3. MATERIALS AND METHODS
3. Material and Methods

3.1. Materials and Suppliers

3.1.1. Drugs

Chlorpheniramine maleate
- Emco Industries, Hyderabad.
ASTEMIZOLE
- German Remedies, Mumbai.

3.1.2. Polymers

Hydroxypropylmethylcellulose (HPMC) (15 cps)
- Genuine Chemicals, Mumbai.
Eudragit RL-100
- Rohm Pharma, GmBH, Weiterstadt.
Hydroxypropylcellulose (HPC)
- Genuine Chemicals, Mumbai.
Microcrystalline cellulose (MCC)
- Genuine Chemicals, Mumbai.
Carbopol 934P
- Goodrich, Germany.
Ethylcellulose
- Genuine Chemicals, Mumbai.

3.1.3. Chemicals

Disodium hydrogen phosphate
- Qualigens Fine Chemicals, Mumbai.
Potassium dihydrogen phosphate
- Qualigens Fine Chemicals, Mumbai.
Succinic acid
- Qualigens Fine Chemicals, Mumbai.
Potassium hydroxide
- Qualigens Fine Chemicals, Mumbai.
Sodium chloride
- New India Chemical Entp., Cochi.
Calcium chloride
- Qualigens Fine Chemicals, Mumbai.
Polyethylene glycol-1000 (PEG-1000)
- Genuine Chemicals, Mumbai.
Span 80
- Genuine Chemicals, Mumbai.
Hydrochloric acid
- Fischer Inorganics & Aromatics, Chennai.
Sodium hydroxide
- Qualigens Fine Chemicals, Mumbai.
Ethanol
- Spectrochem Pvt. Ltd., Mumbai.

3.1.4. Instruments

UV-240 Specrophotometer
- Shimadzu Corporation, Tokyo, Japan.
UV-1201, UV-Vis Spectrophotometer
- Shimadzu Corporation, Tokyo, Japan.
HP 8421 Diode Array Spectrophotometer
- Hewlett Packard, USA.
Tensile Strength Tester
- Gayatri Machine Products, Ahmedabad.
pH Meter
- Control Dynamics, Bangalore.
Single Pan Electrical Balance
Cyclomixer
- Remi Equipments, Mumbai.
Hot Air Oven
- Model, Sc-D, Zenco-Spring Timer, Mumbai.
3. Materials and Methods

3.1.5. Other materials

- Screw Gauge - Mutatoyo, Japan.
- Dialysis Cell - Fabricated locally.
- Tabletting Machine - Cadmach Industries, Ahmedabad.
- Dissolution Test Apparatus (T-MDR-D6) - TAB Machines, Mumbai

3.1.5. Other materials

- Glass/Acrylic Moulds - Fabricated locally.

All the other chemicals and reagents were of analytical grade and were used as obtained.

3.2. Experimental methods

3.2.1. Preparation of buffers and reagents

3.2.1.1. Sorensen's phosphate buffer (pH 6.2)

(i). 

Primary solution (Potassium phosphate solution): 9.078 g of monobasic potassium phosphate was weighed and dissolved in small quantity of distilled water and the volume was made upto one litre with distilled water.

(ii).

Secondary solution (Sodium phosphate solution): 11.876 g of dibasic sodium phosphate was weighed and dissolved in small quantity of distilled water and the volume was made upto one litre with distilled water.

From the fresh solutions prepared above, primary solution and secondary solution were mixed in ratio of 2.8 to get Sorensen's phosphate buffer pH 6.2 solution.

3.2.1.2. Phosphate buffer saline (PBS) (pH 7.4)

2.38 g of disodium hydrogen orthophosphate, 0.19 g of potassium dihydrogen phosphate and 8.0 g of sodium chloride were weighed and dissolved in distilled water. The volume was made upto one litre with distilled water to get the buffer solution with pH 7.4.

3.2.1.3. Buffers of low ionic strength for pKₐ determination using spectrophotometry

All the buffers solutions used in the determination of the pKₐ by spectrophotometric method were prepared as per the procedures described by Perrin (1963).
3.2.2. Analytical methods

In general, UV spectrophotometric estimation of drugs in solutions and dosage forms is quite common practice. This method was employed in the present study.

3.2.2.1. Scanning of chlorpheniramine maleate by UV spectrophotometry

A standard stock solution (1 mg/ml) of chlorpheniramine maleate was prepared in Sorensen's phosphate buffer (pH 6.2). From the standard stock solutions, chlorpheniramine maleate (20 µg/ml) solution was prepared in Sorensen's buffer, pH 6.2 and an UV-scan was taken between the wavelengths of 200-400 nm. The wavelength of 261 nm as λ_max was selected and utilized for further analysis.

Similar scan was taken for chlorpheniramine maleate in phosphate buffer saline, pH 7.4. The λ_max remained same. This procedure was used in certain in vitro studies.

3.2.2.2. Calibration curve of chlorpheniramine maleate in Sorensen's phosphate buffer pH 6.2 by UV spectrophotometry

From the standard stock solution, aliquot volumes were taken into different volumetric flasks and made upto 10 ml with Sorensen's buffer pH 6.2 so as to get concentrations of 2, 4, 6, 8 and 10 µg/ml. The absorbances of these solutions were measured at 261 nm (Table 4). The procedure was performed in triplicate to validate the calibration curve. A calibration graph was plotted (Fig. 2).

<table>
<thead>
<tr>
<th>S. No</th>
<th>Concentration in µg/ml</th>
<th>Absorbance at 261 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0.000</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>0.023</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>0.050</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>0.076</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>0.105</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>0.136</td>
</tr>
</tbody>
</table>

3.2.2.3. Calibration curve of chlorpheniramine maleate in phosphate buffer saline pH 7.4 by UV spectrophotometry

A standard stock solution (1 mg/ml) of chlorpheniramine maleate was prepared in phosphate buffer saline, pH 7.4. From the standard stock solution, aliquot volumes were taken into different volumetric flasks and made up to 10 ml with the same buffer so as to get concentrations of 2, 4, 6, 8, 10, 12, 14, 16 and 18 µg/ml. The absorbances of these solutions were measured at 261 nm (Table 5). The procedure was performed in triplicate to validate the calibration curve. A calibration graph was plotted (Fig. 3).

The absorbance values were taken at low concentrations, because the concentrations of drug in the estimations are in that range. Since sophisticated spectrophotometer was used, the results were accurate and valid. Moreover, the Beer-Lambert's law was valid.
Table 5. Standard Plot of Chlorpheniramine Maleate in PBS pH 7.4

<table>
<thead>
<tr>
<th>S. No</th>
<th>Concentration in µg/ml</th>
<th>Absorbance at 261 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0.000</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>0.032</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>0.059</td>
</tr>
<tr>
<td>4</td>
<td>6</td>
<td>0.090</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>0.119</td>
</tr>
<tr>
<td>6</td>
<td>10</td>
<td>0.140</td>
</tr>
<tr>
<td>7</td>
<td>12</td>
<td>0.162</td>
</tr>
<tr>
<td>8</td>
<td>14</td>
<td>0.190</td>
</tr>
<tr>
<td>9</td>
<td>16</td>
<td>0.219</td>
</tr>
<tr>
<td>10</td>
<td>18</td>
<td>0.245</td>
</tr>
</tbody>
</table>

Fig. 3. Standard plot of chlorpheniramine maleate in PBS pH 7.4.

3.2.2.4. Scanning of astemizole by UV spectrophotometry

A stock solution (1 mg/ml) of astemizole was prepared in methanol. From the standard stock solutions, astemizole (20 µg/ml) solution was prepared in 0.1 N
hydrochloric acid and a UV-scan was taken between the wavelengths of 200-400 nm. The wavelengths ($\lambda_{\text{max}}$) of 277 nm were selected and utilized for further analysis.

Similar scans were taken for astemizole in individual buffers (Sorensen's buffer, pH 6.2 and phosphate buffer saline, pH 7.4). The $\lambda_{\text{max}}$ value of 283 nm was selected and utilized for further analysis. Both buffers had given same absorbance values.

3.2.2.5. Calibration curve of astemizole in 0.1 N hydrochloric acid by UV spectrophotometry

From the standard stock solution (concentration, 1 mg/ml in methanol) of astemizole, aliquot volumes were taken into different volumetric flasks and made up to 10 ml so as to get concentrations of 5, 10, 15, 20, 25, 30 and 35 $\mu$g/ml in 0.1 N hydrochloric acid. The absorbance was measured at 277 nm against a suitable blank (Table 6). The procedure was performed in triplicate to validate the calibration curve. A calibration graph was plotted (Fig. 4).

<table>
<thead>
<tr>
<th>S. No</th>
<th>Concentration in $\mu$g/ml</th>
<th>Absorbance at 277 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0.000</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>0.144</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>0.287</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>0.417</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>0.583</td>
</tr>
<tr>
<td>6</td>
<td>25</td>
<td>0.719</td>
</tr>
<tr>
<td>7</td>
<td>30</td>
<td>0.842</td>
</tr>
<tr>
<td>8</td>
<td>35</td>
<td>0.973</td>
</tr>
</tbody>
</table>
3.2.2.6. Calibration curve of astemizole in Sorensen's phosphate buffer pH 6.2 and phosphate buffer saline (pH 7.4) by UV spectrophotometry

A standard stock solution (1 mg/ml) of astemizole was prepared in Sorensen's buffer (pH 6.2). From this, aliquot volumes were taken into different volumetric flasks and made up to 10 ml so as to get concentrations of 5, 10, 15, 20, 25, 30 and 35 μg/ml in Sorensen's phosphate buffer (pH 6.2). The absorbance was measured at 283 nm against a suitable blank (Table 7). The procedure was performed in triplicate to validate the calibration curve. Similarly, the procedure was repeated with phosphate buffer saline (pH 7.4) and the values were coinciding with that of the drug-Sorensen's buffer solutions. Hence, the same standard plot was used. A calibration graph was plotted (Fig. 5).

<table>
<thead>
<tr>
<th>S. No</th>
<th>Concentration in μg/ml</th>
<th>Absorbance at 283 nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0.000</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>0.101</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>0.230</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
<td>0.347</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>0.464</td>
</tr>
<tr>
<td>6</td>
<td>25</td>
<td>0.585</td>
</tr>
<tr>
<td>7</td>
<td>30</td>
<td>0.704</td>
</tr>
<tr>
<td>8</td>
<td>35</td>
<td>0.818</td>
</tr>
</tbody>
</table>

3.2.3. Determination of pKₐ for astemizole

3.2.3.1. Theoretical prediction of pKₐ of astemizole

Theoretical prediction of pKₐ value was attempted illustrating the methods using substituent constants (Perrin et al., 1981).

3.2.3.2. Determination of pKₐ by spectrophotometry

3.2.3.2.1. Spectral search of different species of astemizole

As the drug is basic, two spectra representing ionised and unionised species were required. Astemizole solution (20 μg/ml) in 0.01 N hydrochloric acid and 0.01 N potassium hydroxide solutions were selected. Since the drug was precipitated in 0.01 N potassium hydroxide solution, 0.001 N sodium hydroxide solution was selected. The spectra were superimposable (Fig. 6). In other words, both ionised and unionised species exhibit same spectra.

![Graph showing standard plot of astemizole in Sorensen's phosphate buffer, pH 6.2.](image)

\[ y = 0.0236x - 0.0073; R^2 = 0.9997 \]
3. Materials and Methods

Since two sites for ionisation are present in the structure, partial ionisation spectra was further considered. Succinic acid (0.1 M) - potassium hydroxide (0.05 M) buffer, pH 6.8 (µ = 0.1) was selected on trial and error method for the ionisation of one site. The spectra of 0.01 N hydrochloric acid and buffer pH 6.8 are different (Fig. 7). Thus, the solutions selected were 0.01 N hydrochloric acid and buffer pH 6.8. Therefore, spectrophotometric method is suitable to determine the dissociation constant of at least one species (Albert and Serjeant, 1971).

3.2.3.2.2. Choice of the analytical wavelength

When the spectra were plotted (Fig. 7), one particular wavelength can be found at which the two species differ the most in absorbances (optical density). The basis of selection is that one species absorb strongly (one site unionised, buffer pH 6.74) and the other has no absorption (completely ionised, 0.01 N hydrochloric acid). This is an ideal condition to get accurate results. In this case, a peak of one species over a trough of other species was identified (Fig. 7). The analytical wavelength of 247 nm was selected.

3.2.3.2.3. Search for an approximate value of pKa of astemizole

Dissociation constant of astemizole can be determined by graphic technique by obtaining the value of pH, when the concentrations of the dissociated and undissociated forms are equal in the mixture. The chemical species particularly in the equilibria are followed closely by observing the variation in the absorption of each species at a particular wavelength. By determining the concentrations of the species in Beer's law and pH of the solutions, pKa can be approximately estimated where the spectra showed continuous variation of absorbance over a pH range (Fig. 8).

The stock solutions were diluted as before, but into buffers of such a pHs (from 5.39 to 6.20; succinic acid (0.1 M)-potassium hydroxide (0.05 M) buffer solution, (µ = 0.1)) so that astemizole was partially ionised. The approximate pKa value of astemizole was 5.309 (Table 8).
Fig. 6. Molecular species spectra of astemizole in low ionic strength buffers (succinic acid (0.1 M) - KOH (0.05 M)).

Fig. 7. Ionization profile of astemizole.

Fig. 8. UV spectra of astemizole.
Table 8. Determination of the Ionisation Constant of Astemizole

<table>
<thead>
<tr>
<th>PH</th>
<th>Optical Density (A)</th>
<th>A-Ai</th>
<th>A-un-A</th>
<th>C = \log \left( \frac{A_{un}}{A_{in}} \right)</th>
<th>pK_a = pH+C</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.39</td>
<td>0.197</td>
<td>0.143</td>
<td>0.138</td>
<td>-0.015</td>
<td>5.375</td>
</tr>
<tr>
<td>5.51</td>
<td>0.236</td>
<td>0.182</td>
<td>0.097</td>
<td>-0.264</td>
<td>5.246</td>
</tr>
<tr>
<td>5.78</td>
<td>0.250</td>
<td>0.196</td>
<td>0.085</td>
<td>-0.363</td>
<td>5.417</td>
</tr>
<tr>
<td>5.88</td>
<td>0.288</td>
<td>0.234</td>
<td>0.047</td>
<td>-0.697</td>
<td>5.183</td>
</tr>
<tr>
<td>6.20</td>
<td>0.302</td>
<td>0.248</td>
<td>0.033</td>
<td>-0.876</td>
<td>5.324</td>
</tr>
</tbody>
</table>

Analytical wavelength: 247 nm; Optical density of ionized molecule (A_i) = 0.054; Optical density of unionized molecule (A_un) = 0.335. Result: pK_a = 5.309.

3.2.3.2.4. Exact determination of pK_a of astemizole

Using this rough estimate, eight buffer solutions of succinic acid (0.1 M)-potassium hydroxide (0.05 M) (ionic strength, \( \mu = 0.1 \)) were made at pH values nearly equal to approximate pK +0, 0.2, 0.4, 0.6, -0.2, -0.4 and -0.6, whose pH ranges from 4.60 to 5.79. A set of eight pK_a values was then obtained from measurement of the spectra of these solutions. The eight values were averaged (see results and discussion for the Table 31). For the scanning of spectra, corresponding blank solutions were used.

3.2.4. Partition coefficient of astemizole

For a variety of drugs, positive correlations have been documented between partition coefficients and in vivo absorption. The choice of the partition systems was made arbitrarily (Beckett and Moffat, 1969). After consulting the literature, the following general method was employed to determine the partition coefficient of astemizole (Subrahmanyam, 2002).

**Method:** A mixture of n-octanol and water, (1:1) ratio, was kept on rocking machine for 16 hours. The mixture was separated to get water-saturated octanol and octanol-saturated water phases.

From these saturated phases, 25 ml of water phase was added to 24 ml of octanol phase. To this 1 ml of drug solution (1 mg/ml in n-octanol) was added and the mixture was kept on rocking machine for 16 hours. The resulting mixture was separated and the amount of drug present in water phase was determined by spectrophotometric method at 277 nm using corresponding blank solutions. The calculation is based on the differences
between initial and final concentrations. Similar procedure was carried out for n-heptane-water solvents pair and other systems. For the purpose, separate standard plot was constructed for astemizole in water.

3.2.5. Buccal absorption test

Sorensen's drug solution (1.0 ml containing 5 mg base) was pipetted into a 100 ml beaker and 24.0 ml of buffer solution (pH 6.2) was added, which represents saliva pH. The pH of the resulting solution was verified. This solution was placed in the subject's mouth and by movement of the cheeks and tongue circulated for 300-400 times round the mouth for 5 minutes after which the solution was expelled. The subjects were instructed to quickly rinse mouth with distilled water (10 ml) for 10 seconds and expelled the rinsing. The expelled solutions were combined and used for analysis after necessary dilutions (Beckett and Triggs, 1967; Beckett and Hossie, 1971). Appropriate blank solutions were simultaneously prepared and used for analysis.

For the buccal absorption test, human volunteers were involved. The drugs used in this study were time tested for its safety and are available in the market. Hence, they were considered safe for the present study protocol. The dose of astemizole administered in the present study was 2 mg, while the prescribed dose is 10 mg. Thus the dose is 5 times lower. The time of contact of drug with the volunteers was 20 minutes, while the entire dose (10 mg) is in contact with the patient for 24 hours, when normal dose administered. Therefore, the exposure period of the drug to the volunteers is further reduced. From these accounts, the protocol study does not cause any adverse reactions to the volunteers.

3.3. Formulation development

3.3.1. Preparation of mucoadhesive films

Buccal mucoadhesive films were prepared using polymer or polymer blends along with the drug and a suitable solvent. The polymer was weighed accurately and dissolved in solvent, ethanol. The drug was then dispersed uniformly into a viscous dispersion with continuous mixing on cyclomixer. The resulting dispersion was then poured into glass mould (4 x 3 cm in size) that was lined with an aluminium foil. The solvent was

Evaporated completely at room temperature for 24 hours (Anders and Merkle, 1989; Baichwal, 1984).

The dried films were removed from the moulds and kept in a dessicator for 6 hours. They are preserved in aluminium foils until further use.

_Incorporation of other additives:_ Plasticizer such as PEG 1000 was used to improve the film characteristics. The preliminary investigations on films showed that the drug distribution was not uniform. Therefore, a dispersing agent, span 80 was employed. Ester noninoic surfactants had no promoting effect. The additives were dissolved in the solvent initially and then the drug was dispersed.

### 3.3.1.1. Chlorpheniramine maleate films

<table>
<thead>
<tr>
<th>Film. I.</th>
<th>HPMC (15 cps)</th>
<th>225 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chlorpheniramine maleate</td>
<td>4 mg</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>7 ml</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Film. II.</th>
<th>HPMC (15 cps)</th>
<th>100 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Eudragit (RL-100)</td>
<td>100 mg</td>
</tr>
<tr>
<td></td>
<td>PEG-1000</td>
<td>100 mg</td>
</tr>
<tr>
<td></td>
<td>Span 80</td>
<td>30 mg</td>
</tr>
<tr>
<td></td>
<td>Chlorpheniramine maleate</td>
<td>4.5 mg</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>7 ml</td>
</tr>
</tbody>
</table>

### 3.3.1.2. Astemizole films

<table>
<thead>
<tr>
<th>Film. I.</th>
<th>HPMC (15 cps)</th>
<th>150 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Astemizole</td>
<td>10 mg</td>
</tr>
<tr>
<td></td>
<td>PEG 1000</td>
<td>100 mg</td>
</tr>
<tr>
<td></td>
<td>Span 80</td>
<td>30 mg</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>10 ml</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Film. II.</th>
<th>HPMC (15 cps)</th>
<th>100 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Eudragit (RL-100)</td>
<td>100 mg</td>
</tr>
<tr>
<td></td>
<td>Astemizole</td>
<td>10 mg</td>
</tr>
<tr>
<td></td>
<td>PEG-1000</td>
<td>100 mg</td>
</tr>
<tr>
<td></td>
<td>Span 80</td>
<td>30 mg</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>7 ml</td>
</tr>
</tbody>
</table>
Since, the total weight of ingredients differs in the two types of films, appropriate content uniformity values were used for all the calculations. These were the formulation necessities.

3.3.2. Buccal tablets

The tablets of drugs were prepared using the conventional procedures.

3.3.2.1. Buccal tablets of chlorpheniramine maleate

The general composition and formula for the chlorpheniramine tablets was given in Table 9. Depending on the dose required (24 mg or 17 mg), the quantities were accordingly calculated and tablets were prepared.

The ingredients were weighed accurately and mixed by triturating in their dry powder forms in glass pestle and mortar. The mix equivalent to 120 mg (equivalent to 24 mg drug) and 85 mg (equivalent to 17 mg drug) were then compressed separately using single punch compression machine. Different amounts of polymers were tested. Flat faced tablets with a diameter of 5 mm and 4 mm thickness were prepared. Tablets containing HPMC polymer were prepared. They did not have sufficient hardness and became soft. During dissolution, the tablet became a gelly viscous mass. This leads to erroneous results. Hence, HPMC is replaced by HPC in the preparation of tablets.

Table 9. Composition of Chlorpheniramine Maleate Buccal Tablets

<table>
<thead>
<tr>
<th>Ingredients, mg</th>
<th>A1</th>
<th>A2</th>
<th>A3</th>
<th>A4</th>
<th>A5</th>
<th>A6</th>
<th>A7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorpheniramine maleate</td>
<td>20.00</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>HPC</td>
<td>47.40</td>
<td>39.5</td>
<td>47.4</td>
<td>39.5</td>
<td>55.3</td>
<td>39.5</td>
<td>47.4</td>
</tr>
<tr>
<td>Carbopol 934P</td>
<td>23.70</td>
<td>23.7</td>
<td>15.8</td>
<td>31.6</td>
<td>15.8</td>
<td>23.7</td>
<td>23.7</td>
</tr>
<tr>
<td>Ethylcellulose</td>
<td>3.95</td>
<td>7.9</td>
<td>7.9</td>
<td>3.95</td>
<td>3.95</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>MCC</td>
<td>3.95</td>
<td>7.9</td>
<td>7.9</td>
<td>3.95</td>
<td>3.95</td>
<td>15.8</td>
<td>7.9</td>
</tr>
<tr>
<td>Magnesium Stearate</td>
<td>1.00</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total weight (mg)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

From the Table 9, different formulations of chlorpheniramine maleate tablets were made at two dose levels, 24 mg and 17 mg. The formulation at 24 mg dose level were correspondingly designated by F1, F2...F7. The formulation at 17 mg dose level were correspondingly designated by G1, G2...G7.
3.3.2.2. **Buccal tablets of astemizole**

Buccal tablets of astemizole were prepared using a similar procedure as described above. The composition of the ingredients was shown in the Table 10. Tablets of 90 mg were compressed and labeled as T1, T2, T3...T7.

<table>
<thead>
<tr>
<th>Ingredients, mg</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
<th>T6</th>
<th>T7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Astemizole</td>
<td>10.00</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>HPC</td>
<td>47.40</td>
<td>39.5</td>
<td>47.4</td>
<td>39.5</td>
<td>55.3</td>
<td>39.5</td>
<td>47.4</td>
</tr>
<tr>
<td>Carbopol 934P</td>
<td>23.70</td>
<td>23.7</td>
<td>15.8</td>
<td>31.6</td>
<td>15.8</td>
<td>23.7</td>
<td>23.7</td>
</tr>
<tr>
<td>Ethylcellulose</td>
<td>3.95</td>
<td>7.9</td>
<td>7.9</td>
<td>3.95</td>
<td>3.95</td>
<td>--</td>
<td>--</td>
</tr>
<tr>
<td>MCC</td>
<td>3.95</td>
<td>7.9</td>
<td>7.9</td>
<td>3.95</td>
<td>3.95</td>
<td>15.8</td>
<td>7.9</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>1.00</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total weight (mg)</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
</tr>
</tbody>
</table>

3.4. Evaluations

3.4.1. **Physical characteristics study**

3.4.1.1. **Thickness uniformity of the films**

The thickness of the each film of size 1 x 1 cm² was measured at different positions of the film for different films and the average was calculated (Baichwal, 1984).

3.4.1.2. **Studies of swelling of the films**

Swelling studies of the films were conducted as per the procedure described by Guo (1994).

3.4.1.2(a). **Area increase due to swelling**: A film of 1 x 1 cm² film was placed in a petridish (of diameter 7 cm). A graph paper was placed beneath the petridish to measure the increase in the area. 50 ml of the phosphate buffer of pH 6.2 was poured into this petridish. The increase in the length and breadth of the film was noted at suitable time intervals and the area was calculated.
3.4.1.2(b). Weight increase due to swelling: A film of $1 \times 1 \text{ cm}^2$ was weighed and kept on a cover slip and again weighed. This was transferred into a petridish and 50 ml of buffer (pH 6.2) was carefully poured. After a predetermined time, film was removed from the petridish and excess water was removed quickly with the help of a blotting paper and weight was taken. The differences in the final and initial weights give the weight increase due to absorption of water and swelling of the film.

3.4.1.3. Tensile strength of the films

This was determined with tensile strength tester (Baichwal, 1984). The sensitivity of the machine is 4 to 10,000 Newton's. It consists of two load cell grips (Fig. 9). The upper one is fixed and the lower one is movable. The test film (of specific size, e.g. 5/2.5 cm.) was fixed between these cell grips and force was gradually applied till the film breaks (standard rate of travel for the material to be tested was checked up). Tensile strength of the film was taken directly from the dial reading in dynes/cm$^2$ that corresponds to the idle pointer.

3.4.1.4. Weight variation of the films

Films of size $1 \times 1 \text{ cm}^2$ were cut from the prepared film. The weight of the each film was taken and the weight variation was calculated.

3.4.1.5. Content uniformity of films

The films were tested for the content uniformity (Samuelov et al., 1979). A size of $1 \times 1 \text{ cm}^2$ films were used for the study.

Procedure: A film of size $1 \times 1 \text{ cm}^2$ was taken and triturated in a mortar with little volume (5 to 10 ml) of Sorensen's phosphate buffer (pH 6.2) for about 10 minutes and transferred to a volumetric flask (50 ml). The volume was made upto 50 ml with buffer (pH 6.2). The dispersion is filtered and the absorbance was measured against the corresponding blank solution at the analytical wavelengths of the respective drugs. The blank solutions were prepared by using a blank film containing the polymer or polymer blends without using the drug and treated similarly.
Fig. 9. Tensile strength tester
3. Materials and Methods

3.4.2. In vitro release studies

3.4.2.1. In vitro release studies of films in Sorensen’s buffer (pH 6.2)

Films containing the drug were evaluated for in vitro release (Borodkin and Tucker, 1974).

A film of 1 x 1 cm² was cut and attached to a glass slide with two drops of buffer (pH 6.2). This slide was kept at an angle into a 250 ml conical flask containing 100 ml of buffer solution. Then the beaker was kept in a water bath (37 °C) during entire release study. A non-agitated system was selected to eliminate any effect of turbulence on the release rate as well as to assure that no disruption of the film occurred. Periodic samples were withdrawn by removing the slide from the beaker. The solution was stirred and a 5 ml of sample was withdrawn using a graduated pipette (whose tip was covered with a muslin cloth). The slide was quickly reintroduced making sure that the film should be completely immersed in the buffer throughout the study. A 5 ml of the buffer was replaced immediately and the beaker was kept covered to prevent evaporation of the fluid.

Samples were taken at predetermined intervals and analysed at λ_{max} 261 and 283 nm, respectively, for chlorpheniramine maleate and astemizole.

3.4.2.2. In vitro release studies of films through porcine buccal mucosa in PBS (pH 7.4)

Buccal films containing known amount of drug were subjected to in vitro release studies (Garren and Repta, 1989; Le Brun et al., 1989). Buccal tissue was taken from pigs at slaughterhouse and kept in normal saline. It was used within several hours (3 hr.) after its removal from the animal body. The epithelial layer was removed from the connective tissue. The porcine buccal mucosa was clamped carefully to one end of the hollow glass tube (dialysis cell). This acts as donor compartment. One hundred ml PBS was taken in a beaker, which was used as receptor compartment. The donor compartment was kept in contact with the receptor compartment. The receptor compartment was stirred magnetically during the study. At every five minutes interval, 10 ml of the sample was withdrawn and immediately replaced with the fresh 10 ml PBS to maintain sink condition. The amount of chlorpheniramine released was analysed at λ_{max} 261 nm. This experiment was done in triplicate and average values are reported.
3.4.2.3. In vitro release (dissolution) studies for buccal tablets

The in vitro release studies of buccoadhesive tablets were conducted by USP XXII paddle method at 50 rpm. The dissolution medium consisted of Sorensen's phosphate buffer pH 6.2 solution at $37 \pm 0.5 \, ^\circ C$. The tablet was placed at the bottom of the glass vessel and pushed with a glass rod to make it adhere to the bottom of the glass vessel, so that one side of the tablet was in contact with the dissolution medium (Christiaens et al., 1996). The drug released was assayed by measuring its absorbance spectrophotometrically at appropriate analytical wavelengths.

3.4.3. In vivo patch test

Anders and Merkle (1989) studied the release of drug from medicated buccal patches of protirelin using human volunteers. The method was known as measurement disappearance. The test requires well-trained and motivated subjects. The subject was not given information about the polymer composition of the patch tested. During the test, the subject was not allowed to drink or eat. Cellophane was fixed as backing layer for the patches ($4 \times 3 \, \text{cm}^2$) containing 10 mg of astemizole. The patches were moistened with a drop of water and applied to the buccal mucosa. It was removed after given intervals of time. Oral cavity was rinsed quickly with 20 ml of the buffer, pH 6.2. The films were dissolved in 20 ml of the buffer, pH 6.2 for 0.5 hour. The washings were mixed to the above solution. The dispersion was allowed to stand for 10 minutes. The supernatant liquid was filtered. The filtrate was analysed at the respective wavelengths using the corresponding blank solution. The results gave the amount of drug remaining unabsorbed.

Absorption kinetics of chlorpheniramine maleate: The above method was suitably modified in the present study to obtain kinetic profiles of chlorpheniramine absorption. The chlorpheniramine maleate patches of $1 \times 1 \, \text{cm}^2$ were used to study the absorption kinetics. About 7 to 8 strips were placed in different locations in the buccal cavity. Each time, one strip was removed from a subject. Therefore, at different time intervals, concentrations can be obtained. The film, which was removed, was dissolved in 20 ml of the buffer, pH 6.2 for 10 minutes. The supernatant liquid was filtered. The filtrate was analysed at the analytical wavelength $\lambda_{\text{max}} \, 261 \, \text{nm}$ for chlorpheniramine.
The duration of the mucosal adhesion was also observed during this study. The duration of mucosal adhesion was the time span required until the adhesive patch completely lost its adhesive contact with mucosa. This was assessed by visual observation of the patch. The patches were found to be intact with the buccal mucosa for the entire period of study without any difficulty.

The regression analysis of the experimental data was done using statistical functions of the MS-Excel program. The graphs were generated through MS-Excel program. These were used to determine the best-fit line, orders of transport and absorption in vitro and in vivo correlation (IVIVC) among the methods.

The results obtained in these methods and the conclusions arrived from them were provided in the following chapter "Results and Discussion".