected around 3445 cm^{-1} for HPMC and the peak corresponding to C-H stretching was around 2894 cm^{-1} for HPMC (Fig. 7b) (Zaltariov *et al.*, 2018). The vibration of asymmetric carbon caused by bending was then measured at 1374 cm^{-1}, with a high peak around 1051 cm^{-1} corresponding to the C-O-C stretching vibration. Furthermore, the strong peak at 1580 cm^{-1} in the absorption spectra of TIB proved the presence of C=N in the molecular structure (Fig. 7c). The existence of benzene's cyclic structures was confirmed by a high peak in the absorption spectra around 1438 cm^{-1}. The presence of C-H bond stretching was shown by the absorption peak with a value of 2972 cm^{-1} (Muhammad *et al.*, 2022b).

The presence of N-H bond stretching at 3449 and 3384 cm^{-1} in the transmission mode of LIG also indicated the presence of amines (Nafisi *et al.*, 2018) as seen in fig. 7d. Moreover, the absorption band at 1654 cm^{-1} in LIG depicted the amide group with C=O. In the absorption spectra, the C=N bond in the chemical structure of LIG was represented by a medium intensity peak at 1670 cm^{-1}, whereas the stretch vibration of the C-H bond was represented by a peak at 2995 cm^{-1} (Hanif *et al.*, 2022). The physical mixture displayed almost similar FTIR spectra as that of individual components such as TIB, LIG, CT, HPMC and SA, with slight shifting of a few peaks compared to the pure drug. This shift of peaks may have occurred due to the development of some weak forces amongst the individual components. LIG peaks demonstrating C=O stretch, C=N bond stretching, C-H bend and N-H bends were observed at 1653, 1670, 2925 and 3450 cm^{-1} in the physical mixture (Fig. 7e). Similarly, TIB demonstrated major peaks of C=N bond stretching vibration, CH_2 vibration, CH vibration and C=O stretch at 1545, 2925, 2855 and 1653 cm^{-1} in the physical mixture.

DSC analysis

The polymeric endotherms of CT and HPMC also matched the published values, displaying endothermic peaks at 91.37 and 78.4 °C, corresponding to their melting points (Hanif *et al.*, 2022) (figs. 8a and 8b). The rapid endothermic peak of LIG was detected at 83.27 °C, which corresponded to the melting point of the drug (fig. 8c) (Wojnarowska *et al.*, 2012). At 75.82ºC, the powder began to melt. TIB, on the other hand, showed a sharp endotherm around 161.4ºC, whereas the powder started melting around 152ºC (Fig. 8d) (Syed *et al.*, 2022b). It was observed that no extra peak was detected when the thermogram of physical mixture was evaluated (fig. 8e).

X-ray powder diffraction (XRPD) analysis

Results of XRPD demonstrated the characteristic peaks for HPMC and CT i.e. 21º (HPMC, CT), 27º (HPMC) (figs. 9a and 9b). While, LIG displayed high intensity peaks at 18°, 25°, 27° and 37° while, TIB also displayed high intensity peaks majorly at 19°, 24°, 28°, 37° and 40°, demonstrating their crystalline nature (Syed *et al.*, 2022b; Hanif *et al.*, 2021b). It indicated that both drugs have shown crystal form as indicated by the sharp diffraction patterns (figs. 9c and 9d). The physical mixture displayed crystalline nature as depicted from sharp peaks at 18º (TIB, LIG), 21º (HPMC, CT), 27º (HPMC), 28º (TIB) and 39º degree (LIG) (Hanif *et al.*, 2022) (Fig. 9e). Similar sharp peaks were also observed in the diffractogram of individual components. It confirmed that in mixed form, there is no interaction between polymer and crystalline drug.

An irregular strain was evident in the peak width when we analyzed the XRPD of the optimized formulation. Sharp peaks were absent in the optimized formulation due to the presence of large amount of polymer and the wider peaks simply described the amorphous nature of the mixture.

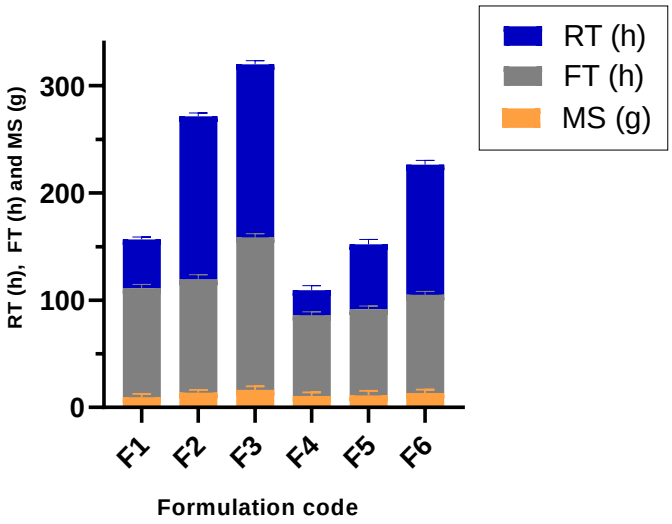

Fig. 6: An assessment of the mucoadhesion properties in the current study. (RT= residence time, FT= ex vivo mucoadhesive flow time and MS= ex vivo mucoadhesive strength).

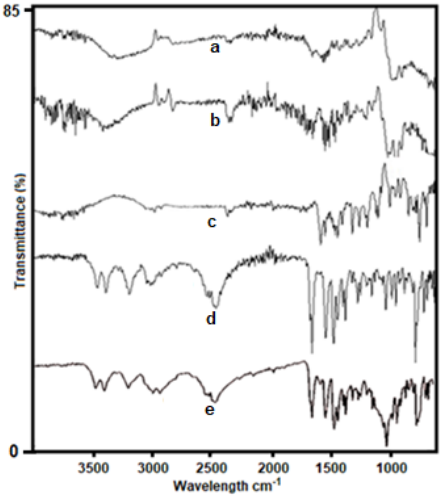

Fig. 7: FTIR spectra of (a) Chitosan (CT); (b) Hydroxypropylmethyl cellulose (HPMC); (c) Tibezonium iodide (TIB); (d) Lignocaine hydrochloride (LIG); (e) Physical mixture.

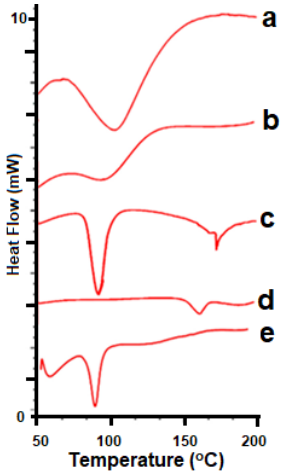

Fig. 8: DSC endotherm of (a) Chitosan (CT); (b) Hydroxypropylmethyl cellulose (HPMC); (c) Lignocaine hydrochloride (LIG); (d) Tibezonium iodide (TIB); (e) Physical mixture.

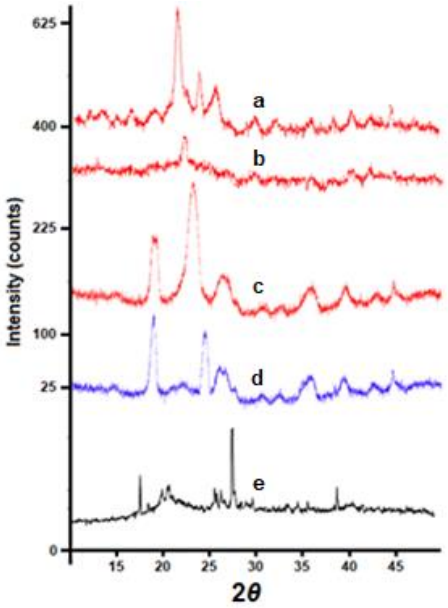

Fig. 9: Diffraction study spectra of (a) Hydroxypropylmethyl cellulose (HPMC); (b) Chitosan (CT); (c) Tibezonium iodide (TIB); (d) Lignocaine hydrochloride (LIG); (e) Physical mixture.

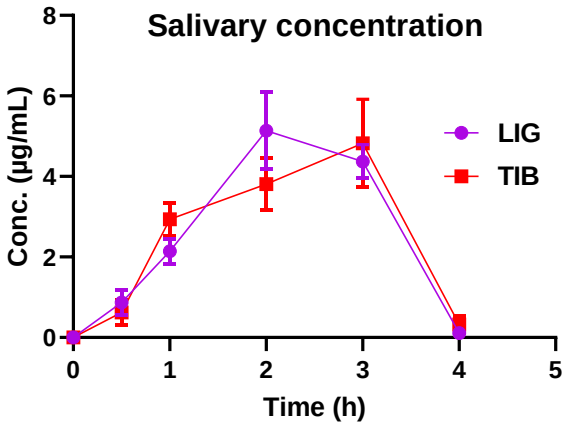

Fig. 10: Salivary concentration of lignocaine hydrochloride (LIG) and tibezonium chloride (TIB) from the optimized formulation.

The crystallinity of the drugs was reduced due to the addition of amorphous polymer in the physical mixture. Also, it might be a possibility that the plane of the rays mainly diffracts the amorphous polymers (Repka *et al.*, 2005). Therefore, the intensity of sharp peaks of crystalline structure was reduced. When disseminated, especially using hot melt extrusion methodology, LIG existed in a non-crystalline form in mucoadhesive formulation in the current study.

Salivary drug concentration

The released amounts of TIB and LIG from formulation F3 in saliva were measured only for the optimized mucoadhesive formulation, with protocols following the procedure prescribed earlier in the methodology section. Maximum salivary concentration was found to be at 2 and 3 h for LIG and TIB, respectively (Fig. 10).

Salivary pharmacokinetic estimation

For pharmacokinetics evaluation, various parameters were estimated, including C_{max}, t_{max}, k_{el}, AUC_{0-t} and $AUC_{0-\infty}$. The respective values for LIG and TIB for the maximum concentration (C_{max}) were 5.14 and 4.82 µg/mL with delivered doses (Fig. 10). However, the rate of elimination (k_{el}) of LIG was greater via buccal region as compared with TIB. Though both for LIG and TIB, the extrapolated $AUC_{t-\infty}$ was insignificant, depicted below 20% value of $AUC_{t-\infty}$ for both drugs (Table 3).

In-vitro salivary drug release kinetics

Various kinetic models were applied to the *in-vitro* release data and the best fit model was shown based on the values of r^2, the regression coefficient. In the case of LIG, the best fit model was Hixson-Crowell (r^2=0.9829, n=0.61) for the optimized formulations.

Table 3: Pharmacokinetic parameters for salivary LIG and TIB release by the optimized buccal formulation.

Parameters	LIG	TIB
Dose (mg)	10	5
C_{max} (µg/mL)	5.14	4.82
t_{max} (h)	2	3
k_{el} (h^{-1})	-1.545	-0.404
AUC_{0-t} (µg.hr/mL)	10.13	7.71
$AUC_{t-\infty}$ (µg.hr/mL)	5.67	2.36
$AUC_{0-\infty}$ (µg.hr/mL)	15.80	10.07
$AUC_{t-\infty}$ (%)	35.90	23.45
Contribution of $AUC_{t-\infty}$	significant	significant

Table 4: Salivary concentration of marketed products containing lignocaine and tibezonium iodide.

Active moiety	Brand®	Labelled composition	Calculated amount (%)	Interpretation	Salivary concentration at 1 h (µg/mL)	Salivary concentration at 3 h (µg/mL)
Lignocaine hydrochloride	Lignocaine Gel®	2.0% w/v	99.75 ± 0.32	USP in limits	0.07	-
Tibezonium iodide	Maxius® Lozenges	5 mg	100.02 ± 0.51	EuPh in limits	0.14	-

Marketed product evaluation

At defined intervals, the concentration of lignocaine from the Lignocaine Gel® was found to be lower (Table 4) as compared to the salivary concentrations of LIG from the optimized formulation (Fig. 9). At 3 h, it was evident that the drug concentration of lignocaine from the Lignocaine Gel® was undetectable, which could not be considered parallel to the mucoadhesive form. Similar behavior was also observed with Tibezonium iodide in the Maxius® lozenge. Since no gel of Tibezonium iodide is marketed under any brand name, the lozenge was used. Other relevant information is added to Table 4 regarding the conventional dosage form.

DISCUSSION

Studies have been reported in the literature that enlightened the sustained action of both drugs in the buccal cavity (Hanif *et al.*, 2021b; Hanif *et al.*, 2023). However, the goal of the current research was to formulate a single-dose combination of lignocaine hydrochloride and tibezonium iodide as an adjunct therapy for patients with sore throat who cannot tolerate the dosage form for prolonged periods. It would, in that case, be clearly advantageous over conventional gels that do not contain mucoadhesive substances for adherence of the dosage form to the buccal mucosa. Similarly, the concentration of the polymer in the formulation was directly associated with the appearance of the gel (Chen *et al.*, 2018). A pH with high acidic or basic value can cause damage to the buccal mucosa. Moreover, the formulation adheres for a longer period during the adhesion process which requires that the surface pH of the formulation should be within physiological buccal pH (Syed *et al.*, 2022b).

The uniformity of gels ensures that the quality and attributes of the gels are uniformly distributed in the polymeric network and can be assessed by the extent of mixing of the active substance. In the current case it was LIG and TIB; the result demonstrated that the drug quantity (%) was ranged between 95-102% in the prepared formulation. It was necessary to characterize the formulation for its mucoadhesive and pharmacokinetic evaluation. If the contents were not supposed to be uniform, it would cause significant variation in the results.

For polymeric gel erosion, high values were observed for all formulations. Results of the SI reflected that SA possesses good swelling properties while HPMC offers poor swelling characteristics. The gel expanded according to its capability with the passage of time, the hydrophilic matrix eroded and eventually released the drugs (Okur *et al.*, 2021). Basically, the addition of HPMC to CT gels did not alter the swelling of the gel formulations. Since the release of TIB was the slowest in F3 compared to all formulations (Fig. 5b), it can be derived that formulation F3 was considered the optimized formulation. The release of LIG in F3 was also found to be slower compared to rest of the formulations. It was a predefined criterion that the more the sustained release of drugs from the gel matrix, the more the capability of the dosage form to release the drug in a controlled manner at the site of action (Pandya *et al.*, 2023).

For local drug release and drug administration through the buccal route, the mucoadhesion is a pivotal phenomenon (Wu *et al.*, 2023). The blend of CT and SA revealed more MS than a single CT gel when used on rabbit buccal mucosa. Although CT can form viscous elastic gels, the characteristics of CT gels improved by incorporating a

better swellable component (Fig. 6). Simultaneously, lower CT concentrations were related to lower MS values in all subcategories of each type of blend of gels. Therefore, the mucoadhesion of the gels was primarily affected by CT concentration; the combination of gels provided greater mucoadhesion than CT alone. Moreover, the adhesive properties of the blend were slightly improved by the addition of HPMC, a finding which was previously endorsed by the authors, where combinations of HPMC with CT were formulated for localized delivery containing similar antiseptic and anesthetic agents (Hanif *et al.*, 2022). The swelling effect of SA reduced the adhesion quality of CT with mucin in gels containing CT and SA, indicating that the mucoadhesive connection of CT with mucin in the cavity, because of electrostatic interaction, was reduced (Diaz-Salmeron *et al.*, 2021). It could possibly be due the fact that once mucoadhesion was formed with mucin, the polymer continued to swell long as it was in contact with the moisture or fluid (Göbel *et al.*, 2021).

The results of RT showed that increasing the amount of CT increased the RT values. At neutral pH, CT interacts constructively with the mucin present in the cavity. Electrostatic attraction, mediated by H-bonding, occurs when CT interacts with mucin (Collado-González *et al.*, 2019).

For compatibility studies, the FTIR absorption region of the various polymers and drugs was analyzed in order to determine each and then compared using FTIR identification of individual ingredients incorporated into the formulation. The FTIR of the optimized mucoadhesive gel formulation comprised peaks from individual components, as shown in fig. 7e. For example, the C-O-C stretching of the HPMC in the optimized formulation was indicated by a significant absorption band at 1057 cm^{-1}. The DSC curves of the physical mixture showed that the ingredients changed individually in the mixture, indicating no degradation due to interaction of the components. A sharp endothermic peak was observed at about 84 °C demonstrating melting point of LIG. While an early endothermic peak observed near 70-80 °C corresponded to CT and HPMC, a small, broadened endothermic peak observed near 190 °C corresponded to TIB. Moreover, glass transitions were also observed in the physical mixture. For XRPD, the diffractogram of physical mixture revealed that the intensity of crystal peaks is reduced, as shown in the fig. 9e. Though no XRPD for TIB has been discovered in the literature, a comparable trend in TIB to LIG behavior has been reported. Research on the inclusion complex with LIG also found that when the medication was physically mixed in dispersion, it lost its crystal characteristics (Soares da Silva *et al.*, 2011).

In the salivary estimation of the drugs, the concentration C_{max} of LIG was, however, comparatively higher than that of TIB. The steady increase in the concentration of LIG was found, where a sharp increase in concentration of LIG was observed at 3 h, as depicted in fig. 10. The release kinetic data indicates the erosion of the gel scaffold from its surface upon introduction into the dissolution medium to release the active drugs (Jafari and Kaffashi, 2016). The value of 'n' was between 0.45 and 0.89, indicating the outflow of LIG is dependent on diffusion as well as erosion mechanisms (Chen *et al.*, 2018). Being hydrophilic, the polymer promotes the sustained diffusion of LIG through the polymer gel matrix (Pereira Camelo *et al.*, 2016). Similarly, the release pattern of TIB was also similar to the release pattern observed for LIG with coefficient values of r^2=0.9984, n=0.54. It follows both diffusion and erosion from the gel surface (Al-Ani *et al.*, 2021). The marketed product evaluation was intended to evaluate whether a mucoadhesive dosage form in the buccal cavity is comparable with the conventional marketed product in terms of salivary concentration. Hence, a conventional non-mucoadhesive lignocaine gel was used and applied to the healthy mucosa of a volunteer. Results of the marketed product versus mucoadhesive formulation can simply be correlated to the mucoadhesive phenomena, which aid mucoadhesion, release and swellability. All these phenomena are typically absent in the conventional dosage form, such as buccal non-mucoadhesive gel.

CONCLUSION

Chitosan-based single-dose mucoadhesive gels for locally acting drugs delivered LIG and TIB successfully with aim to deliver the single dose of both drugs and low polymeric concentrations. The solid-state studies on the physical mixture of polymers and drugs revealed no unusual peaks in the findings of FTIR, DSC and XRPD analysis. Swelling studies revealed that SA based CT blends swelled modestly as compared to the polymeric blend of HPMC and CT blends, where higher swelling was directly associated with increased polymer concentration. Unlikely to swellability findings, optimal MS and FT values were obtained for the formulation (F3) containing a blend of CT (1.5%) and HPMC (0.5%). The salivary drug concentrations were found to be superior to those of conventional dosage forms.

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Authors' contributions
Sana Hanif: Experimentation and writing – original draft; Farhang Hameed Awlqadr, Ijaz Ali, Nariman Shahid, Umaira Rehman, Rouheena Shakir, Syed Hassan Murtaza and Muhammad Ali Syed: Writing – review and editing; Saleha Yasir: Formal analysis and instrumentation. All authors read and approved the final version of the manuscript.

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Data availability statement
All data generated or analysed during the study are included in this published article.

Ethical approval
The study was approved by the Institutional Review Board (or Ethics Committee) from the Department of Pharmacology, Faculty of Pharmacy, the University of Lahore (IREC-2019-125A on 03-02-2020) for studies involving animals. The experiment to estimate the mucoadhesive parameters of gels in the buccal cavity of healthy volunteers was performed after obtaining ethical approval from the Institutional Review Board of the University of Lahore (REC/DPP/FOP/6A). During the experiment, the norms of the Declaration of Helsinki (Directive 2010/63/EU) were observed. This study was performed in adherence with the ARRIVE guidelines. See supplementary file for the ARRIVE checklists.

Conflict of interest
The authors declare no conflict of interest.

Consent to participate
Written informed consent was obtained from all subjects who participated in the study.

Supplementary data

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