Chapter – 3

Transdermal Delivery of Antiasthmatic Drugs through Modified Chitosan Membranes
3.1. TRANSDERMAL DELIVERY OF SALBUTAMOL THROUGH MODIFIED CHITOSAN MEMBRANE

3.1.1. INTRODUCTION

Treatment of chronic diseases such as asthma, rheumatoid arthritis, etc. by transdermal route of drug administration has got several advantages over other conventional routes of drug administration\(^1\text{-}^3\). However, delivery of drugs through transdermal drug delivery system (TDDS) is rather limited due to the difficulty in diffusion of drug through the barriers of skin. Addition of penetration enhancers, modification of membrane delivery system\(^4\text{-}^5\) and alteration in the physicochemical properties\(^6\) of the drug molecule may improve the efficiency of TDDS. Polymeric membrane matrix type of TDDS system has been found to be more effective due to its tailor-made properties\(^1\).

Chitosan is a naturally occurring, biocompatible, cheaply available polymer\(^7\). This polymer has a repeating structural unit of 2-acetamido-2-deoxy-\(\beta\)-D-glucose\(^8\). Chitosan has been proven to be an ideal polymer not only in biomedical but also in various industrial applications. It is insoluble in water but soluble in acidic water. Chitosan membranes, when applied on skin cause irritation due to the presence of traces of acid. Hence it is necessary to wash the films repeatedly with methanol to remove surface bound traces of acid. Chemical modification of chitosan and its applications have been reported\(^9\). Chitosan has been modified by graft copolymerization and by blending with water-soluble polymers\(^9\). Reports are also available on the studies of chitosan.
with polyvinyl alcohol (PVA) membranes. According to Miya et al., chitosan forms a clear homogenous blend with PVA and tensile strength of this membrane was greater than the component value. Uragami, et al. prepared a crosslinked chitosan/PVA blend with a fixed amount of crosslinking agent and studied the active transport of drug through chitosan/PVA membrane.

Grafting of various monomers is a promising method for the preparation of new materials which have the potential and multiple applications due to their improved chemical and physical properties. Many grafting copolymers of chitosan were synthesized and evaluated for flocculant, paper strengthener, drug-releaser and other activities.

In this study, we report the possible usage of chitosan and chemically modified chitosan for transdermal drug delivery. The free amino groups of chitosan were reacted with aldehydes in presence of an acid to form Schiff's bases and thus modified chitosans have been prepared. Selection of aldehydes was made on the basis of film forming capacity of the polymer after the reaction. Based on the preliminary studies, acetaldehyde and propionaldehyde were selected for the preparation of Schiff's bases.

Salbutamol, an antiasthmatic drug has a biological half-life of 4 hours and needs to be administered several times a day. For the chronic management or prophylactic therapy of asthma, a long acting formulation particularly transdermal delivery would be of benefit. Hence, salbutamol was developed as a TDDS and in vitro drug release study through excised rat abdominal skin.
was performed in Keshary-Chien diffusion cell at 37°C using distilled water as dilution media.

**Drug data**

Salbutamol is a Beta- adrenoceptor agonist.

Chemical name : \((RS)-1-(4\text{-hydroxy-3-hydroxymethylphenyl})-2-(\text{tert-butyl amino})\text{ ethanol.}\

Molecular formula : \(C_{13}H_{21}NO_3\)

Molecular weight : 239.30

Chemical structure :

![Chemical structure of salbutamol]

Dose : Orally 6 to 16 mg daily.

Description : Crystalline powder, odorless.

Color : White or almost white.

Solubility : Soluble in ethanol (95%); sparingly soluble in water; slightly soluble in ether.

### 3.1.2. EXPERIMENTAL

**Materials**

The gift sample of salbutamol was supplied by Sun Pharma, Baroda, India. The 75-85% deacetylated chitosan with a viscosity of 200-800 cP
measured by Brookfield viscometer in 1% w/v of chitosan solution, in 1% acetic acid was purchased from Aldrich Chemical Company, USA. Analytical grade chemicals were purchased from s.d.Fine-Chem Ltd. Mumbai, India and used as received.

**Methods**

3.1.2.1. **Chemical modification of chitosan**

Accurately weighed quantity of chitosan (2 g) was dissolved in 100 ml of 1% acetic acid solution. After ensuring complete dissolution of chitosan, the polymer solution was stirred with different concentrations of (2%, 4%, 6% and 8% w/v) acetaldehyde. Stirring was continued for 3 hours and temperature was maintained at 60°C. After completion of the reaction, polymeric solutions were added to acetone to precipitate the chemically modified chitosan (chitosan Schiff's bases). The derivatives were dried in an oven at 40°C and weighed.

A series of Schiff's bases were prepared using propionaldehyde, by adopting the same procedure as explained above (Scheme I).

**Fabrication of transdermal membranes**

Transdermal membranes were prepared by solvent casting technique employing a glass substrate. Solutions of plain chitosan and different Schiff's bases were prepared by dissolving 2 g in 100 ml (1%) acetic acid solution. 20% w/w of salbutamol (dry weight of polymer) was added to these polymeric solutions and stirred for half an hour. The polymeric solutions, containing the
Scheme I

\[ \text{R} = \text{-CH}_3, \quad \text{-CH}_2\text{-CH}_3 \]
drug were poured into glass bangles (10 cm diameter) placed on a mercury surface in petri dishes. Petri dishes were kept in an oven at 40°C for complete drying. Membranes produced were washed with 50% methanol to remove surface bound traces of acid.

3.1.2.2. Determination of tensile strength and percentage elongation

Transdermal membranes were prepared by pouring the polymeric solutions of chitosan and Schiff’s bases (2% w/v) on a plane glass plate. Completely dried films were evaluated for tensile strength and percentage elongation. Membrane strip measuring (10 mm x 50 mm) in dimension and free from air bubbles or any other physical imperfections was held in between two clamps positioned at a distance of 3 cm. During measurement, the film was pulled by top clamp at a rate of 0.5 mm/sec, to a distance of 5 cm before returning to the starting point. The force and elongation were measured when the films broke. The tensile strength and elongation at break were calculated by using the formula,

\[
\text{Tensile strength} = \frac{\text{Breaking force (N)}}{\text{Cross-sectional area of sample (mm}^2)}
\]  

(3.1)

\[
\text{Elongation (\%)} = \frac{\text{Increase in length at breaking point (mm)}}{\text{Original length (mm)}} \times 100
\]  

(3.2)

The results are summarized in Table 3.1.8.
3.1.2.3. Swelling study

Membranes with a specified area (1 cm²) were weighed and put into a watch glass containing water. At regular intervals of time (every 30 minutes), the membranes were taken out from the watch glass, blotted with filter paper and weighed on a digital balance. After attaining equilibrium, the membranes were dried and weighed to calculate any weight loss. The results are given in Table 3.1.8.

3.1.2.4. WVT rate measurement

Water vapor transmission rate (WVTR) studies were carried out using glass vials of equal diameter as the transmission cells. These cells were washed and dried in an oven. About 1g of fused calcium chloride was taken in the cells and the polymeric membranes (1 cm²) were fixed over the brim with the help of an adhesive. Then the cells were accurately weighed and kept in a closed desiccator, containing saturated solution of potassium chloride (200 ml). The humidity inside the desiccator was measured by a hygrometer. The cells were weighed at regular intervals of time (every 5 hours) and the amount of water vapor transmitted was calculated (Table 3.1.8).

3.1.2.5. Drug content

Membranes with a specified area (1 cm²) were weighed and put into a 100 ml volumetric flask. About 50 ml of distilled water was added and kept for 24 hours with occasional shaking. The contents were diluted upto 100 ml using distilled water. Blank was carried out using drug free membrane. The solutions
were filtered and absorbance was measured at 276 nm\textsuperscript{28} using UV spectrophotometer\textsuperscript{29} (Table 3.1.8).

3.1.2.6. In vitro skin permeation study

In vitro drug release study was performed using distilled water in a Keshary-Chien diffusion cell. Appropriate sized polymeric membranes were mounted with excised rat abdominal skin in between donor and receptor compartments of the diffusion cell and were held tightly by springs. The donor compartment was empty, whereas the receptor compartment was filled with distilled water. The magnetic stirrer was set at 100 rpm and temperature was maintained at 37°C. The amount of drug released was determined by withdrawing 5 ml aliquots at regular time intervals (every 30 minutes). The volume withdrawn was replaced with an equal volume of fresh, prewarmed (37°C) distilled water. Samples were analyzed using UV spectrophotometer\textsuperscript{30}. Amount of drug released was calculated using the calibration curves, constructed from the reference standards (Fig. 3.1.11).

3.1.2.7. Skin irritancy test

The primary skin irritation test was performed on seven healthy male albino rabbits weighing between 2 to 3.5 kg. Adhesive tape (Johnson plast) was used as control patch. Adhesive material containing the drug and 5 cm\textsuperscript{2} in area were used as test patches. The control patch was placed on the left dorsal surface of each rabbit whereas the test patch was placed on the identical site on the right dorsal surface of the rabbit. The patches were removed after a period of 24 hours
with the help of alcohol swab and the skin was examined for erythema/edema\textsuperscript{31}. The results are given in Table 3.1.9.

3.1.2.8. Stability studies

The polymeric membranes were stored at different temperatures like 28°, 37° and 45°C for a period of 3 months. The samples were withdrawn at weekly intervals and analysed for the drug content\textsuperscript{32}. The results are shown by Fig. 3.1.12 and Fig. 3.1.13 for Polymer-I and Polymer-II respectively.

3.1.3. RESULTS AND DISCUSSION

As explained under experimental, membranes were prepared using different concentrations of acetaldehyde and propionaldehyde. Since all the membranes, except those prepared with 2% w/v of acetaldehyde and 2% w/v propionaldehyde were found very brittle in nature and not easily removable from the glass plate. Hence only those membranes prepared using 2% w/v acetaldehyde (Polymer-I) and 2% w/v propionaldehyde (Polymer-II) were used for further analysis.

Elemental analysis and melting point of chitosan and Schiff's bases are given in Table 3.1.1 and Table 3.1.2 respectively.

3.1.3.1. Spectral details

A novel and an inexpensive method was developed for the production of chitosan transdermal membranes. These membranes were characterized by FTIR spectroscopy, \textsuperscript{13}C NMR spectroscopy, DSC and TGA techniques.
Table 3.1.1. Elemental analysis of chitosan

![Chemical structure of chitosan](image)

<table>
<thead>
<tr>
<th>Name of the compound</th>
<th>Nature</th>
<th>m.p. (°C)</th>
<th>Molecular formula (Chitosan)</th>
<th>Elemental analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitosan</td>
<td>White powder</td>
<td>265</td>
<td>C₆H₁₁O₄N</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>42.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(42.62)</td>
</tr>
</tbody>
</table>
Table 3.1.2. Elemental analysis of chitosan Schiff’s bases

![Chemical Structure](image)

<table>
<thead>
<tr>
<th>S. No.</th>
<th>R</th>
<th>Nature</th>
<th>m.p. (°C)</th>
<th>Yield (%)</th>
<th>Molecular formula</th>
<th>Elemental analysis</th>
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<td></td>
<td></td>
<td>C</td>
</tr>
<tr>
<td>1</td>
<td>-CH₃</td>
<td>Brown flakes</td>
<td>&gt;300</td>
<td>70</td>
<td>C₈H₁₃O₄N</td>
<td>51.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(51.33)</td>
</tr>
<tr>
<td>2</td>
<td>-CH₂-CH₃</td>
<td>Brownish yellow flakes</td>
<td>&gt;300</td>
<td>75</td>
<td>C₉H₁₅O₄N</td>
<td>53.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(53.73)</td>
</tr>
</tbody>
</table>
FTIR Spectral studies

FTIR spectral data were used to confirm the intactness of drug molecule in the membrane matrix. FTIR spectra of chitosan polymer, pure salbutamol, chitosan Schiff's bases and polymeric membranes loaded with salbutamol were compared and given in Fig. 3.1.1 and Fig. 3.1.2.

In case of pure chitosan, the characteristic band at 3425 cm⁻¹ was observed and was due to O-H stretching (Table 3.1.3). Amino groups of chitosan were converted into imine group (C=NH) when treated with aldehydes, which was confirmed by the appearance of new peak at 1641 cm⁻¹.

Salbutamol showed a principal peak at 3388 cm⁻¹ due to O-H stretching. The bands at 1616 cm⁻¹ and 1443 cm⁻¹ were due to C=C aromatic stretching vibrations. It showed a band at 1375 cm⁻¹ due to C-N stretching.

When salbutamol was incorporated into membranes of chitosan and Schiff's bases, along with all the characteristic peaks of the Schiff's bases, some additional peaks have also appeared due to the presence of salbutamol. In the drug loaded matrices, the characteristic peaks of salbutamol have appeared at 2974 cm⁻¹ due to C-H stretching and the peak due to C=C aromatic stretching at 1616 cm⁻¹ without any change. This indicates that the incorporated salbutamol has not undergone any chemical changes while producing the membranes.
Table 3.1.3. FTIR Spectral data of chitosan derivatives

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name of the compound</th>
<th>C=N</th>
<th>-NH₂-</th>
<th>O-H</th>
<th>C=C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chitosan</td>
<td>--</td>
<td>2926</td>
<td>3425</td>
<td>--</td>
</tr>
<tr>
<td>2</td>
<td>Polymer -I</td>
<td>1641</td>
<td>--</td>
<td>3431</td>
<td>--</td>
</tr>
<tr>
<td>3</td>
<td>Polymer -II</td>
<td>1641</td>
<td>--</td>
<td>3438</td>
<td>--</td>
</tr>
</tbody>
</table>
| 4     | Salbutamol                    | --  | --    | 3388 | 1616
|       |                               |     |       |      | 1443|
| 5     | Salbutamol loaded Polymer -I  | 1647| --    | 3450 | 1616|
| 6     | Salbutamol loaded Polymer -II | 1641| --    | --   | 1616|
Fig. 3.1.1. FTIR Spectra of (1) Chitosan, (2) Polymer-I and (3) Polymer-II
Fig. 3.1.2. FTIR Spectra of - (1) Salbutamol, (2) Salbutamol loaded Polymer-I and (3) Salbutamol loaded Polymer-II
$^{13}$C NMR Spectral studies

Further confirmation of all the derivatives of chitosan was substantiated with $^{13}$C NMR spectroscopic studies. Chitosan (Fig. 3.1.3) showed the peaks at chemical shift values of 45.9 and 80.9 due to C$_4$ and C$_5$ carbons respectively. The peaks at 55.6 and 77.3 were accounted for C$_2$- and C$_3$- carbons respectively. The peak observed at 66.4 was due to C$_6$ carbon. Chitosan displayed its anomeric carbon peak at 102.3 ppm (Table 3.1.4).

As shown in Fig. 3.1.4 and Fig. 3.1.5, Polymer I and Polymer II (the chitosan Schiff's bases) exhibited peaks due to the imine (C=N) carbons, at δ values of 161.1 and 162.2 respectively, and are absent in the chitosan. It is because of the presence of sp$^2$ hybridized carbon atom in C=N group.

In case of Polymer I, the peak due to C$_1$- carbon of -CH$_3$ appeared at 16.1 whereas Polymer II showed peaks at 25.1 and 10.2 due to C$_1$ and C$_2$- carbons of -CH$_2$ -CH$_3$ (Table 3.1.5).
Table 3.1.4. $^{13}$C NMR Spectral data of chitosan
(Chemical shift, in $\delta$ ppm)

![Chemical structure of chitosan](image)

<table>
<thead>
<tr>
<th></th>
<th>C$_1$</th>
<th>C$_2$</th>
<th>C$_3$</th>
<th>C$_4$</th>
<th>C$_5$</th>
<th>C$_6$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>102.3</td>
<td>55.6</td>
<td>77.3</td>
<td>45.9</td>
<td>80.9</td>
<td>66.4</td>
</tr>
</tbody>
</table>

Table 3.1.5. $^{13}$C NMR Spectral data of chitosan Schiff's bases
(Chemical shift, in $\delta$ ppm)

![Chemical structure of Schiff's bases](image)

<table>
<thead>
<tr>
<th>Sample</th>
<th>R</th>
<th>C$_1$</th>
<th>C$_2$</th>
<th>C$_3$</th>
<th>C$_4$</th>
<th>C$_5$</th>
<th>C$_6$</th>
<th>N=C</th>
<th>C$_1^\prime$</th>
<th>C$_2^\prime$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymer-I</td>
<td>-CH$_3$(C$_1^\prime$)</td>
<td>102.3</td>
<td>61.4</td>
<td>67.3</td>
<td>51.0</td>
<td>70.9</td>
<td>69.6</td>
<td>161.1</td>
<td>16.1</td>
<td>-</td>
</tr>
<tr>
<td>Polymer-II</td>
<td>-CH$_2$-CH$_3$ (C$_1^\prime$ C$_2^\prime$)</td>
<td>102.3</td>
<td>61.4</td>
<td>67.3</td>
<td>51.0</td>
<td>70.6</td>
<td>69.6</td>
<td>162.2</td>
<td>25.1</td>
<td>10.2</td>
</tr>
</tbody>
</table>

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DSC Studies

Differential scanning calorimetry was employed for the determination of melting point and glass transition temperatures (Tg) of pure drug, polymer and drug loaded polymer membranes. The thermograms are given in Fig. 3.1.6 and Fig. 3.1.7.

The polymorphism of drug and transition temperature of the polymer was studied before and after drug loading. The results are presented in Table 3.1.6. Chitosan showed an endothermic peak at 50°C, which corresponds to its Tg and a peak at 261°C corresponding to its melting point. After reacting with aldehydes, the endothermic peak of chitosan shifted to higher temperatures, i.e. from 50°C to 61°C and 63°C, for Polymer-I and Polymer-II respectively. Chitosan was found to decompose above 310°C.

The endothermic peak for salbutamol appeared at its melting point 205°C, which did not appear in the DSC thermogram of salbutamol-loaded polymeric membranes. This confirms the molecular level dispersion of drug in the polymer matrix.
Table 3.1.6. Thermal analysis data

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name of the compound</th>
<th>DSC peaks (°C)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Endothermic</td>
<td>Exothermic</td>
</tr>
<tr>
<td>1</td>
<td>Chitosan</td>
<td>50 and 255</td>
<td>300</td>
</tr>
<tr>
<td>2</td>
<td>Polymer -I</td>
<td>61</td>
<td>300</td>
</tr>
<tr>
<td>3</td>
<td>Polymer -II</td>
<td>63</td>
<td>318</td>
</tr>
<tr>
<td>4</td>
<td>Salbutamol</td>
<td>205</td>
<td>225</td>
</tr>
<tr>
<td>5</td>
<td>Salbutamol loaded Polymer -I</td>
<td>60</td>
<td>No heat changes were observed at the temperature of m.p. of salbutamol</td>
</tr>
<tr>
<td>6</td>
<td>Salbutamol loaded Polymer -II</td>
<td>58</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 3.1.6. DSC Thermograms of - (1) Chitosan, (2) Polymer-I and (3) Polymer-II
Fig. 3.1.7. DSC Thermograms of - (1) Salbutamol, (2) Salbutamol loaded Polymer-I and (3) Salbutamol loaded Polymer-II
TGA studies

Effect of Schiff's base formation and drug loading on thermal decomposition of chitosan was studied by thermogravimetry. TGA curves obtained for chitosan and Schiff's bases of chitosan are shown in Fig. 3.1.8. As depicted in Table 3.1.7, thermogravimetric analytical curves and the corresponding data indicated that the compounds decompose in two steps. For chitosan and the Schiff's bases, the first step involves a small weight loss indicating the loss of few fragments attached at the periphery. The destruction of chitosan moiety occurs in the second step.

The drug, salbutamol showed about 8% weight loss between 80°C-150°C and sudden decomposition occurs at 180°C, which showed about 99% weight loss (Fig. 3.1.9).

Schiff's bases of chitosan showed a lesser weight loss as compared to pure chitosan and drug loaded membranes showed a higher weight loss compared to chitosan. Since the melting point of drug was not altered, indicates the absence of any chemical interaction with the polymer.
Table 3.1.7. TGA data of chitosan and its derivatives

<table>
<thead>
<tr>
<th>Name of the Compound</th>
<th>Decomposition temperature (°C)</th>
<th>Mass loss</th>
<th>Probable mode of Decomposition and fragments lost</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% Found</td>
<td>% Calcd.</td>
</tr>
<tr>
<td>Chitosan</td>
<td>42-164 164-500</td>
<td>11.01</td>
<td>10.55</td>
</tr>
<tr>
<td></td>
<td></td>
<td>59.89</td>
<td>60.76</td>
</tr>
<tr>
<td>Polymer-I</td>
<td>44-178 178-449</td>
<td>10.00</td>
<td>9.77</td>
</tr>
<tr>
<td></td>
<td></td>
<td>58.61</td>
<td>58.96</td>
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<tr>
<td>Polymer-II</td>
<td>47-170 170-470</td>
<td>14.99</td>
<td>9.04</td>
</tr>
<tr>
<td></td>
<td></td>
<td>55.55</td>
<td>56.95</td>
</tr>
<tr>
<td>Salbutamol</td>
<td>60-147 147-276</td>
<td>5</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>99.12</td>
<td></td>
</tr>
<tr>
<td>Salbutamol loaded Polymer-I</td>
<td>23-138 138-456</td>
<td>10.233</td>
<td>9.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60.920</td>
<td>61.99</td>
</tr>
<tr>
<td>Salbutamol loaded Polymer-II</td>
<td>40-141 141-477</td>
<td>8.92</td>
<td>9.95</td>
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<tr>
<td></td>
<td></td>
<td>60.56</td>
<td>61.12</td>
</tr>
</tbody>
</table>
Fig. 3.1.8. TGA curves of Chitosan (—), Polymer-I (---) and Polymer-II (- - - )
Fig. 3.1.9. TGA curves of Salbutamol (---), Salbutamol loaded Polymer-I (---) and Salbutamol loaded Polymer-II (----)
Characterization of membranes

3.1.3.2. Determination of tensile strength and percentage elongation

Pure chitosan showed highest tensile strength (0.198 kg/cm²) and percentage elongation (10.8) as compared to the chemically modified chitosan (Table 3.1.8). This may be due to degradation of chitosan after chemical modification, which led to decreased molecular weight, thereby less film forming capacity.

3.1.3.3. Swelling study

Swelling of membrane plays an important role in controlling the drug transport. Membranes were analyzed for water absorption ability by soaking in water. Since chitosan, Polymer-I and Polymer-II are insoluble in water, the membranes were not dissolved, but only swollen. Using water absorption data, diffusion coefficient (D) was calculated using the Eqn. (3.3)

\[
D = \left( \frac{h \theta}{4M_\infty} \right)^2 
\]  

(3.3)

where \(h\) is thickness of the membrane and \(\theta\) is slope obtained by plotting \(M_t/M_\infty\) vs t. \(M_t\) is the amount of water absorbed at time \(t\) and \(M_\infty\) is the total amount of water absorbed.

As shown in Table 3.1.8, the D value was high for chitosan (6.11 \(\times\) 10⁻⁸ cm²/s) than Polymer-I (3.28 \(\times\) 10⁻⁸ cm²/s) and Polymer-II (2.08 \(\times\) 10⁻⁸ cm²/s).

The percentage water uptake was found maximum in chitosan membrane, which may be due to higher chain relaxation capacity and increased
entanglement of hydrophilic polymeric chain. The modified chitosan membranes showed lesser extent of water absorption, because of the transformation of hydrophilic amine to the hydrophobic imine group.

3.1.3.4. WVT rate measurement

The permeability of membranes to water vapor is an important parameter from which permeability of drug can be predicted. The WVT rate for different polymeric membranes were calculated using Eqn. (2.3)\textsuperscript{27}.

The summary of the results of tensile strength, % elongation, swelling study and WVT measurements are presented in Table 3.1.8. All the membranes were permeable to water vapor. Polymer-I and Polymer-II showed lesser permeability compared to pure chitosan. A graph of WVTR vs time for different chitosan derivatives was plotted (Fig. 3.1.10), which showed that WVT rate follows zero-order kinetics.

3.1.3.5. Drug content

The membranes were analyzed for % drug entrapment efficiency using UV spectrophotometry and these data are presented in Table 3.1.8. The content of salbutamol was found highest in chitosan membrane (1.83mg/cm\textsuperscript{2}) as compared to Polymer-I (1.44mg/cm\textsuperscript{2}) and Polymer-II (1.22mg/cm\textsuperscript{2}).

The entrapment efficiency represents the amount of drug entrapped in the matrix, which varies considerably with the type of network.
Fig. 3.1.10. Water Vapour Transmission Rate through membranes of - (●) Chitosan, (■) Polymer–I and (▲) Polymer - II
Table 3.1.8. Results of tensile strength, % elongation, % drug content, WVT rate, diffusion coefficient (D) and $T_{50}$ of different chitosan derivatives

<table>
<thead>
<tr>
<th>Name of the compound</th>
<th>% Drug content (mg/cm$^2$)</th>
<th>WVTR (g.cm$^2$/day) $10^{-4}$</th>
<th>D (cm$^2$/s)$10^{-8}$</th>
<th>$T_{50}$ (min)</th>
<th>Tensile strength (kg/cm$^2$)</th>
<th>% Elongation</th>
<th>% Swelling</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitosan</td>
<td>1.83</td>
<td>8.10</td>
<td>6.11</td>
<td>62</td>
<td>0.198±0.01</td>
<td>10.8 ± 0.32</td>
<td>33.4 ± 0.02</td>
</tr>
<tr>
<td>Polymer-I</td>
<td>1.44</td>
<td>5.18</td>
<td>3.28</td>
<td>88</td>
<td>0.212±0.02</td>
<td>11.18 ± 0.10</td>
<td>30.4 ± 0.1</td>
</tr>
<tr>
<td>Polymer-II</td>
<td>1.22</td>
<td>3.53</td>
<td>2.08</td>
<td>118</td>
<td>0.251±0.01</td>
<td>11.68 ± 0.22</td>
<td>27.9±0.11</td>
</tr>
</tbody>
</table>

$T_{50}$: Time for 50 % drug release
Chitosan is a hydrophilic polymer and entraps higher amount of drug in the free space available within the matrix. Conversion of chitosan to Schiff's bases has led to the formation of rigid polymeric networks and thereby leading to lower % entrapment efficiency.

3.1.3.6. In vitro skin permeation study

*In vitro* permeation of salbutamol through excised rat abdominal skin from membranes of chitosan, Polymer-I and Polymer-II are shown in Fig. 3.1.11. The permeation was highest through the chitosan membrane compared to Polymer-I and Polymer-II.

The time taken for permeation of 50% of salbutamol ($T_{50}$) was 62, 88 and 118 minutes for chitosan, Polymer-I and Polymer-II respectively (Table 3.1.8). Formation of Schiff's bases has increased the time taken for drug release. This may be attributed to lesser affinity of Schiff's bases for water, which results in decreased thermodynamic activity of the drug in the membrane and decreased drug release.

The size of the polymer matrix is found to decrease gradually with time, and towards the end of the drug release, the matrix disintegrates into pieces. This indicates that the erosion takes place from the surface as well as from the bulk of the matrix\textsuperscript{33}. 

\hfill 107
Fig. 3.1.11. *In vitro* release profile of Salbutamol from membranes of 
(●) Chitosan, (■) Polymer–I and (▲) Polymer – II
3.1.3.7. Skin irritancy test

The results of primary skin irritation test performed on rabbits, using different polymeric membranes are given in Table 3.1.9. Johnson Plast was used as a control. The transdermal membranes produced very slight erythema. There was no evidence of edema either with the control or test membranes. This indicates chitosan polymer is compatible with skin and chitosan and its Schiff's bases can suitably be used as carriers for transdermal drug delivery system.

3.1.3.8. Stability studies

Whenever a new formulation is developed, it is very essential to establish that the therapeutically active drug has not undergone any chemical change. The drug and additives are subjected to multifarious processing steps during the development of a new formulation. Any interactions between the ingredients and drug might take place, which has to be detected. To confirm the stability, the formulations are subjected to accelerated stability studies\(^{34}\).

In the present study, both salbutamol loaded Polymer-I (Fig. 3.1.12) and salbutamol loaded Polymer-II (Fig. 3.1.13) exhibited stability at all different storage temperatures.
Table 3.1.9. Results of primary skin irritancy test

<table>
<thead>
<tr>
<th>(Rabbits)</th>
<th>Polymer-I</th>
<th></th>
<th>Polymer-II</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Test</td>
<td>Control</td>
<td>Test</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>1</td>
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<td>5</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>2</td>
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<tr>
<td>6</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
</tbody>
</table>
Fig. 3.1.12. Stability studies of Salbutamol loaded Polymer-I at different temperatures

(*) 27°C, (▲) 37°C and (■) 60°C
Fig. 3.1.13. Stability studies of Salbutamol loaded Polymer-II at different temperatures

(●) 27°C, (▲) 37°C and (■) 60°C
3.2. TRANSDERMAL DELIVERY OF TERBUTALINE SULPHATE THROUGH MODIFIED CHITOSAN MEMBRANE

3.2.1. INTRODUCTION

This chapter discusses the possible usage of chitosan and chemically modified chitosan using two different aldehydes, acetaldehyde and propionaldehyde. The free amino groups of chitosan were reacted with aldehydes in presence of acid to form Schiff’s bases. Selection of aldehydes was made on the basis of their film forming capacity with the polymer.

The drug selected for this study was Terbutaline sulphate (TS) an antiasthmatic, which is widely used in the treatment of asthma. Terbutaline sulphate has a short duration of action, low peak plasma level of 1.2 μg/ml and poor bioavailability of only 14.8%. Bioavailability is the amount of drug that is available to exert a pharmacological action. Inhalation therapy is also not suitable, since only 10-20% of the inhaled dose reaches the lungs. Therefore, long acting formulation of TS is needed for bronchial asthma, particularly for the treatment of all types of reversible airway obstruction to inhalation therapy. The effective half-lives of many drugs can be increased substantially by the use of drug containing polymers. Transdermal films of TS, formulated using various cellulose polymers have already been reported. Various methods have been reported for quantification of TS in single as well as in combined dosage form. Hence it is a potential drug for the development of transdermal membrane.
Drug data

Terbutaline sulphate is a Beta-adrenoceptor agonist.

Chemical name: (RS)-2-(tert-butylamino)-1-(3,5-dihydroxyphenyl)ethanol sulphate.

Molecular formula: C_{24}H_{40}N_{2}O_{10}S

Molecular weight: 548.65

Chemical structure:

\[
\begin{align*}
\text{OH} & \\
\text{CH}_{3} & \\
\text{CH}_{2}NH & \\
\text{CH}_{3} & \\
\text{CH}_{3} & \\
\text{CH}_{3} & \\
\end{align*}
\]

Description: Crystalline powder; odourless.

Color: White.

Solubility: Freely soluble in water; slightly soluble in ethanol (95%); practically insoluble in chloroform and in ether.

Dose: Orally upto 15 mg daily.

3.2.2. EXPERIMENTAL

The materials used and detailed experimental procedures are explained in 3.1.2.

3.2.3. RESULTS AND DISCUSSION

The transdermal membranes produced were thin, flexible, smooth and transparent. The method adopted for film casting was quite satisfactory to produce films of uniform thickness.
3.2.3.1. Spectral details

FTIR studies

FTIR spectral data were used to confirm the intactness of drug in the polymer matrix. FTIR spectra of TS and membranes loaded with TS were compared as shown in Fig. 3.2.1.

Amino groups of chitosan were converted into imine (C=N) group when treated with aldehydes. This was confirmed by the appearance of peak at 1641 cm⁻¹. TS showed characteristic bands at 3332 cm⁻¹ and 2968 cm⁻¹ due to O-H stretching and N-H stretching respectively. The peaks at 1604 cm⁻¹ and 1492 cm⁻¹ were due to C=C aromatic stretching, as presented in Table 3.2.1.

FTIR spectra of TS loaded polymer matrix showed peaks at 1610 cm⁻¹ and 1455 cm⁻¹ due to C=C aromatic stretching due to TS. This indicates that TS is not involved in any chemical reactions either with the polymer or an aldehyde.

Table 3.2.1. FTIR spectral data

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name of the compound</th>
<th>C=N</th>
<th>O-H</th>
<th>N-H</th>
<th>C=C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>TS</td>
<td></td>
<td>3332</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1604</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1492</td>
</tr>
<tr>
<td>2</td>
<td>TS loaded Polymer -I</td>
<td>1653</td>
<td>3388</td>
<td>2981</td>
<td>1610</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1455</td>
</tr>
<tr>
<td>3</td>
<td>TS loaded Polymer -II</td>
<td>1641</td>
<td>3432</td>
<td>2925</td>
<td>1615</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1452</td>
</tr>
</tbody>
</table>
Fig. 3.2.1. FTIR Spectra of (1) TS, (2) TS loaded Polymer-I and (3) TS loaded Polymer-II
DSC Studies

The differential scanning calorimetric (DSC) thermograms of different chitosan derivatives were compared, as shown in Fig. 3.2.2. TS showed a sharp endothermic peak at 276°C, corresponding to its melting point (Table 3.2.2). This peak is absent in the DSC thermograms of TS loaded polymer matrix. This confirms a uniform dispersion of drug in the membranes.

Table 3.2.2. Thermal analysis

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name of the compound</th>
<th>DSC peaks (°C)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Endothermic</td>
<td>Exothermic</td>
</tr>
<tr>
<td>1</td>
<td>TS</td>
<td>276</td>
<td>290</td>
</tr>
<tr>
<td>2</td>
<td>TS loaded Polymer -I</td>
<td>64</td>
<td>No heat changes were observed at the m.p. of TS</td>
</tr>
<tr>
<td>3</td>
<td>TS loaded Polymer -II</td>
<td>70</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 3.2.2. DSC thermograms of - (1) TS, (2) TS loaded Polymer-I, and (3) TS loaded Polymer-II
TGA studies

Effect of Schiff's base formation and drug loading on thermal decomposition of chitosan was studied by thermogravimetry. TGA experiments were carried out on TS, Schiff's bases of chitosan and TS loaded polymer membranes (Fig. 3.2.3).

TS showed less than 2% weight loss below 220°C. It decomposes above 260°C, producing more than 85% weight loss at 300°C. Schiff's bases of chitosan showed a lesser weight loss compared to chitosan and drug loaded membranes showed a higher weight loss compared to pure chitosan (Table 3.2.3). Since the melting point of drug was not altered, indicates the absence of any chemical interactions with the polymer.

Characterization of membranes

3.2.3.2. WVT rate measurement

WVT rates of chitosan, Polymer-I and Polymer-II membranes were determined and results are given in Table 3.1.8.

3.2.3.3. Drug content

Entrapment efficiency is the amount of drug entrapped in the matrix. The membranes were analyzed for the drug content using UV spectrophotometer and the data are presented in Table 3.2.4. Content of TS was found maximum in chitosan membrane (2.91 mg/cm²) and least in Polymer-II (1.42 mg/cm²).
Table 3.2.3. TGA data of various chitosan derivatives

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name of the Compound</th>
<th>Decomposition temperature (°C)</th>
<th>Mass loss</th>
<th>Probable mode of Decomposition and fragments lost</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>% Found</td>
<td>% Calcd.</td>
</tr>
<tr>
<td>1</td>
<td>TS</td>
<td>53-210 210-330</td>
<td>5</td>
<td>99.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>TS loaded Polymer-I</td>
<td>47-230 230-459</td>
<td>12.756</td>
<td>62.861 66.89</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>TS loaded Polymer-II</td>
<td>45-132 132-470</td>
<td>9.001</td>
<td>61.98 64</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig. 3.2.3. TGA curves of TS (——), TS loaded Polymer-I (---) and TS loaded Polymer-II (---)
Table 3.2.4. Results of % Drug content and T_{50} of different chitosan derivatives

<table>
<thead>
<tr>
<th>Name of the Sample</th>
<th>% Drug content (mg/cm²)</th>
<th>T_{50} (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitosan</td>
<td>2.91</td>
<td>62</td>
</tr>
<tr>
<td>Polymer-I</td>
<td>2.03</td>
<td>90</td>
</tr>
<tr>
<td>Polymer-II</td>
<td>1.42</td>
<td>120</td>
</tr>
</tbody>
</table>

T_{50}: Time for 50% drug release
The % drug content reflects the composition and rigidity of the membrane matrix. It depends upon the space available within matrix and hydrophilicity of the polymer.

3.2.3.4. *In vitro* skin permeation study

Results of *in vitro* permeability of TS from membranes of chitosan, Polymer-I and Polymer-II, through excised rat abdominal skin is displayed in Fig. 3.2.4. The permeation was high through chitosan than Polymer-I and Polymer-II. The membranes developed were hydrophilic in nature and were partly converted into hydrophobic by reacting with aldehydes. As shown in Table 3.2.4, the time taken for permeation of 50 % of drug ($T_{50}$) was 62, 90 and 120 minutes for chitosan, Polymer-I and Polymer-II respectively.

To find out the nature of the release kinetics and mode of drug diffusion, the *in vitro* release data were obtained for about 150 minutes. Results of *in-vitro* permeation suggest that these polymeric membranes are suitable for prolonged regimen of controlled drug delivery through transdermal route.

3.2.3.5. Stability studies

All the membranes were stable at room temperature and 37°C for a period of 3 months. Membranes showed slight change in color at 60°C, indicating gradual degradation of the formulation at higher temperatures. The results are shown in Fig. 3.2.5 and Fig. 3.2.6.
Fig. 3.2.4. Permeation of TS through rat skin from membranes of
(▲) Chitosan, (■) Polymer-I, and (●) Polymer-II
Fig. 3.2.5. Stability study of TS loaded Polymer-I at

(a) 27°C,   (▲) 37°C, and   (●) 60°C
Fig. 3.2.6. Stability study of TS loaded Polymer-II at
(a) 27°C, (▲) 37°C and (●) 60°C
References


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28. Indian Pharmacopoeia, Published by the Controller of Publications, Delhi, 2, 668(1996).


