Colon Specific Chronotherapeutic Drug Delivery for Nocturnal Asthma: Effect of Eudragit Enteric Coating on Matrix Tablets of Salbutamol Sulphate

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Abstract: Objective: The purpose of this study was to prepare Eudragit S-100 coated salbutamol sulphate matrix tablet for chronotherapeutic drug delivery system for the treatment of nocturnal asthma.

Methods and Material: This study is designed to analyze the effect of Eudragit S-100 coating on the drug release from hydroxypropyl methylcellulose matrix to achieve the time and pH dependent chronotherapeutic drug delivery system of salbutamol sulphate. Hydroxypropyl methylcellulose matrix tablets of salbutamol sulphate were prepared by wet granulation method and coated with Eudragit S-100 using dip coating method. Then the tablets were evaluated for different physical parameters, compatibility studies and in vitro dissolution.

Results: Matrix tablets of salbutamol sulphate have been characterized for weight variation, hardness, friability and drug content. HPMC matrix tablet failed to control the drug release in initial hrs. and shows 68 ± 0.71% of the drug release. Formulation FMS 5 (HPMC K-100) selected for coating with Eudragit S-100 as it shows significant drug content uniformity and consistent drug release. Eudragit S-100 coated formulations control the drug release in first hrs. at pH 1.2 and 6.8 and shows burst release at pH 7.4 after 7 hrs. This is due to pH dependent nature of eudragit polymer. Compatibility studies revealed that there was no interaction between drug and the polymers.

Conclusion: From observations mentioned in the results, it is obvious that the developed salbutamol sulphate enteric coated matrix tablets are suitable for chronotherapeutic drug delivery system.

Key words: Salbutamol Sulphate, HPMC, Eudragit, Matrix tablet, Chronotherapeutic.

Introduction

Chronotherapeutics means a clinical practice of synchronizing drug delivery in a manner consistent with the body’s circadian rhythm including disease state to produce maximum health benefit and minimum harmful effects [1-2]. The pulsatile release system is an excellent way for chronotherapeutic drug delivery. Pulsatile release can be described as the fast release of a certain amount of drug within a short time period after a lag time [3]. For chronotherapeutic time controlled system, when a lag time is needed, enteric coated formulations are utilized [4-6].

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Asthma may be the most common disease with the largest circadian variations. The activity of the lung exhibits a circadian rhythm with a maximum around 4 am and minimum around 4 am. In asthmatic patient, the intensity of variation in lung function is as much as 50% in a day.[7] Nocturnal asthma is defined as sleep-related worsening in reversible airway disease. Symptoms generally include shortness of breath or wheezing at night. Asthma was approximately 70 fold more frequent between 4 and 5 am during indented night time sleep than between 2 and 3 am middle of the day time activity span. It has been reported that 94%, 74%, 64% & 39%, patient under asthmatic treatment disturbed their night time sleep at least one time per month, one time per week, three nights per week and every night respectively. It is reported that 13 of 19 (68%) asthma deaths occurred nocturnally, between 12 am and 6 am[8-9]

All the current sustained release formulation have a short coming of inability to maintain high blood levels fort long period with high disease intensity. This may leave the patient unprotected against the worst event of nocturnal asthma Thus a chronotherapeutic drug delivery that is administered before sleep and maintains high blood levels for longer period (from midnight to 8 am in the morning during which maximum intensity of disease occurs) could be very much beneficial for proper management of nocturnal asthma[10-11]

Colonic delivery useful to improve treatment of disease also depends on upon diurnal asthma such as asthma and arthritis. Coating with pH dissolution dependent polymers such as Eudragit S100 and L100 is one important approach used for designing colon targeted based pulsatile drug delivery system[12]

The potential benefits of the matrix system include increased bioavailability, predictable reproducible and short gastric residence time, no risk of dose dumping, reduced risk of local irritation and the flexibility of blend pellets with different composition or release pattern because of their smaller size. These systems are capable of passing through GIT easily leading to less intra-subject variability[13].

Salbutamol sulphate which is short acting β 2 agonists used to treat asthma was selected as a model drug. The shorter duration of action has left most patient without bronchodilation at early morning during which intensity of asthma is maximum.[14]

Materials and Methods

Materials

Salbutamol Sulphate was obtained as a gift sample from Cipla (Rangpoo, Sikkim India). Eudragit S-100 was procured from Loba Chemicals (Mumbai, India). Various grades of HPMC were procured from Ambica Scientific Pvt. Ltd. (Ambala, India). All other chemicals used were of analytical grade.

Methods:

Preparation of matrix tablets

Wet granulation method was adopted to prepare the HPMC matrix tablets. SAL, HPMC and excipients other than glidants and lubricant were accurately weighed, passed through 60 mesh sieves and mixed in a poly bag for 5-10 min, and then granules were prepared with the addition of 10% polyvinyl pyrrolidone in alcohol as binding agent, dried at 40°C for 15 min and sieved to obtain uniformly sized granules (18 mesh and 22 mesh sieves used for granulation and sieving of dried granules respectively). The obtained granules were lubricated with t alc and magnesium stearate by blending for another 5 min. The resultant mixture was directly compressed into tablets using 5000 kg compression pressure with 12.70 mm round flat punches using 8-station rotary tableting machine (Sri. Kadambni, Ahmedabad, India). The final weight of the tablet was adjusted to 300 mg. The compositions of the matrix tablets were given in Table 1.[15]

Pre-compression Characteristics:

Angle of repose:

Flow property of granules for 100 mg salbutamol sulphate was determined. Angle of repose can be determined by the fixed funnel and free standing cone methods, the method employed a funnel that was secured with its tip at a given height, H above the graph paper that was placed on a flat horizontal surface. Powder or granules were carefully poured through the funnel until the apex at the conical pile just touched the tip of the funnel. Thus, with R being the radius of the base of the conical pile tan1 = H/R. Where, H and R are the height and radius of the powder cone.[16]
Bulk Density

Apparent bulk density ($\rho_b$) was determined by pouring the blend into a graduated cylinder. The bulk volume ($V_b$) and weight of powder ($M$) was determined. The bulk density was calculated using the formula

$$\rho_b = \frac{M}{V_b}$$

Where $M=$Mass; $V_b =$ Bulk Volume

Tapped Density

The measuring cylinder containing a known mass of blend was tapped 100 times using density apparatus. The constant minimum volume ($V_t$) occupied in the cylinder after tapings and the weight ($M$) of the blend was measured. The tapped density ($\rho_t$) was calculated using the formula

$$\rho_t = \frac{M}{V_t}$$

Where $M=$Mass; $V_t =$ True Volume

Compressibility Index

The simplest way for measurement of flow of the powder was its compressibility, an indication of the ease with which a material can be induced to flow. It is expressed as compressibility index ($I$) which can be calculated as follows

$$I = \left( \frac{\rho_t - \rho_b}{\rho_t} \right) \times 100$$

Where, $\rho_t =$ Tapped density; $\rho_b =$ Bulk density.

Hausner’s Ratio

Hausner’s ratio (HR) is an indirect index of ease of powder flow. It was calculated by the following formula

$$HR = \frac{\rho_t}{\rho_b}$$

Where, $\rho_t$ is tapped density and $\rho_b$ is bulk density.

Lower Hausner’s ratio (<1.25) indicates better flow properties than higher ones.

Table 1. Formulation development and optimization of matrix tablet

<table>
<thead>
<tr>
<th>COMPOSITION</th>
<th>FORMULATION CODE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FMS1</td>
</tr>
<tr>
<td>SALBUTAMOL SULPHATE</td>
<td>100</td>
</tr>
<tr>
<td>HPMC E100</td>
<td>50</td>
</tr>
<tr>
<td>HPMC E50</td>
<td>---</td>
</tr>
<tr>
<td>HPMC K4M</td>
<td>---</td>
</tr>
<tr>
<td>HPMC K15M</td>
<td>---</td>
</tr>
<tr>
<td>HPMC K100M</td>
<td>---</td>
</tr>
<tr>
<td>PVP K30</td>
<td>30</td>
</tr>
<tr>
<td>LACTOSE MONOHYDRATE</td>
<td>100</td>
</tr>
<tr>
<td>MAGNESIUM STEARATE</td>
<td>5</td>
</tr>
<tr>
<td>TALC</td>
<td>5</td>
</tr>
<tr>
<td>ISOPROPYL ALCOHOL</td>
<td>1ml</td>
</tr>
</tbody>
</table>
Total porosity of granules:

Total porosity of the granules was determined by measuring the volume occupied by a selected weight of a powder ($V_{\text{bulk}}$) and the true volume of granules (the space occupied by the powder exclusive of spaces greater than the intermolecular space $V_I^{[17]}$)

Porosity ($\%$) = $\frac{V_{\text{bulk}} - V}{V_{\text{bulk}}} \times 100$

Preparation of calibration curve:

From the stock solution, 20, 40, 60, 80, 100µg/ml solutions of Salbutamol sulphate were prepared in pH 1.2, 5.8, 6.8 and 7.4 phosphate buffer solution. The absorbance was measured at 276 nm and a graph of concentration versus absorbance was plotted. Standard plot data of Salbutamol sulphate in pH 1.2 hydrochloric acid and buffer solutions pH7.4 is reported in fig.1 and 2 respectively

![Calibration Curve of Salbutamol in pH 1.2](image1)

**Fig.1: Calibration curve of salbutamol sulphate in acidic buffer pH 1.2**

![Calibration curve of Salbutamol sulphate in phosphate buffer pH 7.4](image2)

**Fig.1: Calibration curve of salbutamol sulphate in phosphate buffer pH 7.4**
Post compression characterization of matrix tablet:

Granules of salbutamol sulphate with different grades of HPMC were prepared separately and dried. These dried granules were lubricated separately with talc and magnesium stearate. 300 milligrams of salbutamol SR granules per tablet was taken and compressed on a 08 station lab press compression machine (Sri. Kadambini Pvt. Ltd., Ahmedabad, India) using D tooling round punches.

Physical properties of matrix tablets:

The % age friability of tablet was determined immediately after the formulation. The weight variation of the 20 tablets was accomplished according to guidelines mentioned in I.P. 1996 using an electronic balance. Friability of 10 tablets was evaluated by Roche type friabilator for 4 min at the rate of 25 rpm.

The hardness of 10 tablets was evaluated using Monsanto hardness tester. The thickness of the 10 tablets was measured by Vernier caliper. As the formulations are matrix tablet so there is no scope for disintegration test.

Drug-polymer compatibility studies: [18-19]

FTIR characterization

The FTIR analysis of the Salbutamol Sulphate, HPMC and mixture of drugs and polymer was carried out to study the interactions between drug and polymer. The pellet of approximately 01 mm diameter of the samples was prepared by grinding 3-5 mg of sample with 100-150 mg of potassium bromide in pressure compression machine. The sample pellet was mounted in FTIR (8400S, Shimadzu) compartment and scanned at wavelength 4000 – 500 cm-1.

DSC Thermogram

To analyze any drug excipients incompatibility, thermal analysis of pure drug and selected formulation were performed using DSC-TA system (Perkin Elmer). All samples were sealed in a crimped Aluminum pan and heated at a rate 20°C/ min from 70 to 300°C in an atmosphere of nitrogen gas by passing at a flow rate 60 ml/min. An empty aluminum pan was used as reference.

Assay of matrix tablet

Twenty tablets of the formulation were crushed into a fine powder by mortar and pestle; powder equivalent to 100 mg of salbutamol sulphate was take in a volumetric flask and diluted in with methanol up to 100 ml. After sonication for 15 min the diluted solution was filtered. The total amount of drug for each tablet was analyzed after the proper dilution of test solution by using the U.V spectrophotometric method against the reference solution of pure drug powder prepared in the same procedure [19].

Dissolution study of matrix tablet

Drug release of individually six tablets were measured using USP 1 (basket type) apparatus (Lab India,) using media of 900 ml 0.1 (N)HCl for first 2 h and the rest of hours at pH 6.8 and 7.4 phosphate buffers. The dissolution media were maintained at a temp of 37°C. The sample was withdrawn at the interval of 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12 hrs according to pre-programmed manner. At every withdrawal the sample was replaced with 5 ml of fresh media. All the solutions of samples were analyzed by using U.V spectrophotometer [20].

In vitro release kinetics

The data obtained from the in vitro dissolution studies was fitted to zero order, first order and Higuchi models to explain the pattern and the release mechanism from the formulations. Koresmeyer–Peppas model is one of the mathematical expressions, used to understand the mechanism of drug release from these formulations. The mean dissolution time (MDT) is defined as the sum of different release fraction periods (release areas) during dissolution studies divided by the initial loading dose. T10% and T80% (time in hours to take 10% and 80% drug release, respectively) were calculated to clarify the colon-specific release from matrix tablets.
Selection of optimized matrix formulation:

Formulation FMS 5 (HPMC-K100) was selected for further study as the formulation shows significant hardness, drug content and consistent drug release in different pH medium.

Coating of matrix tablet (FMS 5) with Eudragit S-100

The enteric coating solution was prepared by dissolving Eudragit S100 in acetone at 10% w/v. Coating of optimized matrix formulation (FMS5), which shows significant drug release during the initial period of dissolution was performed by dip coating method. Samples were taken, weighed, and the mean coat weight calculated. The process was repeated until the desired amount of coating per tablet was achieved.

Roentgenography study.

The evaluation of dosage form in animal model renders support to the in vitro studies. To closely simulate the human physiological environment of the colon, rabbits were selected as animal model for evaluating the colon specific delivery. Roentgenography study; a comparatively safer technique was carried out in healthy male albino rabbits to access the in vivo performance of the selected batch. The study was carried out using barium sulphate as X ray opaque material. Formulation (ECF6) containing barium sulphate for the selected batch was formulated and administered to rabbits by gastric intubation. Images were taken at definite intervals in order to trace the performance of the formulation and result was graphically represented in fig. [15]

Results and discussion:

Determination of flow properties of granules:

Table 2. shows the results of different formulations of salbutamol sulphate with various grades of HPMC. The results of angle of repose were ranged between 23.31 ± 0.05to 25.09 ± 0.03 which indicates good flow properties of powder. The compressibility index values were found to be in the range of 19.39 ±0.03% to 23.30 ±0.02%. These findings indicated that the powder mixture of all batches of formulation exhibited good flow.

Physical properties of matrix tablet:

All the formulated tablets containing the active drugs were evaluated to find the physical properties like hardness, thickness, friability and drug contents. In a weight variation test, the pharmacopoeial limit of percentage deviation for tablets whose weight is more than 250 mg is ±5%. The average percentage deviation of all the tablets was found within the limit. The drug content was also found uniform and within the prescribed limit. (Table 3)

Table 2: Micromeritic properties of salbutamol sulphate granules

<table>
<thead>
<tr>
<th>Formulation code</th>
<th>Angle of repose</th>
<th>Bulk density (gm/ml)</th>
<th>Tapped density (gm/ml)</th>
<th>Compressibility Index (%)</th>
<th>Total porosity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMS1</td>
<td>23.19 ± 0.07</td>
<td>0.392 ±0.09</td>
<td>0.509±0.08</td>
<td>22.98 ±0.08</td>
<td>22.02±0.13</td>
</tr>
<tr>
<td>FMS2</td>
<td>22.67 ± 0.04</td>
<td>0.402 ±0.04</td>
<td>0.529±0.10</td>
<td>24.00 ±0.11</td>
<td>24.06±0.11</td>
</tr>
<tr>
<td>FMS3</td>
<td>25.19 ± 0.09</td>
<td>0.428 ±0.03</td>
<td>0.531±0.06</td>
<td>19.39 ±0.03</td>
<td>23.09±0.18</td>
</tr>
<tr>
<td>FMS4</td>
<td>25.09 ± 0.03</td>
<td>0.405 ±0.10</td>
<td>0.522±0.09</td>
<td>22.41 ±0.08</td>
<td>22.08±0.09</td>
</tr>
<tr>
<td>FMS5</td>
<td>24.31 ± 0.05</td>
<td>0.395 ±0.01</td>
<td>0.515±0.06</td>
<td>23.30 ±0.02</td>
<td>24.02±0.06</td>
</tr>
<tr>
<td>FMS6</td>
<td>23.31 ± 0.05</td>
<td>0.403 ±0.01</td>
<td>0.513±0.06</td>
<td>23.30 ±0.02</td>
<td>24.02±0.06</td>
</tr>
</tbody>
</table>

Mean ± Standard Deviation, n=6
Table 3: Physical properties of Salbutamol Sulphate matrix tablets

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Thickness (mm)</th>
<th>Weight variation (mg)</th>
<th>Hardness (Kg/cm²)</th>
<th>Friability (%)</th>
<th>Drug content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FMS1</td>
<td>5.1 ±0.47</td>
<td>300.06 ±0.18</td>
<td>5.8 ±0.21</td>
<td>0.53 ±0.47</td>
<td>92.03 ± 0.12</td>
</tr>
<tr>
<td>FMS2</td>
<td>4.6 ±0.39</td>
<td>300.09 ±0.31</td>
<td>4.7 ±0.83</td>
<td>0.48 ±0.71</td>
<td>90.09 ± 0.26</td>
</tr>
<tr>
<td>FMS3</td>
<td>4.8 ±0.52</td>
<td>299.98 ±0.16</td>
<td>5.2 ±0.33</td>
<td>0.67 ±0.39</td>
<td>93.12 ± 0.37</td>
</tr>
<tr>
<td>FMS4</td>
<td>4.7 ±0.26</td>
<td>300.03 ±0.19</td>
<td>5.2±0.44</td>
<td>0.63 ±0.57</td>
<td>96.03 ± 0.41</td>
</tr>
<tr>
<td>FMS5</td>
<td>5.4 ±0.33</td>
<td>300.00 ±0.20</td>
<td>5.6 ±0.31</td>
<td>0.56 ±0.37</td>
<td>99.09 ± 0.19</td>
</tr>
<tr>
<td>FMS6</td>
<td>5.9 ±0.22</td>
<td>300.09 ±0.30</td>
<td>5.1 ±0.61</td>
<td>0.41 ±0.18</td>
<td>94.18 ± 0.56</td>
</tr>
</tbody>
</table>

Mean ± Standard Deviation, n=20 (For wt. variation, content uniformity & friability) & n=10 (For thickness and hardness)

Drug-Polymer interaction study:

Straight line in FTIR spectra of salbutamol sulphate and HPMC K-100 was observed after spectral calculation. This shows that there are no interactions between the drug and polymer.(Fig.3)

![FTIR spectra of salbutamol sulphate and HPMC K-100 mixture.](image)

**Fig 3:** FTIR spectra of salbutamol sulphate and HPMC K-100 mixture.

DSC Thermogram:

The thermogram shows a sharp melting point peak with onset at 100.00°C similarly thermogram of drug and polymer mixture showed a depressed endotherm at near 110.00°C, which could be due to the dilution effect of amorphous polymers (Fig. 4.)
Dissolution study of matrix tablet:

In vitro release of salbutamol sulphate from matrix tablet

Mean cumulative % release of salbutamol sulphate at different time intervals are shown in Fig. 2. The dissolution profile of the formulations was found to be different from batch to batch. But the formulation of FMS5 was found to be the most desired release profile for the formulation and selected further for coating with Eudragit S-100. The release of formula FMS5 was most consistent, accurate and complete.

Kinetic of drug release from HPMC matrix tablet:

Comparing the correlation coefficient given in table a good fitting Higuchi model could be anticipated for all formulations. When data was analyzed according to peppas model, the release exponents were found to be in the range of 0.4 to 0.6. It is assumed that release of Salbutamol from matrix tablet occurred due to swelling of the polymer as a result of formation of gel. This is followed by drug dissolution and further diffusion through the gel. The Higuchi model showed the most appropriate model to describe the kinetic of core formulation. Thus drug release mechanism was assumed to be controlled by diffusion. When analyzed according to power
law model, the release exponent was found to be in the range of 0.4 -0.6, therefore the diffusional release was found to follow Fickian diffusion.

Table 4: Kinetic of drug release from HPMC matrix tablet:

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Zero order</th>
<th>First Order</th>
<th>Higuchi</th>
<th>Pappas</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r²</td>
<td>K₀</td>
<td>r²</td>
<td>Kᵣ</td>
</tr>
<tr>
<td>FMS1</td>
<td>0.98</td>
<td>2.712</td>
<td>0.920</td>
<td>0.141</td>
</tr>
<tr>
<td>FMS2</td>
<td>0.947</td>
<td>3.245</td>
<td>0.959</td>
<td>0.114</td>
</tr>
<tr>
<td>FMS3</td>
<td>0.978</td>
<td>3.221</td>
<td>0.941</td>
<td>0.069</td>
</tr>
<tr>
<td>FMS4</td>
<td>0.976</td>
<td>3.308</td>
<td>0.945</td>
<td>0.062</td>
</tr>
<tr>
<td>FMS5</td>
<td>0.971</td>
<td>3.632</td>
<td>0.960</td>
<td>0.062</td>
</tr>
<tr>
<td>FMS6</td>
<td>0.947</td>
<td>3.245</td>
<td>0.959</td>
<td>0.114</td>
</tr>
</tbody>
</table>

Effect of Eudragit S-100 coating:

In order to evaluate the effect of the coating level, release profile under pH-gradient, formulation FMS5 tablets were coated with Eudragit S-100 in amounts varying from 5, 10, and 15% w/w (ECF1, ECF2 and ECF3 respectively). The result shows that drug release from coated matrix tablet depends on the pH of the dissolution medium. Complete absence of drug release was observed from the tablet in medium of pH 2 after time period of 2 hrs. When pH was gradually increased from 1.2 to 5.8, there was 3.6 to 5.6% drug release from tablet coated with 5 and 10% of the Eudragit S-100. However complete absence of release was found from the tablet coated with 15% concentration of Eudragit S-100, small fraction of drug released in pH 6.8. When pH was gradually increased up to pH 7.4 formulation shows initial 82% burst release from the coated matrix. Formulation sustained the level of drug up to 15 hrs, which will be helpful to maintain the drug level in blood to achieve maximum therapeutic benefit. [21]

X-ray imaging studies (Roentgenography study):

Roentgenography study; a comparatively safer technique was carried out in healthy male albino rabbits to access the in vivo performance of the selected batch. The tablet was intact and slight swelling was observed in the first 2hrs. This observation is similar to in vitro drug release as drug release was absent during this period.
After 4 hrs. the tablet still appeared to be intact with further swelling showing the increasing tendency of swelling in higher pH. At 7 hrs. As the tablet, disintegration was observed (fig 7.)

Fig. 7: X-Ray photograph of formulation ECF3 at 0 hr. (a), 2 hrs. (b), 4 hrs. (c) and 7 hrs. (d).

Conclusion:

The present investigation was carried out with significant plan to develop Salbutamol Sulphate matrix tablets. Chronotherapeutic matrix tablets using different HPMC grade, coated with Eudragit S-100 were decreased the drug release in the upper region of GIT, but gave good amount of drug release in colon. Formulations ECF1, ECF2 and ECF3 coated with different concentration of Eudragit showed the significant level of drug release in the colon with negligible loss in stomach and small intestine. From the drug release kinetics, above formulation followed zero order profile and the mechanism of drug release followed by Fickian diffusion. Drug-Polymer compatibility studies proved no interaction between them. The in vivo x-ray imaging study in albino rabbit proved that the tablets (ECF3) reached the colon without disintegrating in the upper GIT. All in all, development of Eudragit S100 coated HPMC matrix tablets using combination of pH-sensitive and time dependent approaches is a great methodology for chronotherapeutic delivery of Salbutamol Sulphate.

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Abbreviations:

SAL : Salbutamol sulphate
HPMC : Hydroxy Propyl Methyl Cellulose
ECF : Eudragit coated formulation
DSC : Differential Scanning Calorimetry
FTIR : Fourier Transform Infrared Spectroscopy

********Conflict of interest “None”**********

References


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