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FORMULATION AND *INVITRO* EVALUAITON OF ANTI-TUBERCULAR DRUG LOADED MICROSPHERES BY PRECIPITATION METHOD

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ABSTRACT

The present research objective is to formulate and evaluate antitubercular drug loaded microspheres by precipitation technique by varying the concentration of polymer and homogenization and ultrasonication time. Primary optimization of microspheres was done by particle size, zetapotential, polydispersity index. A SEM study was carried out for the optimized formulation and the results show a uniform spherical, smooth surfaced and micron sized particles. Further Percentage Yield, Entrapment Efficiency, Drug Content, *Invitro* drug release, *Invitro* release kinetics studies and stability studies are carried out for all the formulation. Among all the formulation F5 Rifampicin microsphere shows best physical and release characteristics. The Particle size, Zetapotential and Polydispersity index of F5 was found to be 890.2 d.nm, -37.9 mV and 0.702, which shows the particle size was in range with good stable surface charge distribution around the particle. Thus it was concluded that Precipitation method with High speed homogenizer speed of 1000 RPM and ultrasonication pulse with 20 min was an optimized factorial parameter for formulation of Rifampicin loaded microsphere.

Key Words:- Microspheres, Precipitation method, High speed homogenizer, Ultrasonication, Chitosan.

INTRODUCTION

In recent years a wide variety of newer oral drug delivery system like sustained/ controlled release dosage forms are designed and evaluated in order to overcome the limitation of conventional therapy. These products are able to maintain steady drug plasma levels for extended periods of time as a result the variation of the drug levels in the blood are prevented and minimized drug related side effects (Chein YW, 1992).

Microsphere is a term used for small spherical particles, with diameters in the micrometer range (typically 1µm to 1000µm (1mm)). Microspheres are sometimes referred to as microparticles. Microspheres can be manufactured from various natural and synthetic materials. Glass Microspheres, polymer Microspheres and ceramic Microspheres are commercially available. Solid and hollow Microspheres vary widely in density and, therefore, are used for different applications. Hollow Microspheres are typically used as additives to lower the density of a material. Solid Microspheres have numerous applications depending on what material they are constructed. The Microspheres were characterized by shape, size, surface morphology, size distribution, incorporation efficiency, and *in vitro* drug release studies. The outer surfaces of the core and coated Microspheres, which were spherical in shape, were rough and smooth, respectively. The size of the core Microspheres ranged

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from 22 to 55 µm, and the size of the coated Microspheres ranged from 103 to 185 µm (Vyas SP & Khar RK, 2002).

Preparation of microspheres should satisfy certain criteria like The ability to incorporate reasonably high concentrations of the drug, Stability of the preparation after synthesis with a clinically acceptable shelf life, Controlled particle size and dispersibility in aqueous vehicles for injection, Release of active reagent with a good control over a wide time scale, Biocompatibility with a controllable biodegradability, Susceptibility to chemical modification (Jaspreet KS *et al*., 2003).

The objective of this research work is to formulate and evaluate antitubercular drug loaded microspheres by precipitation technique by varying the concentration of polymer, homogenization and ultrasonication time and to select the best optimized formulation.

MATERIALS AND METHODS

Rifampicin was received as a gift sample from Micro labs pvt Ltd, Hosur. PLGA was obtained from Medzone laboratories, Pondicherry. All other chemicals and solvents used for the research are of analytical grade and procured from an authorized dealer. Other solvents and materials were obtained from by Himedia and Loba Chemie Mumbai.

Preparation of Rifampicin Microspheres by Precipitation method

Rifampicin was dissolved in an aqueous solution of acetic acid (2% v/v); chitosan and sodium alginate at different concentration were also added. A solution of sodium sulphate (20% w/v) was added in drop wise, during stirring with High speed homogenizer at 500, 1000 and 1500 rpm followed by ultrasonication in a Probe type Ultrasonicator for 10, 20, 30 minutes. After addition of sodium sulphate, in some formulations, a solution of Glacial Acetic acid (25% w/w) was also added to evaluate the influence of cross-linking agents. Microspheres were purified by centrifugation for 15 minutes at 3000 rpm. The obtained sediment is washed with formaldehyde and then was suspended in water. These two purification steps were repeated twice. All purified particles then were lyophilized (Jaspreet KS *et al*., 2003).

Drug polymer interaction study (DSC)

Differential Scanning Calorimetry (DSC) analysis was performed using Nietzsche DSC 200PC (Nietzsche, Selb, Germany). The instrument was calibrated with indium (calibration standard, >99.999%) for melting point and heat of fusion. A heating rate of 100˚C/min was employed in the range of 25–2000˚C. Analysis was performed under nitrogen purge (20mL/min). The samples were weighed into standard aluminium pans and an empty pan was used as reference and the reading are noted (Lakshmana Prabu S *et al*., 2009).

Particle Size Analysis

All the Microspheres were evaluated with respect to their size and shape using optical microscope fitted with an ocular micrometer and a stage micrometer. The particle diameters of more than 100 Microspheres were measured randomly by optical microscope and particles are measured with the help of stage micrometer and the readings are noted and particle size was calculated by using the zero correction value (Mohanraj P *et al*., 2009; Manna Niranjan K *et al*., 2010; Cho S-M, Choi H-K, 2005; Veena B *et al*., 2009).

Zeta Potential Measurements

Particle size and zeta potential were measured by Malvern Zeta sizer respectively. The nanocrystals formulations were diluted with triple distilled water for the dynamic lighter scattering analysis. Measurements were made in triplicate at 25 ± 1 °C. Optical properties of the sample were defined as follows: refractive index 1.84 and absorption 0.02. The samples were diluted until they were transparent so as to ensure free diffusion and unhindered Brownian motion of the nanocrystals was obtained and zetapotential was readed (Mohanraj P *et al*., 2009; Veena B *et al*., 2009; Vaden P *et al*., 2010; Naoki N *et al*., 1998).

In vitro release studies

The dissolution test for Microspheres is carried out using USP apparatus II with 500ml of 0.1 N HCL , The microspheres were filled in the capsule, which was placed in the basket and the basket is rotated at 50 RPM for the first 24 hours. Samples are collected at different time interval say 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 hours. The collected samples are analyzed by using UV spectrophotometer (Naoki N *et al*., 1998; Anand G *et al*., 2011; Aritomi H *et al*., 1996; Kawashima Y *et al*., 1992; Sambathkumar R *et al*., 2011; Singla AK & Dhawan S, 2003).

In vitro release kinetics

In order to understand the mode of release of drug from Microspheres, the release data were fitted to Peppas equation ($Q = kpt^{n}$). Where, Q is the percent of the drug release at time t. K is the release constant and *n* is the release exponent indicative of the release mechanism. The n value is used to characterize different release

mechanism. The value for *n* is ≤ 0.45 for Fickian release, > 0.45 and < 0.89 for Non-fickian release, 0.89 for case II release and > 0.89 for super case II type release [5, 8, 11].

RESULTS AND DISCUSSION

DSC of Rifampicin showed a sharp endothermic peak at 187.34^{0}C (melting point). The physical mixture of Rifampicin and other excipients also showed the same thermal behavior as the individual component i.e., a blunt peak at 189.45^{0}C (melting point). Results are shown in Figure 1. DSC results also revealed that the physical mixture of Rifampicin with excipients showed superimposition of the thermogram. There was no significant change observed in melting endotherm of physical mixture of Rifampicin and excipients. From the DSC studies, it was found that there were no interaction between Rifampicin and the other ingredients.

Particle Size Analysis:

The microspheres are uniform in size with a mean size range of 890.4 to 1278.0 µm which fall in the arbitrary particle size range of 5 - 5000 µm. The particle size ranges are shown in Table 2.Among the six formulation F5 shows the best control of particle size ,may be due to the ratio of polymer, homogenizer RPM rate and ultrasonication Pulse.

Zeta potential and PDI

Zeta potential of Rifampicin loaded microspheres was in the range of -10.20 to -37.9 mV and Polydispersity index (PDI) was found to be between 0.260 to 0.702. From the results it shows that as homogenization time, sonication time and polymer concentration increases with decrease in particle size to nanometric range. If homogenization time increases with decrease in Poly Dispersibility index which shows good dispersibility particles and stability by increasing the concentration of tween 80.The results are shown in Table 2.

Scanning Electron Microscopy (SEM)

From the optimized particle size analysis data F5 formulations was selected and SEM studies were carried out. Shape and surface morphology of the Microsphere prepared with optimized parameters was observed by research microscope and scanning electron microscopy. The study revealed that microspheres were fairly spherical in shape, the surface of the particle showed a characteristic smoothness, and that the particle size was in the micrometric range, as depicted by SEM. Some of the particles were found to be in clusters as shown in the Figure 2.

Percentage Yield, Drug Content, Entrapment efficiency and Percentage moisture loss Percentage yield of the formulations was found in the range 84.23 ± 2.84 to 86.88 ± 2.94 .In case of drug content study, all the formulation was in the range of 85 – 115% w/v. This may due to increase in concentration of polymer leads to increase in drug content of microspheres. Uniform homogenous time and surfactant concentration plays a vital role in enhancing the drug content and encapsulating the drug into the microsphere. Percentage of moisture loss of the formulation was found in the range 1.69-4.57%.The results are shown in table 3.

***In vitro* release studies**

Release pattern for formulation F1-F6 are shown in figure 4. From the *in vitro* release studies it was confirmed that there was significant relation between percentage drug release and concentration of cross linker sodium sulphate used in the Microsphere formulation. This was confirmed by the F 3, F 4 and F 5 formulation, i.e. drug release was controlled well by cross-linking agent. This shows that the cross linker plays important role in the drug release from the Microsphere hydrogel formulation

***In vitro* release kinetics:**

The values of *n* and the coefficient of determination (r^2) obtained are listed in Table 4.It shows that by fitting the percentage drug release values of optimized formulation F5 in zero order release kinetics datas, it show the regression coefficient value (R^2) as 0.999 and in Peppas fitting curve it shows the release exponent value ‘n’ as 0.860, which confirms that the drug release from the formulation F5 obeys zero order and Nonfickian diffusion mechanism.

Stability studies

The Optimized formulation were analyzed and checked for changes in physical appearance and drug content at regular intervals The objective of stability studies is to predict the shelf life of a product by accelerating the rate of decomposition, preferably by increasing the temperature and percentage relative humidity. The optimized formulations (F5) were subjected to stability studies as per ICH guidelines by storing at 25 ºC/60 % RH and 40 ºC/75 % RH for 90 days. These samples were analyzed and checked for changes in physical appearance and drug content at regular intervals and the results are shown in table 5.

Table 1. Formulations of Rifampicin Microspheres

Sl. No	Ingredients	Rifampicin Microspheres					
		F1	F2	F3	F4	F5	F6
	Formulation variable						
1	Rifampicin (in ratio)	1	1	1	1	1	1
2	Chitosan (in ratio)	1	2	3	-	-	-
3	Sodium alginate	-	-	-	1	2	3
4	Sodium sulphate	20%	20%	20%	20%	20%	20%
5	Acetic acid	2%	2%	2%	2%	2%	2%
6	GA	25%	25%	25%	25%	25%	25%
7	Formaldehyde	1%	1%	1%	1%	1%	1%
8	Distilled water	5ml	5ml	5ml	5ml	5ml	5ml
	Process variable						
1	High speed homogenizer (RPM)	500	1000	1500	500	1000	1500
2	Ultrasonicator pulse (min)	10	20	30	10	20	30

Table 2. Particle size analysis data, Zeta potential and Polydispeersibility index for microspheres formulation F1 – F6

Formulation	Average Particle size (µm)	Zeta Potential (mV)	Poly dispersibility Index (PDI)
F 1	1235.6	- 10.2	0.345
F 2	1278.0	- 14.5	0.260
F 3	1124.2	- 32.4	0.502
F 4	1065.6	- 32.8	0.544
F 5	890.2	- 37.9	0.702
F6	1024.2	- 34.2	0.488

Table 3. Percentage Yield, Entrapment Efficiency, Drug Content of Microbeads (n=3)

Formulation code	Percentage yield	% Encapsulation efficiency	% Drug loading	Drug content (%w/v)	%Moisture loss
F 1	86.23 ± 1.02	68.44 ± 1.092	82.11 ± 1.41	96.29 ± 1.32	4.57 ± 0.02
F 2	85.51 ± 1.98	77.36 ± 1.071	72.98 ± 1.37	95.87 ± 1.28	1.69 ± 0.12
F 3	84.23 ± 2.84	79.64 ± 1.069	75.24 ± 1.28	96.10 ± 1.40	4.63 ± 0.29
F 4	86.88 ± 2.94	73.23 ± 1.084	86.26 ± 1.24	99.06 ± 1.59	3.97 ± 0.11
F 5	85.13 ± 1.03	77.74 ± 2.078	74.24 ± 1.18	95.69 ± 1.87	2.92 ± 0.08
F6	85.40± 2.03	75.40 ± 1.04	78.94 ± 1.08	94.92± 1.08	1.92 ± 0.22

Table 4. *Invitro* Release Kinetics studies for microsphere formulation F1-F6

Formulation	Zero order R^2	First order R^2	Higuchi R^2	Peppas	
				R^2	n value
F1	0.857	0.952	0.959	0.960	0.423
F2	0.808	0.991	0.947	0.902	0.508
F3	0.994	0.824	0.956	0.905	0.567
F4	0.7719	0.986	0.965	0.942	0.412
F5	0.999	0.86	0.932	0.983	0.860
F6	0.942	0.826	0.968	0.980	0.548

Table 5. Stability study parameter of the optimized formulation F5

Stability condition	Sampling interval (days)	Physical appearance	% Drug content F5 (mean ± S.D*)
25°±2°C/60±5% RH	0	No change	94.34 ± 0.015
	15	No change	93.13 ± 0.045
	45	No change	92.47 ± 0.087
	90	No change	92.23 ± 0.025
40°±2°C/75±5% RH	0	No change	89.34 ± 0.015
	15	No change	88.21 ± 0.067
	45	No change	87.87 ± 0.089
	90	No change	87.07 ± 0.092

Figure 1. Overlay DSC Spectrum showing both Physical mixture and pure drug

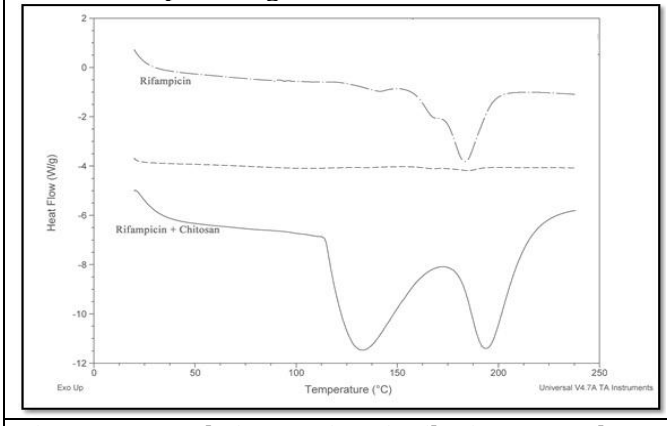


Figure 2. SEM studies showing the surface morphology of Microspheres-F5

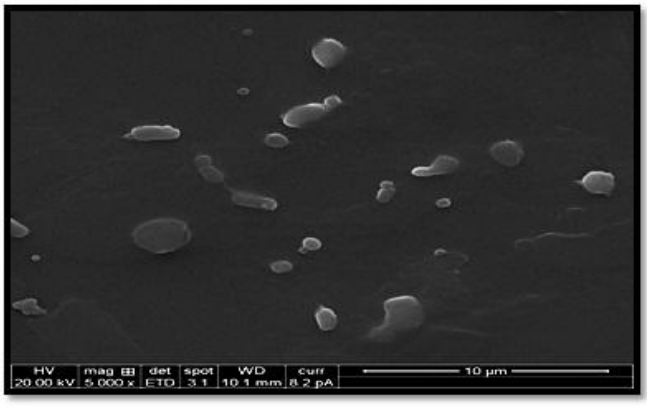


Figure 3. Formulation F5 Size Distribution Report by intensity of the peak (Malvern Instrument ltd. - Zeta Sizer version 6.0)

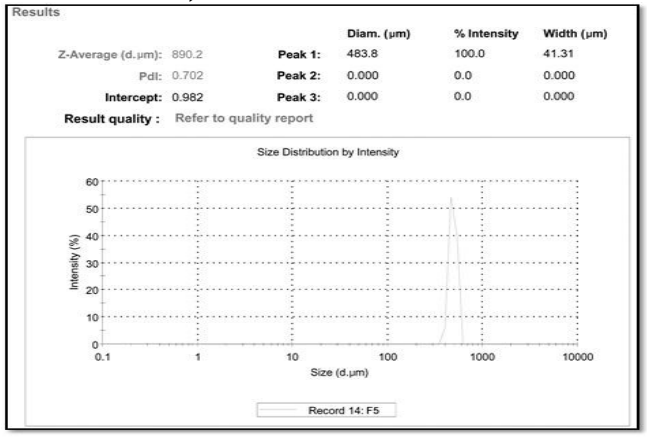
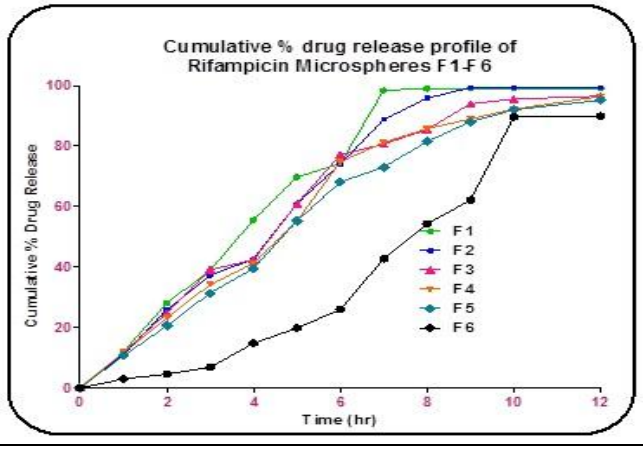


Figure 4. Cumulative % drug release profile of Rifampicin Microspheres F1-F6



CONCLUSION

From the above results and discussion it was confirmed that F5 microsphere formulated by precipitation method shows best results. Thus it was concluded that Precipitation method with 1:2 ratio of Drug: Polymer, High speed homogenizer speed of 1000 RPM and Ultrasonication pulse with 20 min was an optimized factorial parameter for formulation of microspheres.

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