PREPARATION AND IN-VITRO CHARACTERIZATION OF PCL MICROSPHERES OF RIFABUTIN

Manoj Kumar

SLT Institute of Pharmaceutical Sciences, Guru Ghasidas Vishwavidyalaya, Bilaspur, C.G.

Correspondence to: E. mail: mrmanojkumar1@yahoo.co.in

ABSTRACT

Tuberculosis (TB) is a contagious, granulamatous communicable bacterial infectious disease caused by tubercle bacillus resides in the pulmonary tissues of the lungs. As Mycobacterium tuberculosis (MTB) infects mostly in the alveolar macrophage (M) cells, direct delivery of anti-tuberculosis agents to the MTB-infected M cells expected to exert effective bactericidal activity without severe systemic side effects associated with prolonged oral treatment. RB is clinically used as a standard component of a combination regimen for tuberculosis treatment in HIV infected patients where rifampin therapy is contraindicated. PCL is one of the biocompatible and biodegradable polymers having high hydrophobicity, to minimal toxicity. Therefore, it is widely used as controlled delivery of various drugs. In this connection I have developed RB loaded microsphere with PCL. The developed formulation was evaluated for particle size, surface morphology, drug content and in-vitro drug release. The content of PCL, drug content, stirring rate influence the size, drug loading and entrapment efficiency. Regardless of drug loading, the release profiles exhibited a rapid release phase followed a slow release phase. 7-8% of the entrapped drug was released within 8 h.
Keywords: Tuberculosis, rifampin, hydrophobicity.

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1. INTRODUCTION

Tuberculosis (TB) is a contagious, granulamatous communicable bacterial infectious disease caused by tubercle bacillus resides in the pulmonary tissues of the lungs. As Mycobacterium tuberculosis (MTB) infects mostly in the alveolar macrophage (M) cells, direct delivery of anti-tuberculosis agents to the MTB infected M cells expected to exert effective bactericidal activity without severe systemic side effects associated with prolonged oral treatment. [1, 2]. Rifabutin (RB) is a potent semisynthetic drug derived from rifamycin-S having high permeability and low solubility. It is active against MTB, including rifampicin resistant strains, and atypical mycobacteria, further RB is clinically used as a standard component of a combination regimen for tuberculosis treatment in HIV infected patients where rifampin therapy is contraindicated [3]. In order to improve the therapeutics efficacy of RB, many ways have been explored, like inhalable microparticles and liposomal drug delivery etc [4]. Microencapsulation with biodegradable polymer can provide a feasibility to overcome systemic side-effects and to target pulmonary tissues particularly alveolar macrophages [5].

PCL is one of the biocompatible and biodegradable polymers having high hydrophobicity, to minimal toxicity [6].Therefore, it is widely used as controlled delivery of various drugs [7,8]. Further Solvent evaporation technique for preparation of microsphere is one of the wide methods for microencapsulation. In the present study RB load. PCL microspheres were prepared in order to improve its therapeutic efficacy and reduced systemic side effects due to prolonged use.

2. MATERIAL AND METHODS

2.1. MATERIALS

RB was a gift from M/s Lupin laboratories (Aurangabad, India). PCL (MW 50,000) was supplied by Polyvinyl alcohol, Tween 80 and dichloromethane were obtained from Sigma Aldrich.

2.2. PREPARATION OF MICROSPHERE

RB loaded PCL microspheres were prepared by solvent evaporation technique [9]. Briefly 300 mg of micronized drug was dispersed in 3ml of dichloromethane containing PCL (300-750 mg). This dispersion was added drop wise into 40 ml of 1% (w/v) PVA aqueous solution. The resulting emulsion was stirred with a double-bladed propeller continuously for 40 min under ambient pressure followed by another 20 min under reduced pressure. Finally, microspheres were collected by filtration, washed with deionized water and dried in a vacuum 371esiccators at room temperature. The blank PCL microspheres were prepared by simple o/w solvent evaporation method, and the variables were kept constant during the preparation.

2.3. PARTICLE SIZE ANALYSIS

Particle size of microspheres was measured by a particle size analyzer (SLAD-2201, Shimadzu). For the analysis, the sample was prepared by suspending 50 mg of microspheres in 5ml of 0.2m filtered distilled water containing 2% (w/v) Tween 80 and then sonicating in a water bath for 3 min to prevent aggregation between microspheres. The particle size was expressed as the volume mean diameter in micrometer.

2.4. MORPHOLOGY OF MICROSPHERES

The surface morphology of microspheres was observed with scanning electron microscope (SEM) (JSM-6610, JEOL, Japan). Dried microspheres were mounted onto stubs using double-sided adhesive tape with conductive effect and analyzed with SEM.

2.5. ANALYSIS OF DRUG CONTENT

The drug content of RB loaded PCL microspheres were determined by UV-Visible spectrophotometer (UV-1800, Shimadzu, double beam). To 20 mg of microsphere in 1.2 ml of acetonitrile was added, then polymer was precipitated by addition of 8.8 ml of purified water. The resulting solution was centrifuged for 10 min at 10,000 rpm and the supernatant was collected for UV analysis. The absorbance was taken at 281 nm in photometric mode. The linearity of the response was verified over the concentration range of 10-100µg/ml (r^2 = 0.998). The content of RB was determined by extrapolation method. Drug loading and entrapment efficiency were calculated as follows: Drug loading = Mass of drug in microspheres/ Mass of microspheres × 100% and Encapsulation efficiency= Actual drug loading/ Theoretical drug loading × 100%

2.6. IN VITRO RELEASE BEHAVIOR

30 mg of microspheres were placed in 3ml of 0.05M phosphate buffer (PB) adjusted to pH 7.4 and incubated in a horizontal-shaker at 37 ∘C. At predetermined intervals, microspheres were centrifuged for 2 min at 500 rpm, then 0.1 ml of supernatant was extracted and 0.1 ml of fresh buffer was added. The extracted supernatant was diluted with 0.9 ml of mobile phase for UV analysis.

3. RESULTS AND DISCUSSION

3.1. ENCAPSULATION EFFICIENCY AND PARTICLE SIZE

Among several parameters of the formulation, the polymer concentration played an important role in the encapsulation of RB at a constant feed ratio of PCL to RB and the fixed stirring rate, the encapsulation efficiency of RB increased with the increasing concentration of the polymer. The increase in entrapment efficiency was mainly attributed to the increased viscosity of dispersion, which prevented the leakage of drug into the aqueous phase during the hardening stage. The viscosity of dispersion increased with the increasing concentration of the polymer, and the more viscous the dispersion was, the higher entrapment efficiency was achieved. This result is in agreement with other studies [10]. The increased polymer concentration can accelerate the solidification of the phase-separated polymer domain, which answers for the increase in particle size as well as the decrease in drug loss. In addition, the

increasing amount of drug addition led to an increase in encapsulation efficiency, shown in table-1. The probable reason for the increase in encapsulation efficiency is that the polymer was not overloaded with the amount of the drug [11].

Table-1: Particle size, drug loading and entrapment efficiency of microspheres (n=3)

Batch	PCL %	RB in mg	Stirring rate	Particle size	D.L(%±SD)	E.E(%±SD)
1	15	300	800	41.20±1.20	6.64±0.12	56.01±1.05
2	20	300	800	43.12±1.23	6.69±0.23	57.12±1.36
3	25	300	800	46.36±1.56	7.01±0.15	58.12±1.56
4	30	300	800	51.12±2.10	7.15±0.13	59.12±2.01
5	15	300	1000	39.24±1.25	6.69±0.45	60.12±1.25
6	20	300	1000	42.36±1.46	7.03±0.48	63.24±2.01
7	25	300	1000	44.13±2.13	7.24±0.49	69.01±1.29
8	30	300	1000	49.23±2.01	7.49±0.25	70.12±2.05
9	15	300	1200	36.25±2.12	7.31±0.15	75.01±1.59
10	20	300	1200	40.12±2.10	7.36±0.26	78.96±1.26
11	25	300	1200	43.56±1.37	7.9±0.56	80.12±1.56
12	30	300	1200	48.24±2.04	8.1±0.66	81.56±2.06

Finally, the effect of stirring rate was taken into account. Higher stirring rate led to smaller mean diameter and lower encapsulation efficiency. Which was due to the fact that higher stirring rate resulted into smaller emulsion droplets, facilitating the leakage of drug into the water phase [12].

3.2. MORPHOLOGY OF MICROSPHERES

The surface morphology and internal structure of microspheres were observed by SEM. The drug-loaded microspheres appeared spherical with diameters in the range of 10-250µm (Fig-1), and were easily found to be coarse and porous at a high magnification. The coarseness and porosity was commonly considered as a result of solvent removal during the terminal stage of the formulation [13].

However, the blank PCL microspheres prepared under the same conditions revealed the smooth surface without pores. Thus, the coarseness and porosity should not be caused by the preparation procedure itself, including the operation of reducing pressure. The marked morphology were probably due to the physicochemical characteristics of the model drugs, because the drug in salt form not only can generate osmotic pressure, but also easily leads to a phase separation phenomenon during the polymer coacervation [14].In order to confirm this explanation, an attempt to encapsulate varied drugs or compounds was made under the same conditions. These results supported the explanation that the coarseness and porosity was a result of the salt form of the model drug.
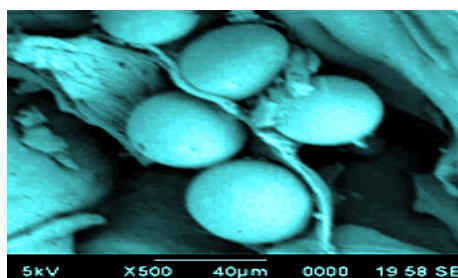

Figure 1: SEM photomicrographs of rifabutin loaded microsphere of PLC

3.3. IN VITRO RELEASE BEHAVIOR

In vitro release profiles of RB from the microspheres of different drug loading in phosphate buffer (0.05 M, pH 7.4) were observed. Regardless of drug loading, the release profiles exhibited a rapid release phase followed a slow release phase. 7-8% of the entrapped drug was released within 8 h. It is due to leaching of surface adsorbed drug into medium. Hydrophobicity of drug attributed to further sustained release of drug. The drug release profiles can be divided into a rapid release phase and a slow release phase. During the rapid release phase, the release of RB is caused by a combination of diffusion and osmotic pressure, whereas during the slow release phase, the drug release was mainly governed by diffusion.

4. CONCLUSION

This study demonstrates that it is feasible to encapsulation of RB drug in the polymeric microspheres employing the solvent evaporation technique. An investigation into modifying both the microspheres morphology and the drug release has been carried out, and the results were discussed. In-vivo studies in RB loaded PCL microspheres are underway and results will be published in future.

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