A NOVEL APPROACH FOR THE MANAGEMENT OF TUBERCULOSIS

Sefeera A.A., Prof. Junise V., Prof. Dr. T.N.K. Suriya Prakash, Hafsa P.V. and Ajith Chandran

ABSTRACT

The present study deals with the formulation of Chitosan Microsphere loaded with Rifampicin with a view to developing an extended release formulation. In this preparation, polymers (chitosan and sodium alginates) were used with a fixed concentrations of Rifampicin [(1:1:1), (1:1:2), (1:1:3), (1:1:4), (1:2:1), (1:3:1), (1:4:1) and (1:2:2)] by ionotropic gelation/external gelation technique. All formulations show good flow properties and compatibility studies show that there was no interaction between drug and carrier used. The principle involved was the cation-induced gelation of alginate, for the simultaneous encapsulation of rifampicin. Ionotropic gelation was applied to prepare microspheres using combinations of chitosan and Ca$$^{2+}$$ as cationic components and alginate as the anion. The prepared Rifampicin microspheres are shown red color with elegant appearance, before drying and on drying, it appeared as reddish brown color. SEM and particle size analysis reported that the size of the particles was in micrometer range and all formulation showed average particle size below 10$$\mu$$m and with good % yield, good %drug content and %encapsulation efficiency. Results of dissolution studies show that formulation 3 (RM-3) gives good release profile compared to other formulations. A n d pharmacokinetic evaluation shows all the formulation shows zero-order kinetics with the Non-fickian mechanism of release.

INTRODUCTION

Tuberculosis (TB) is one of the most common causes of morbidity and mortality in the world today. Despite all the efforts, the disease is not coming to grip. Mycobacterium tuberculosis kills about 3 million people each year and in 1993, the World Health Organization declared
tuberculosis a global emergency. Tuberculosis (TB) is an infectious disease caused by Mycobacterium tuberculosis and rarely by other organisms of the “tuberculosis complex”. It is usually transmitted by inhalation of infected droplet nuclei which are discharged into the air when a patient with untreated sputum positive TB coughs or sneezes.[1,9,10]

Rifampicin is bactericidal to Mycobacterium tuberculosis and many other Gram positive and gram negative bacteria. The standard dose of Rifampicin in T.B treatment is 10mg /kg of body weight, corresponding to 600 mg in most populations. A Number of research works were conducted on rifampicin microsphere using different types of polymers.[1,9,10]

The main aim of my work was to modulate the release of rifampicin in the stomach and their by minimize the degradation of rifampicin in acidic medium. The proposed formulation has released the drug into the body for an extended period of time. In the case of multi-drug therapy, Rifampicin shows rapid degradation in the acidic condition of the stomach and the rate of degradation is increased when it used in combination therapy, conversion of rifampicin into microspheres will provide an extended release of drug in intestinal pH and reduce the rate of degradation in acidic condition.[9,14,16,18]

EXPERIMENTAL MATERIALS AND METHODS: MATERIALS
Rifampicin was brought from Yarrow Chem products Mumbai-421201 (India). Chitosan was purchased from Marine chemicals- Cochin, Kerala. Sodium alginate and calcium chloride were purchased from Nice-chemicals Pvt. Ltd, Cochin, Kerala and all other chemicals used were analytical grade.

1. PREFORMULATION STUDIES
1.1 MELTING POINT TEST
The deviation from the melting point of a sample is an indication of the impurities in the sample. A Small amount of Impurity can be determined by lowering as well as the widening of the melting point range. The melting point of Rifampicin was determined by the open capillary method.[17,18]

1.2 COMPATIBILITY (DRUG-POLYMER) STUDY
a) Fourier Transform Infrared Spectroscopic (FTIR) Analysis.
The study was carried out to find out the possible interaction between selected Drug Rifampicin and the polymer Chitosan and Sodium alginate. Samples of pure drug, pure
polymer and prepared microspheres were kept in a sample holder and scanned from 4000 cm$^{-1}$ to 400 cm$^{-1}$ in FT-IR spectrophotometer. The spectra obtained for these samples were compared and interpreted.$^{[2,5]}$

2. METHOD OF PREPARATION$^{[5,8,12]}$

Rifampicin microspheres prepared by using chitosan/sodium alginate as coat material, employing ionotropic/external gelation technique. 100 mg of drug was dissolved in 4 mL distilled water containing methanol (methanol/water) for the complete solubilization of rifampicin. 2 drops of Ascorbic acid is added in order to prevent oxidation of drug. To it was added 4 ml of sodium alginate solution (25 g/L). After thorough mixing, 20–30 min was allowed to elapse in order to make the solution bubble-free. The mixture was passed through a 2.5ml hypodermic syringe and allowed to fall dropwise at 60 drops/min into 150 ml of 0.1 M calcium chloride solution containing 4ml chitosan (25 g/L - which is previously dissolved in 0.5% acetic acid) (i.e. drug/alginate/chitosan 1:1:1). At pH 4 to 5, the beads formed instantaneously and were left as such for 4-6 h at room temperature. Subsequently, the beads were recovered by filtration, washed twice with distilled water and dried at room temperature.

3. CHARACTERIZATION OF RIFAMPICIN MICROSPHERES

3.1 PARTICLE SIZE DETERMINATION OF DIFFERENT BATCHES OF RIFAMPICIN MICROSPHERES BY USING OPTICAL MICROSCOPY

The Eyepiece of the microscope was fitted with a micrometer. The eyepiece micrometer was calibrated using a standard stage micrometer. The sample was prepared as a suspension in a suitable vehicle. The sample of the suspension was mounted on a slide and placed on a mechanical stage. The size of the particle was estimated with the help of the eyepiece micrometer. The diameter of 100 particles was determined by the number of divisions of eyepiece micrometer. This is then converted to microns. The particles were arranged on the basis of size ranges. The number of particles in each size range was then converted and tabulated.

Results are represented in Table No: 1

3.2 DETERMINATION OF PERCENTAGE YIELD OF RIFAMPICIN MICROSPHERE$^{[10]}$

Percentage yield of all formulations was calculated by the formula,
3.3 ESTIMATION OF DRUG CONTENT\(^{[10]}\)

The rifampicin content in the microsphere was determined by a digestion method. The rifampicin microspheres (10mg) were pulverized and incubated in 150 ml phosphate buffer (7.4) at room temperature for 24 h. The suspension was then centrifuged at 2000 rpm for 30 min. The supernatant with suitable dilution was assayed spectrophotometrically for rifampicin content at the wavelength of 475 nm. The absorbance of the solutions was measured using double beam UV-spectrophotometer against supernatant from the empty microcapsules was taken as blank. All samples were analyzed in triplicate.

\[
\text{% Drug content} = \frac{\text{drug content}}{\text{label claim}} \times 100
\]

Results are represented in Graph No: 1.

3.4 ESTIMATION OF ENTRAPMENT EFFICIENCY

A small portion of the Microsphere dispersion was centrifuged at 5,000 rpm for 1 hr. using Microcentrifuge (Remi). The supernatant was removed and the amount of unincorporated drug was measured by taking the absorbance of the appropriately diluted supernatant solution at 475nm using UV spectrophotometer against blank/control Microsphere dispersion. Drug entrapment is calculated using following equations:

\[
\text{% Entrapment efficiency} = \left(1 - \frac{M_{\text{supernatant}}}{M_{\text{total}}} \right) \times 100
\]

\(M_{\text{total}}\) → Mass of total drug,
\(M_{\text{supernatant}}\) → Mass of drug in supernatant solution, Results are represented in Graph No: 2.

3.5 MORPHOLOGICAL STUDY USING SCANNING ELECTRON MICROSCOPE

Scanning electron microscopy has been used to determine surface topography, texture, and to examine the morphology of fractured. SEM is probably the most commonly used method for characterizing of microspheres; SEM studies were carried out by using scanning electron microscope. Dry Rifampicin microspheres were placed on an electron microscope brass stub and coated within an ion sputter. A since vacuum field is necessary for images generation SEM. Photomicrograph of Rifampicin microspheres was taken by random scanning of the
stub. JSM-6390 Instrument with different accelerating voltage was used in this study and analytical techniques were carried out in SAIF- Technical Analysis Center- Cochin. The SEM photomicrograph of Rifampicin microspheres are shown in Figure No: 2 and 3.

3.6 IN VITRO DRUG RELEASE STUDY OF MICROCAPSULES

The in vitro dissolution studies were performed at two different pH values:

- pH(1.2) - simulated gastric fluid pH
- pH (7.4) - which is simulated intestinal fluid pH
- An accurately weighed sample was responded in dissolution media consisting 900 ml of 0.1 N (pH 1.2) HCl by using rotating basket method specified in USP XXIV. An amount of microcapsules equivalent to 100mg of rifampicin were taken in the basket. A speed of 75 rpm and temperature of 37±0.5°C was maintained throughout the experiment and the dissolution was done for 2 hours.

At fixed time intervals, aliquots (5ml) of the sample was withdrawn and replaced with fresh dissolution media and the withdrawn samples were diluted if required and then estimated for rifampicin concentration at 475 nm spectrophotometrically (Shimadzu Pharm spec UV-1700 series, Japan) against blank.

- Release study in phosphate buffer(pH 7.4)
- An accurately weighed sample was responded in dissolution media consisting 900 ml simulated intestinal fluid pH. (7.4 pH- phosphate buffer) by using the same method described above and dissolution was performed for 13 hours. At fixed time intervals, aliquots (5ml) of the sample was withdrawn and replaced with fresh dissolution media and the withdrawn samples were diluted if required and then estimated for rifampicin concentration at 475 nm spectrophotometrically (Shimadzu Pharm spec UV-1700 series, Japan) against blank.

RESULT AND DISCUSSION

1. PREFORMULATION TESTS

1.1 Melting Point Test

The melting point determination of Rifampicin was carried out by the open capillary method. The Melting point of Rifampicin was found to be 186°C-187°C.
FTIR – ANALYSIS

FT-IR Spectrum shows characteristic peaks of rifampicin within the formulation spectra and spectral reports showed that there is no incompatibility between the drug and polymer.

2. IMAGES OF PREPARED MICROSPHERE BEFORE DRYING

![Image](image.png)

Figure No: 1

3.1 PARTICLE SIZE AND SHAPE DETERMINATION OF DIFFERENT BATCHES OF RIFAMPICIN MICROSPHERES

a) By using optical microscope

<table>
<thead>
<tr>
<th>SI NO</th>
<th>FORMULATION CODE</th>
<th>AVERAGE SIZE (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>RM-1</td>
<td>2.3±0.9</td>
</tr>
<tr>
<td>2</td>
<td>RM-2</td>
<td>2.6±1</td>
</tr>
<tr>
<td>3</td>
<td>RM-3</td>
<td>3.0±1</td>
</tr>
<tr>
<td>4</td>
<td>RM-4</td>
<td>6±1</td>
</tr>
<tr>
<td>5</td>
<td>RM-5</td>
<td>7.75±1</td>
</tr>
<tr>
<td>6</td>
<td>RM-6</td>
<td>5.35±1</td>
</tr>
</tbody>
</table>
3.1 PERCENTAGE YIELD OF RIFAMPICIN MICROSPHERES

<table>
<thead>
<tr>
<th></th>
<th>RM-7</th>
<th>RM-8</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>7.3±1.3</td>
<td>9.55±1</td>
</tr>
</tbody>
</table>

Graph No: 3, Graphical representation of % yield of Rifampicin Microspheres.

Results showed that the percentage Yield of Rifampicin microspheres was within the range 33±0.2% to 60±0.11%.

3.2 ESTIMATION OF DRUG CONTENT

Graph No: 2 Graphical representation of % Drug content

Rifampicin Microspheres shows percentage drug content within the range of 75% to 96%, and formulation RM-3 shows maximum (95.83) % Drug content.
3.3 ESTIMATION OF ENTRAPMENT EFFICIENCY.

![Graph No: 3 Determination of percentage entrapment efficiency](image)

The result showed that the Rifampicin Microspheres had percentage entrapment Efficiency within the range of 83.4 ± 1% to 89.12± 1.2% Formulation RM-3 shows maximum percentage entrapment Efficiency.

3.4 MORPHOLOGICAL STUDY USING SCANNING ELECTRON MICROSCOPE

![SEM image of RM-3](image)

SEM reports show that the shape of rifampicin microspheres are spherical and smooth surfaced and with particle diameter are in the micrometer range.
3.5 IN VITRO DRUG RELEASE STUDY OF MICROSPHERES

Reports of in-vitro release kinetic study in (PH1.2 and PH 7.4) were indicated that about 8% to 10% drug is released within 2.5 hours at PH (1.2) and in PH (7.4) about 10±0.2% to 95.42±0.12% drug is released within 13 hours. All formulation shows zero -order kinetics with Non- Fickian mechanism of drug release.

SUMMARY

In this work, polymers (chitosan and sodium alginates) were used with a fixed concentrations of Rifampicin[(1:1:1), (1:1:2), (1:1:3), (1:1:4), (1:2:1), (1:3:1), (1:4:1) and (1:2:2)]. Microspheres were prepared for all these concentrations by ionotropic gelation/ external gelation technique. All formulations were showed good flow properties. Interaction studies (FTIR-Spectra) show that there was no interaction between drug and carrier used. The principle involved was the cation- induced gelation of alginate, for the simultaneous encapsulation of rifampicin. The prepared Rifampicin microspheres were shown red color with elegant appearance, before drying and on drying, it appeared as reddish brown color. SEM and particle size analysis showed that the size of the particles was in micrometer range, and all formulation shows average particle size in between 2 to 10µm and % yield in between 33±0.2% to 60±0.11%.% and 75% to 96% drug content and %encapsulation efficiency of RM-3 formulation shows maximum (89.12±0.016)%. In in-vitro dissolution studies formulation 3 (RM-3) gives good release profile (ie 95.42±0.2%) compared to other formulations and pharmacokinetic evaluation shows all the formulation shows zero-order kinetics with Non-fickian mechanism of release.
AKNOLEDGMENTS

I express sincere thanks to my family, friends and all the teaching and nonteaching staffs of Al Shifa College of Pharmacy, Perinthalmanna for their support and help throughout my work and also convey my thanks to Nishka labs- Hyderabad, SAIF analysis center Cochin, and Yarrow chem products Mumbai, for their analytical support in this work. Above all, I thank and submit my work in to the hands of ‘God’ the Almighty, who shows the path to the ladder of success.

REFERENCE

1. Book Reference

2. Journal reference
3. Fact sheet reference

4. Report reference

5. Patent reference

6. External Links- Reference
15. Prescribing Information For Rifadin By Sanofi-Aventis.