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Formulation and Evaluation of Polymeric Nanoparticles of Rifampicin for Anti-Tubercular Therapy

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Abstract

Polymeric Nanoparticles of Rifampicin were prepared by emulsion solvent evaporation technique using poly methyl methacrylate as polymer matrix and Poly vinyl alcohol as surfactant. Drug entrapped free flowing nanoparticles of Rifampicin were obtained after optimization using 32 factorial design and characterized for entrapment efficiency, particle size distribution, differential scanning calorimetry (DSC), X-ray diffraction (XRD), scanning electron microscopy (SEM) and in vitro and stability studies. The PMMA nanoparticles had a small size (213 ± 0.72 nm), uniform size distribution. The effects of dependent variables drug-polymer ratio and surfactant concentration on particle size and encapsulation efficiency were studied. The drug and polymer were not interacting with each other. SEM studies revealed the spherical shape of nanoparticles and in vitro release studies showed sustained drug release. RIF-polymeric nanoparticles drug delivery system proved to be promising for anti-tubercular therapy.

Keywords: Rifampicin; Polymeric nanoaprticles; Poly methyl methacrylate; Particle size.

1. Introduction

Tuberculosis is a chronic infectious disease caused by the infection of *Mycobacterium tuberculosis*, which is the foremost cause of death worldwide and its incidence is increasing particularly in association with AIDS pandemic. Now a days multiple drug chemotherapy forms the backbone of antituberculotic therapy. Particularly, rifampicin is the first choice drug in the treatment of tuberculosis. But the current treatment of tuberculosis involves prolonged oral administration of large systemic doses of combined antibiotics, which are associated with unwanted side effects and poor patient compliance [1]. The polymeric nanoparticles (PNPs) are prepared from biocompatible and biodegradable polymers where the drug is dissolved, entrapped, encapsulated or attached to a nanoparticle matrix. PNPs are promising vehicles for drug delivery by easy manipulation to prepare carriers with the objective of delivering the drugs to specific target, such an advantage improves the drug safety. Polymer-based nanoparticles effectively carry drugs, proteins, and DNA to target cells and organs [2].

Even though the development history of polymeric nanoparticles-based antimicrobial drug delivery systems is relatively shorter than other nanoparticle systems such as liposomes and SLNs. Nanoparticles have shown great therapeutic potentials.

1.1. Advantages of Polymeric Nanoparticles [3, 4]

- Increases the stability of any volatile pharmaceutical agents, easily and cheaply fabricated in large quantities by a multitude of methods.
- They offer a significant improvement over traditional oral and intravenous methods of administration in terms of efficiency and effectiveness.
- Delivers a higher concentration of pharmaceutical agent to a desired location.
- The choice of polymer and the ability to modify drug release from polymeric nanoparticles have made them ideal candidates for cancer therapy, delivery of vaccines, contraceptives and delivery of targeted antibiotics.
- Polymeric nanoparticles can be easily incorporated into other activities related to drug delivery, such as tissue engineering.

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In the present study, we examined the potential of polymeric nanoparticles for oral anti-tubercular drug delivery. The aim of our study was to formulate nanoparticles incorporating the lipophilic drug Rifampicin and evaluate its release and absorption from these *in vitro*.

2. Materials & Methods

2.1. Materials

Rifampicin was obtained as a gift sample from Lupin Pharma Pvt. Ltd. Aurangabad. Polu methyl methacrylate was received as a gift sample from Prachi Enterprises Pvt. Ltd., Mumbai. Poly vinyl alcohol was a gift sample from BASF, Mumbai. All other chemicals and reagents were of analytical grade and were used without further purification.

2.2. Methods

2.2.1. Preparation of RIF- Nanoparticles

Polymeric Nanoparticles were prepared by Emulsion solvent evaporation method. Required quantity of polymer and drug were weighed & dissolved in 12ml of dichloromethane. Quantity of PVA was mixed with 30ml of water & this solution was kept in another beaker.Both the phases were kept for sonication for 15 min. until it become clear. Solution containing drug and polymer were added drop wise to aqueous phase under continues stirring. The formed nanoparticles suspension were homogenized at 18000 rpm for 15min then followed by magnetic stirring for 3hr. The suspension was centrifuged at 9,000 rpm. for 45 min. The samples were added to glass vials & freeze-dried with mannitol 2% (w/v) as cryprotectant in a lyophilizer.

2.2.2. Total Drug Content (TDC) and Entrapment Efficiency (EE)

The total amount of drug per unit volume was determined by suitably disrupting 1ml of the dispersion in 10ml suitable solvent volumetrically. Total drug content was determined by using the equation given below:

EE was determined by analyzing the clear supernatant obtained by centrifuging the developed SLN dispersions using Remi Ultracentrifuge. The EE was calculated as follows:

Where D_f = Amount of drug in clear supernatant fluid.

2.2.3. Particle Size Analysis

Nanoparticles formulation was characterized for particle size using Malvern 2000. Double distilled water was used as a dispersant medium.

2.2.4. Particle Shape and Surface Morphology

Scanning Electron Microscopy (SEM) analysis of the prepared nanoaprticles was carried out to study the morphology like sphericity and aggregation. Sample was examined by SEM (Jeol, JSM-6360).

2.2.5. FT-IR Spectroscopy

Drug was characterized by FT-IR spectroscopy. The spectrum was recorded using FT-IR spectrophotometer (Jasco, FT/IR-4100). The scanning range was 4000 to 400cm^{-1}. The spectrum of RIF is shown in Figure No. 2. The samples were previously dried and mixed thoroughly with Potassium bromide in 1:5 (Sample:KBr) were obtained at a resolution of 2 cm^{-1} from 4000 to 400 cm^{-1}.

2.2.6. Differential Scanning Calorimetry (DSC)

DSC measurements were performed on differential scanning equipped with an intra-cooler (DSC Mettler STAR SW 9.01). Inert atmosphere was maintained by purging Nitrogen gas at a flow rate of 50ml/min. RIF and RIF-Nanoparticles, approximately 3-5mg of each sample was heated in aluminium pan from 40 ^{0}C to 240 ^{0}C at heating rate of 10 ^{0}C/min under a stream of Nitrogen at flow rate of 20ml/min.

2.2.7. X-Ray Diffraction (XRD) Study

X-Ray diffraction patterns of the powdered samples of the drug RIF and carrier were recorded using Philips PW3710 Analytical XRD B.V. X-Ray diffractometer using CU K 2α-rays with a voltage of 40kV and a current of 25mA. Samples were scanned for 2θ from 5 to 50^{0}C. Diffraction pattern for RIF was obtained.

2.2.8. In vitro Drug Release Study

Rifampicin loaded nanoparticles formulation equivalent to drug dose (250 mg) was filled in (size“0”) capsule. The quantitative in vitro release test was performed in two different GI environment i.e. 0.1N HCl (stomach), 6.8 pH buffer. At first two hours, in vitro release test was performed in 900 mL (0.1N HCl). This study was then continued for four hours in 900 ml (6.8 pH buffer solution) maintained at 37 ± 0.5°C using USP type I dissolution apparatus. The basket were rotated at 50 rpm. 5 ml aliquots was collected periodically (30, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360, 390, 420, 450, 480) and replaced with fresh dissolution medium. Aliquots, after filtration through whatman filter paper #45 and diluted . Analysis was carried out using UV spectrophotometer. Results were compared with pure rifampicin. The dissolution experiments were carried out in triplicate, and data were expressed as mean ± S.D.

2.2.9. Anti-Microbial Study

The antimicrobial study of formulated rifampicin nanoparticles was carried out by using agar gel diffusion method. In which weighed 9 gm of Agar-Agar and 5 gm of nutrient broth mixed it into 300 ml of DW. The prepared nutrient medium placed in Autoclave and start sterilization cycle according to IP 2007, in which holding time becomes 30 min to 60 min at 15 psi pressure and temperature reaches up to 115^0C to 138^0C after completion of cycle, nutrient medium was transferred into sterile petri-plates for cooling. Cultured microorganism *Bacilus subtilis (B. subtilis), Staphylococcus aureus (S. aureus)* etc. were spread on nutrient medium then the prepared suspension of nanoparticles and pure drug were poured into the well and kept it into the freeze for 20 min. then petriplates were incubated for 12 hrs and finally observed the plates for calculating zone of inhibition.

3. Result & Discussion

3.1. Characterization of RIF-Polymeric Nanoparticles

Nanoparticles were prepared using poly methyl methacrylate and Poly vinyl alcohol (Surfactant). The Nanoparticles formulation exhibited a small particle size below 213nm and a TDC of >95% (w/v) with Entrapment efficiency 96.04%.

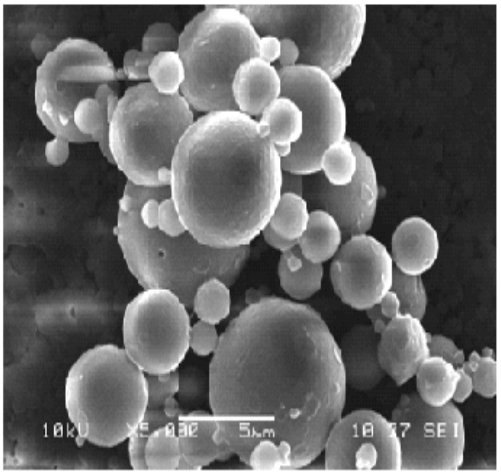
Table-1. Experimental runs with results of response

Batch	X_1 polymer (mg)	X_2 Surfactant (%)	Drug (mg)	Y_1 Entrapment Efficiency (%)	Y_2 Particle size (nm)
F1	750	1.5	100	91.08 ± 0.23	723 ± 0.43
F2	1000	2.5	100	78.95 ± 0.48	831 ± 0.82
F3	750	2	100	89.82 ± 0.82	527 ± 0.29
F4	1000	2	100	76.8 ± 0.56	713 ± 0.81
F5	500	2.5	100	89.43 ± 0.21	384 ± 1.03
F6	500	2	100	90.67 ± 1.04	324± 0.89
F7	500	1.5	100	92.04 ± 0.63	540 ± 0.39
F8	750	2.5	100	96.04 ± 1.03	213 ± 0.72
F9	1000	1.5	100	80.12 ± 0.87	921 ± 0.41

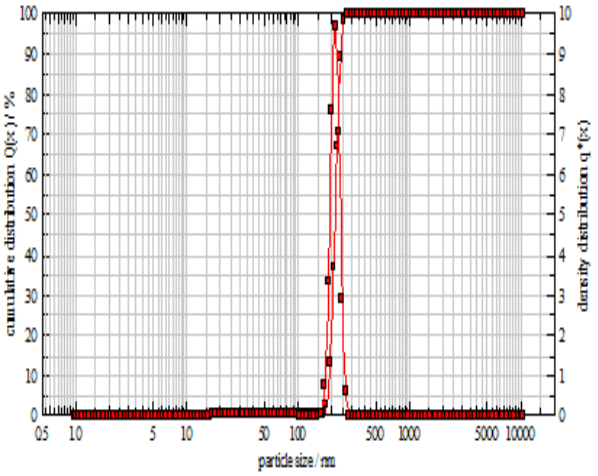
3.2. Particle Shape and Surface Morphology

The morphology of nanoparticles is similar as they appear generally well formed and characterized by a spherical shape. Shown in Figure No. 1 (A) below:

Figure-1. (A) SEM of Optimized Formulation F-8, (B) Particle size distribution for Optimized Formulation F-8



(A)



(B)

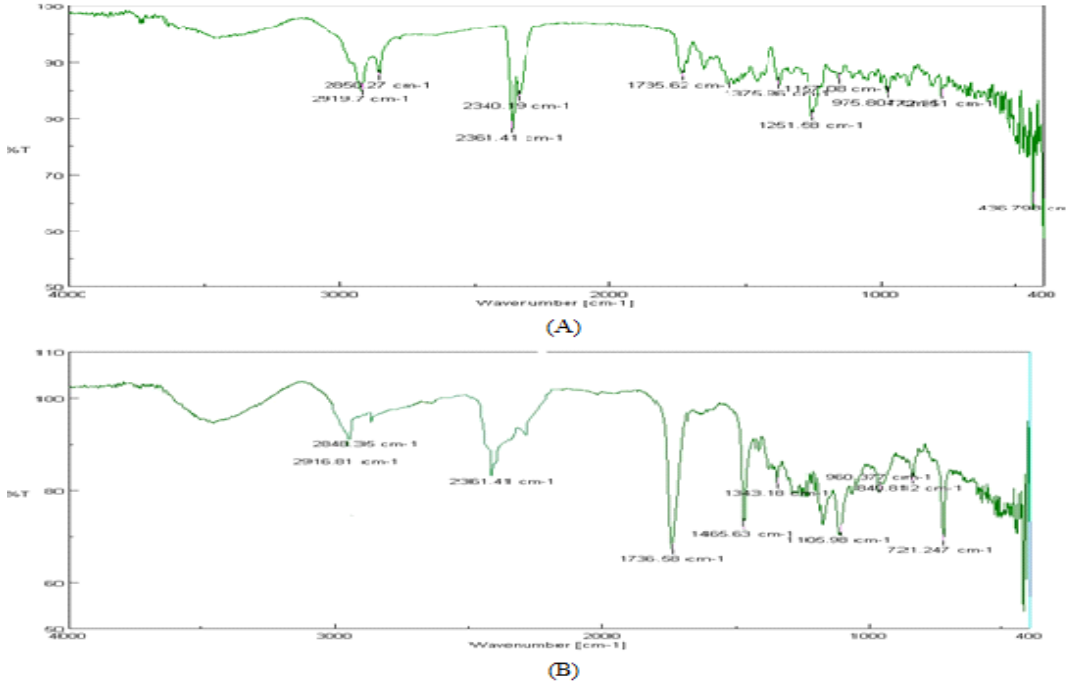
3.3. Particle Size

The mean particle size of the RIF-Nanoparticles formulation was 213nm showed in Figure No. 1 (B).

3.4. Infrared Spectroscopy

IR spectra of pure drug RIF and formulation showed no interaction with the drug and prominent peaks of the drug were not affected. Hence drug-excipients compatibility was established suggesting that there is no interaction between RIF and other excipients or no degradation of drug molecule.

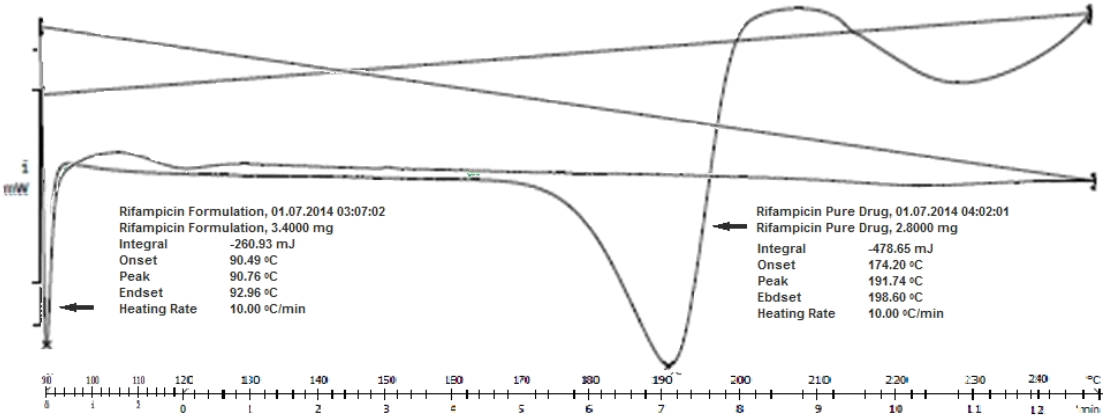
Figure-2. FTIR Spectrums of (A) Pure drug RIF, (B) Formulation batch F8



3.5. Differential Scanning Calorimetry

DSC of RIF observed has been shown in Figure No. 3.

Figure-3. Overlay of DSC curve of Drug and Formulation

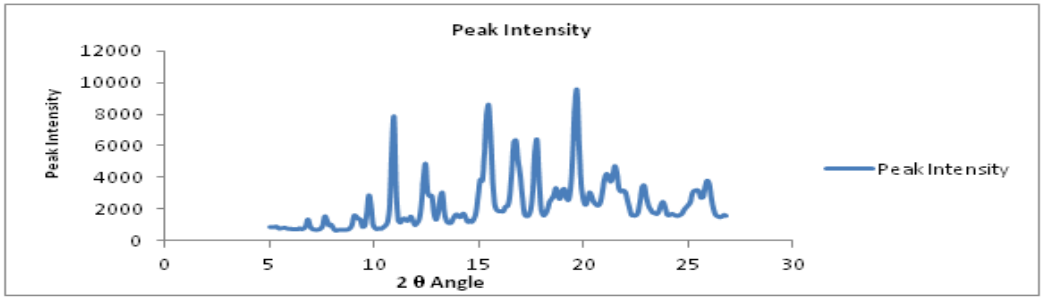


DSC curve presents an endothermic peak with T_{onset} at 174.20^{0}C, T_p (temperature of peak) at 191.74^{0}C and T_f (temperature of extrapolated end set) at 198.60^{0}C. The sharp endothermic peak T_p at 191.74^{0}C (actual M.P. 186^{0}C) corresponds to the melting point of RIF which confirms with value mentioned in the literature.

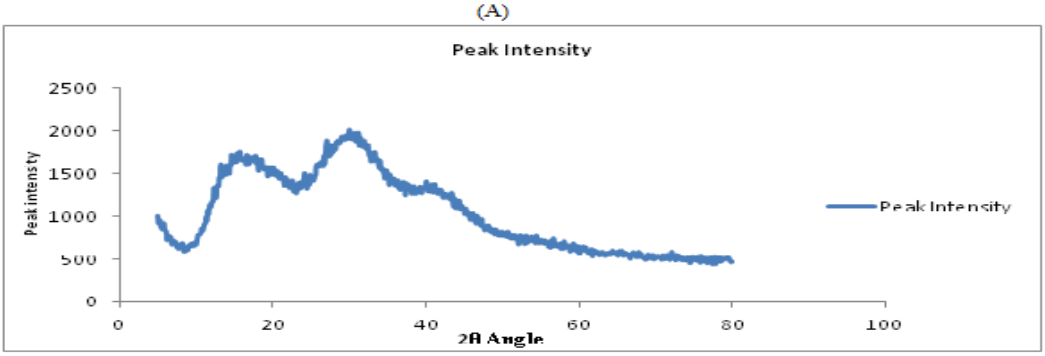
3.6. X-Ray Diffraction Study

As XRD was performed to confirm the findings obtained by DSC, the X-ray patterns of Rifampicin and RIF-Nanoparticles are shown in Figure No. 4.

Figure- 4. X-ray Diffraction Patterns of (A) Pure Drug RIF,(B) Optimized Formulation F-8



(A)

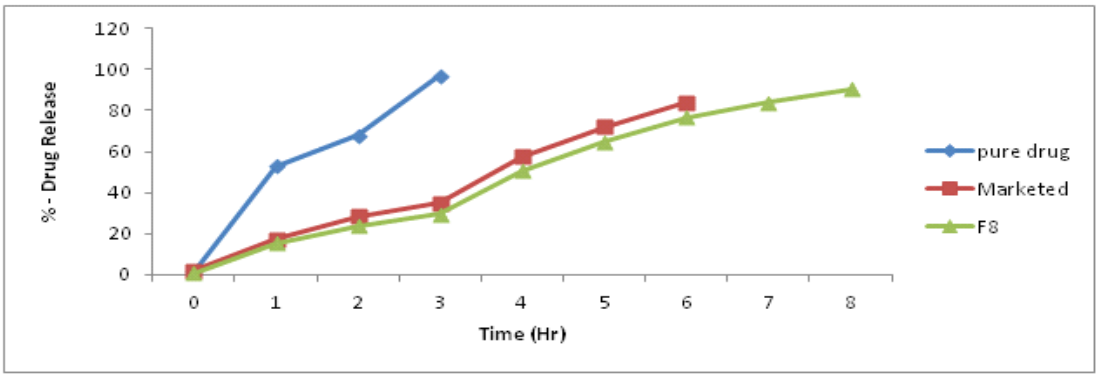


(B)

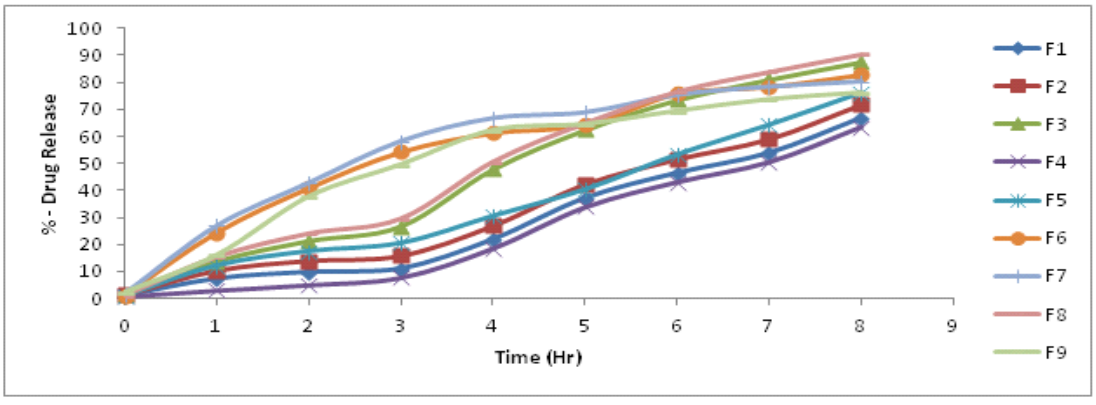
3.7. In vitro Drug Release Study

The *in vitro* drug release of F8 formulations is shown in Figure No. 5.

Figure-5. (A) *in vitro* drug release profile comparison (B) *In vitro* drug release graph for F-1 to F-9



(A)



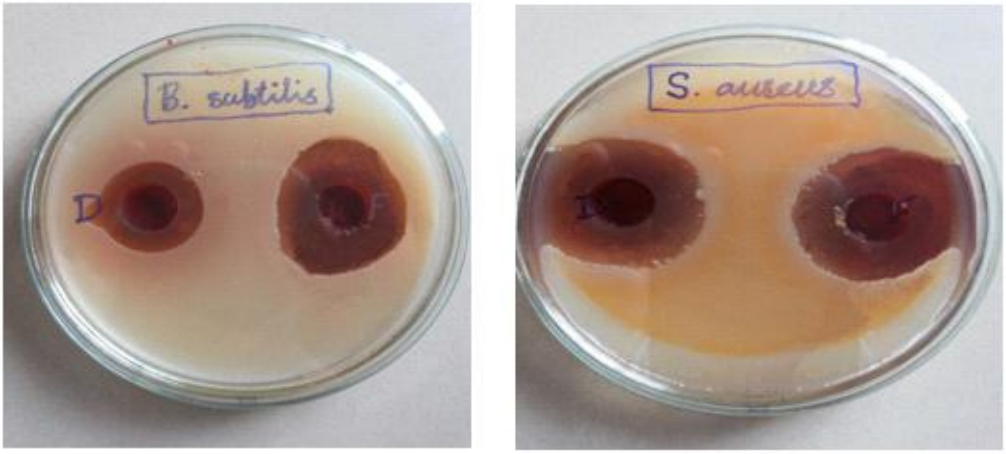
(B)

From Figure No. 5, *In vitro* drug releases from formulation F-1 to F-9 were carried out. From the above drug release study it is found that formulation F-8 showed better drug release (90.48%) in 8hrs compared to others.

3.8. Anti-Microbial Study

Antimicrobial activity of RIF-Nanoparticles was carried out with the individual component by using agar gel diffusion method. In that method *Bacilus subtilis (B. subtilis), Staphylococcus aureus (S. aureus)* cultured microorganisms were used. From the Figure No. 6, the zone of inhibition of each component was observed, in that zone of inhibition of formulation and the pure drug were clearly observed. The above observation might be concluded that the RIF-Nanoparticles had great antimicrobial potential which may further apply for various medical applications.

Figure-6. Antimicrobial activity of RIF-Nanoparticles on microorganism (A) *B. subtilis*, (B) *S. aureus*



(A) (B)

Table-2. Observation Table for Microbial Assay

Microorganism	**Diameter**	
	Plain Drug	**Formulation**
Staphylococcus aureus	3.2 ± 0.05cm	3.5 ±0.23 cm
	3.3 ± 0.15cm	3.6 ±0.057 cm
	3.2 ± 0.057cm	3.6 ± 0.20 cm
Bacilus subtilis	2.5 ± 0.15cm	2.9 ± 0.15cm
	2.1 ±0.15cm	2.7 ± 0.208cm
	2.2 ± 0.15cm	2.3 ± 0.15cm

4. Conclusion

In the present study, polymeric nanoparticles of Rifampicin were developed to a satisfactory level in terms of particle size, drug entrapment, drug release and antimicrobial activity. The particles were found to be spherical and smooth. The drug release data shows satisfactory results when compared with drug and marketed formulation. The antimicrobial study shows good zone of inhibition when compared with plain drug. So these drug delivery systems have potential to treat tuberculosis.

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