An increasing interest in the development of novel mucoadhesive buccal dosage form meant for systemic delivery as well as local targeting have been seen in recent years. Buccal delivery leading to transmucosal absorption of a drug into the systemic circulation offers a number of advantages for drugs that suffer from extensive first pass metabolism and poor bioavailability. Higher bioavailability, administration of lower doses, avoidance of liver and/or gastrointestinal metabolism and irritation of the gastrointestinal membrane, high permeability due to rich blood supply, reduced risk of overdose, non-invasive administration, convenience of self-medication, improved patient compliance, feasibility of beneficial adjunct product to an existing product and reduced risk of infectious disease makes buccal mucosa an attractive alternative route for systemic delivery of drugs\cite{1,2}. The pH of the medium and pKa of the drug may affect the release profile\cite{3}. Weakly basic drugs like ondansetron hydrochloride with pH-dependent solubility can experience problem on release from controlled release dosage forms\cite{4}. Penetration of fluid with higher pH may cause conversion of more ionizable drug to a less soluble base and therefore diffusion of the drug from the matrix. Because of which, formulation of weakly basic drugs for oral administration can be expected to result in particularly variable release rates with changes in pH of the surrounding fluids\cite{5}. The objective of transmucosal formulation designs for weakly basic drug is enhancing the bioavailability and reduce variability. Polymeric film coating is frequently used to control drug release from solid pharmaceutical dosage forms\cite{6-10}. An anionic polymer, sodium alginate has been reported to produce pH-independent release of a basic drug, verapamil HCl from a hydrophilic HPMC-based matrix tablet where...
sodium alginate has altered the pH in the matrix tablets[11]. The permeability of a non-ionized drug is likely to increase across an epithelial barrier, and this may be achieved by a change in pH of the drug delivery system[12-14]. Absorption of some drugs via the buccal mucosa is found to increase when carrier pH is lowered[4]. Increasing levels of pH modifiers progressively enhance drug release. The incorporation of pH modifiers such as citric, fumaric or sorbic acid is a common approach employed with matrix and coated systems[15-18], but not very common with buccal adhesive drug delivery systems[19]. Release of weakly basic drug from swellable tablets prepared with hydrophilic polymers was enhanced by incorporating pH modifiers, such as succinic, fumaric or adipic acid[18,20,21]. The addition of fumaric acid to drug/alginate-based matrix systems have decreased the microenvironmental pH within the tablets and resulted into increase in the solubility of the weakly basic drug at higher pH[4].

Ondansetron is a serotonin (5-hydroxytryptamine) subtype 3 (5-HT3) receptor antagonist used in the management of nausea and vomiting associated with cancer chemotherapy, radiotherapy and surgery. Ondansetron has been reported to produce adverse events such as, headache, constipation and diarrhea, which are mild to moderate and rarely require treatment. Following oral administration, it is absorbed rapidly but extensively metabolized by liver. Antiemetic drugs tend to be discharged by vomiting[22], but on the contrary, intravenous administration renders rapid effects to a patient, but the onset is too rapid to cause undesirable effects with local pain[23].

Therefore, in an attempt to prepare buccal mucoadhesive formulations for rapid delivery devoid of first pass metabolism, this study was undertaken to formulate a buccal mucoadhesive drug delivery system for ondansetron. Investigations were further directed to evaluate the effect of pH modifiers, citric acid (CA) and sodium bicarbonate (SB), on in vitro permeation, drug release and formulation properties.

**MATERIALS AND METHODS**

Ondansetron HCl was received as a generous gift from commercial suppliers (Ellis Pharma Pvt. Ltd., Ahmedabad, India). Carbopol-934 (C), gelatin (G), sodium alginate (SA), magnesium stearate, microcrystalline cellulose (Loba Chemie Laboratory Chemicals Ltd., India); citric acid, sodium bicarbonate (RFCL Fine Chemicals Ltd., India) used were of either analytical or pharmaceutical grade.

**Tablet preparation:**

The three polymers Carbopol-934, sodium alginate and gelatin (C:SA:G) were used in combinations to prepare bioadhesive tablets of ondansetron HCl by direct compression method using a 10 station rotary tableting machine (Rimek Minipress-I, India). All the ingredients were sieved before use (No. 200, 75 μm). Accurately weighed quantities of drug (8 mg/tablet), polymer (varying drug: polymer ratio from 1:1 to 1:5) and diluent microcrystalline cellulose with added magnesium stearate (2%) were mixed and compressed, using 6.0 mm standard concave punches keeping the weight of tablet constant to 100 mg. Prepared tablets were evaluated for weight and content uniformity, hardness, thickness, friability and disintegration characteristics as per pharmacopoeial (IP-96/ USP-24) specifications. In addition to the above properties prepared tablets, formulations were also evaluated for microenvironmental pH, in vitro release, bioadhesion, water uptake and in vitro permeation.

**Microenvironmental pH:**

The release of the drug exhibiting pH-dependent solubility is largely dependent upon the pH of the microenvironment[24] and release of weakly basic compound has been enhanced by incorporating adipic acid and fumaric acid[16,18,21,25]. Microenvironmental pH of tablets was determined by allowing the tablets to swell for 2 h in 4 ml distilled water (pH 6.5±0.05) in a fabricated glass tube. A pH-electrode was kept in contact with the tablet surface, equilibrated for 1 min and microenvironmental pH was determined by potentiometry[26].

**In vitro bioadhesion test:**

Weight required for detachment of tablets from the porcine buccal mucosa was determined by using a bioadhesion test assembly[27]. Fresh porcine buccal mucosa obtained from the slaughter house was freed from underlying fat and tissue, washed with distilled water and then with phosphate buffer 6.8 at 37°C. Mucosa cut into pieces was placed in phosphate buffer pH 6.8 for 5 min. Each individual piece was tied to a previously balanced (with 5 g) teflon block keeping mucosal side upward which is then lowered into the beaker containing phosphate buffer pH
6.8 maintained at 37±0.5. Tablets under test were moistened and stuck to the hanging cylinder on left hand side. The balance beam was raised with the removal of 5 g weight from right pan lowering the teflon cylinder along with the tablet with a force of 5 g and kept in this position for 3 min. The weight was increased gradually, till the tablet separated from the mucosa. The weights added in the right pan represent the bioadhesive force required to separate from mucosa.

**In vitro water uptake studies:**
Water uptake of tablets of each formulation was evaluated using a 1% w/v agar gel plate. Twenty-four tablets divided into six groups, each group consisting of four tablets were weighed and average weight of four tablets was calculated. These tablets were placed on the gel surface in six Petri dishes, each containing four tablets and kept in an incubator at 37±1°C. Each Petri dish was removed at one-hour interval for 6 h. The excess water on the surface of each tablet was blotted using a Whatman filter paper and the swollen tablets in each Petri dish were weighed. The average weight of the swollen tablets was calculated. Water uptake was calculated using the formula, Water uptake (g) = (W1-W2)/W1, where, W2 is the average weight of four tablets and W1 is the average weight of the swollen tablets.

**In vitro release studies:**
In vitro drug release was determined by USP method-II at a temperature of 37±1°C and paddle speed of 50 rpm using 500 ml phosphate buffer. Six tablets were selected from each formulation and placed in each vessel. Ten milliliters of sample were withdrawn at 1 h interval for 10 h. The sample was then filtered and analyzed for ondansetron HCl spectrophotometrically at 310 nm.

**In vitro permeation studies:**
Due to comparable water permeability and morphological similarities with human buccal mucosa, porcine buccal mucosa can be used for evaluation of drug permeability. In vitro permeation of ondansetron HCl from tablets through the excised porcine buccal membrane was studied. Porcine buccal tissue of domestic pigs was obtained from slaughter house and stored in phosphate buffer pH 6.8 at 4°C. The membrane was mounted over Franz diffusion cell and tablet placed on the membrane and compartments were clamped together. The donor and receptor compartment was filled with buffer of 6.8 and 7.4, respectively at 37°C. One milliliter sample was withdrawn from receptor compartment at predetermined time and estimated UV spectrophotometrically.

**Effect of pH modifiers:**
The pH specificity of the drug or the formulation may often affect the controlled release profile. Weakly basic drugs with pH-dependent solubility can experience problems on release from controlled release dosage forms in oral cavity. However, this effect is dependent on pKa of the drug and related to pH of the surrounding fluids. Therefore an attempt was made to study the effect of an organic acid and base on the release and permeability of buccal adhesive ondansetron tablets.

On the basis of microenvironment pH, in vitro drug release and in vitro bioadhesive strength, formulation (C:SA:G, 2:0.5:6.5) was selected for evaluating the effect of pH modifiers on formulation characteristics. Citric acid and sodium bicarbonate sieved (Sieve No. 200) and incorporated in concentrations 1-5% w/w mixed intimately and compressed. These prepared tablets were then evaluated for microenvironment pH, bioadhesive strength, water uptake, in vitro release and permeation.

Statistical analysis of the data obtained from the studies was carried out with either one way analysis of variance (ANOVA) and post hoc Dunnett test or two way ANOVA followed by post hoc Bonferroni comparisons.

**RESULTS AND DISCUSSION**

Tablets of ondansetron HCl prepared with different polymer combinations were evaluated for friability, drug content, hardness, disintegration time, thickness, weight uniformity and their values complied with pharmacopoeial limit ranging from 0.12 to 0.40%, 7.25±0.40 to 8.06±0.32 mg/tablet, 5.00±0.50 to 7.33±0.76 kg/cm², 130 to 170 min., 5.0 to 5.5 mm, 96.50±3.73 to 100.10±1.94 g, respectively. Hardness of buccal adhesive tablets of ondansetron HCl prepared by using different polymer proportions of C, SA and G in combination was found to increase with increase in the concentration of all three polymers but the effect of polymer concentration on hardness was found to be more pronounced with
Bioadhesion and microenvironmental pH are determinant of the performance and acceptability of buccal adhesive tablet. Polymers with high bioadhesion used in the formulation often result in decreased microenvironmental pH and therefore cause less irritation. Hence, in the present study the blends of three different polymers were used to have optimum bioadhesion and microenvironmental pH as well. Microenvironment pH determines the drug release and is determinant of buccal irritation. The mean microenvironment pH for the formulation containing value C:SA:G (1:1:1) was 4.99±0.1. Buccal transmucosal tablets prepared with different proportions of all three polymers for acceptable microenvironmental pH with optimum bioadhesive strength.

The release of the drug exhibiting pH-dependent solubility is largely dependent upon the microenvironment pH\(^{[24]}\) and release of weakly basic compound has been enhanced by incorporating adipic acid and fumaric acid\(^{[16,18,21,25]}\). Microenvironment pH increased with increase in sodium alginate C:SA:G (1:1:1 to 1:1:5) from 4.99±0.11 to 5.39±0.15 (F=2.313, \(P=0.1286\)) and 4.99±0.11 to 5.84±0.25 (F=7.456, \(P=0.0047\)), respectively (Table 1). Sodium alginate and gelatin is commonly used as antacid into increased irritation and this results are in well agreement with earlier reports\(^{[37]}\). It is likely that acidic groups present with carbopol might have decreased the microenvironmental pH. Tablets prepared with higher proportion of gelatin C:SA:G (1:1:1 to 1:1:5) produce desired pH values and therefore it may cause less irritation to buccal mucosa (F=7.456, \(P=0.0047\)).

The mean bioadhesive strength for formulation containing C:SA:G (1:1:1) was 23.28±0.86 as depicted in Table 1. Increase in the content of carbopol-934 concentration (1:1:1 to 5:1:1) increases the bioadhesive strength from 23.28±0.86 to 31.53±1.63 (F=30.05, \(P<0.05\)), whereas increased sodium alginate concentration from 1:1:1 to 1:5:1 decreases bioadhesive strength from 23.28±0.86 to 21.25±1.16 (F=1.162, \(P=0.40\)). Increased proportion of gelatin from 1:1:1 to 1:1:5 seemed to have significant effect on bioadhesive strength (F=0.140, \(P=0.963\)). Formation of secondary mucoadhesive bonds with mucin because of rapid swelling and interpenetration of the polymer chains in the interfacial region is responsible for greater bioadhesion by carbopol-934, while other polymers undergo only superficial bioadhesion\(^{[38]}\) and gelatin, a non-ionic polymer in the treatment of esophageal reflux\(^{[36]}\) and the basic nature of these polymers contributes in the increase pH with increase in their concentration. However increased carbopol concentration C:SA:G (1:1:1 to 5:1:1) have been found to decrease the microenvironment pH (4.99±0.11 to 3.91±0.26, F=3.868, \(P=0.0377\)) (Table 1) which may result into increased irritation and this results are in well agreement with earlier reports\(^{[37]}\). It is likely that acidic groups present with carbopol might have decreased the microenvironmental pH. Tablets prepared with higher proportion of gelatin C:SA:G (1:1:1 to 1:1:5) produce desired pH values and therefore it may cause less irritation to buccal mucosa (F=7.456, \(P=0.0047\)).

<table>
<thead>
<tr>
<th>Formulation (C:SA:G)</th>
<th>Hardness (Kg/cm²) (Mean±SD, n=3)</th>
<th>Friability (%) (Mean±SD, n=3)</th>
<th>Disintegration time (h.min)</th>
<th>Microenvironmental pH (Mean±SD, n=3)</th>
<th>Bioadhesion (Mean±SD, n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:1:1</td>
<td>5.50±0.50</td>
<td>0.12±0.01</td>
<td>2.20</td>
<td>4.99±0.11</td>
<td>23.28±0.86</td>
</tr>
<tr>
<td>1:2:1</td>
<td>5.85±0.76</td>
<td>0.35±0.011</td>
<td>2.10</td>
<td>5.14±0.16</td>
<td>22.39±1.78</td>
</tr>
<tr>
<td>1:3:1</td>
<td>6.35±0.76</td>
<td>0.23±0.020</td>
<td>2.30</td>
<td>5.21±0.24</td>
<td>21.97±1.48</td>
</tr>
<tr>
<td>1:4:1</td>
<td>7.00±1.00</td>
<td>0.25±0.015</td>
<td>2.20</td>
<td>5.34±0.22</td>
<td>21.54±0.98</td>
</tr>
<tr>
<td>1:5:1</td>
<td>5.15±0.29</td>
<td>0.30±0.005</td>
<td>2.40</td>
<td>5.39±0.15</td>
<td>21.25±1.16</td>
</tr>
<tr>
<td>2:1:1</td>
<td>5.00±0.50</td>
<td>0.25±0.015</td>
<td>2.30</td>
<td>4.14±0.42*</td>
<td>28.81±0.28*</td>
</tr>
<tr>
<td>3:1:1</td>
<td>5.65±0.29</td>
<td>0.20±0.015</td>
<td>2.10</td>
<td>4.05±0.47*</td>
<td>30.64±1.50*</td>
</tr>
<tr>
<td>4:1:1</td>
<td>5.00±0.50</td>
<td>0.31±0.036</td>
<td>2.40</td>
<td>3.98±0.53*</td>
<td>31.10±0.21*</td>
</tr>
<tr>
<td>5:1:1</td>
<td>5.50±0.50</td>
<td>0.21±0.02</td>
<td>2.50</td>
<td>3.91±0.26</td>
<td>31.53±1.63*</td>
</tr>
<tr>
<td>1:1:2</td>
<td>6.33±0.58*</td>
<td>0.24±0.017</td>
<td>2.25</td>
<td>5.39±0.24</td>
<td>23.06±2.07</td>
</tr>
<tr>
<td>1:1:3</td>
<td>7.03±0.50</td>
<td>0.26±0.005</td>
<td>2.30</td>
<td>5.45±0.25</td>
<td>22.98±2.18</td>
</tr>
<tr>
<td>1:1:4</td>
<td>7.33±0.76</td>
<td>0.25±0.005</td>
<td>2.40</td>
<td>5.75±0.18*</td>
<td>22.75±0.39</td>
</tr>
<tr>
<td>1:1:5</td>
<td>7.03±1.00</td>
<td>0.40±0.20</td>
<td>2.45</td>
<td>5.84±0.25*</td>
<td>22.46±0.75</td>
</tr>
</tbody>
</table>

Evaluation of mucoadhesive tablets of ondensetron hydrochloride prepared with carbopol-934 (C), sodium alginate (SA) and gelatin (G) in different ratio (C:SA:G) (*P<0.05, when compared with control formulation 1:1:1; one way ANOVA followed by Dunnett test).
relative to carbopol-943, being an anionic polymer shows better bioadhesion\textsuperscript{37}.

For the formulation (C:SA:G; 1:1:1) the water uptake value was 1.2±0.12 after 6 h (fig. 1a,2a,3a). Water uptake was 1.41±0.13 to 1.78±0.20 g (fig. 2a) that found to be increased with increasing the concentration of carbopol-934 (F=0.989, P=0.431). With increased sodium alginate concentration the water uptake enhanced insignificantly (F=0.389, P=0.8145) (fig.1a) but the effect was less as compare to carbopol-934 and this may be due to more hydrophilic nature of carbopol-934\textsuperscript{39}.

Ondansetron HCl was found to release more rapidly from the formulation with lower concentrations of carbopol-934 (fig. 2a) as compare to formulation with similar contribution of other polymers. An increase in the polymer concentration increases viscosity of the gel as well as forms gel layer which increases the diffusional path. This could cause a decrease in the effective diffusion coefficient of the drug and therefore a reduction in release rate of drug\textsuperscript{39}. Increased gelatin resulted in decreased ondansetron release (fig. 3b). The release of ondansetron could be prolonged and controlled by carbopol and gelatin in a concentration dependent manner. Similar results are reported by Mohammed and Khedr\textsuperscript{36}.

Based on the above mentioned studies on microenvironment pH, \textit{in vitro} bioadhesion, water uptake and \textit{in vitro} release, tablet formulation
containing C:SA:G in proportions 2:0.5:6.5 of ondansetron HCl were prepared and used to investigate the influence of citric acid and sodium bicarbonate on formulation characteristics.

The mean microenvironment pH values after 2 h decreased (Table 2) with the increasing concentration of citric acid from 1% to 5% w/w (F=28.26, P<0.05) and increased with the increasing sodium bicarbonate concentration (F=19.31, P<0.05). This increase in the microenvironment pH could be attributed to increase sodium bicarbonate concentration which being a base has pH of 8.3 (freshly prepared 0.1 M aqueous solution at 25º)[22]. Earlier reports are available on the incorporation of acidic pH modifiers enhanced the drug release by creating a more acidic microenvironment, increasing the solubility and dissolution rates. The enhanced release of weakly basic drugs by incorporated pH modifiers occurs mainly through modulation of the pH[20].

The mean bioadhesive strength values (Table 2) were not significantly affected by citric acid (F=0.1855, P=0.9626) whereas sodium bicarbonate containing formulations showed a decrease in bioadhesion with increasing concentrations (F=1.768, P=0.1940).

For citric acid formulations, the mean water uptake values after 6 h decreased (F=1.047, P=0.3958) with the increasing concentration (1-5% w/w, fig. 4a) whereas with sodium bicarbonate formulations the mean water uptake increased (F=18.94, P<0.05)
TABLE 2: EFFECT OF CITRIC ACID AND SODIUM BICARBONATE (5-20% W/W) ON MICROENVIRONMENTAL pH AND BIOADHESIVE STRENGTH

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Concentration</th>
<th>Microenvironment pH Mean±SD (n=3)</th>
<th>Bioadhesive strength Mean±SD (n=3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control formulation</td>
<td>0%w/w</td>
<td>5.48±0.28</td>
<td>24.86±2.09</td>
</tr>
<tr>
<td>Citric acid</td>
<td>1%w/w</td>
<td>4.87±0.27*</td>
<td>24.67±0.31</td>
</tr>
<tr>
<td></td>
<td>2%w/w</td>
<td>4.74±0.20*</td>
<td>24.47±0.88</td>
</tr>
<tr>
<td></td>
<td>3%w/w</td>
<td>4.61±0.10*</td>
<td>24.41±1.14</td>
</tr>
<tr>
<td></td>
<td>4%w/w</td>
<td>4.48±0.08*</td>
<td>24.53±1.44</td>
</tr>
<tr>
<td></td>
<td>5%w/w</td>
<td>4.37±0.08*</td>
<td>24.24±0.72</td>
</tr>
<tr>
<td>Sodium bicarbonate</td>
<td>1%w/w</td>
<td>5.72±0.08</td>
<td>23.99±0.92</td>
</tr>
<tr>
<td></td>
<td>2%w/w</td>
<td>5.92±0.16*</td>
<td>23.32±0.79</td>
</tr>
<tr>
<td></td>
<td>3%w/w</td>
<td>6.04±0.21*</td>
<td>23.33±0.71</td>
</tr>
<tr>
<td></td>
<td>4%w/w</td>
<td>6.23±0.10*</td>
<td>22.12±3.56</td>
</tr>
<tr>
<td></td>
<td>5%w/w</td>
<td>6.37±0.16*</td>
<td>22.19±3.56</td>
</tr>
</tbody>
</table>

Effect of citric acid (CA) and sodium bicarbonate (SB) on microenvironment pH and bioadhesive strength of ondansetron HCl buccal mucoadhesive tablets formulation containing carbopol-934, sodium alginate and gelatin (C:SA:G) in 2:0.5:6.5 proportion (*-P<0.001 when compared with control formulation; one way ANOVA followed by Dunnett test)

Fig. 5: Effect of SB on water uptake and cumulative percent release of ondansetron from

Effect of sodium bicarbonate (SB) at 0% (<—●—>, 5% (<—□—>, 10% (<—▲—>), 15% (<—▼—>) and 20% (<—▲—>) on (a) water uptake and (b) in vitro release of ondansetron HCl from buccal mucoadhesive formulation containing carbopol-934 (C), sodium alginate (SA) and gelatin (G) in 2:0.5:6.5 proportions. *P<0.05 when compared with control formulation 2:0.5:6.5; two way ANOVA followed by post hoc Bonferroni test

with the increasing concentration (1-5%w/w, fig. 5a), respectively. Most of the frequently used pH modifiers are more soluble at higher pH as compared to most basic drug compounds. The pH modifiers diffuse out more rapidly as compared to drug and therefore it is likely that the effect on pH within and in the interface of the dosage form may be decreased[20]. It is probable that increase water uptake with sodium bicarbonate might show decreased bioadhesion.

In vitro release of ondansetron HCl decreased significantly from citric acid (fig. 4b) containing formulation (C:SA:G; 2:0.5:6.5) (F=1989, P<0.05) whereas increased with sodium bicarbonate (fig. 5b) in concentration dependant manner (F=942.5, P<0.05). Release effect was in accordance with the water uptake. Higher water uptake by sodium bicarbonate containing formulation may be a key determinant controlling the release of weakly basic ondansetron HCl.

The drug permeation was slow and steady and 54.93±2.23% of ondansetron could permeate through the buccal membrane in 10 h with an average flux of 112.87 μg/h/cm² from control formulation (C:SA:G; 2:0.5:6.5). Drug permeation was reduced to 20.68±1.42% and flux of 40.96 μg/h/cm² from citric acid containing formulation and no significant effect of sodium bicarbonate was observed on permeation (59.33±2.15%) and flux of 121.88 μg/h/cm² of ondansetron from tablets. Drug permeation increases with increased unionized form through biological membrane. Ondansetron, a weakly basic drug is labile for ionization by citric acid and hence the
significant reduction in permeation (fig. 6a) through porcine buccal mucosa might have observed. Sodium bicarbonate increases the permeation of ondansetron HCl but to a less significant level (fig. 6b). The insignificant effect observed with sodium bicarbonate may be due to the lesser concentration used in the formulation and for pronounced effect on ionization of ondansetron HCl higher concentration of sodium bicarbonate might be required.

Increasing sodium bicarbonate concentration increases the microenvironment pH and water uptake and decreases the bioadhesive strength whereas increased carbopol-934 relative to sodium alginate and gelatin decreases the microenvironment pH and increase bioadhesive strength and water uptake. Increased gelatin lead to increased microenvironment pH to more satisfactory levels and decreased bioadhesive strength and water uptake.

Addition of citric acid to the formulation (C:SA:G; 2:0.5:6.5) decreases microenvironment pH, water uptake and drug permeation in a concentration-dependent manner. Increased sodium bicarbonate was found to increase the microenvironment pH, water uptake, drug permeation but decreases the bioadhesive strength. The buccal delivery system of ondansetron HCl formulated in this study was feasible for buccal administration, and use of pH modifier can be used for controlled and desired release profile.

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