Natural Gellan Gum (Phytagel®) Based Novel Hydrogel Beads of Rifampicin for Oral Delivery with Improved Functionality

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ABSTRACT

Background: Despite exhaustive global efforts, tuberculosis (TB) continues to higher rates of infection and drug-resistance rates are also raising to alarming levels. Sustained release dosage forms are gaining higher acceptance over conventional dosage forms in the treatment of several chronic conditions including TB. The aim of this study was to investigate whether the novel particulate system improved the drug release profile of rifampicin when formulated with natural polymer based hydrogel beads along with beta cyclodextrin as a solubility enhancer.

Materials and Methods: Particulate hydrogel beads were prepared by ionotropic gelation method using divalent calcium cations. Prepared beads were coated with chitosan through polyelectrolyte complexation. Formulations were evaluated for drug content, particle size, swelling index and in-vitro dissolution, and further characterized by Scanning Electron Microscopy, Fourier Transfer Infrared Spectroscopy, Differential Scanning Calorimetry and X-Ray Diffractometry.

Results: All the formulations showed sustained drug release but the formulation G2 exhibited highest amount of drug release among all. While considering the formulations with cyclodextrin the release rate is slightly increased at initial hours. The formulations with chitosan coating showed sustained release up to 24 h.

Conclusion: Utilization of natural polymers was proved to be effective in formulating a novel particulate sustained release solid dosage form of rifampicin and presence of cyclodextrin proved to be useful in solubility enhancement which can achieve higher drug release.

Key words: Microbeads, Hydrogels, Tuberculosis, Ionotropic gelation, Chitosan.

INTRODUCTION

The higher rates of tuberculosis (TB) infection and multidrug resistance are rising to alarming levels despite of exhaustive global efforts. Even discovering new agents for treating this bacterial pathogen imparts new challenges, but these challenges have been faced throughout the entire history of research into anti-infective.1 Mycobacterium tuberculosis is bacteria which can lead to serious infections of lungs, genitourinary tract, skeleton and meninges. Treating TB as well as other mycobacterial infections involves therapeutic problems. The organisms grow slowly and the disease may take six months to two years for the treatment. Resistant organisms readily emerge, particularly in patients who have had prior therapy or who fail to adhere to the treatment protocol. It is currently estimated that about one third of the world’s population is infected with M. Tuberculosis with thirty million people having active disease. Worldwide, eight million new cases occur and two million people die of the disease each year.2 New advancements in the drug delivery strategies for the treatment of TB are reported to be minimizing the unwanted toxicities and improving the efficacy of the treatment.3 Sustained release dosage forms are gaining higher acceptance over conventional dosage.
forms in the treatment of several chronic conditions including TB. In recent years there is great interest in developing sustained drug delivery systems by using biopolymers to provide many advantages such as reduced side effects, improved drug utilization and decreased dosing frequency when compared with conventional dosage forms.\(^3,^5\) Hence, sustained release dosage forms are designed to achieve a prolonged therapeutic action with continuous release of medication over an extended period of time after a single dose. The encapsulation of drugs using different biodegradable and biocompatible polymers has been paid much attention in recent years.\(^6,^7\) Encapsulation of drugs into a suitable matrix that can protect during exposure to the harsh condition of the human gastrointestinal tract. In addition, encapsulation also will help in attaining the sustained release over a long period of time. Hydrogel microbead prepared by ionotropic gelation method using natural based biocompatible polymers has been widely accepted as an oral sustain release dosage form.\(^8,^11\) Gellan gum (Phytagel\(^9\)) hydrogel beads are prepared either by its cross linking or polymerization of monomers and cross linking with poly functional monomers. The release of drug involves a simultaneous absorption of water and desorption of drug via a swelled controlled mechanism. Gellan gum forms a complex with chitosan, hence, the porosity of the beads will get decreases and thereby reduces the leakage of the encapsulated drugs.\(^12\) Cycloextrins (CDs) have been used as complexing agents to increase the aqueous solubility and stability of poorly soluble drugs. In addition, CDs have also been used to reduce gastrointestinal or ocular irritation, to mask unpleasant taste and prevent drug-drug and drug-additive interactions. CDs have now to constitute a powerful tool as permeation enhancer by decreasing the resistance of aqueous barrier or by modification of the structurally lipid layer of biological membranes.\(^13\)

Even though there are extensive works are published on the sustained release dosage forms such as matrix tablets and gastro retentive beads of rifampcin. There is lack of scientific data which explains the use of natural based gellan gum hydrogel microbeads of rifampcin. In the present study, an attempt was made to design sustained particulate hydrogel based drug delivery system along with utilization of hydroxy-propyl \(\beta\)-cyclodextrin (HPBCD) as a solubility enhancer in the formulation, in order to improve the release property of poorly soluble rifampcin from matrix formulations.

**MATERIALS AND METHODS**

**Materials**

Rifampicin (Micro Labs Ltd. Hosur, Tamil Nadu, India), chitosan (Indian seafoods Ltd., Cochin, India), and hydroxy-propyl \(\beta\) cyclodextrin (Gangwal Chemical, Mumbai, India) were obtained as gift samples. Gellan gum (Phytege\(^9\)) was purchased from Sigma-aldrich, Bengaluru, India. All other chemicals and solvents of analytical grade were purchased locally and used with further purification.

**Methods**

**Preparation of Microbeads**

The experimental setup requires a beaker containing calcium chloride and chitosan solution placed on a magnetic stirrer with arrangement for hypodermic syringe \(^{\#20}\) above beaker at fixed height filled with drug and polymer solution. Rifampicin loaded hydrogel beads were prepared employing ionotropic gelation technique\(^{11}\) using gellan gum a natural matrix forming polymer and chitosan as a complexation agent. Ionotropic gelation technique was applied to prepare cross linked hydrogel beads using Ca\(^{2+}\) as cationic component, chitosan as a polyelectrolyte complexing agent. The required quantity of gellan gum was soaked in distilled water (12 ml) for 12 h and the mixture was heated at 60°C to get a uniform solution of coating material. HPBCD also dissolved in sufficient amount of distilled water. The rifampicin solution in ethanol was mixed with HPBCD solution. The resultant solution was then added to aqueous solution of gellan gum with constant stirring to get complete homogenous solution. The bubble-free solution obtained was kept on rest for 30 min. The mixture was dropped using a \#20 hypodermic syringe drop wise into 50 ml of 3% w/v calcium chloride solution. Formulations with chitosan also prepared in the similar manner using solution of chitosan previously prepared in 0.5% v/v acetic acid. The beads formed were washed twice with distilled water, filtered and dried for 2-3 h in a hot air oven at 40°C temperature (Table 1). The beads thus obtained were packed in the aluminum foils and placed in a decicator till further use.

**Evaluation Parameters of Microbeads**

**Visual appearance**

The visual appearance of prepared beads was recorded by visual observation about the colour, shape and physical texture.
Percentage yield

The percentage yield of prepared beads was calculated to know about feasibility of the methodology adopted for the preparation of beads. Percentage yield was calculated using the following formula:\(^14\)

\[
\text{Percentage Yield} = \frac{\text{Actual weight of beads}}{\text{Theoretical weight of beads}} \times 100
\]

Drug content estimation

The rifampicin content in the microbeads was determined by a digestion method. The active drug-loaded beads (10 mg) were crushed and incubated in 10 ml methanol/phosphate buffer (pH=7.4) at room temperature for 24 h. The suspension was then filtered. The filtrate was assayed by spectrophotometric method at 467.6 nm. Supernatant from the dummy microbeads (without rifampicin) was taken as blank. All samples were analyzed in thrice for robustness. The drug content was determined using standard calibration curve and percentage was calculated by formula.\(^15\)

\[
\text{Percent drug content} = \frac{\text{Estimated drug content}}{\text{Theoretical drug content}} \times 100
\]

In-vitro drug release studies

The *in vitro* drug release studies were performed in both simulated gastric fluid (SGF) pH 1.2 and simulated intestinal fluid (SIF) pH 7.4. An accurately weighed beads equivalent to 50 mg of rifampicin was placed in rotating basket (USP-I Dissolution apparatus) dipped into 900 ml of SGF as a dissolution medium initially for 2 h followed with SIF up to 24 h. 200 mg ascorbic acid was added as an antioxidant\(^16\) to the dissolution media in order to avoid the decomposition of rifampicin by oxidation. The basket was rotated at 75 rpm and temperature was maintained at 37 ± 0.5°C throughout the experiment. At fixed time intervals, aliquots (5 ml) was withdrawn and suitably diluted. The fresh media was replaced after each sample withdrawal to maintain the constant volume. The amount of drug present in the withdrawn sample was estimated using UV-visible spectrophotometer\(^14\) against blank. The studies were carried out in triplicate to maintain robustness. The percentage drug release at various time intervals was calculated and plotted against time.\(^17\)

Calculation of release kinetics

In order to understand the mechanism and kinetics of drug release, the data of *in vitro* drug release study were fitted into various kinetic models such as zero order (percent drug release \(vt\) time). In order to define a model which will represent a better fit for the formulation, drug release data was further analyzed by Koare-mayer Peppa sequation. \(R^2\) values were calculated for the linear curves obtained by regression analysis of the above plots.

Characterization of Microbeads

Particle size determination

The size of the prepared hydrogel beads was measured by the optical microscopy method using a calibrated stage micrometer. Fifty randomly chosen beads were taken to measure their individual size. Beads were visualized under 10X magnification. Particle size was calculated by using the following formula.\(^18\)

\[
\text{Average particle size} = \frac{\text{Snd}}{\text{Sn}}
\]

Where, \(n = \) total number of microbeads, \(d = \) midpoint of the size range.

Determination of swelling index

The swelling property of prepared beads was determined in SIF. 50 mg of beads (\(W_o\)) were placed in a glass vial containing 10 ml of SIF and allowed to swell at 37°C. The swollen beads were periodically removed, blotted with filter paper to remove the excess moisture and weight was determined (\(W_t\)). The study was carried out in triplicate to maintain robustness. The percentage swelling of beads was calculated from the formula as below:\(^19,20\)

\[
\text{Swelling Index} = \frac{(W_t - W_o)}{W_o}
\]

Where, \(W_o = \) initial weight and \(W_t = \) final weight

Surface morphology determination

The particle size, shape and surface morphology of the microbeads were examined by Scanning Electron Microscopy (SEM). The SEM analysis was carried out using SEM instrument coupled EDAX Model-JEOI-SEM 6360. A small amount of microbeads was spread on glass stub. The stub was placed in the SEM chamber and coated with platinum using a sputter coater. The microphotographs of beads were taken at the acceleration voltage of 5 Kv, chamber pressure of 0.01 mmHg.\(^21,22\)

FTIR spectroscopy

FTIR spectroscopy is a qualitative analytical technique, which offers the possibility of detecting chemical interactions between drug and excipients in the formulation.
Infrared spectra of the samples were recorded on Fourier Transform Infrared Spectrometer (Bruker Alpha-T). The spectra of rifampicin and its different formulations were recorded and evaluated for compatibility within the formulations.23

**Thermal analysis**

Differential Scanning Calorimetry (DSC) is an important tool used to detect the drug-excipient compatibility, because it shows changes in the appearance, shift of melting endotherms/exotherms and/or variation in the corresponding enthalpies of reaction. Thermal behavior of the beads was examined by using a thermal analyzer (Mettler Toledo, Japan). The DSC thermograms of pure rifampicin and its formulations were recorded on a thermal analyzer. The thermal analysis was performed at a heating rate of 10°C/min over temperature range of 50-400°C under a nitrogen atmosphere in a micro calorimeter and then thermograms were obtained.24

**X-ray diffraction study (XRD)**

XRD technique is useful tool to get information about change in crystalline nature of drug as well as excipients. The optimized formulations were subjected for XRD study using D8 advance diffractometer.25,26

**RESULTS AND DISCUSSION**

**Evaluation Parameters**

The microbeads were subjected for evaluation parameters such as visual appearance, percentage yield, drug content and *in vitro* drug release profile. All the formulations appeared to be reddish brown free flowing microbeads with nearly spherical in shape. The percentage yield was in the range of 82 to 99.14%. The percent drug content was found to be in the rage of 72.35-100.20. Drug release from the different microbeads was studied for first 2 h in simulated gastric fluid (phosphate buffer pH 1.2) followed by in simulated intestinal fluid (phosphate buffer pH 7.4) for up to 24 h. The results of *in vitro* studies indicated that the rate and extent of drug release were decreased significantly with an increase in polymer concentration, which may be attributed to increase in the density of polymer matrix followed by increasing diffusional path length for drug molecules. Initially the *in vitro* drug release was found to be high. This initial high rate of release may be due to less swollen thin polymer film present on the surface of microbeads. The drug release from formulation G1 and G2 with HPBCD exhibited 67.21% and 71.49% respectively, which is highest percent of release at the end of 24 h among all the formulations. This may be due to presence of HPBCD in the formulation enhanced the solubility of drug. Formulation G4, G5, and G6 with both HPBCD and chitosan showed 67.20, 63.86 and 52.90% drug release respectively. The formulation G7, G8 and G9 with chitosan showed least amount of release among all formulations.

Formulation G4 showed relatively good release extended period may be due to presence of HPBCD and chitosan coating on the surface of microbeads. Formulation G7 showed least release among all the formulations may be due to absence of HPBCD. Formulation G4, G5, and G6 although contain HPBCD exhibited less release with extended period of time. That may be due to chitosan coating on the surface of beads. The microbeads prepared with gellan gum and coated with chitosan exhibited excellent matrix forming and release retarding properties and found suitable for development of a once-daily sustained release particulate formulations of rifampicin.27,28 Dissolution profiles of all formulations were shown in Figure 1. The release rate kinetic data for all the models is shown in Table 3. Drug release data of formulation G4 showed good fit into zero order and the Higuchi equation (R²=0.890, and R²=0.971, respectively). The formulation G2 showed high linearity with Korsmeyer-peppas equation (R²=0.916) and indicated combined effect of diffusion and erosion mechanisms for controlled drug release.29,30

**Characterization Parameters**

The microbeads were characterized by various characterization tools. The size of microbeads was measured by optical microscopy method and which is found in the range of 660 to 980 μm. The particle size of higher micron range may be due high viscosity of the dropping solution (Table 2). The percent swelling index of formulations was found to be in the range of 12 to 27. The swelling index of formulation G2, G5 and G8 was found to be relatively highest may be due higher proportion of gellan gum used in these formulations. The formulation G1, G4 and G7 showed average swelling index as these formulations comprise equal core-coat ratio. Whereas, formulation G3, G6 and G9 exhibited the least swelling index may be due to less amount of coating material used (Table 2). Figure 2 shows SEM microphotographs of pure rifampicin and microbeads prepared with and without chitosan. The microphotographs revealed that beads obtained were nearly spherical in shape with rough surface associated with dentations and pores in case of microbeads prepared without chitosan, whereas the surface was found relatively smooth with lesser dentation and pores in case of microbeads coated with chitosan. Figure 3 shows SEM
microphotographs of hydrogel formulations taken at different time intervals from dissolution media in order to observe and identify the mechanism of drug release based on surface morphology of beads. Formulation G1 was selected for the SEM analysis based on its performance of other studied parameters. Sample G1 was taken out from the dissolution media at the intervals of 0, 2, 6 and 24 h and allowed to dry at room temperature, the microphotographs were taken. There were no remarkable changes found in the surface morphology of beads after two hour interval, indicated that drug release followed swelling mechanism. The SEM micropho-
In an effort to investigate the possible incompatibilities between drug and polymer, we have carried out FTIR analysis. The spectrum of pure rifampicin shows characteristic peaks at 3480 cm\(^{-1}\) (-OH bonded), 2970 cm\(^{-1}\), 2930 cm\(^{-1}\) (-CH\(_3\)), 1715 cm\(^{-1}\) (C=O acetyl), 1640 cm\(^{-1}\) (C=O furanone), 1620 cm\(^{-1}\) (amide I), 1540 cm\(^{-1}\) (amide II), 1250 cm\(^{-1}\) 1040 cm\(^{-1}\) 1020 cm\(^{-1}\) (-C-O-C- acetyl) (Figure 4). The spectra of formulations retained all the characteristic peaks of rifampicin indicating that the drug is intact in the formulation and there is no interaction between drug and polymer. As shown in the Figure 5 the DSC thermogram of rifampicin showed a sharp endothermic peak at 189.46°C which corresponds to the melting point of pure drug. DSC thermogram of formulation G1, G4 and G7 showed broad endothermic peaks with slight shifting towards higher and lower level may be due to high and low calorie consumption. The broad endothermic peak exhibited by the above formulations indicated that the drug is intact and perfectly dispersed in the molecular level in polymer matrix. Crystallinity of a compound can be studied with X-ray diffraction analysis. As rifampicin is highly crystalline in nature it can easily observed in X-ray diffraction (XRD) pattern as shown in Figure 6. X-ray dfractograms of pure drug has shown its own characteristic crystal peaks between 20 of 10\(^{\circ}\) to 20\(^{\circ}\) (Figure 6). This characteristic needle like sharp incensed peaks indicated the highly
crystalline nature of pure rifampicin alone. Whereas, these characteristic peaks of drug were absent in prepared microbeads. The formulations showed undefined, broad diffused peaks with the low intensities indicated that the drug is present in amorphous form due to formation of complexation with HPBCD.35

**CONCLUSION**

As conclusion, rifampicin encapsulated gellan gum hydrogel based microbeads were successfully prepared by ionotropic gelation method and coated with another natural polymer chitosan through polyelectrolyte complexation. Some of the formulations were developed with cyclodextrin as a solubility enhancer for poorly soluble rifampicin in the polymer matrix. The encapsulation technique in preparing hydrogel based beads was found to be simple, mild, easily controllable, and reproducible. In addition, the natural polymers used for the formulation such as gellan gum and chitosan are biodegradable and biocompatible. Encapsulation through ionotropic gelation and coating through polyelectrolyte

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>G1</th>
<th>G2</th>
<th>G3</th>
<th>G4</th>
<th>G5</th>
<th>G6</th>
<th>G7</th>
<th>G8</th>
<th>G9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rifampicin (gm)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.5</td>
<td>1.0</td>
<td>1.5</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.5</td>
</tr>
<tr>
<td>Gellan gum (gm)</td>
<td>1.0</td>
<td>1.5</td>
<td>1.0</td>
<td>1.0</td>
<td>1.5</td>
<td>1.0</td>
<td>1.5</td>
<td>1.0</td>
<td>1.5</td>
</tr>
<tr>
<td>Ethanol (mL)</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Chitosan (mg)</td>
<td>-</td>
<td>-</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>250</td>
<td>250</td>
</tr>
<tr>
<td>HPBCD (gm)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CaCl₂ (%)</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
<td>3.0</td>
</tr>
<tr>
<td>Distilled water</td>
<td>q.s.</td>
<td>q.s.</td>
<td>q.s.</td>
<td>q.s.</td>
<td>q.s.</td>
<td>q.s.</td>
<td>q.s.</td>
<td>q.s.</td>
<td>q.s.</td>
</tr>
</tbody>
</table>

**Table 2: Physico-chemical parameters of microbeads**

<table>
<thead>
<tr>
<th>Batch</th>
<th>Visual appearance</th>
<th>% yield</th>
<th>Size (µm)</th>
<th>% drug content</th>
<th>Swelling Index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>Reddish brown</td>
<td>82.66</td>
<td>660</td>
<td>95.86 ± 0.26</td>
<td>0.20</td>
</tr>
<tr>
<td>G2</td>
<td>Reddish brown</td>
<td>99.14</td>
<td>920</td>
<td>78.23 ± 0.29</td>
<td>0.24</td>
</tr>
<tr>
<td>G3</td>
<td>Reddish brown</td>
<td>97.71</td>
<td>980</td>
<td>83.52 ± 0.31</td>
<td>0.14</td>
</tr>
<tr>
<td>G4</td>
<td>Reddish brown</td>
<td>71.66</td>
<td>830</td>
<td>98.70 ± 0.23</td>
<td>0.18</td>
</tr>
<tr>
<td>G5</td>
<td>Reddish brown</td>
<td>84.28</td>
<td>860</td>
<td>98.25 ± 0.25</td>
<td>0.26</td>
</tr>
<tr>
<td>G6</td>
<td>Reddish brown</td>
<td>78.28</td>
<td>980</td>
<td>72.35 ± 0.26</td>
<td>0.14</td>
</tr>
<tr>
<td>G7</td>
<td>Reddish brown</td>
<td>91.50</td>
<td>920</td>
<td>100 ± 0.20</td>
<td>0.16</td>
</tr>
<tr>
<td>G8</td>
<td>Reddish brown</td>
<td>86.40</td>
<td>960</td>
<td>86.47 ± 0.21</td>
<td>0.22</td>
</tr>
<tr>
<td>G9</td>
<td>Reddish brown</td>
<td>90.80</td>
<td>980</td>
<td>99.68 ± 0.23</td>
<td>0.12</td>
</tr>
</tbody>
</table>

**Table 3: In-vitro drug release kinetics from different microbeads**

<table>
<thead>
<tr>
<th>Batch</th>
<th>Regression coefficient (R²)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Zero Order</td>
</tr>
<tr>
<td>G1</td>
<td>0.809</td>
</tr>
<tr>
<td>G2</td>
<td>0.853</td>
</tr>
<tr>
<td>G3</td>
<td>0.870</td>
</tr>
<tr>
<td>G4</td>
<td>0.890</td>
</tr>
<tr>
<td>G5</td>
<td>0.810</td>
</tr>
<tr>
<td>G6</td>
<td>0.859</td>
</tr>
<tr>
<td>G7</td>
<td>0.777</td>
</tr>
<tr>
<td>G8</td>
<td>0.793</td>
</tr>
<tr>
<td>G9</td>
<td>0.811</td>
</tr>
</tbody>
</table>

Values in parenthesis indicate 'n' values.
complexation found to be helpful in formulating once daily sustained release dosage form which were capable of maintaining constant drug level throughout 24 h. This can be expected to reduce the frequency of administration and improve the patient compliances. Inclusion of cyclodextrin proved to be useful in solubility enhancement of rifampicin. This hydrogel based microbeads formulation found to be better particulate drug delivery system over conventional dosage forms available in the market. However, more in vivo studies are required in order to confirm these observations.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

Authors declared no conflict of interest.

REFERENCES


SUMMARY

- Natural gellan gum based hydrogel beads of rifampicin were prepared by ionotropic gelation method and coated with chitosan through polyelectrolyte complexation.
- Prepared hydrogel beads of rifampicin were evaluated and characterized by battery of parameters.
- Natural based biocompatible polymer was proved to be effective in formulating a novel particulate sustained release solid dosage form of rifampicin.
- Presence of HPBCD in the particulate drug formulation proved to be enhanced the drug release profile from sustained release dosage forms.
- Chitosan coating was worked to further enhance the sustained release effect of the formulations.
- This system found to be useful in formulating once daily dosage form in order to reduce the frequent dosing and thereby enhance the patient’s compliance.

ABBREVIATIONS USED


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