DEVELOPMENT AND EVALUATION OF TRAMADOL HYDROCHLORIDE MICROSPHERES AS COLON TARGETED DRUG DELIVERY SYSTEM

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ABSTRACT
Colon targeted drug delivery system is capable of protecting the drug in route to the colon i.e. drug release and absorption does not occur in stomach and small intestine, but only released and absorbed once it reaches the colon. A multiparticulate system combining pH sensitive property and biodegradability has been investigated to prepare and evaluate Eudragit S-100 coated Sodium Alginate microspheres for colon targeting of Tramadol hydrochloride. Uncoated Tramadol Hydrochloride microspheres were prepared by Ionotropic Gelation Technique using different ratios of Tramadol Hydrochloride and Sodium Alginate. Coated Tramadol Hydrochloride microspheres were prepared by Coacervation Phase Separation Technique using different ratios of uncoated Tramadol Hydrochloride and Eudragit S-100. Uncoated and coated Tramadol Hydrochloride microspheres were evaluated for percentage yield, particle size, surface morphology, flow properties, drug content and entrapment efficiency and were found to be within the acceptable range. The uncoated microspheres sustained the release up to 8 hrs whereas coated microspheres sustained the release up to 12 hrs in a pH progression medium mimicking the condition of GIT. The drug release from MC4 formulation coated with Eudragit s-100 (1:4) showed desired rate as there was no drug release observed up to 4-5 hrs, while in colonic fluid controlled drug release was observed releasing about 69.66 % after 12 hrs. The release kinetics followed Peppas showing Super Case II transport. Stability studies suggested formulations were stable. It is concluded from the present investigation that Eudragit S-100 coated Sodium Alginate microspheres are promising controlled release carriers for colon targeted delivery of Tramadol Hydrochloride.

KEY WORDS: Colon targeted drug delivery system, Tramadol Hydrochloride, Sodium alginate, Eudragit S-100.
Treatment of IBS consists primarily of medications to control constipation, diarrhoea, and abdominal pain [2].

Tramadol Hydrochloride is a synthetic centrally acting aminocyholaxal analgesic that acts as an opioid agonist with selectivity for the μ receptor have demonstrated that this drug is an effective agent for moderate to severe chronic pain, for the treatment of functional GI disorders such as IBS and non-cardiac chest pain. The most commonly occurring adverse side effects during treatment of pain with Tramadol preparations are gastrointestinal upsets, nausea and vomiting. It has mean plasma half-life of 5 hrs. A dose of 25-50 mg appears to provide effective relieve from the symptoms of IBS but higher frequency of administration of drug may lead to high plasma concentration, resulting into systemic side effects like decreased heart rate and blood pressure [3-5]. Controlled release oral drug delivery systems are designed to achieve therapeutically effective concentrations of drug in the systemic circulation over an extended period of time, thus achieving better patient compliance and allowing a reduction of both the total dose of drug administered and the incidence of adverse side effects. Considering this aspect, it is desirable to develop a 12-16 hrs sustained release formulation of Tramadol Hydrochloride.

MATERIALS AND METHODS

Tramadol Hydrochloride, Sodium alginate and Eudragit S-100 were procured from Yarrow chemical, Mumbai. All the reagents used were of analytical grade.

Preparation of uncoated Tramadol Hydrochloride microspheres

The Sodium alginate microspheres were prepared by Ionotropic Gelation Technique. Tramadol Hydrochloride was dispersed in an aqueous solution of Sodium Alginate with stirring to produce a viscous form. Then polymer drug solution was added drop wise by using syringe of 22 G in diameter from a height of about 5 cm into a beaker containing 10% w/v solution of calcium chloride with continuous stirring by magnetic stirrer at 500 rpm. Then the solution containing gel formed microspheres was filtered by whatman filter paper no. 1. The microspheres were allowed to dry at 30-40 °C and kept in an airtight container for further studies. Sodium alginate microspheres were prepared using different ratios of Tramadol Hydrochloride: Sodium Alginate [6-7].

Evaluation of uncoated Tramadol Hydrochloride microspheres

Percentage Yield

The percentage yield of the prepared microspheres was determined by using the formula:

$$\text{Percentage yield} = \left( \frac{\text{Practical Yield}}{\text{Theoretical Yield}} \right) \times 100$$

Particle Size

Determination of average particle size was carried out by optical microscopy in which stage micrometer was employed. A minute quantity of microspheres was spread on a clean glass slide and average size of 150 microspheres was determined in each batch by the given equations:

$$\text{Size of Individual particles (µm)} = \frac{\text{Number of Division on Eyepiece} \times \text{Calibration factor}}{\sum \text{Size of Individual Particles}}$$

Average Particle Size (µm) = \frac{\sum \text{Size of Individual Particles}}{150}

Surface Morphology

Scanning electron microscopy has been used to determine particle size distribution, surface topography, texture and to examine the morphology of fractured or sectioned surface. SEM studies were carried out by using JEOL-JSM 6380LA analytical scanning electron microscope. The samples of SEM were prepared by lightly sprinkling the microspheres powder on a double adhesive tape, which was stuck on an aluminium stub. The photomicrographs were taken with the help of SEM analyzer.

Flow Properties

Flow properties of microspheres were studied by measuring the angle of repose of the formulation by employing fixed funnel method. Microspheres were weighed and passed through the funnel, which was kept at a height of ‘h’ from the horizontal surface. The passed microspheres formed a pile of a height ‘h’ above the horizontal surface and the radius ‘r’ of the pile was measured and the angle of repose was determined for all the batches by using the formula:

$$\theta = \tan^{-1} \frac{h}{r}$$

θ = angle of repose

h = height of the pile

r = radius of the pile

Drug Content

Microspheres were accurately weighed equivalent to 50 mg of Tramadol Hydrochloride, triturated and digested in 10 ml buffer solutions (pH 1.2, pH 4.5 and pH 7.4) separately and kept overnight for extraction of drug. The digested homogenate was centrifuged and supernatant was collected. After appropriate dilution of supernatant with same buffer solutions, aliquots were assayed by UV spectrophotometer at λ_max 274 nm for acidic buffer pH 1.2 and at λ_max 273 nm for acetate buffer pH 4.5 and phosphate buffer pH 1.2. Corresponding drug concentrations in the sample were calculated from the standard calibration curve.

Drug Entrapment Efficiency

Efficiency of drug entrapment for each batch was calculated in terms of percentage drug entrapment (PDE) as per the following formula:

$$\text{Drug Entrapment Efficiency} \% = \left( \frac{\text{Drug Content} \times 100}{\text{Drug Added}} \right)$$
The coated Tramadol Hydrochloride microspheres were evaluated for percentage yield, particle size, surface morphology, flow properties, drug content, drug entrapment efficiency and in-vitro drug release similar to the procedure carried out for evaluation of uncoated Tramadol Hydrochloride microspheres as mentioned above. In addition kinetic studies were carried out [8-10].

**Kinetic Studies**

In order to describe the kinetics of the release process of drug in the different formulations, models were fitted to the dissolution data of optimized formulations using linear regression analysis.

**a. Zero order kinetics**

Drug dissolution from pharmaceutical dosage forms that release the drug slowly, assuming that area does not change and no equilibrium conditions and are represented by the equation:

\[ Q_t = Q_0 + K_0 t \]

where:
- \( Q_t \) is the amount of drug dissolved in time \( t \),
- \( Q_0 \) is the initial amount of drug in the solution,
- \( K_0 \) is the zero order release constant.

**b. First order kinetics**

The application of this model to drug dissolution studies used to describe absorption and/or elimination of drugs. To study the first order release rate kinetics the release rate data were fitted to the following equation:

\[ \log Q_t = \log Q_0 + K_1 t / 2.303 \]

where:
- \( Q_t \) is the amount of drug released in time \( t \),
- \( Q_0 \) is the initial amount of drug in the solution,
- \( K_1 \) is the first order release constant.

**c. Higuchi model**

Higuchi developed several theoretical models to study the release of water soluble and low soluble drugs incorporated in semi-solid and/or solid matrices. Mathematical expressions were obtained for drug particles dispersed in a uniform matrix behaving as the diffusion media, the equation is:

\[ Q_t = K_H \cdot t^{1/2} \]

where:
- \( Q_t \) is the amount of drug released in time \( t \),
- \( K_H \) is higuchi dissolution constant.

Higuchi describes drug release as a diffusion process based in the Fick’s law, square root time dependent.

**d. Korsmeyer and Peppas model**

This model is generally used to analyse the release of pharmaceutical polymeric dosage forms, when the release mechanism is not well known or when more than one type of release phenomena could be involved.

\[ M_t / M = K \cdot t^n \]

where:
- \( M_t / M \) is the fraction of drug release
- \( K \) is the release constant
- \( t \) is the release time
- \( n \) is the diffusion exponent for the drug release that is dependent on the shape of the dosage form.
The results obtained from *in-vitro* studies were plotted adopting five different mathematical models of data treatment as follow:

- Cumulative percent drug release v/s time (Zero order rate kinetics).
- Log cumulative percent drug retained v/s time (First order rate kinetics).
- Cumulative percent release v/s square root time (Higuchi matrix).
- Log of cumulative percent drug released v/s log time (Peppas exponential equation).

**Stability studies**

Selected formulation was subjected to stability studies for a period of 2 months at an accelerated condition at 40±2 °C/75±5% RH due to lack of time to carry out for 6 months as per new ICH guidelines. Microspheres were evaluated for drug content, entrapment efficiency and *in-vitro* drug release studies [11].

**RESULTS AND DISCUSSION**

**Evaluation of uncoated Tramadol Hydrochloride microspheres**

**Percentage Yield**

The percentage yield of formulations, M1-M4, was found to be 72.7, 76.6, 79.9 and 84.8% respectively. The results indicated that as the amount of polymer increases, the number of microspheres formed increases and hence the percentage yield increases. The results obtained are given in Table 5.

**Particle Size**

The particle sizes of formulations, M1-M4, were found to be 25.5, 26.2, 27.6 and 29.0 µm respectively. The results indicated that with increase in polymer concentration in the microspheres from M1-M4, the particle size of the microspheres increases. This increase in particle size can be attributed to an increase in viscosity with increase in polymer concentration, which resulted in larger emulsion droplets and finally in greater microsphere size. The results obtained are shown in Table 5.

**Surface Morphology**

The SEM studies of uncoated Tramadol Hydrochloride microspheres showed that they were spherical in shape and with a smooth surface. Very less particulate matter of the drug were seen on the surface of the microspheres indicating uniform distribution of the drug in the polymer network. SEM photographs of formulation M4 are shown in Figure 1.

**Flow Properties**

The values of angle of repose of formulations, M1-M4, were found to be 25° 12", 26° 34", 26° 52" and 27° 77" respectively, which indicate good flow properties of the microspheres. The results obtained are given in Table 5.

**Drug Content**

As the polymer ratio was increased, the drug content increased with 32.85, 34.45, 36.67 and 39.02 mg, for formulations M1-M4 respectively. High drug content was observed for M4 formulation, thereby indicating that the polymer concentration plays a major role in drug content. The results obtained are depicted in Table 5.

**Drug Entrapment Efficiency**

As the polymer ratio was increased, the entrapment efficiency increased with 65.70, 68.90, 73.38 and 78.05% for formulations M1-M4 respectively. High entrapment efficiency was observed for M4 formulation, thereby indicating that increase in polymer concentration resulted in the formation of larger microspheres entrapping greater amount of drug. The results obtained are listed in Table 5.

**In-vitro drug Release**

The *in-vitro* release profiles obtained for all formulations, M1-M4, are shown in Figure 2.

A high burst release of 40.24% was observed from the formulation M1, which contain 1:1 drug polymer ratio; whereas a less burst release of 20.62% was observed from M4, which contain 1:4 drug polymer ratio. Within 4 hrs 60-90% of drug was released from the formulations M1-M4. These results indicated that formulation with lesser drug-polymer ratio shows higher drug release.

This type of high burst release in stomach and small intestine is not satisfactory for a formulation, which is supposed to release its contents in the colon. In order to prevent the drug release in stomach and small intestine these uncoated Tramadol Hydrochloride microspheres were coated with ES-100, which shows solubility at a pH ≥7. Since M4 formulation showed high drug entrapment efficiency and controlled drug release pattern, it is selected for coating process.

**Evaluation of coated Tramadol Hydrochloride microspheres**

**Percentage Yield**

The percentage yield of formulations, MC1-MC4, was found to be 79.8, 83.7, 88.9 and 90.8% respectively. The results indicated that as the amount of polymer increases, the number of microspheres formed increases and hence the percentage yield increases. The same are shown in Table 6.

**Particle Size**

The particle sizes of formulations, MC1-MC4, were found to be 120.00, 122.47, 124.02 and 126.00 µm respectively. The results indicated that with increase in polymer concentration in the microspheres from MC1-MC4, the particle size of the microspheres increases. This increase in particle size may be due to corresponding increase in the polymer concentration that results in larger emulsion droplets. The results obtained are shown in Table 6.
Surface Morphology

The SEM studies of coated Tramadol Hydrochloride microspheres showed that coated Tramadol Hydrochloride microspheres exhibited smooth surface and spherical shape. SEM photographs of formulation MC4 is shown in Figure 3.

Flow Properties

The values of angle of repose of formulations, MC1-MC4, were found to be 23° 67”, 22° 99”, 22° 07” and 21° 91” respectively, which indicate good flow properties of the microspheres compared to uncoated Tramadol Hydrochloride microspheres. The results obtained are shown in Table 6.

Drug Content

As the polymer ratio was increased, the drug content increased with 44.40, 45.89, 46.57 and 48.50 mg considering 20 mg for formulations, MC1-MC4 respectively. High drug content was observed for MC4 formulation thereby indicating that the polymer concentration plays a major role in drug content. The results obtained are depicted in Table 6.

Drug Entrapment Efficiency

As the polymer ratio was increased, the entrapment efficiency increased with 88.80, 91.78, 93.14 and 97.00% for formulations MC1-MC4 respectively. High entrapment efficiency was observed for MC4 formulation thereby indicating that the polymer concentration plays a major role in drug content. The results obtained are depicted in Table 6.

In-vitro drug Release

The in-vitro release profile obtained for all formulations, MC1-MC4, are shown in Figure 4 and Figure 5. The cumulative percent drug release after 12 hrs was found to be 95.73, 85.83, 78.00 and 69.66% for formulations MC1-MC4 respectively.

As expected, there was no measurable drug release observed at acidic pH 1.2 and acetate pH 4.5 for 4 hrs, this may be due to coating of uncoated Tramadol Hydrochloride microspheres with ES-100 polymer. The results showed that release of Tramadol Hydrochloride slowed down as the concentration of coating polymer increased. This could be attributed to an increase in the density of the polymer matrix and the diffusional path length that the drug has to traverse.

The cumulative percentage drug release from MC4 formulation coated with ES-100 (1:7) showed the desire rate, as there was no drug release observed up to 4 hrs in acidic buffer pH 1.2 and acetate buffer pH 4.5. While in colonic fluid controlled drug release was observed due to dissolution of the Eudragit coat at pH 7.4 and the sodium alginate were degraded on exposure to the colonic fluid, thus resulting in drug release.

Kinetic Studies

In order to study the exact mechanism of drug release from coated Tramadol Hydrochloride microspheres, drug release data were fit into various mathematical models, zero order, first order, higuchi matrix and peppas and regression co-efficient are depicted in Table 7.

These values were compared with each other for model fitting equation. Based on the highest regression values (r), the best fit model for MC1-MC4 was Peppas.

Further Korsmeyer and Peppas equation resulted into the values of n > 1, which appears to indicate that the release from the coated Tramadol Hydrochloride microspheres was by Super Case II transport, indicating that the release mechanism is dominated by the erosion and swelling of the polymer.

Stability Studies

The results of the stability studies indicated that the microspheres did not show any changes in the drug content and entrapment efficiency during the stability study period. The percentage cumulative drug release after 6 months showed 70.23% after 12 hrs indicating no significant changes. The results obtained were depicted in Table 8 and Figure 6.

Table 1. Preparation of uncoated Tramadol Hydrochloride microspheres

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Drug: Polymer Ratio</th>
<th>CaCl₂ Solution</th>
<th>Speed of Rotation</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>1:1</td>
<td>10% w/v</td>
<td>500 rpm</td>
</tr>
<tr>
<td>M2</td>
<td>1:2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M3</td>
<td>1:3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M4</td>
<td>1:4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2. Angle of Repose as an Indication of Powder Flow Properties

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Angle of Repose</th>
<th>Type of Flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&lt; 20</td>
<td>Excellent</td>
</tr>
<tr>
<td>2</td>
<td>20 – 30</td>
<td>Good</td>
</tr>
<tr>
<td>3</td>
<td>30 – 34</td>
<td>Passable</td>
</tr>
<tr>
<td>4</td>
<td>&gt; 34</td>
<td>Very Poor</td>
</tr>
</tbody>
</table>
Table 3. Preparation of coated Tramadol Hydrochloride microspheres

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Drug: Polymer Ratio (ES-100)</th>
<th>Conc. Of Span 80</th>
<th>Speed of Rotation</th>
</tr>
</thead>
<tbody>
<tr>
<td>MC1</td>
<td>1:4</td>
<td>0.2%</td>
<td>600 rpm</td>
</tr>
<tr>
<td>MC2</td>
<td>1:5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MC3</td>
<td>1:6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MC4</td>
<td>1:7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Kinetic studies

<table>
<thead>
<tr>
<th>Release Exponent (n)</th>
<th>Drug Transport Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>n = 0.5</td>
<td>Fickian diffusion or square root of time kinetics</td>
</tr>
<tr>
<td>0.5&lt;n&lt;1</td>
<td>Anomalous (non-Fickian) diffusion</td>
</tr>
<tr>
<td>n = 1</td>
<td>Case – II transport</td>
</tr>
<tr>
<td>n&gt;1</td>
<td>Super Case II transport</td>
</tr>
</tbody>
</table>

Table 5. Evaluation of uncoated Tramadol Hydrochloride microspheres

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Evaluation parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Yield (%)</td>
</tr>
<tr>
<td>M1</td>
<td>72.7</td>
</tr>
<tr>
<td>M2</td>
<td>76.6</td>
</tr>
<tr>
<td>M3</td>
<td>79.9</td>
</tr>
<tr>
<td>M4</td>
<td>84.8</td>
</tr>
</tbody>
</table>

Table 6. Evaluation of coated Tramadol Hydrochloride microspheres

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Evaluation parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Yield (%)</td>
</tr>
<tr>
<td>MC1</td>
<td>79.8</td>
</tr>
<tr>
<td>MC2</td>
<td>83.7</td>
</tr>
<tr>
<td>MC3</td>
<td>88.9</td>
</tr>
<tr>
<td>MC4</td>
<td>90.8</td>
</tr>
</tbody>
</table>

Table 7. Kinetics release study of coated Tramadol Hydrochloride microspheres

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Zero Order</th>
<th>First Order</th>
<th>Higuchi Matrix</th>
<th>Peppas Plot</th>
<th>Best Fit Model</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r² values</td>
<td>‘n’ values</td>
<td>Peppas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MC1</td>
<td>0.9939</td>
<td>0.8396</td>
<td>0.6086</td>
<td>0.9942</td>
<td>2.1474</td>
</tr>
<tr>
<td>MC2</td>
<td>0.9801</td>
<td>0.9324</td>
<td>0.6345</td>
<td>0.9905</td>
<td>2.3009</td>
</tr>
<tr>
<td>MC3</td>
<td>0.9816</td>
<td>0.9383</td>
<td>0.5959</td>
<td>0.9961</td>
<td>2.5849</td>
</tr>
<tr>
<td>MC4</td>
<td>0.9853</td>
<td>0.9352</td>
<td>0.5065</td>
<td>0.9988</td>
<td>2.3948</td>
</tr>
</tbody>
</table>

Table 8. Evaluation of MC4 during stability study

<table>
<thead>
<tr>
<th>Drug Content (mg)</th>
<th>Time (Days) (Accelerated condition at 40±2 °C &amp; 75±5 % RH)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Entrapment Efficiency (%)</td>
<td>97.00</td>
</tr>
</tbody>
</table>

Figure 1. SEM of M4 formulation of uncoated Tramadol Hydrochloride microsphere

Figure 2. Percentage CDR of uncoated Tramadol Hydrochloride microspheres
CONCLUSION

The present study has been a satisfactory attempt to formulate microparticulate system for colon targeted delivery of Tramadol Hydrochloride using natural polymer (Sodium alginate) and pH sensitive polymers (ES-100 and EL-100). From experiments, it can be concluded that in order to prevent the drug release in stomach and small intestine these uncoated Tramadol Hydrochloride microspheres were coated with ES-100. The % CDR from MC4 formulation coated with ES-100 (1:7) showed desire rate, as there was no drug release observed up to 4 hrs, while in colonic fluid controlled drug release was observed.

REFERENCES