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Preparation, characterization and *in vitro* evaluation of rifampicin loaded biodegradable microsphere

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ABSTRACT

The aim of this study was to prepare Rifampicin-loaded Polycaprolactone (PCL) microspheres by emulsion solvent evaporation technique with different drug-to-polymer ratios (1:5), (1:10), (1:15), (1:20) and (1:25), characterize and evaluate the in vitro performance. The microspheres were characterized for particle size, surface morphology, drug excipient compatibility, percentage yield, drug entrapment, and in vitro release kinetics. Of five formulations prepared, MP 4, i.e., 1:20 (drug-polymer) ratio was selected as the optimized formulation based on particle size, particle shape, and the release behavior. The size of microspheres was found to be ranging from 1.9 to 2.3 μm. The shape of microspheres was found to be spherical by SEM. Among the four formulations, MP 4 (1:20) showed a maximum percentage yield of 79% ± 3.4%. There was no interaction between drug and polymer by FT-IR study. In the in vitro release study, formulation MP 4 showed 75.6 % drug release and was found to be sustained for 15 days. The microsphere formulations were able to sustain the release of drug in vitro.

KEYWORDS

In vitro release;
Microspheres;
Polycaprolactone (PCL);
Rifampicin (RIFA).

INTRODUCTION

PCL is one of the biodegradable, biocompatible and water insoluble polymer suitable for controlled drug delivery due to a high permeability to many drugs and at the same time being free from toxicity and low cost when compared to other biodegradable polyesters. It has the ability to form compatible blends with other polymers. Biodegradation of PCL is very slow in comparison to other polymers, so it is most suitable for long-term delivery. Release rate of drug from PCL depends on the type of formulation, method of preparation, PCL content, size and percent of drug loaded in the microcapsules. Within the last decades, PCL polymers have been major areas of concern to develop controlled delivery systems especially for peptides and proteins. Despite considerable research efforts and impressive progress made in recent years, the question of feasibility of injectable PCL microspheres as protein/peptide or a vaccine delivery system remains open to debate. Microencapsulation techniques have been developed to allow incorporation of sensitive proteins into PCL polymers under mild conditions. The successful use of these polymers in pharmaceutical applications has naturally led to the evaluation of other aliphatic polyesters such as poly (caprolactone) (PCL), a biocompatible semi-crystalline polymer with a very low glass transition temperature[1]. The interest of PCL has been re-

cently highlighted as a platform for sustained delivery of antigen and low molecular weight drugs[2].

MATERIAL AND METHOD

Material

Polycaprolactone (Av. $M_{w\ 60,000}$) was purchased from Sigma-Aldrich (Mumbai, INDIA), Rifampicin was a gift sample from Macleod Pharmaceuticals, Mumbai. Polyvinyl Alcohol was purchased from Qualichens, New Delhi.

Preparation of PCL microspheres

Microspheres of Rifampicin (RIFA) using biodegradable polycaprolactone as the polymer were prepared by emulsion-solvent evaporation method[3]. Five different formulations MP 1, MP 2, MP 3, MP 4, MP 5 containing drug-polymer in the ratio of 1:5, 1:10, 1:15 1:20 and 1:25 respectively, were prepared. Dichloromethane (10 ml) was taken as the organic phase in which polymer and drug were dissolved. The drug content was always 20 mg in all the batches. The aqueous phase contained polyvinyl alcohol (PVA) solutions in water at 2.5% w/v (40 ml). The organic phase was added to the aqueous phase drop by drop while the aqueous phase was kept for stirring on a magnetic stirrer. The resultant emulsion was homogenized for 10 min with an Ultraturax® homogenizer at 2000 rpm. Subsequent evaporation of the DCM was carried out with mechanical stirring at room temperature till complete evaporation of dichloromethane occurred[4]. As the organic phase evaporates, precipitation of the polymer and drug occurs due to which drug gets entrapped in the polymer and stirring results in size reduction as well as a spherical particle formation. Microparticles were collected by centrifugation and washed by dispersion in water with subsequent centrifugation; this step was repeated three times. Microspheres were then air dried.

Characterization of RIFA-loaded PCL microspheres

Particle sizing and morphology

The microspheres were analyzed for their size and polydispersity index on Zetasizer Nano ZS, Malvern instruments, based on photon correlation spectroscopy, at a scattering angle of 90° and temperature of 25°. Measurements were carried out both for fresh and air-dried samples. Before counting, the samples were diluted with a 0.05% (w/v) tween 80 water solution in order to prevent precipitation during the measurements. The results were the means of triplicate experiments.

Surface charge (Zeta-potential)

The surface charge of the microspheres was determined with Zetasizer Nano ZS, Malvern instruments. The measurements were carried out in an aqueous solution of KCl 0.1N. Immediately before the determinations microspheres were diluted with KCl solution. The measured values were corrected to a standard reference for temperature of 20°. The results are the means of triplicate experiments.

Particle morphology

The surface morphology (roundness, smoothness) and the size of microparticles formulations were studied by scanning electron microscope (SEM). The samples were diluted with distilled water and measured at room temperature with a scattering angle of 90°. The data obtained after the observation were analyzed accordingly.

Measurement of entrapment efficiency of RIFA in PCL microspheres

A series of rifampicin solutions of known concentrations in acetonitrile were prepared, and absorbances were measured in order to generate a calibration curve. The rifampicin content of each lot of microspheres was determined by first extracting the rifampicin and quantifying the amount of drug spectrophotometrically. The drug encapsulation efficiency (EE %) was calculated as the percentage of drug entrapped in microspheres compared with the initial amount of drug recovered in unpurified samples. The concentration of rifampicin contained in each sample was determined by measuring the absorbance on a spectrophotometer at 485 nm.

Release studies

In vitro release of RIFA from PCL microspheres was determined using as the release mediums, phosphate buffer pH 7.4. Air-dried formulations were suspended in 500 ml of the dissolution medium. There was 0.1% w/v of citric acid added to dissolution medium to

prevent the degradation of RIFA during the process. The experiments were carried out at 37 ±0.5°C at a rotation speed of 100 ±2 rpm. A measure of 1 ml samples was withdrawn at appropriate time intervals and centrifuged at 10000 RPM. Supernatants were diluted suitably with acetonitrile and absorbance of the resulting solution was measured at 485 nm. The residue (after centrifugation) was redispersed in 1 ml of the fresh dissolution medium and replaced back into the dissolution apparatus. The cumulative amount of RIFA was obtained from the calibration curves of RIF in acetonitrile. The stock standard solution of RIFA (2 mg/ml) was prepared by dissolving the drug in acetonitrile and storing at 4°C. A standard calibration curve was built up by using standard solutions prepared by dilution of the stock standard solution with acetonitrile.

EE= {Experimental drug content - free drug content/ Experimental drug content} *100

Drug Loading = (Mass of drug determined / Mass of MP produced) *100

Statistical analyses

All experiments were repeated at least three times. Results are expressed as means ± standard deviation. A difference between the means was considered significant if the p value was less than or equal to 0.05.

Kinetic of drug release

In order to understand the mechanism and kinetics of drug release, the result of the in vitro dissolution study of microspheres were fitted with various kinetic equations, such as zero-order (percentage release versus time), first-order (log percentage of cumulative drug remaining versus time) and Higuchi's model (percentage drug release versus square root of time. Correlation coefficient (R^2) values were calculated for the linear curves obtained by regression analysis of the above plots[5].

RESULT AND DISCUSSION

Preparation of RIFA-loaded PCL microspheres

Solvent evaporation method is the most popular and simple technique of preparing PCL microparticles. It involves emulsifying a drug-containing organic polymer solution into a dispersion medium. The o/w method was used in this work. For this technique, drug is dissolved or dispersed in a solution of the polymer in a water-immiscible and volatile organic solvent dichloromethane (DCM). This dispersion is emulsified into an aqueous phase. The organic solvent then diffuses into the aqueous medium and finally evaporates into the air. In the solvent evaporation method, polyvinyl alcohol (PVA) is widely used as an emulsifier in the external aqueous phase and dichloromethane is the most commonly used solvent to dissolve the polymer.

Physico-chemical evaluation of microsphere.

The microspheres prepared by emulsion solvent evaporation method were evaluated for the following characteristics. Drug-excipients compatibility study was carried out by the FTIR analysis of pure polymer (polycaprolactone), micro particular system (RIFA micro particle), and placebo microparticles

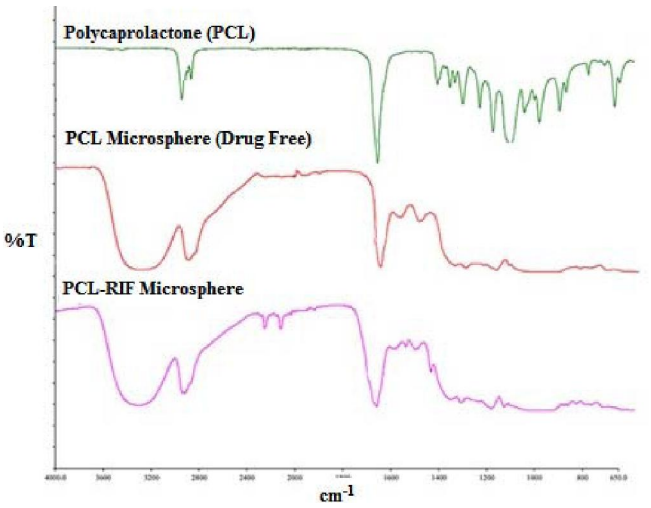

Figure 1: Showing FTIR Spectra

(polycaprolactone) Figure 1.

The effect of polymer concentration on properties of micro-particles

Polymer concentration is also a key factor. In this study, two PCL concentrations, 100 to 800 mg/10 ml, were taken in the preliminary test. The concentrations showed the best result in preliminary test is 100 to 500 mg/10 ml. The encapsulation efficiency was increased by increasing PCL concentration from 3 to 5 g/100 ml Figure 2. By increasing the polymer concentration in the organic phase, the viscosity of the solution was increased. Increasing viscosity can decrease the RIFA diffusion into the aqueous phase and thus increase the

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RIFA incorporation into the microparticles[6]. Consequently, the encapsulation efficiency of micro-particles was increased by increasing the polymer concentration. Conversely, RIF loading (%) was decreased by increasing PCL concentration Figure 3. Increasing viscosity increased the total mass of PCL in the micro-particles i,e % yield Figure 4.

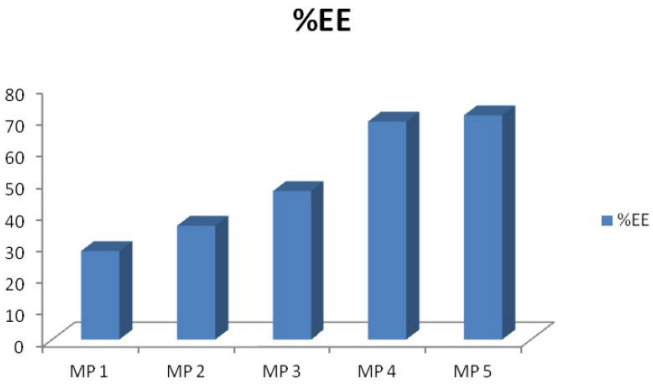

Figure 2 : Effect of PCL concentration on Entrapment Efficiency

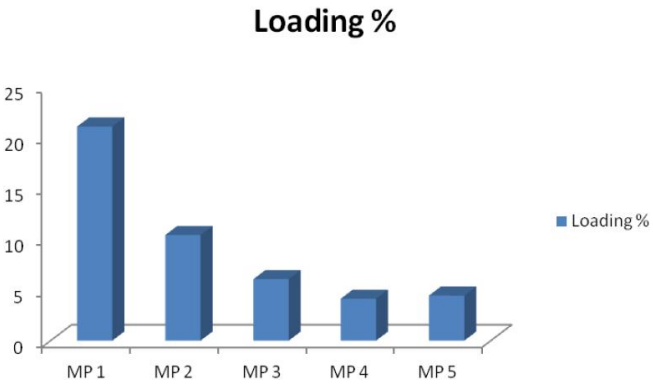

Figure 3 : Effect of PCL concentration on Drug Loading

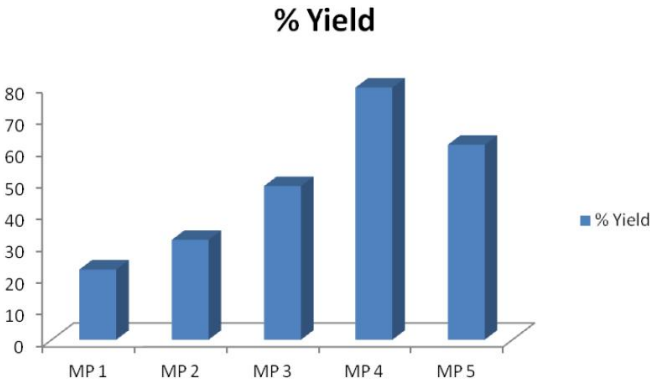

Figure 4 : Effect of PCL Concentration on % Yield

Size and morphological properties of PCL microspheres

RIFA-loaded PCL microspheres were obtained in the size range around 2 μm and good polydispersity index as shown in table Figures 5,6. PCL microparticles size is normally affected by the presence of an emulsifier. In this case PVA was used to prevent aggregation of the emulsion droplets and polymer sticking during stirring. 2.5% of PVA were able to stabilize particles also during their storage. This is confirmed by the polydispersity index that was very low because aggregation did not occur and particles maintained a narrow distribution, as confirmed also by SEM study. When working with microparticulate systems; it is often helpful to visualize particle shapes and surface characteristics in order to correlate with other properties such as surface area and size distribution. RIFA-loaded PCL microspheres prepared using the solvent evaporation method, were spherical in shape with a very smooth surface, as shown in Figures 7.

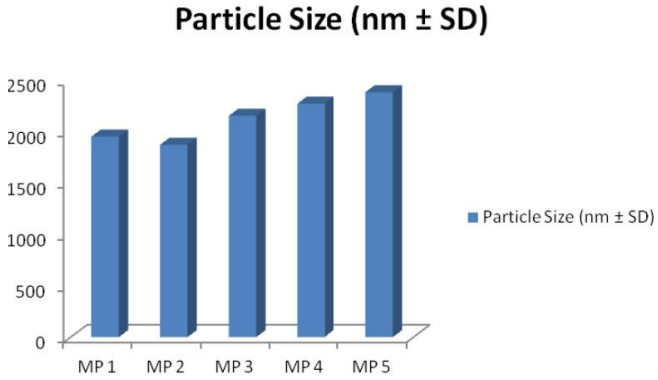

Figure 5 : PCL Micro Particle size distribution

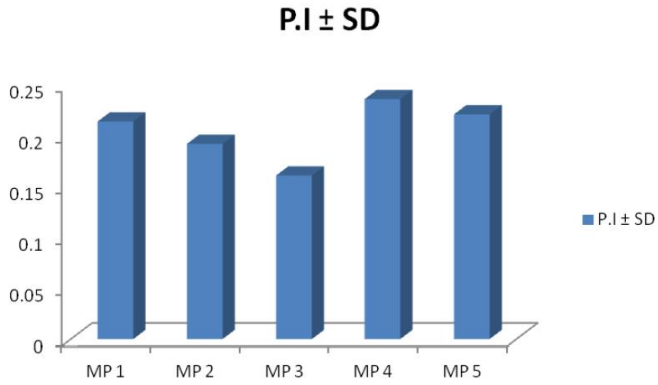

Figure 6 : Polydispersity Index of PCL Microparticles formulation

Surface charge

Microparticle formulations were characterized also in term of zeta potential because, as well known, it can

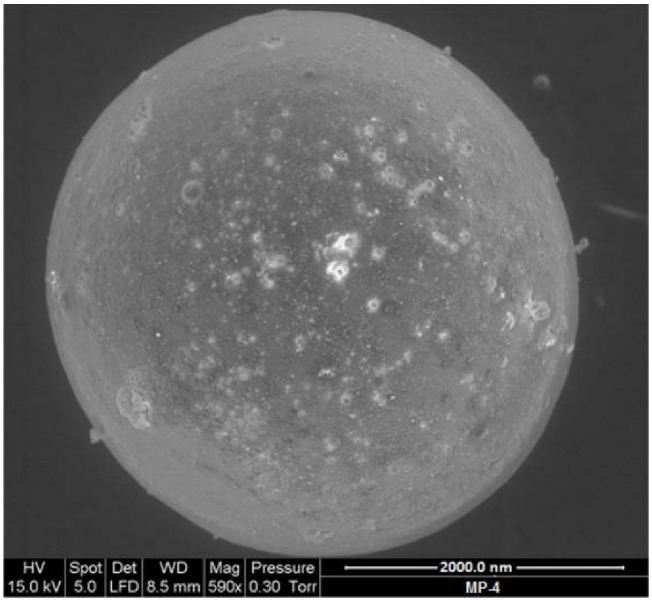

Figure 7 : SEM Micrographs of PCL Microspheres

influence particle stability. In theory, more pronounced zeta potential values, being positive or negative, tend to stabilize the particle suspension. The electrostatic repulsion between particles with the same electric charge prevents the aggregation of the spheres. The PCL particles made by the solvent evaporation method were negatively charged as can be seen in TABLE 1 and in Figure 8.

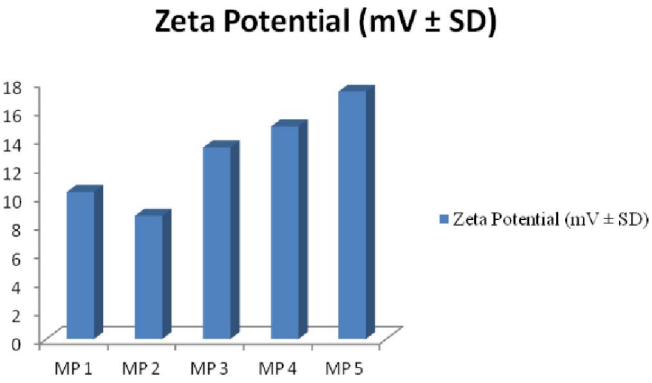

Figure 8 : Zeta potential of PCL Microparticles

TABLE 1: Properties of PCL Micro Particle

Formulation Code	Particle Size (nm ± SD)	P.I ± SD	Zeta Potential (mV ± SD)
MP 1	1952 ± 34	0.214 ± 0.30	10.31 ± 0.6
MP 2	1873 ± 29	0.192 ± 0.08	8.64 ± 0.57
MP 3	2155 ± 21	0.161 ± 0.02	13.43 ± 0.21
MP 4	2274 ± 49	0.236 ± 0.04	14.91 ± 0.81
MP 5	2385 ± 43	0.221 ± 0.09	17.35 ± 0.95

Entrapment efficiency (E%)

Encapsulation efficiency (EE %) is an important index to evaluate drug-loaded microspheres as it is more economical when high encapsulation efficiency can be obtained. An O/W emulsion technique is mostly used for the encapsulation of drugs. Figure 1 shows the EE % of the prepared microspheres. As can be seen, significant difference was found between the formulations.

Release studies

In vitro drug release profile reveals fundamental information on the structure (e.g., porosity) and behavior of a formulation on a molecular level, as well as possible interactions between drug and polymer, and their influence on the rate and mechanism of drug release and model release data. Release studies were carried out by using phosphate buffer at pH 7.4 and acetic acid buffer at pH 4.4, in order to evaluate the effect of pH on RIFA release from PCL microspheres. The release profiles were very similar in the two different release media. Normally, the release medium pH is able to affect the drug release pattern from the PCL-based microparticles. In this case it was found the same behavior at pH 7.4 and at pH 4.4 during the 15 day's experiments. In Figure 9, RIFA release profiles from PCL microspheres at pH 7.4 buffer solutions are shown. The main reason that can explain this behavior correlates to the transition temperature (Tg) of the polymer. An initial burst effect demonstrated, after this initial burst, PCL microspheres released RIFA at a lower rate Formulation MP 4 was selected as the optimized formula-

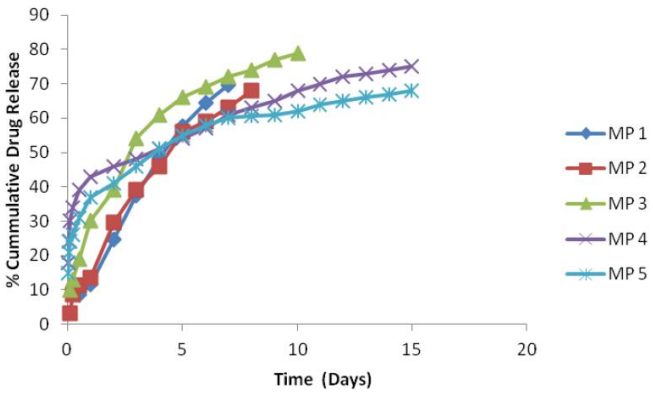

Figure 9 : Cumulative % release of PCL Microsphere in Phosphate Buffer pH 7.4

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Results

			Size (d. nm):	% Intensity:	Width (d.nm)
z-Average (nm):	2274.4	Peak 1:	2290.3	100	2.929
PdI:	0.236	Peak 2:	0.000	0.0	0.000
Intercept:	0.986	Peak 3:	0.000	0.0	0.000
Result quality:	Good				

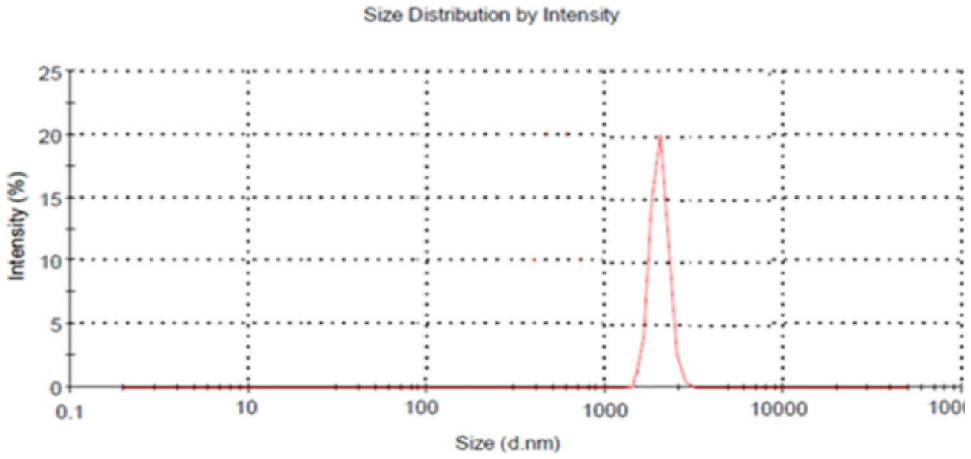

Figure 10 : Particle size distribution

Results

			Mean (mV):	Area (%):	Width (mV)
Zeta Potential (mV):	-14.91	Peak 1:	-15.17	100	2.145
Zeta Deviation (mV):	0.194	Peak 2:	0.000	0.0	0.000
Conductivity (mS/cm):	0.961	Peak 3:	0.000	0.0	0.000
Result quality:	Good				

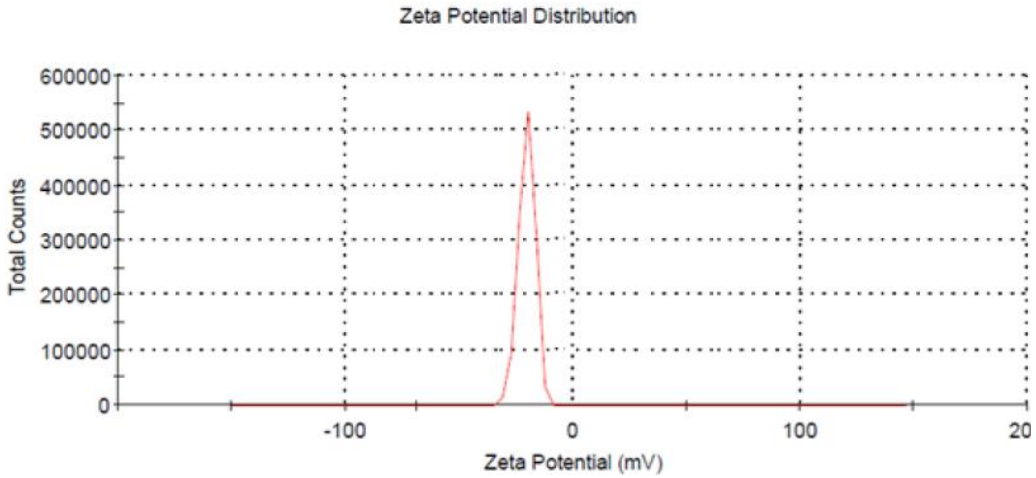

Figure 11 : Zeta Potential Distribution

TABLE 2 : Dissolution Profile

Formula	Zero Order	First Order	Hixon Crowell	Higuchi Plot	Korsmeyer Pappas
MP 1	0.991	0.994	0.997	0.998	0.993
MP 2	0.98	0.996	0.992	0.997	0.984
MP 3	0.939	0.982	0.97	0.998	0.995
MP 4	0.942	0.982	0.972	0.989	0.985
MP 5	0.912	0.951	0.94	0.98	0.975

tion which having a size of 2274 mm Figure 10 and zeta potential of 14.91 mg Figure 11 and having a prolonged and sustained release period of 15 days following the Huguchi equation i.e. Initial burst and then sustained release.

CONCLUSIONS

The results obtained during this study have shown that solvent evaporation process to prepare PCL microspheres easy and reproducible manner which can lead RIFA with very good yields. These summarizing the results of this study, we may conclude that the PCL polymer can be used, for the formation of microparticles that could release RIFA in a sustained manner for prolong period which may alter the patient non compliance in the treatment of tuberculosis..

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