International Journal of PharmTech Research
CODEN (USA): IJPRIF ISSN : 0974-4304
Vol.2, No.3, pp 2116-2123, July-Sept 2010

PLGA Nanoparticles of Anti Tubercular Drug: Drug Loading and Release Studies of a Water In-Soluble Drug

Akansha Tripathi*, Ranjana Gupta, Shubhini A. Saraf

Faculty of Pharmacy, BBDNITM, Sector-I, Dr. Akhilesh Das Nagar, Faizabad Road, Lucknow, 227105 (UP), India

Corres.author: ranjanagunja20@gmail.com

Abstract: Poly lactic-co-glycolic-acid (PLGA) nanoparticles are often produced using the single emulsion solvent evaporation method. In most cases poly vinyl alcohol (PVA) is used as stabilizer of the emulsion. The rationale of this study was to develop PLGA nanoparticles loaded with rifampicin, intended to be intravenously administered and able to improve the therapeutic index of the drug. The influence of the concentration of PVA and the polymers was tested on particle size of prepared particles. PLGA-based rifampicin nanoparticles were prepared by single and double evaporation method, solvent diffusion and ionic interaction method. The incorporation efficiency of Rifampicin was higher with the single emulsion evaporation method in the nanosize range particles. The processing parameters involved in the method were optimized, including drug / polymer ratio, concentration of surfactant, phase ratio (organic phase/ aqueous phase) and sonication time to obtain small nanoparticles with maximum drug entrapment. The release behaviour of rifampicin exhibited a biphasic pattern characterized by an initial burst (11.26 % in 1 days) release followed by a slower and continuous release (more than 30 days). Therefore, Rifampicin loaded PLGA-nanoparticles may be considered as an effective antitubercular drug delivery system for therapy.

Key Words: Rifampicin, Poly (D,L-lactic-co-glycolide) (PLGA), Poly vinyl Alcohol (PVA), Antitubercular drugs (ATDs).

Introduction

Drugs do not deliver themselves[1]. For a molecule to reach the target site from the site of administration in sufficient concentration, and to maintain therapeutic levels for a sufficient period of time, a delivery system is needed. The delivery system is as important as the therapeutic moiety itself. Controlled and novel drug delivery, which was only a dream or at bests a possibility, is now a reality. During last decade and half, pharmaceutical and other scientist have carried out extensive and intensive investigation in the field of drug research[2]. Among those, nanoparticulte drug delivery systems from biodegradable and biocompatible polymer are interesting option for controlled drug delivery and drug targeting. Poly (lactide-co- glycolide) has gained attention for preparation of wide variety of delivery systems containing several drugs due to their biodegradable and biocompatible properties and low toxicity. Because of their biodegradability and biocompatibility, Polylactic acid and its copolymers with glycolic acid (PLGA) are widely employed for the preparation of sustained release preparations[3] and PLGA microparticles have successfully employed as Antitubercular drug (ATD) carrier [4-7]. With injectable PLGA microparticle a sustained drug release has been observed for 6-7 weeks in mice[4-5]. The formulation was subsequently explored as an ATD-carrier in order to avoid the discomfort associated with subcutaneous injection. However, oral PLGA microparticle suffered from several drawbacks such as low drug encapsulation, high polymer composition, sustained drug release for 3-4 days[6] and a partial therapeutic benefit[7]. It is possible to circumvent these drawbacks by developing PLGA nanoparticles (PLGA-NP) encapsulated three front line antitubercular drugs, i.e. rifampicin (RIF), isoniazid (INH) and pyrazinamide (PZA) which resulted in a reduced dosing frequency in a murine TB model[8]. The administration routes of PLGA nano / micro particle may vary from parenteral,

oral, dermatological, pulmonary and nasal to ocular[9]. Recently, diclofenac sodium-loaded PLGA nanoparticles were developed for ocular use and found good biocompatibility with eye[10]. Sparfloxcin loaded PLGA (50:50) nanoparticles were also studied extensively in various pharmacological aspects for their application in treatment of conjunctivitis and proved to have good stability and ocular tolerance[11].

Tuberculosis (TB), a ubiquitous, high contagious chronic granulomatous bacterial infection caused by the Mycobacterium tuberculosis that infects over 8 million people worldwide and is responsible for 2 million deaths annually[12]. Although an effective therapeutic regimen is available, patient non-compliance (because of the need to take antitubercular drugs (ATDs) daily or several times a week) results in treatment failure as well as the emergence of drug resistance. The study also reveals that fabrication of a polymeric once-daily oral multiparticulate fixed dose combination of the principal antitubercular drugs, which attains segregated delivery of rifampicin and isoniazid for improved rifampicin bioavailability, could be a step in the right direction in addressing issues of treatment failure due to patient non-compliance[13]. Patient compliance can be improved by the use of ATD formulations, which reduce the dosing frequency of the drugs. Thus the purpose of the present study was to prepare Rifampicin loaded PLGA nanoparticles for sustained action.

Materials and Methods

Materials

Rifampicin was obtained from M/s LI TAKA Drugs Ltd. Pune, India, as a gift sample. Poly (lactide-co-glycolide) (50:50) was obtained from Boehringer Ingelheim Pharma, Germany as a gift sample. All other ingredients used were of analytical grade.

Method

Rifampicin – loaded nanoparticles were prepared through single emulsion evaporation method[14]. 160mg of PLGA and 40mg of rifampicin were dissolved in 2ml of dichloromethane. This solution was added drop wise to an aqueous 20ml PVA (3%, w/v) solution under sonication for 20 min (in pulsed manner, 40% intensity) using a probe sonicator (Lapsonic® P, Sartorious Biotech GmbH, Germany) over an ice bath. The solvent was evaporated at room temperature (28˚C) for 12h, under magnetic stirring. Rifampicin loaded nanoparticles were isolated by 30 min centrifugation at 35,000 rpm. After centrifugation, the supernatant was recovered and assayed for unentrapped drug and sediment was washed using the same amount of distilled water as of the supernatant and again centrifuged at 35,000 rpm for 20 min. The washing process was repeated 3 times. All the washings were collected and assayed for unentrapped drug.

Optimization of Drug/Polymer ratio with regard to per cent drug entrapment and average particle size

For optimization of Drug/polymer ratio the nanoparticles were prepared with 1:1, 1:2, 1:3, 1:4 1and 1:5 w/w ratio of drug and PLGA and other parameters were kept constant. The average particle size was determined using Laser Particle Size Analyzer (Malvern UK) (Table 1).

Optimization of surfactant concentration with regard to per cent drug entrapment and average particle size

In order to optimize the concentration of aqueous PVA solution, the nanoparticles were prepared by using PVA concentration of 0.5 to 3.5% and other parameters were kept constant. The Average particle size was determined using Laser Particle Size Analyzer (Malvern UK) (Table 2).

Optimization of Phase Ratio (Organic/ Aqueous Phase) with regard to per cent drug entrapment and average particle size

Phase ratio was optimized in regard to average particle size and %drug entrapment and other parameters were kept constant. For optimization the nanoparticles were prepared with 1:1 to 1:20 (v/v) ratio of organic phase to aqueous phase and determined their particle size and % drug entrapment (Table 3).

Optimization of Sonication time with regard to per cent drug entrapment and average particle size

Nanoparticle formulation with optimized drug/ polymer ratio, surfactant concentration and phase ratio was selected (NP IV F-3). Nanoparticles were again prepared by same procedure however; with a varying sonication time 4, 8, 12, 16, 20 and 24 minutes. Average particle size and drug entrapment were determined and recorded (Table 4).

Nanoparticle Characterization

Size and shape morphology of nanoparticles

The morphology of Rifampicin loaded PLGA nanoparticles were analyzed using a scanning electron microscope (Hitachi High Technology, Pleasanton, CA). Samples were prepared from dilutions in distilled water of particle suspensions and dropped onto stubs. After air drying, particle were coated with a thin layer of gold and then examined by scanning electron microscopy. (Figure: 1). Average particle size and polydispersity index of nanoparticles were measured by Laser Particle size analyzer after suitable dilution. Data of size and polydispersity were discussed earlier

(Table no. 1- 4). Particle size density was also measured by Zetasizer (Malvern, UK).

Zeta Potential Study

The surface charge of nanoparticles was determined by the eletrophoretic mobility of nanoparticles in a U type tube at 25˚C, using a zetasizer (Malvern, UK). The zeta potential was found to be –11.6.

Drug Loading, Encapsulation Efficiency, Drug Content and Process Yield

The loading efficiency of drug in PLGA nanoparticles was determined as described below. Nanoparticles were separated from the aqueous medium by ultracentrifugation at 35,000 rpm for 30 min. The amount of drug present in the nanoparticles was determined as the difference between the total amount of drug used to prepare the nanoparticles and the amount of drug present in the aqueous medium.

In vitro release studies

100 mg of PLGA/Rifampicin nanoparticles were redispersed in 2.0 ml of pH 7.4 PBS and kept in an incubator at 37^0C (without agitation). The supernatant obtained after centrifugation (35,000 rpm, 30 min) of the suspension was collected daily for 30 days to determine the release of drug. The buffer solution was changed with fresh one every day and the rifampicin concentration in the dispersing medium was spectrophotometrically (UV1700 Shimadzu, Japan) measured at 475 nm. Results were expressed as concentration of rifampicin released in the buffer daily. The release study was carried out with n = 3 for 30 days (Table 6).

$$\text{Drug content (\% w/w)} = \frac{\text{(Mass of the total drug} - \text{Mass of free drug)} \times 100}{\text{Mass of nanoparticles}}$$

$$\text{Drug entrapment (\%, w/w)} = \frac{\text{(Mass of the total drug} - \text{Mass of free drug)} \times 100}{\text{Mass of total drug}}$$

$$\text{Drug loading (\%, w/ w)} = \frac{\text{(Mass of the total drug} - \text{Mass of free drug)} \times 100}{\text{Mass of total polymer}}$$

$$\text{Process Yield (\%)} = \frac{\text{(Mass of nanoparticles)} \times 100}{\text{Total mass of drug + polymer}}$$

Table 1: Optimization of Drug/Polymer ratio with regard to per cent drug entrapment average particle size

Formulation Code	Drug/ Polymer Ratio (w/w)	% Drug Entrapment	Avg Particle Size(nm)	Polydispersity Index
NP I	1:1	38.5	920	0.894
NP II	1:2	47.9	700	0.813
NP III	1:3	65.3	640	0.939
NP IV*	1:4	71.6	430	0.417
NP V	1:5	69.7	560	0.448

Table 2: Optimization of surfactant concentration with regard to per cent drug entrapment and average particle size

Formulation Code	Concentration of aq. PVA solution (% w/v)	% Drug Entrapment	Average Particle Size(nm)	Polydispersity Index
NP IV A	0.5	51.2	796	0.738
NP IV B	1.0	58.4	622	0.648
NP IV C	1.5	60.7	568	0.526
NP IV D	2.0	65.2	521	0.513
NP IV E	2.5	69.4	440	0.467
NP IV F*	3.0	72.5	380	0.313
NP IV G	3.5	71.4	390	0.379

Table 3: Optimization of Phase Ratio (Organic/ Aqueous Phase) with regard to per cent drug entrapment and average particle size

Formulation Code	Phase Ratio	% Drug Entrapment	Avg Particle Size(nm)	Polydispersity Index
NP IV F- 1	1:1	52.8	720	0.618
NP IV F- 2	1:5	65.6	696	0.543
NP IV F- 3*	1:10	74.0	373	0.396
NP IV F- 4	1:15	68.7	440	0.487
NP IV F- 5	1:20	63.7	560	0.534

Table 4: Optimization of Sonication time with regard to per cent drug entrapment and average particle size

Formulation Code	Sonication Time (min)	% Drug Entrapment	Avg Particle Size(nm)	Polydispersity Index
NP- T1	4	48.6	882	0.816
NP-T2	8	50.3	694	0.748
NP-T3	12	56.9	560	0.626
NP-T4	16	68.7	438	0.536
NP-T5*	20	75.8	360	0.304
NP-T6	24	75.1	390	0.315

Table 5: Characterization parameters of rifampicin nanoparticles

Characterization parameters	Result (Mean ± S.D., n=3)
Drug content (%, w/ w)	26.69±0.14
Drug Entrapment (%, w/w)	75.8±2.16
Drug Loading (%, w/w)	18.95±1.65.
Process Yield (%)	56.8±2.67

Table 6: *In vitro* release study of rifampicin nanoparticles

Time (days)	% Drug Release*	% Cumulative Drug Release*
1	11.266±0.102	11.266±1.152
2	6.858±0.278	18.124±1.681
3	5.224±0.136	21..348±1.09
5	5.656±0.169	31±0.791
10	3.594±0.092	51.535±0.989
15	2.23±0.047	65.72±0.982
20	0.883±0.033	69.362±1.584
25	0.528±0.021	72.201±1.691
30	0.632±0.063	75.49±1.294

Fig 1 - SEM image of Rifampicin nanoparticles

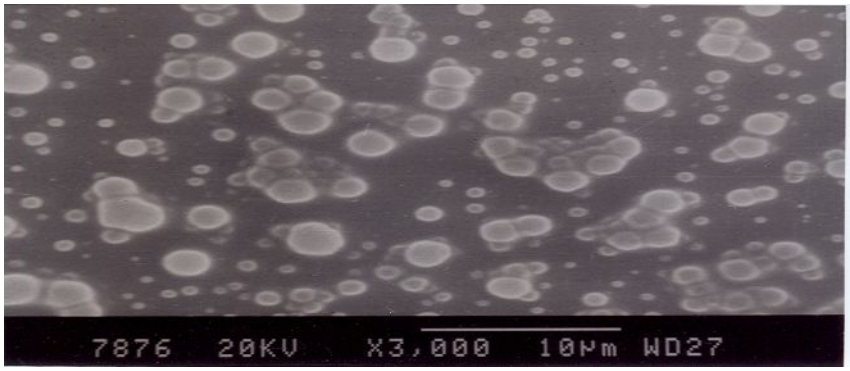


Fig 2: Effect of drug / polymer ratio w/w on % drug entrapment and average particle size

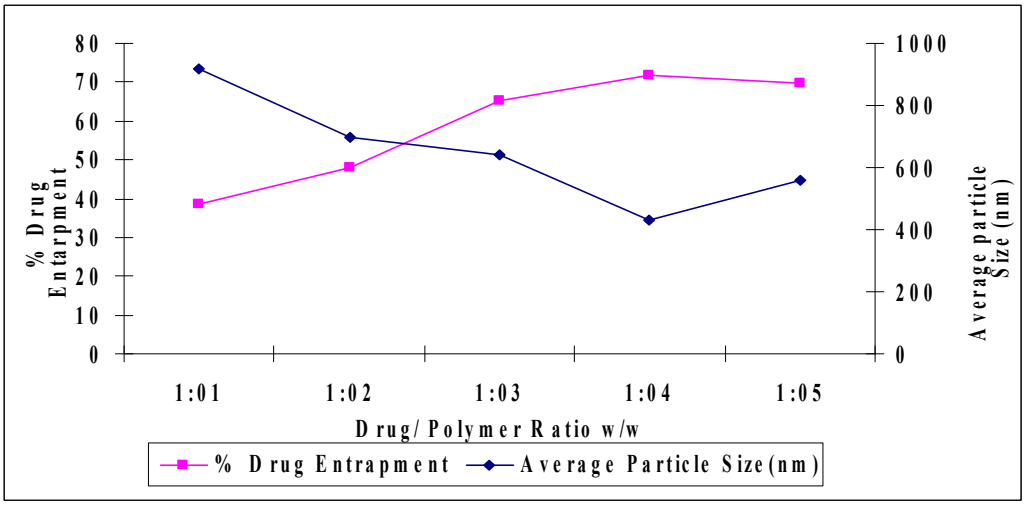


Fig 3: Effect of surfactant concentration on %drug entrapment and average particle size

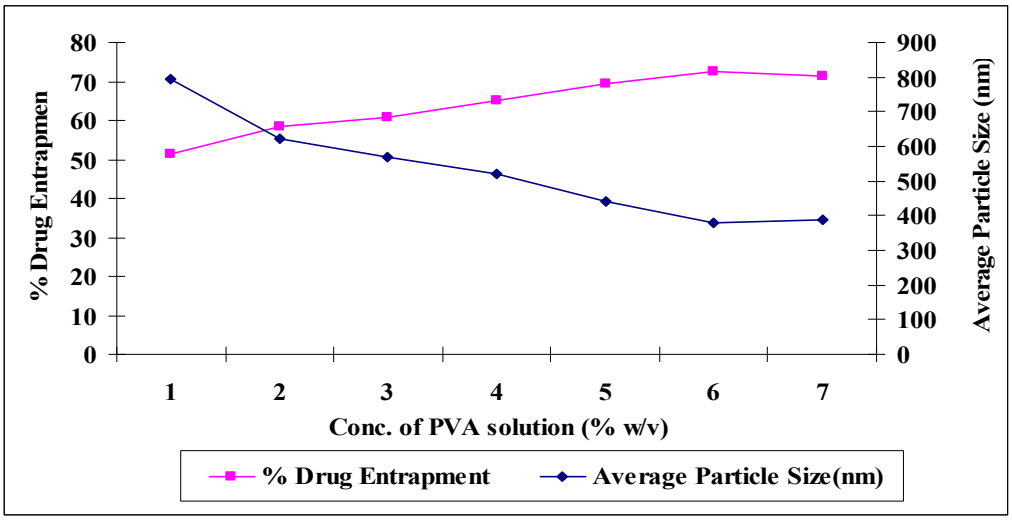


Fig 4: Effect of Phase ratio on % drug entrapment and average particle size

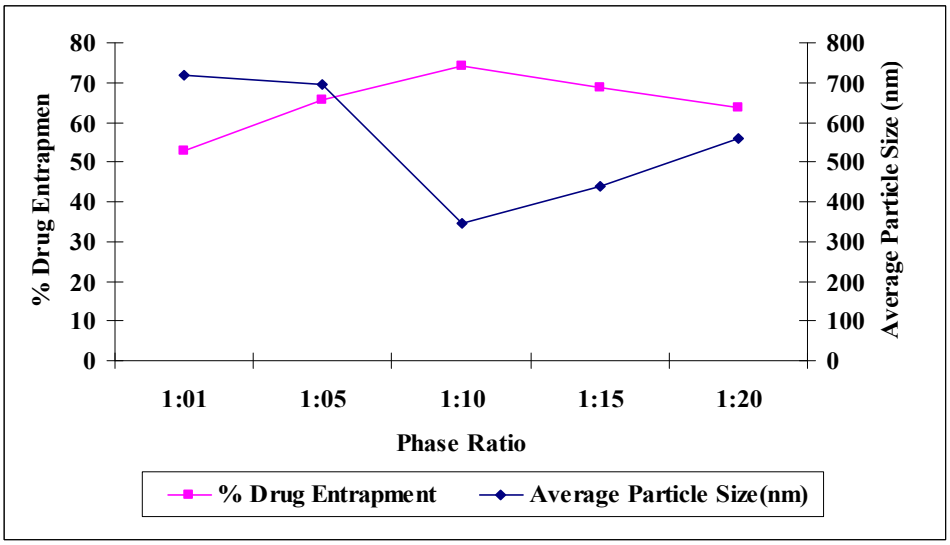


Fig 5: Effect of sonication time on average particle size and % drug entrapment

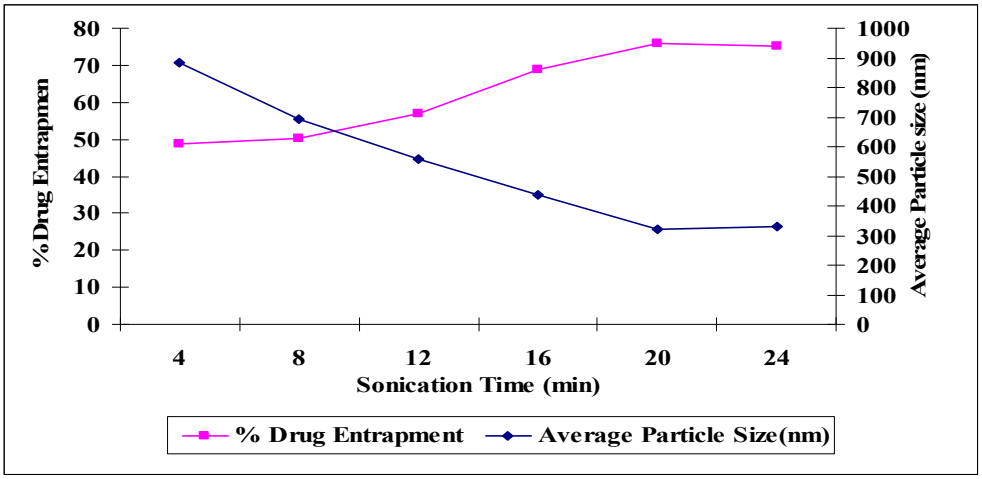
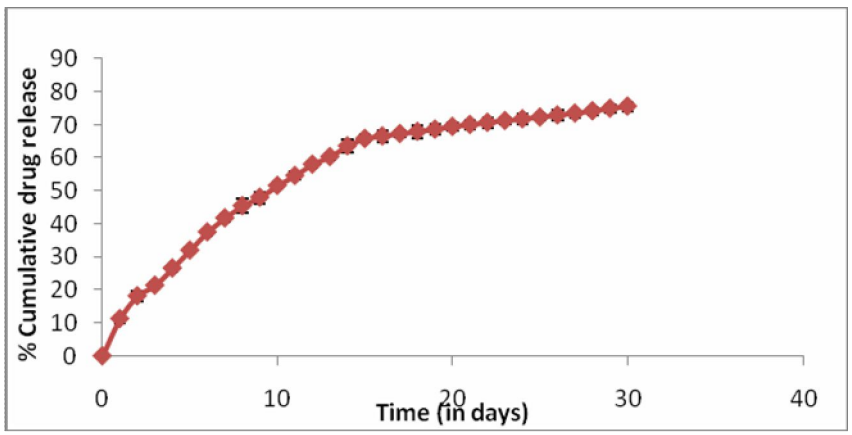


Fig 6: % Cumulative release of drug in 30 days



Result and Discussion

Preparation and optimization of nanoparticles

On the basis of particle size, single emulsion evaporation techniques were optimized in single and double evaporation method, solvent diffusion and ionic interaction technique. Microemulsions are clear, thermodynamically stable, optically isotropic systems, obtained spontaneously by mixing surfactant, co-surfactant. The nanoparticles size is affected by the composition of the micro emulsion system, particularly by the surfactant and co-surfactant used, as well as by the experimental parameter. The nanoparticles size is affected processing parameters such as drug /polymer ratio, concentration of surfactant, phase ratio (organic phase/ aqueous phase) and sonication time.

A drastic decrease in particle size and increase in percent drug entrapment was observed by increasing drug /polymer ratio. The size of nanoparticles decreased from 920nm to 430nm and per cent drug entrapment increased from 38.5% to 71.6% as on increasing drug/ polymer ratio from 1:1 to 1:4 w/w. There was no significant increase in particle size and percent drug entrapment as polymer concentration was increased. The results indicate the optimal drug/ polymer ratio to be 1:4 w/w for maximal nanoparticles formulation (Table 1, Fig. 2). Although a satisfactory yield was obtained at PLGA concentrations lower than 1:4 w/w, the value decreased with the increase in the PLGA concentration. This was probably caused by the increasing viscosity and hence resulting poor dispersibility of PLGA solution into the aqueous phase.

The concentration of surfactant was optimized in order to obtain small nanoparticles with maximum percent drug entrapment. At concentration 3% w/v of PVA a minimum average particle size 380nm and maximum per cent drug entrapment 72.5% were recorded (Table 2). The mean nanoparticles size was found to decrease with increasing PVA concentration. The polydispersity index also decreased with increasing PVA concentration. It can be concluded that with increasing PVA concentration more PVA molecules overlap the surface of the droplets, providing increased protection of the latter against coalescence and resulting in smaller emulsion droplets. There was no significant difference in particle size and percent drug entrapment with PVA concentration 3.5% (w/v). Therefore 3% (w/v) PVA was recorded as optimized surfactant concentration (Fig. 3).

The phase ratio (organic/ aqueous phase) was optimized in order to obtain nanoparticles selectively. At phase ratio 1:10 (v/v) of organic phase to aqueous phase a minimum average particle size 373nm and maximum per cent drug entrapment 74.08 % were recorded (Table 3). Upon increasing the phase ratio from 1:10 to 1:20 (v/v) an increase in average particle size 560nm was recorded, where as the per cent drug entrapment decreased considerably to 63.7% (Fig 4). It may be due to formation of some aggregates in the formulation.

Sonication time was also optimized in order to achieve stable formulation with minimum average particle size and maximum per cent drug entrapment. Emulsification is carried out under high-shear stress to reduce the size of the emulsion droplets (directly related to the final size of nanoparticles). A stable nanoparticulte formulation was achieved after sonicating the formulation for 20 minutes in a pulsed manner with minimum average particle size and maximum percent drug entrapment, i.e. 360nm and 75.8% respectively (Table 4). A further increase in sonication time (24 minutes) resulted in an increase in particle size (390nm) and decrease in per cent drug entrapment (75.1%) (Fig 5) This may be due to the agglomeration of particles due to generation of surface charge. Nanoparticles were prepared by single emulsion evaporation method using optimized drug/ polymer ratio (1:4 w/w), PVA concentration (3% w/v), phase ratio (1:10 v/v) with sonication time 20 minutes (pulsating, 40% intensity).

Size and shape morphology of nanoparticles

Shape and surface morphology of prepared nanoparticles were evaluated by SEM. The study revealed that most of the nanoparticles were fairly spherical in shape. The surface of the particles showed a characteristic smoothness (Fig 1). The polydispersity index is a measure of the distribution of nanoparticles. Laser particle size analyzer yields the diameter of the bulk population (average) and a polydispersity index gives the distribution range from 0.000 to 0.500. Polydispersity index greater than 0.5 indicate the aggregation of particles. With increase in PVA concentration and sonication time, particle size and polydispersity index decrease. Therefore 3% (w/v) PVA concentration with sonication time 20 minutes (in apused manner) was used.

Zeta Potential Study

In general, particle aggregation is less likely to occur for charged particles (high zeta potential) due to electric repulsion. Lower zeta potential facilitates aggregation. The zeta potential of nanoparticles was found to be –11.6, which would not allow aggregation.

Drug Loading, Encapsulation Efficiency, Drug Content and Process Yield

Percent drug entrapment was found to be 75.8±2.16 %. Per cent drug entrapment increases with increase in surfactant concentration. This may be due to the stability of emulsion droplets. Drug content, drug loading and process yield was found to be

26.69±0.14% (w/w), 18.95±1.65% (w/w) and 56.8±2.67% (w/w) respectively and recorded in Table 5.

In vitro release Studies

The in-vitro release profile was biphasic with an initial burst release (7.266%) in 1 days attributed to surface associated drug, followed by a slower release phase as the entrapped drug slowly diffused out into the release medium (Figure 6) and 75.49% drug was released after 30 days. A low burst release of the drug was observed due to washing of prepared PLGA nanoparticles. There was a sustained release of drug at a constant rate. The concentration of drug was above MIC (in-vitro) even after 30 days. The adsorbed molecules on the surface of particles are rapidly desorbed when in contact with the dissolution medium. The diffusion of the drug, the erosion and swelling of polymer matrix and the degradation of polymer are the main mechanism for the drug release. Since degradation of PLGA is slow, the release of rifampicin from nanoparticles would mainly depend on the drug diffusion, in the time of experimental studies.

Conclusion

Conclusion drawn from the present study is that the nanoparticles may be a suitable device for administration of rifampicin. The encapsulation method was suitable for the preparation of nanoparticles because the system has nanometric size and regular shape. The development of a system whereby drugs could be administered in a single dose, that maintain active levels of the drug for a prolonged period would be the ideal system. Such a system has been developed in this study in the form of PLGA nanoparticles. The technology would improve patient compliance, the lack of which is the major reason for the development of multi-drug resistant strains of mycobacterium. Further studies, such as confocal microscopy and study of accumulation of drugs in infected macrophages can be done and is suggested as future scope of this work.

References

1. Davis S.S. and Illum L., Int, J. Pharm., 1998, 176, 1-8.
2. Bala I., Hariharan S. and Kumar R., Crit. Rev. Ther. Drug. Carrier System, 2004, 21, 5, 387-422.
3. Anderson J.M. and Shive M.S., Biodegradation and biocompatibility of PLA and PLGA microspheres, Adv. Drug. Delivery Rev., 1997, 28, 5–24.
4. Dutt M., Khuller G.K., Chemothearpy of *Mycobacterium tuberculosis* infection in mice with a combination of isoniazid entrapped in poly (DL-lactide-co-glycolide) microparticle. J. Antimicrob. Chemother., 2001, 4, 829-835.
5. Dutt M., Khuller G.K., Therapeutics efficacy of poly (DL-lactide-co-glycolide)-encapsulated antitubercular druds against *Mycobacterium tuberculosis* infection induced in mice, Antimicrob. agent and Chemother., 2001, 45, 363-366.
6. Ain Q., Sharma S., Khuller G.K., Role of poly (DL-lactide-co-glycolide) in development of a sustained oral drug delivery system for antitubercular drugs(s), Int. J. Pharm., 2002, 239, 37-46.
7. Ain Q., Sharma S., Khuller G.K., Chemotherapeutic potential of orally administered microparticles containing isoniazid, rifampicin and pyrazinamide against experimental tuberculosis, Antimicrob. Agents Chemother., 2003, 47, 3005–7.
8. Pandey R., Zahoor A., Sharma S., Khuller G.K., Nanoparticle- encapsulated antitubercular drugs as a potential oral drug delivery system against experimental murine tuberculosis, Tuberculosis (Ed-inb), 2003, 83, 373–8.
9. Vandervoort J. and Ludwig A., Biocompatible stabilizers in the preparation of PLGA nanoparticles: a factorial design study, Int. J. Pharm., 2002, 238, 79–92.
10. Agnihotri S.M. and Vavia P.R. Diclofenac-loaded biopolymeric nanosuspension for ophthalmic application, Nanomedicine: NBM, 2009, 5, 90-95.
11. Gupta H., Aqil M., Khar R.K., Ali A., Bhatnagar A., Mittal G., Sparfloxacin-loaded PLGA nanoparticles for sustained ocular drug delivery, Nanomedicine : NBM, 2010, 6, 324-333.
12. Pandey R., Zahoor A., Sharma S., Khuller G.K., Tuberculosis, 2003, 83, 373-378.
13. Pillay V., Toit L.S., Danckwerts M.P., Tuberculosis chemotherapy: current drug delivery approaches, Respiratory Research, 2006, 7, 1-18.
14. Govender J., Stolnik S., Garnett M.C., Illum L., Davis S.S., J. Control Rel., 1999, 57, 171-185.

*****