Design, *in vitro* and *in vivo* evaluation of buccal patches for mucosal delivery of analgesics and antiseptics for the treatment of oral mucositis

Farzana Perveen[1], Jahanzeb Mudassir[1]* Ikhlaq Hussain[2] and Abdul Majeed[1]
[1]Department of Pharmaceutics, Faculty of Pharmacy, Bahauddin Zakariya University, Multan, Pakistan
[2]Health and Population Department Punjab

Abstract: **Background**: Oral mucositis is an inflammatory condition of the oral mucosa and causes pain associated with oral mucositis, leading to impaired quality of life. Localized drug delivery systems may provide effective treatment while avoiding the drawbacks of systemic administration. **Objective**: The purpose of this study was to formulate a buccal patch of lidocaine, fentanyl, and cetylpyridinium chloride using chitosan (CS), glycerol (G) and propylene glycol (PPG) to treat oral mucositis as a safe alternative to systemic administration. **Methods**: Solvent casting was used to create mucoadhesive buccal patches. Several characteristics were evaluated to optimize the buccal patch, including folding endurance, thickness measurement, mucoadhesion study, drug release, cell viability, permeation study and pharmacokinetic study. In addition, physicochemical interaction between CS, G and PPG was examined using FTIR, DSC and TGA. **Results**: The optimized buccal patch BP4 showed a swelling index of 70%. All of the bioadhesive patches showed surface pH ranging from 6.2 ± 0.18 to 7.2 ± 0.18. Further, the BP4 had an adhesion force of $69 \pm 3.06 \times 10^{-3}$ N. The *in vitro* release of cetylpyridinium chloride, fentanyl and lidocaine from BP4 was 85%, 65% and 75%, respectively, for 12 hours. *Ex vivo* penetration study revealed 70%, 58%, and 78% penetration from three drugs, lidocaine, fentanyl, and cetylpyridinium chloride, respectively, from optimized buccal patches (BP4). When compared to suspension, the buccal administration of fentanyl and lidocaine in rabbits verified a notable increase in the bioavailability of the drugs. **Conclusion**: The developed mucoadhesive buccal patch represents a promising and safe localized delivery system for analgesic and antiseptic agents in the treatment of oral mucositis, offering sustained drug release and improved bioavailability.

Keywords: Buccal patches; Cetylpyridinium chloride; Fentanyl; Lidocaine

INTRODUCTION

Drug administered buccally and sublingually are quickly absorbed before being emptied into the systemic circulation (Sarkhejiya *et al*.). The buccal, sublingual, palatal and gingival regions of the oral cavity are the four possible sites for drug administration (Birudaraj *et al*., 2005, Schwarz *et al*., 2013). Compared to invasive or parenteral medication administration, this method is more practical for the delivery of therapeutic substances because it is non-invasive (Barua *et al*., 2016). Painless procedure, low enzymatic activity, high patient compliance and easy to take out the dosage forms are additional advantages that make this route more proper and acceptable for the delivery of medication (Gilhotra *et al*., 2013). Furthermore, the buccal mucosa has a higher bioavailability because the drug bypasses the hepatic first pass metabolism (a drug degradation phenomenon where a medication's level is drastically lowered when it enters the bloodstream) and has direct access to the systemic circulation without acid hydrolysis in the gastrointestinal tract (Shirvan *et al*., 2019). However, the primary obstacles to drug absorption through the buccal route include the mucosa's barrier characteristics, small surface area, relatively short

residence period and substantial drug loss. A number of methods, including drug-polymer conjugation and bioadhesive compounds, have been used to get past barriers and increase the drugs' bioavailability (Caon *et al*., 2015). The buccal mucosa makes up 1/3 of the entire oral mucosa surface (Rossi *et al*., 2005). The basement membrane, submucosa, lamina propria and squamous stratified epithelium and several sensory receptors make up the oral mucosa (Venkatalakshmi *et al*., 2012).

An inflammatory disease of the mucosa of the mouth, mucositis is brought on by chemotherapy for cancer, especially bone marrow conditioning regimens for bone marrow transplants and radiation therapy for the head and neck, especially when treating oral cancer. Mucosal damage brought on by dosage causes painful ulcers, issues with speaking, eating and swallowing, as well as a higher risk of infections (Sankar *et al*.). Lidocaine-containing buccal products, such as mucoadhesive films, bilayer tablets, discs and patches, have become more and more popular. This relates to pharmacokinetic advantages by avoiding intestinal and liver first-pass metabolism as well as popularity among patients because of the simplicity of use, such as eliminating injections (Kottke *et al*.). Additionally, CPC inhibits the growth and accumulation of bacterial biofilms, which helps to lessen and manage

Corresponding author: e-mail: jahanzebmudassir@bzu.edu.pk

plaque and gingivitis. Patches would therefore have a decent residence time and the best kill time.

The rationale of this work is to describe a buccal mucoadhesive film or patches for the treatment of oral mucositis employing analgesics and antiseptics. Glycerol would be used as a plasticizer and Carbopol 971P as a mucoadhesive polymer to create buccal films. The mucoadhesive films/patches would be characterized using FTIR, DSC, TGA, swelling experiments and pharmacokinetic study using an animal model.

MATERIALS AND METHODS

Materials
Lidocaine HCl, Fentanyl and Cetylpyridinium chloride were gifted by Remington Pharmaceuticals Pvt. Ltd., Lahore, Pakistan. Chitosan (CS), Glycerol (G), Propylene glycol (PPG), methanol, and acetonitrile were purchased from Sigma-Aldrich GmbH, Darmstadt, Germany. All other materials used were of analytical grades.

Preparation of buccal patches
Solvent double casting technique was utilized to formulate the patches containing lidocaine HCl, fentanyl and cetylpyridinium chloride. A 10 cm-diameter glass plate was filled with a backing layer solution consisting of glycerol (G), polypropylene glycol (PPG) as a plasticizer and chitosan (CS) as a polymer. After that, it was oven-dried at 55 °C for two hours. The second matrix solution, consisting of glycerol, polypropylene glycol and lidocaine HCl+ fentanyl+ cetylpyridinium chloride (Table 1), was immediately poured on top of the pre-cast dry CS-G-PPG backing layer and allowed to dry for 12 hours at 55 °C. The patches were placed in a desiccator until they were used for further study after drying and were wrapped in aluminium foil. After being cut to a 20 mm diameter, the patches were stored in a desiccator until they were required for further study (Jaipakdee *et al.*, 2018).

Physical characterization
Measurement of thickness
An electronic digital micrometer (model: PK-1012E, Mitutoyo, Japan) was used to determine the thickness at five separate points (the center and four corners) before calculating the average thickness (Abouhussein *et al.*, 2020).

Swelling study
The buccal patches were assessed independently for swelling behaviour and were placed on separate 2% agar gel plates. Incubated at 37°C ± 1°C and were examined for changes in appearance. After three hours, the patches were removed from the gel plates, and the remaining surface water was gently cleaned off using filter paper. In addition to the weight rise, the mean of the three experiments was computed (Abouhussein *et al.*, 2020).The Swelling Index (S.I.)was determined using following formula:

$$S.I = \frac{Wt - Wo}{Wo}$$

Where: W_t: film weight at time t and W_o: film weight at initial time

Folding endurance
One patch was folded repeatedly at the same spot until it broke, or folded up to 200 times without breaking, to test a patch's folding endurance. The folding durability of a film is an indicator of its mechanical properties (Mundhey *et al.*, 2021). Folding endurance was calculated as the total number of repetitions the film was able to wrap in the exact same place with no splitting or breaking (Adhikari *et al.*, 2010).

Mucoadhesion study
The patches' mucoadhesive characteristics were investigated by means of a texture analyzer (Stable Micro Systems). Freshly excised cow buccal mucosa was divided into 2 mm thick slices for mucoadhesiveness tests. To simulate the oral mucosa, a pH 6.8 buffered saline was used to moisten the mucosa that was connected to the analyzer's top probe. The material was then adhered firmly to the instrument probe. After lowering the probe at a speed of 0.5 mm/s and exerting 0.5 N forces for 120 s to bring it into contact with the mucosa, the probe separated the specimen and mucosa interfaces by moving up vertically from the mucosal face at a speed of 0.5 mm/s. F_{max}, or the maximum force of separation, was calculated. Three duplicates of each experiment were conducted (Özkahraman *et al.*, 2022).

Chemical characterization
FTIR analysis
FTIR was used to recognize potential interaction between the drug and the polymeric components of the patches (Palem *et al.*, 2011). An ATR-FTIR spectrophotometer (Bruker-Alpha-Germany) was used to record the FTIR spectra. After being cut, each sample was put in a sample holder and the materials' spectra (4000 to 650 cm^{-1} at a resolution of 4 cm^{-1}) were recorded (Jaipakdee *et al.*, 2018).

DSC analysis
Using a differential scanning calorimeter (DSC822, Mettler Toledo, Switzerland), the samples' DSC curves were captured. 3 to 5 mg of each sample was precisely weighed into a 50 µL open aluminum pan. The measurements were made between 0 and 500 °C with a heating rate of 10 °C per minute (Jaipakdee *et al.*, 2018).

TGA analysis
The TGA analyzer (Mettler Toledo, TGA/DSC1 HT) was used to determine the thermal characterization in the range of 0-500ºC with a heating ramp of 10 ◦C/min in a nitrogen atmosphere. The weights of the samples ranged from 5 to 10 mg (Ozbas *et al.*, 2022).

In vitro drug release

The in-vitro release upto 12 h was performed from drug-loaded patches. Patches were sliced into 1×1 mm^2 pieces and were added to a shaking water bath at 37 °C. 10 mL of synthetic saliva with a pH of 6.8 was used for the release experiments. At predetermined intervals, the 3 mL aliquots were swapped out for new 3 mL of buffer solution. A Shimadzu UV-1800, Japan, UV-Vis Spectrophotometer was used to measure the drug concentrations in the aliquots at a fixed wavelength of 242 nm. The following formula was used to calculate the cumulative release (%),

$$Cummulative\ release\ (\%) = \left[Cn + \frac{3}{10}\ \Sigma\ Cn - 1\right] \times 100$$

Where C_n and C_{n-1} represent the drug release amounts at particular times n and n-1, respectively. Three runs of each experiment were conducted and the mean value was reported (Ozbas *et al.*, 2022).

Cell viability assay

The L929 cell line was used in indirect MTT test for *in vitro* cytotoxicity investigations. In the tests, Passage L-929 cells were employed. Dulbecco's modified Eagle's medium (DMEM), which contained 10% (volume/volume) fetal bovine serum (FBS), 1% (volume/volume) penicillin–streptomycin and 1% (volume/volume) L-glutamine, was utilized as the culture medium.

The samples were sterilized on both sides using UV radiation for the MTT test. After that, sterile samples were incubated for 24 hours at 37 °C with 5% CO_2 after culture media was supplied at a rate of 1 cm^2/mL. Following the incubation period, the same volume of DMEM was added to the culture medium that contained the samples. After seeding 1×10^4 cells per well in 96-well plates, 200 μL of culture media was added and the cells were incubated for the entire night at 37 °C with 5% CO_2. Following the addition of the incubating media to the wells, the samples were incubated for a further six and twenty-four hours, respectively. After the period of incubation, 200 μL of DMEM and 20 μL of MTT solution were added to each well and they were incubated for three hours at 37 °C with 5% CO2. 300 μL of DMSO was incorporated to the medium after 20 minutes of incubation and the result was measured at 570 nm using a microplate reader (Ozkahraman *et al.*, 2022).

Permeation study of buccal patch

The drug released from buccal patch was examined for permeation study using a Franz type glass diffusion cell at 37 ± 0.5 °C. The donor and receptor compartments were separated by freshly applied buccal mucosa. The compartments were securely fastened together and the patch was positioned with the core toward the mucosa. It contained 1 milliliter of phosphate buffer (pH 6.8) in the donor tank. Phosphate buffer (pH 6.8) was added to the receptor compartment and with a magnetic bead spinning at 50 rpm, the hydrodynamics within the receptor region were kept stable. At predetermined intervals, a 1 mL sample was taken out and subjected to a UV spectrophotometer to assess its drug content at 290 nm (Cavallari *et al.*, 2013).

Pharmacokinetics

Healthy rabbits were used for pharmacokinetic investigations. For two weeks before the trial, the animals chosen for it were not given any medicine. Before the test patch was applied, a 25 mg/kg intramuscular ketamine injection was used to sedate the rabbits. The prepared test patch was inserted into the buccal cavity and adhered directly to an ethyl cellulose backing layer (Kaur and Kaur, 2012). During the night prior to the dose, the rats were divided into two groups and given only water to drink. Group 1 received the BP4 formulation (test), while Group 2 received the drug suspension. For both groups, the dosage of the drug was 1 mg/kg. Blood samples were taken at predetermined intervals and centrifuged for 20 min at 4000 rpm. The blood samples were collected at 0.5, 1, 2, 4, 6, 8, 12, 24 and 36 hours for all three groups. The sample is injected into UV for analysis (Hanif *et al.*, 2020).The non-compartmental analysis using PK Solver Excel based sheets was used to calculate the pharmacokinetic parameters.

RESULTS

All of the patches had surface pH values close to 6, therefore the buccal cavity shouldn't become irritated by them (Kaur and Kaur, 2012).The test was performed in distilled water to ascertain whether water was consumed during the entire procedure of adhering the films to the buccal mucosa. Hydrodynamic free volume and hydrophilic functional groups allow water to form hydrogen bonds and raise the swelling of a film, which is what determines how well a film absorbs water (Abouhussein *et al.*, 2020).The prepared patches were homogeneous in thickness and drug content and had a smooth look. The findings demonstrated that the mucoadhesion of patch to the buccal mucosa was influenced by the quantity of the polymer used. In other words, the force required to remove the patches from the mucosal membrane rose in proportion to the amount of polymer in the formulation matrix. BP4 was selected as optimized adhesive patch showing release of incorporated drugs at mucoadhesion site (Ozkahraman *et al.*, 2022).

Physical characterization
Thickness measurements
The patch's thickness varied from 0.42 ± 0.48 to 0.74 ± 0.66 mm. The buccal patches' drug content of lidocaine, cetylpyridinium chloride and fentanyl ranged from 32 ± 1.21 to 43 ± 1.3, 34 ± 1.05 to 48 ± 1.59 and 39 ± 1.01 to 57 ± 1.29, respectively, while the mucoadhesive patches' surface pH ranged from 6.2 ± 0.18 to 7.2 ± 0.18 as shown in Table 2.

Swelling study

Fig. 1 shows the distilled water used to calculate the films' swelling Index. In swelling index, five formulations (BP1 to BP5) are given. The BP4 exhibits 70% swelling in mucoadhesive buccal patch as shown in Fig. 1, suggesting that it is a stable formulation.
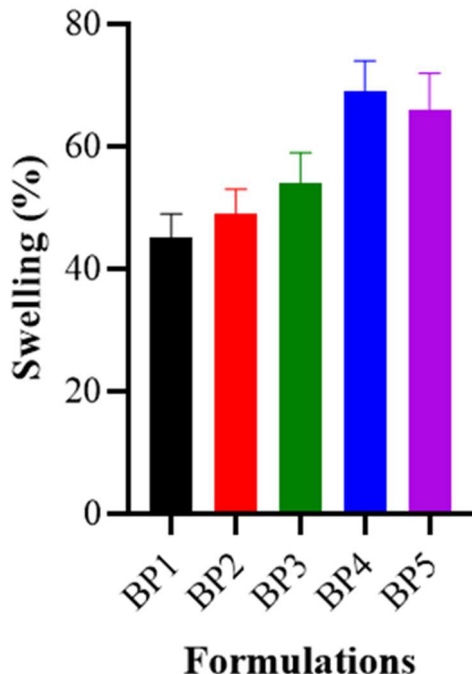

Fig. 1: Swelling studies of formulation BP1 to BP 5

Folding endurance

There is good folding endurance in the patches range from 180 to 204 (Table 2) (Kaur and Kaur, 2012). The developed films had a surface pH ranged from 6.6 ± 0.21 to 7.1 ± 0.25. Table 2 displays the surface pH (Abouhussein *et al.*, 2020).

Mucoadhesion study

One of the most crucial variables in determining mucoadhesive behaviors is the force of adhesion, or maximum force F_{max}. We measured the force needed to separate the buccal patches from the buccal mucosa in order to assess the buccal patches' in vitro mucoadhesion. Table 3 displays the results that were collected. $52 \pm 2.98 \times 10^{-3}$ N, $56 \pm 3.01 \times 10^{-3}$ N, $63 \pm 2.04 \times 10^{-3}$ N, $69 \pm 3.06 \times 10^{-3}$ N and $68 \pm 2.78 \times 10^{-3}$ N were the force of bioadhesion of the BP1 through BP5 samples, respectively.

Chemical characterization

FTIR analysis

The FTIR spectra of lidocaine HCL (brown), fentanyl (green) and cetylpyridinium chloride (purple), chitosan (CS) (red), glycerol (G) (blue), propylene glycol (PPG) (black) and formulation BP4 (zinc) are shown in the Fig. 2.

DSC and TGA analysis

Figs. 3 and 4 show the DSC and TGA curves for lidocaine HCl, fentanyl, cetylpyridinium chloride, chitosan (CS), glycerol (G), propylene glycol (PGG) and the loaded formulation BP4, respectively.
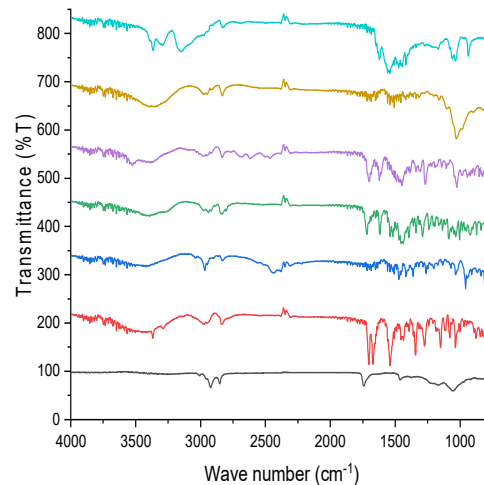

Fig. 2: FTIR spectra of Lidocaine (brown), fentanyl (green), cetylpyridinium chloride (purple), CH (red), G (blue) and PPG (black), and BP4 (zinc)
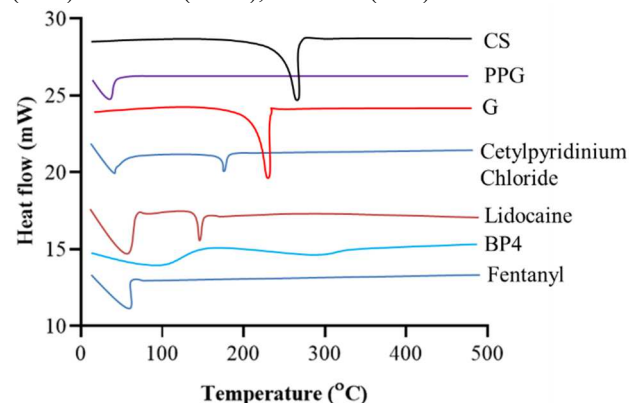

Fig. 3: DSC thermogram of lidocaine (maroon), fentanyl (dark blue), cetylpyridinium chloride (zinc), chitosan (black), glycerol (red), propylene glycol (purple) and BP4 (sky blue)
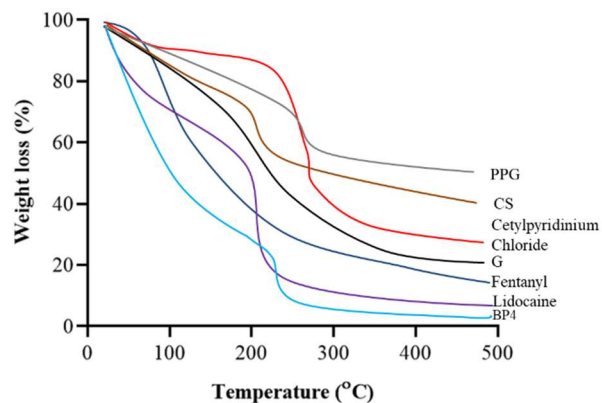

Fig. 4: TGA thermo gram of lidocaine (purple), fentanyl (zinc), cetylpyridinium chloride (red), chitosan (brown), glycerol (black), propylene glycol (grey), and BP4 (sky blue)

Table 1: Composition of buccal patches containing same amount of lidocaine, fentanyl and cetylpyridinium chloride

Sr. no.	Glycerol (G) in gram	Propylene glycol (PPG) in gram	Chitosan (CS) in gram
BP1	50	25	30
BP2	60	35	40
BP3	70	45	50
BP4	80	55	60
BP5	90	65	70

lidocaine, fentanyl and cetylpyridinium chloride was added in same quantity in all patches

Table 2: Physical characterization of buccal patch containing lidocaine, cetylpyridinium chloride and fentanyl

Formulations	Thickness (mm)	Drug content (%) (Lidocaine, cetylpyridinium chloride, Fentanyl)	Surface Ph	Folding endurance
BP1	0.63 ± 0.52	34±2.51 37±2.09 41±1.89	6.9 ± 0.12	221 ± 13
BP2	0.51 ± 0.32	32±1.21 34±1.05 39±1.01	6.6 ± 0.21	179 ± 12
BP3	0.58 ± 0.53	41±2.08 46±2.01 49±1.05	6.7 ± 0.17	175 ± 29
BP4	0.69 ± 0.19	43±1.31 48±1.59 57±1.29	7.0 ± 0.31	219 ± 11
BP5	0.65 ± 0.18	40±1.05 42±1.04 51±1.08	7.1 ± 0.25	193 ± 12
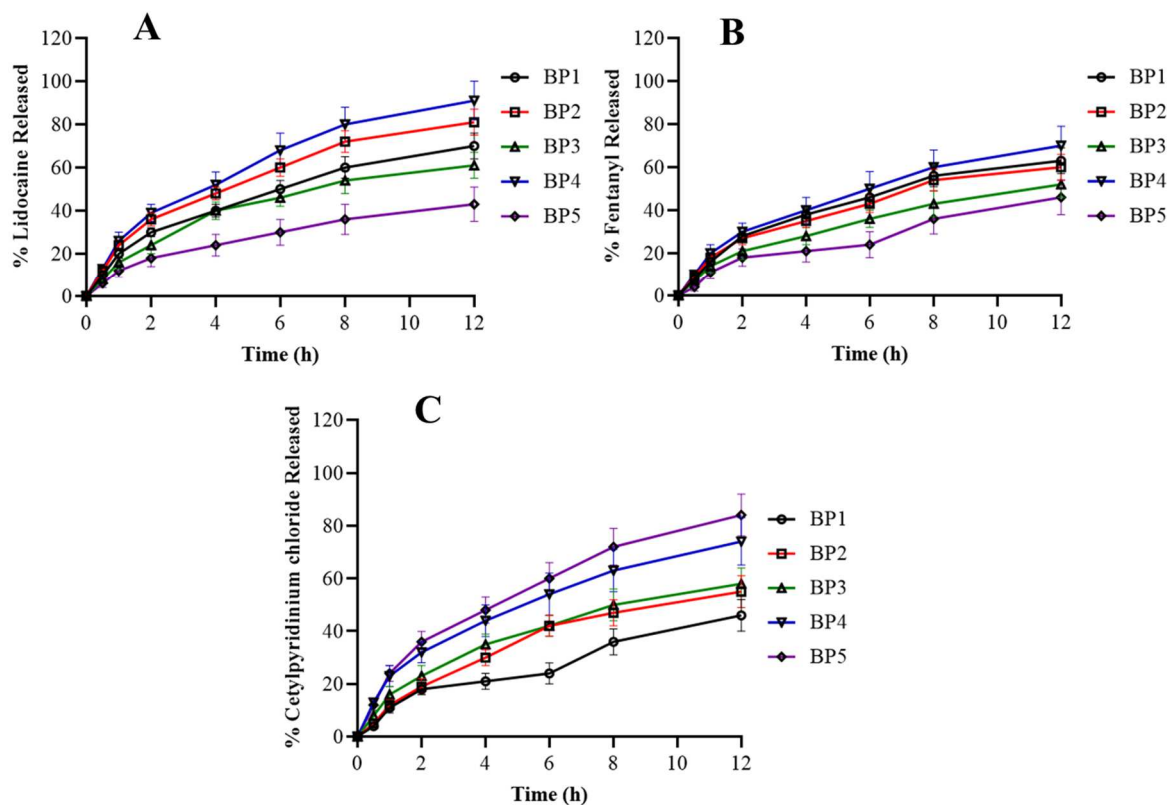
Fig. 5: Comparative release pattern for lidocaine from BP1 to BP5 (A), fentanyl from BP1 to BP5 (B) and cetylpyridinium chloride from BP1 to BP5 (C)

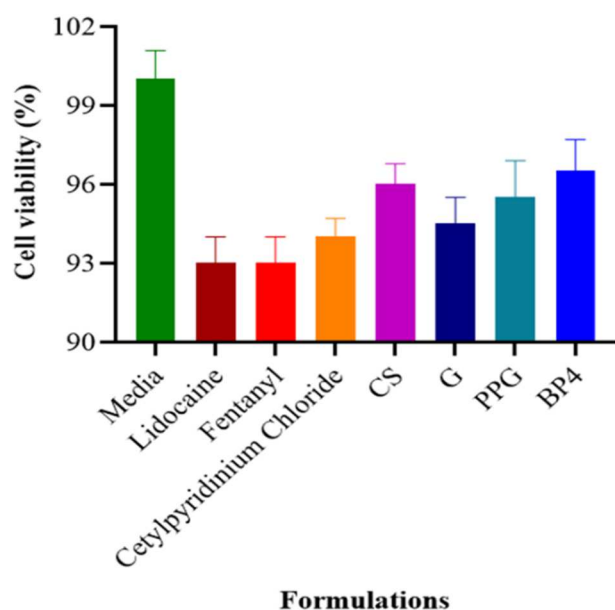**Table 3**: Mucoadhesion study and force of bioadhesion of the BP1 to BP5

Sample	Force of bioadhesion, F_{max} (10^{-3} N)
BP1	52±2.98
BP2	56±3.01
BP3	63±2.04
BP4	69±3.06
BP5	68±2.78

**Table 4**: Pharmacokinetic parameters of lidocaine and fentanyl from suspensions and test buccal patches

Parameters	Reference suspensions		Test (Buccal patch)	
	Lidocaine	Fentanyl	Lidocaine	Lidocaine
C_{max} (µg/mL)	6.5±1.23	35±2.48	13±1.87	62±2.34
T_{max} (h)	4±1.04	4±1.01	8±1.86	8±2.08
AUC $_{0-t}$ (µg.h/mL)	25.25±2.89	141.32±4.24	53.23±2.08	282.12±6.06
AUC $_{0-\infty}$ (µg.h/mL)	31.40±2.08	151.08±4.21	64.23±2.98	291.24±6.24
K_{el}	0.15±0.03	0.19±0.04	0.30±0.07	0.39±0.06
$t_{1/2}$ (h)	11.2±1.21	12.4±1.29	23.5±1.92	25.9±2.05

In vitro drug release

Fig. 5 showed the release profiles of the buccal patches for all the formulation (BP1 to BP5). It was found that each of the obtained patches had an equilibrium time of roughly 12 h. The release of lidocaine form BP1, BP2, BP3, BP4 and BP5 was shown in Fig. 5A and have release levels of 65, 78, 59, 85 and 39 %, respectively, during a 12 h period. The release of fentanyl form BP1, BP2, BP3, BP4 and BP5 was shown in Fig. 5B and have release levels of 59, 58, 45, 65 and 40 %, respectively, during a 12 h period. The release of Cetylpyridinium chloride form BP1, BP2, BP3, BP4 and BP5 was shown in Fig. 5C and have release levels of 42, 58, 59, 75 and 82 %, respectively, during a 12 h period.

Cell viability assay

This study evaluated each sample's cytotoxicity to the L929 cell line using the indirect MTT test. Fig. 6 presented the outcomes of the experiment. The cells' viability was monitored and was approximately 1000 % for media, 93 % for lidocaine and fentanyl. Cetylpyridinium chloride has a cell viability of around 93.5 %.

Fig. 6: Cell viability assay of media, lidocaine, fentanyl, cetylpyridinium chloride, CS, G, PPG, and BP4
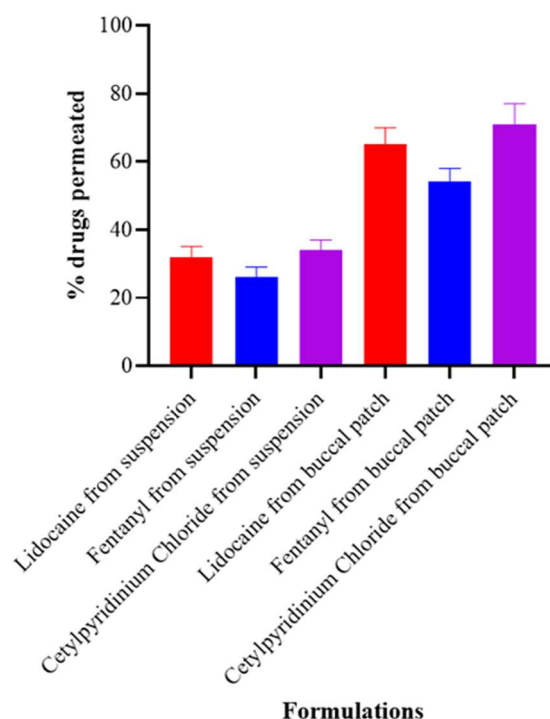
Fig. 7: Permeation profile of system across buccal mucosa

### Permeation study of buccal patch

The patch formulations underwent a permeation test. After the patch was applied using a Franz cell onto a section of

rabbit buccal mucosa, the drugs (lidocaine, fentanyl and cetylpyridinium chloride) absorbed over time are plotted, in which permeation of drugs from suspension and buccal patches are shown in Fig. 7. Lidocaine, fentanyl and cetylpyridinium chloride from suspension show 38%, 30% and 39% permeation. Lidocaine, fentanyl and cetylpyridinium chloride from buccal patch show 70%, 58% and 78% permeation.

Pharmacokinetics study
Fig. 8 shows the average plasma concentration of lidocaine and fentanyl in rabbits at various points in time after the buccal patch was applied and after an oral lidocaine and fentanyl were administered. The average steady-state drug level decreased for up to 35 hours after the fentanyl and lidocaine plasma concentration progressively increased and reached a maximum. After applying a buccal patch, the AUC total was greater for fentanyl and lidocaine buccal patches (test) as compared to suspension (reference). The buccal formulation selected for the *in vivo* investigation that increased the bioavailability of lidocaine and fentanyl in buccal patches in contrast to suspension. Cetylpyridinium chloride only penetrates the mucosa and cannot be absorbed orally. Rabbits were subsequently administered the suspension and BP4 formulation via the oral route.
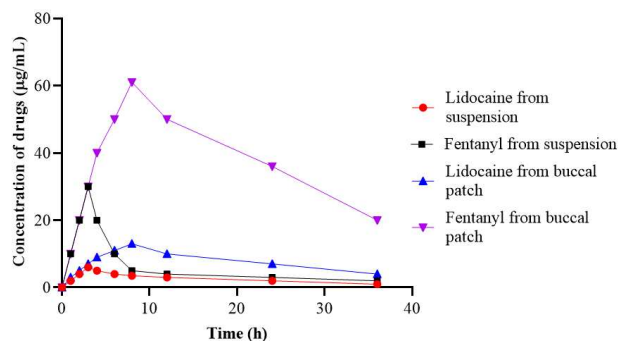
Fig. 8: Plasma concentration profile of lidocaine and fentanyl suspension and buccal patches

DISCUSSION

The prepared patches had no noticeable flaws, were homogeneous in thickness and drug content and had a smooth look. The findings clearly demonstrated that the mucoadhesion of patch to the buccal mucosa is influenced by the quantity of the polymer used. In other words, the force required to remove the patches from the mucosal membrane rose in proportion to the amount of polymer in the formulation matrix. BP4 was selected as the best adhesive patch for the release of drugs, research and medicinal activity assessment based on mucoadhesion.

FTIR spectra
The (C=O) of amide I group was seen at 1670 cm⁻¹, (C-N) of amide II at 1458 cm⁻¹, the NH stretching at 3385 cm⁻¹ and the stretch of C-H at 3001 cm⁻¹ in the powdered lidocaine HCl (Jaipakdee *et al*., 2018). The carbonyl amide groups of Fentanyl exhibited a stretching band at 1,622 cm⁻¹, while In terms of vibration mode; the stretch of C-O was located at 1647 cm⁻¹. The FTIR spectrum of Cetylpyridinium chloride presented the peak at 3523 cm⁻¹ which indicated the bending of the N-H group and at 3300 cm⁻¹ indicated the presence of the -OH group. Characteristic infrared absorption bands associated with the CPC were observed at 1029 cm⁻¹, which indicated the existence of the C-N group (Karikalan *et al*., 2018). The FTIR spectra of CS revealed that the C–H bending was discovered at 1436 cm⁻¹, while the stretching vibrations of NH, –OH and C = O were observed at 3366 cm⁻¹, 2972 cm⁻¹ and 1707 cm⁻¹, respectively (Abbas *et al*., 2022). In the FTIR spectrum of glycerol, the peaks at 3631 cm⁻¹ and 1300-1400 cm⁻¹ are coupled with O-H and C-H vibrations, respectively. The bands in the 2950-2850 cm⁻¹ range correspond to stretching C-H vibrations, while a sharp and intense band at 1110-1030 cm⁻¹ corresponds to C-O stretching vibrations (Gómez-Siurana *et al*., 2013). There are distinct peaks in the PPG spectra at 1077, 1361, 1400, 2854, 2959 and 3450 cm⁻¹. The C–H stretching and bending modes exhibit peaks at 2854 and 1400 cm⁻¹, respectively, but the stretching of the H-bonded OH functional group is evidently responsible for the absorption band at 3450 cm⁻¹. The FTIR spectra of formulation BP4 showed its possible stretching peaks at 3423 cm⁻¹, 3300 cm⁻¹, 2999cm⁻¹, 1700cm⁻¹, 1029cm⁻¹.

DSC thermogram
Following an endothermic peak at 75 °C, lidocaine HCl exhibited a boiling and volatilization peak that started at 169 °C in its DSC thermogram. The TGA thermogram showed the weight loss of lidocaine HCl as a first stage of decomposition below 100°C, which may have been caused by moisture elimination. The weight loss of lidocaine HCl was in the 150-250°C temperature range, with Tmax = 220.24°C for 90% decomposition (Jaipakdee *et al*., 2018). Fentanyl's DSC thermogram revealed a distinct endothermic peak at 83°C, which is also its melting point (Ogawa *et al*., 2010). The TGA thermogram showed the weight loss of fentanyl below 200°C. Two endothermic peaks were observed in the DSC thermogram of Cetylpyridinium chloride: one at 199°C and the other beginning at roughly 72°C. The sudden appearance of the first event and the mass loss at the same temperature shown in the TG curve indicate that it corresponds to a melting peak and decomposition, respectively. The DSC curve confirmed similar events at the temperature shown by the TGA curves, which showed a slight mass loss between 79°C and 88°C (2.5%), corresponding to the loss of hydrated molecules and following decomposition comparable to 90% at 250°C (de Aquino *et al*., 2023). Because of the amine units' thermal breakdown, the DSC thermogram of chitosan (CS) showed a single broad endothermic peak at 279°C (Abbas *et al*., 2022).

TGA

CS's TGA thermogram revealed weight loss that began below 100ºC followed by a major loss between 220-350 °C and continued till 500 °C(Abbas *et al.*, 2022). In the TGA thermogram of glycerol (G), show early and sharp degradation starting near 75°C and a major weight loss step, primarily due to glycerol evaporation, showed a TGA-peak at 250°C (Gómez-Siurana *et al.*, 2013). As a non-crystalline polymer, PPG doesn't exhibit a melting point. A peak is shown at around 55°C in its DSC thermogram. The two thermal events are seen in the TGA thermogram of PPG. At 150°C, PPG begins to degrade and complete degradation occurs at 399°C (Loh *et al.*, 2008). The DSC thermogram of the formulation BP4 showed a broad endothermic transition at nearly 200°C indicating possible melting and composite behavior. The TGA thermogram of formulation BP4 showed a very rapid degradation between 150 and 250°C, followed by stabilization, indicating the complex with multiple degradation steps. *In vitro* drug release and CS concentration can be linked: a boost in CS concentration led to an increase in the drug's release pattern from CS patches, with polymer also playing a role (Ozbaş *et al.*, 2022).

Cell viability study

Chitosan has about 96.5% cell viability and the cell viability of G and PPG was 95% and 95.9%. The formulation BP4 showed the maximum cell viability and was about 96.9%. After 24 hours of culture, the results indicated that none of the samples were cytotoxic to L929 cells, with differences that were not significant from the control groups ($p > 0.05$). From results, all the samples were determined to be biocompatible and could be a viable option for treating oral mucositis (Ozkahraman *et al.*, 2022).

Cetylpyridinium chloride can penetrate the skin, but it will never absorb into the bloodstream in Fig. 7. The profile is nearly linear and, more intriguingly, the rate of permeation is roughly equal to that of release. This indicates that the patch does not decrease the mobility of drugs within the film. It also confirms the drug's good permeating ability, which may lead to a quick start of anesthetic effect, antiseptic effect and analgesic effect when using the current patches. The backing membrane's effectiveness in preventing the release of drug was also assessed by this test; the results of the investigation showed that during the course of the 120 minutes, no drug had been released in the donor compartment of the diffusion cell. This suggests that the backing layer's integrity was unaffected by the enlargement of the mucoadhesive layer. As a result, it was discovered that the patch effectively released drugs through the buccal mucosa (Cavallari *et al.*, 2013).

A non-compartmental method was then used to compute the pharmacokinetic parameters Cmax (µg/mL), tmax (h), AUC 0-t (µg/ml.h) and t1/2 (h). The results are shown in Table 4. The C max mean ± SEM after buccal administration of the BP4 formulation was greater than the reference formulation's mean ± SEM (13 ± 1.87 and 62 ± 2.34 µg/mL ($p = 0.001$) and 6.5 ± 1.23 and 35 ± 2.48 µg/mL, respectively). The formulas' varying compositions were blamed for the discrepancy. The BP4 formulation's greater C max value is mostly attributable to its more precisely defined and regulated release. As indicated in Table 4 and Fig. 8, the t1/2 of the BP4 and standard formulations further supported the designated release. Additionally, the test formulation (BP4) showed a better bioavailability than the reference formulation. The statistical analysis indicated that the value of p was <0.05, as indicated in Table 2, indicating that the results are statistically significant. T max post-administration values showed a significant change ($P < 0.05$) (Vasisht *et al.*, 2010).

CONCLUSION

The buccal patches based on CS, G and PPG containing lidocaine HCl, fentanyl and Cetylpyridinium chloride demonstrated acceptable mucoadhesive and physicomechanical properties. Patches offered sustained buccal delivery for extended periods of time in the treatment of oral mucositis. The optimized BP4 showed 70%, 58% and 78% of lidocaine HCl, fentanyl and Cetylpyridinium chloride release. Further, BP4 shown 70% swelling index and $69\pm3.06 \times 10^{-3}$ N mucoadhesion. The in vitro and in vivo investigations, revealed the potential of patches in treating oral mucositis because of releasing loaded drugs at adhesion site. The bioadhesive capability of, buccal patches prolonged the retention period in the oral cavity and supplied drug concentrations above their minimal inhibitory concentration as compared to the suspension.

Authors' contributions
Farzana Perveen: Conceptualization, methodology, investigation, writing - original draft; Jahanzeb Mudassir: Project administration, supervision, writing - review and editing; Ikhlaq Hussain: Investigation, data curation, writing - original draft; Abdul Majeed: Data analysis, manuscript writing, manuscript revision.

Data availability statement
All data generated and analyzed during this research study are included in this published.

Ethical approval
Animal protocols were reviewed and approved by the Ethical Committee, Department of Pharmaceutics, Faculty of Pharmacy, Bahauddin Zakariya University, Multan, with reference No.345/PEC/2024 dated 30-12-2024.

Conflict of interest
The authors declare no conflict of interest.

Supplementary data

REFERENCES

Abbas G, Rasul A, Fakhar-E-Alam M, Saadullah M, Muzammil S, Iqbal O, Atif M, Hanif M, Shah S and Ahmad S (2022). Nanoparticles of thiolated chitosan for controlled delivery of moxifloxacin: *In-vitro* and *in-vivo* evaluation. *J. King Saud Univ. Sci*., **34** (7): 102218.

Abouhussein D, El Nabarawi MA, Shalaby SH and El-Bary AJ (2020). Cetylpyridinium chloride chitosan blended mucoadhesive buccal films for treatment of pediatric oral diseases. *J. O. D. D. S. & Technology*., **57**(1): 101676.

Adhikari SNR, Nayak BS, Nayak AK and Mohanty B (2010). Formulation and evaluation of buccal patches for delivery of atenolol. *AAPS Pharm Sci Tech.,* **11**(3): 1038-1044.

Barua S, Kim H, Jo K, Seo CW, Park T, Lee KB, Yun G, Oh K and Lee JI (2016). Drug delivery techniques for buccal route: Formulation strategies and recent advances in dosage form design. *J. Pharm. Investig.,* **46**(7): 593-613.

Birudaraj R, Mahalingam R, Li X and Jasti BR (2005). Advances In: Buccal drug delivery. *Therapeutic Drug Carrier Systems*., **22**(3): 295-330.

Caon T, Jin L, Simões CM, Norton RS and Nicolazzo JA (2015). Enhancing the buccal mucosal delivery of peptide and protein therapeutics. *J. Pharm. Res.,* **32**(1): 1-21.

Cavallari C, Fini A and Ospitali F (2013). Mucoadhesive multiparticulate patch for the intrabuccal controlled delivery of lidocaine. *Eur J Pharm Biopharm.,* **83**(3): 405-414.

De Aquino DA, Oliveira AS, Amorim MV, Gomes A. PB, Veríssimo LM and Ferrari M (2023). Thermal behavior of cetylpyridinium hydrochloride and its association with sugar alcohols and flavoring agents: A preformulation study. *J. Therm. Anal. Calorim.,* **148**(18): 9477-9488.

Gilhotra RM, Ikram M, Srivastava S and Gilhotra NJ (2013). A clinical perspective on mucoadhesive buccal drug delivery systems. *J. Biomed. Res.,* **28**(2): 81.

Gómez-Siurana A, Marcilla A, Beltrán M, Berenguer D, Martínez-Castellanos I and Menargues S (2013). TGA/FTIR study of tobacco and glycerol–tobacco mixtures. *Thermochim. Acta.,* **573**: 146-157.

Hanif M, Shah S, Rasul A, Abbas G, Zaman M, Amjad M. W, Abdul Ghafoor Raja M, Khan HU, Ashfaq M and Iqbal O (2020). Enhancement of oral bioavailability of ibandronate through gastroretentive raft forming drug delivery system: *In vitro* and *in vivo* evaluation *Int. J. Nanomed.,* **4847**(15): 4858.

Jaipakdee N, Pongjanyakul T and Limpongsa E (2018). Preparation and characterization of poly (Vinyl Alcohol)-Poly (Vinyl Pyrrolidone) mucoadhesive buccal patches for delivery of lidocaine HCL. *Int. J. Appl. Pharm.,* **10**(1): 115-123.

Karikalan V, Panneerselvam A and Vallalperuman K (2018). Physico—chemical analysis on cetylpyridinium chloride (CPC) with alcohol solution at different temperatures—ultrasonic, UV And FTIR Analysis. *Dig. J. Nanomater. Bios.,* **13**(1): 115-128.

Kaur A and Kaur G (2012). Mucoadhesive buccal patches based on interpolymer complexes of chitosan–pectin for delivery of carvedilol. *Saudi Pharm. J.,***20** (1): 21-27.

Kottke D, Majid H, Breitkreutz JR and Burckhardt BB (2020). Development and evaluation of mucoadhesive buccal dosage forms of lidocaine hydrochloride by *ex-vivo* permeation studies. *Int. J. Pharm.,* **15**(581): 119293.

Loh XJ, Sng K and Li J (2008). Synthesis and water-swelling of thermo-responsive poly (Ester Urethane) S containing poly (E-Caprolactone), poly (Ethylene Glycol) and poly (Propylene Glycol). *Biomaterials.,* **29**(22): 3185-3194.

Mundhey D, Sapkal N and Daud AJ (2021). Fabrication of microemulsion loaded sublingual film for rapid absorption of fentanyl citrate in transient breakthrough pain. *Int J App Pharm* ., **13**(3): 233-238.

Ogawa N, Higashi K, Nagase H, Endo T, Moribe K, Loftsson T, Yamamoto K and Ueda H (2010). Effects of cogrinding with B-cyclodextrin on the solid state fentanyl. *J. Pharm. Sci.,***99** (12): 5019-5029.

Ozbaş Z, Ozkahraman B, Akgüner ZP and Bal-Ozturk A (2022). Evaluation of modified pectin/alginate buccal patches with enhanced mucoadhesive properties for drug release systems: *In-vitro* and *Ex-vivo* Study. *J Drug Deliv Sci Technol.,* **67**(1): 102991.

Ozkahraman B, Ozbaş Z, Yaşayan G, Akguner ZP, Yarımcan F, Alarçin E and Bal-Ozturk A (2022). Development of mucoadhesive modified kappa-carrageenan/pectin patches for controlled delivery of drug in the buccal cavity. *J. Biomed. Mater. Resh. Part B: Applid. Biomaterials.,***110**(4): 787-798.

Palem CR, Gannu R, Doodipala N, Yamsani V and Yamsanim MR (2011). Transmucosal delivery of domperidone from bilayered buccal patches: *In vitro*, ex vivo and *in vivo* characterization. *Arch. Pharm. Res.,* **34**(10): 1701-1710.

Rossi S, Sandri G and Caramella CM (2005). Buccal drug delivery: A challenge already won? *Drug Discovery Today: Technologies Drug Discov. Today Technol.,* **2**(1): 59-65.

Pak. J. Pharm. Sci., Vol.39, No.4, April 2026, pp.1063-1072

1071

Sankar V, Hearnden V, Hull K, Juras D V, Greenberg M, Kerr A, Lockhart P, Patton L, Porter S and Thornhill M (2011). Local drug delivery for oral mucosal diseases: Challenges and opportunities. *Oral Disease.,* **17**(1): 73-84.

Sarkhejiya NA, Sodha HP, Kapdia YD and Patel VP (2013). Review on oral mucosal drug delivery system. *Pharma Science Monitor.,* **15**(4): 281-310.

Schwarz JC, Pagitsch E and Valenta CJ (2013). Comparison Of ATR–FTIR spectra of porcine vaginal and buccal mucosa with ear skin and penetration analysis of drug and vehicle components into pig Ear. *Eur. J. Pharm. Sci.*, **50**(5): 595-600.

Shirvan AR, Bashari A and Hemmatinejad N (2019). New insight into the fabrication of smart mucoadhesive buccal patches as a novel controlled-drug delivery system. *Euro. Poly. J.,* **119**(1): 541-550.

Vasisht N, Gever LN, Tagarro I and Finn AL (2010). Single-dose pharmacokinetics of fentanyl buccal soluble film. *Pain Medicine.,* **11**(7): 1017-1023.

Venkatalakshmi R, Sudhakar Y, Chetty MC, Sasikala C and Varma MM (2012). Buccal drug delivery using adhesive polymeric patches. *Int. J. Pharm. Sci. Res.*, **3**(1): 35-41.

1072

Pak. J. Pharm. Sci., Vol.39, No.4, April 2026, pp.1063-1072