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RESEARCH ARTICLE

PREPARATION AND CHARACTERIZATION OF ISONIAZID CHITOSAN LOADED NANOPARTICLES

Sharma Prashansa*, Sharma Pramod Kumar, Mishra Vidya Bhushan, Akanksha Bhandari

Department of Pharmacy, School of Medical and allied sciences, Galgotias University, Greater Noida, U. P. (India)

**corresponding Author's E-mail- prashansa51@gmail.com, Contact- 9310179107*

ABSTRACT

Although the part that we live in, is an age of creative technology and modernization, infectious diseases, like Tuberculosis (TB), still remain to be one of the greatest health challenges worldwide. The objective of this study was to load first line anti-tubercular drug, isoniazid in chitosan nanoparticles in order to enhance its bioavailability and to reduce the dosing frequency. The isoniazid nanoparticles were prepared by Ionic gelation method with addition of suitable polymer (Chitosan) in acidic aqueous solution at various concentrations. The percentage yield obtained for the various formulations was 52-70 % which shows the suitability of ionic gelation technique for preparation of nanoparticles of isoniazid and the entrapment efficiency for the formulations F1-F5 was found to be in the range of 50-70% depending upon the polymer concentrations. It was observed that with an initial increase in polymer i.e. chitosan concentration entrapment efficiency increases, however it dips as concentration of chitosan is increased further because of increased solution viscosity which decreases the loading capacity of nanoparticles. After the SEM studies, it was observed that the formulation F3 nanoparticles was the best being spherical and more regular in shape and size range between 124-220 nm. The F3 isoniazid nanoparticle formulation had a mean particle size of 1.81 nm and zeta potential in (mV) is 52.4. During the *in-vitro* diffusion studies, it was observed that all the formulations show good control over the release of drug from the nanoparticles matrix. F3 formulation shows 87% drug release in 24 hours. It was observed that F3 follows the Korsmeyer Peppas model (r^2 value=0.9864) indicating that the mechanism of drug release from the prepared F3 formulation is non fickian diffusion.

Key words: Isoniazid, Chitosan, Entrapment efficiency, Mean particle size, Zeta potential, Tuberculosis.

1. INTRODUCTION

Nanotechnology is a multi-disciplinary scientific field which should be undergoing explosive development. Parts of this field, development of nano-scale drug delivery devices are done. Nanoparticles have been developed as an important strategy to deliver conventional drugs, recombinant proteins, vaccines and most recently nucleotides [1].

Nanotechnology is a rapidly growing field due to the ever-growing increase in technology and science. Because of the new inventions and the latest technology made available day by day, a lot of new avenues are bring opened which were impossible to even dream about in the yester-days.

In the past few years nanotechnology has grown by leaps and bounds, and this multi-disciplinary scientific field is undergoing explosive development day by day with no limits [2-5].

A complete list of the potential applications in nanotechnology is too vast and diverse to discuss, and its detail is too lengthy, but without doubt, one of the best values of nanotechnology will be in the development of new and effective medical treatments and different devices [6-10].

Nanoparticles (NP) have been studied extensively as particulate carriers in several pharmaceutical and medical fields. Nanoparticles, can be used to provide targeted (cellular/ tissue) delivery of drugs, to sustain drug effect in target tissue, to improve oral bioavailability of solubilize drugs for intravascular delivery and to improve the stability of therapeutic agents against enzymatic degradation [11].

These are the future benefits of the nanoparticles

- It can protect drugs from degradation.
- It can improve the physical properties of drug.
- Reduction in number of doses required.
- Make treatment a better experience and reduce treatment expenses.
- Allow delivery of insoluble drugs.
- It should not be loss their activity and therapeutic efficacy while in circulation.
- Improve the oral bioavailability of the agents that are not effectively used orally.

In the present study, we explored the potential of isoniazid nanoparticle in improving the oral bioavailability of isoniazid.

1.1 MAIN OBJECTIVE OF WORK:

The aim of the present project work was to prepare and evaluate isoniazid nanoparticles for pulmonary drug delivery.

Nanoparticle drug delivery system has received great attention through the last decade. Nanoparticle as a drug delivery system can deliver certain drug to systemic circulation in more convenient and effective way than possible with conventional dosage form. A drug incorporated nanoparticle can release the drug to greater extent with high concentration to the target site with low therapeutic dose.

For pulmonary administration formulation includes biodegradable polymers nanoparticles, which are degraded in the body into carbon dioxide and water which are biocompatible. Poor soluble drugs bioavailability can be increased by reducing their particle size into submicron size.

The main objectives of the project work were to develop a polymeric nanoparticle loaded with anti-tubercular drug to target the lungs with in a size range of 1-5µm. This provides control over the release pattern of drug.

- To prepare polymeric nanoparticles of Isoniazid.
- To optimize.
- Pulmonary drug delivery using nanoparticles in the size range of 1-5 µm resulting in the effective distribution of nanoparticles in respiratory tract.
- To extent the release of drug from the nanoparticles.
- To improve the safety profile of the drug and localized stable drug concentration achieve and reduce the dosing frequency.

2. MATERIALS AND METHODS

2.1 Materials

Isoniazid was obtained as a gift sample from Amsal Chemical Private Ltd, Gujrat, India. Chitosan was provided by Indian Research Products, Chennai, India. Acetic acid and Sodium tri-polyphosphate are obtained from Spectrum Reagents and Chemicals Pvt. Ltd, Cochin, India. All other reagents are used in this study were of analytical grade.

2.2 Methods

The isoniazid nanoparticles were prepared by Ionic gelation method with addition of suitable polymer (Chitosan) in acidic aqueous solution at various concentrations. Chitosan was dissolved in acetic acid aqueous solution at various concentrations. Drug was dispersed in chitosan solution and agitated over magnetic stirrer at room temperature for a period of thirty minutes. The Tri-polyphosphate aqueous solution with various concentrations was added drop wise to the above solution. Tri-polyphospate was added as a cross linking agent for the chitosan nanoparticles to achieve sustained drug release. Then the prepared nanoparticles were separated from the solution by sonication followed by centrifugation. [12]

2.2.1 Preparation of isoniazid nanoparticles

Drug (Isoniazid) and Polymer (Chitosan) at various concentrations {0.25, 0.5, 0.75, 1, and 1.25 (w/v)} dissolved in Acetic acid **{**for all formulations the concentration of Acetic acid aqueous solution was 2 ml (v/v)} by help of magnetic stirrer and allowed to stand 30 minutes and solution of drug and polymer was formed and this solution was kept over magnetic stirrer at specific room temperature. Various concentration of Sodium tri-polyphosphate solution is added in drop wise manner in prepared solution of drug and polymer and suspension of Isoniazid-Chitosan-Sodium tri-polyphosphate nanoparticles was formed. It was kept for Sonication for 25 minutes. After sonication nanoparticle suspension was centrifuged at 19,000 RPM for 20 minutes. The supernatant was discarded Pellets were resuspended in De-ionized water Followed by Sonification, centrifugation this process was repeated three times. The obtained particles were dried by using lyophilizer (VIRTIS FREEZE DRYER Maker-SP industries INP, Model-virtis advantage plus EL-85 and collected and store in well close container.

2.2.2 Characterization of prepared nanoparticles

All the method of characterization was important parameter for nanoparticle preparation. Several parameters which have to be considered in characterization are as follow.

2.2.2.1 Fourier transformed infrared (FTIR) spectroscopic analysis

FTIR spectra were recorded with a (Alpha-T Bruker IR instrument) IR spectrometer in range 400–4000 cm^{-1} using a resolution of 4 cm^{-1} and 10 cm^{-1}.Weighed amount of drug 3 mg was mixed with 12 mg of potassium bromide. The mixture was taken and compressed less than 10 ton pressures in a hydraulic press to form a transparent pellet. The spectra were recorded in order to evaluate the molecular states of micronized isoniazid and nano-isoniazid and also for the drug interaction studies of pure isoniazid, Chitosan and both.

2.2.2.2 Evaluation of drug encapsulation

2.2.2.2.1 Percentage yield

The percentage yield of prepared nanoparticles of drug and polymer was calculated by using following equation

$$\text{Percentage yield} = \frac{\text{Total weight of obtained nanoparticle}}{\text{Total weight of drug and polymer}} \text{ X } 100$$

2.2.2.2.2 Entrapment Efficiency

Entrapment efficiency was determined by crushed nanoparticles (1 mg Equivalent drug) add in 10 ml methanol; allow this suspension for 24 hrs. Then filter the suspension through (0.2 µm nylon filters) and supernatant collected was for assayed for drug content by UV spectroscopy (Shimadzu 1700, Japan) at the wavelength of 262 nm.

The entrapment efficiency of nanoparticles for the drug was calculated by using the following equation

$$\text{Entrapment efficiency} = \frac{\text{Amount of drug in nanoparticles}}{\text{Amount of drug added in nanoparticles}} \text{X} 100$$

2.2.2.3 Scanning Electron Microscopy (SEM):

The SEM analysis of prepared nanoparticles formulation, Scanning Electron Microscopy (SEM MU) was used for morphological studies. The formulation was scattered on an aluminium stub, the latter surface covered with clear double sided adhesive tape. . In order to obtain uniformly scattered sample the aluminium stab was tapped gently on its edge with a spatula. The particles were then coated with a 20nm layer of gold using a sputter coater on an argon atmosphere at 50 mA for 50 seconds.

2.2.2.4 Measurement of Particle size and Zeta potential:

Particle size distribution of isoniazid nanoparticle was determined by laser scanning technique using (Malvern Instrument) after appropriate dilution with KCl solution . The measurements were carried out in an aqueous solution of 0.1 N KCl. The mean particle size and zeta potential were calculated for each formulation maintained at 20˚C.

2.2.2.5 *In-vitro* release studies

In-vitro release study of Isoniazid nanoparticles was performed on a USP Type II dissolution test apparatus (Electro lab, USP TDT 06P). A dialysis membrane pouches are made by sealing with clips used for filling 5 mg isoniazid nanoparticles and attached on paddle by thread for dissolution test. The experiments were carried out at 37 ± 0.3°C at a rotation speed of 100 ± 2 rpm using 900 ml phosphate buffer (pH 7.4) as the release media, in order to simulate the condition in the lungs. A measure of 5 ml of the sample was withdrawn at appropriate time intervals (0, 1, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24) and centrifuged at 10000 rpm for 5 minutes. Supernatants were diluted suitably with methanol and absorbance of the resulting solution was measured at 262 nm in a UV spectrophotometer (Shimazdu UV-1700, Japan). The residue (after centrifugation) was redispersed in 1 ml of the fresh dissolution medium and replaced back into the dissolution apparatus. [13]

2.2.2.6 Mechanism of *in-vitro* drug release kinetics by using mathematical models:

Various models were tested for investigate the mode of release explaining the kinetics of drug release form isoniazid nanoparticles, the release data were analyzed with the following mathematical models. To analyze the mechanism of the drug release rate kinetics of the dosage form, the obtained data were fitted into zero-order, first order, Higuchi's and Korsmeyer-Peppas release model. Drug release rate kinetic of isoniazid nanoparticles was calculated by using DDS over, A Microsoft Excel Add-in.

3. RESULTS AND DISCUSSION

3.1 Fourier transforms infrared spectroscopy:

Fourier transform infrared (FTIR) spectrometry is a useful tool for identifying both organic and inorganic chemicals. It can be used to identify some components of an unknown mixture and can be utilize to analyse liquids, solids, or gases. FTIR Spectroscopy was used to ensure that no chemical interaction occurs between the drug and polymer used. This is given in Table 1:

Table 1: FTIR Studies for drug and polymer

S. NO.	FTIR STUDIES
1	Isoniazid (Drug)
2	Chitosan (Polymer)
3	Isoniazid + Chitosan

FTIR Spectra interpretation the following results were obtained and shown in Table 2:

Table 2: FTIR Vibrations for Isoniazid

S. NO.	VIBRATIONS	RANGE (cm^{-1})
1	N-H_2	3304
2	C-H	2866
3	C=O	1667
4	N-H	1220
5	C-O	1411
6	C-N	1556
7	N-N	1140

The IR spectrum of the pure drug shows a broad absorption in near 3304 cm^{-1} due to presence of (N-H_2) amine group and other peaks C-H on 2867 cm^{-1}, C=O on 1667 cm^{-1}, N-H on 1221 cm^{-1}, this signifying that this structure is Isoniazid and its present in pure form.

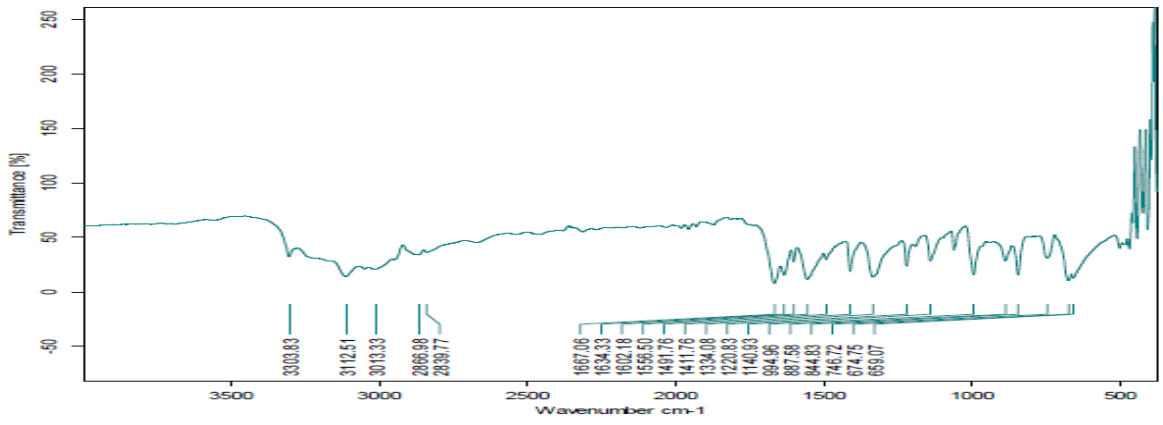

Figure 1: FTIR Spectrum of pure Isoniazid

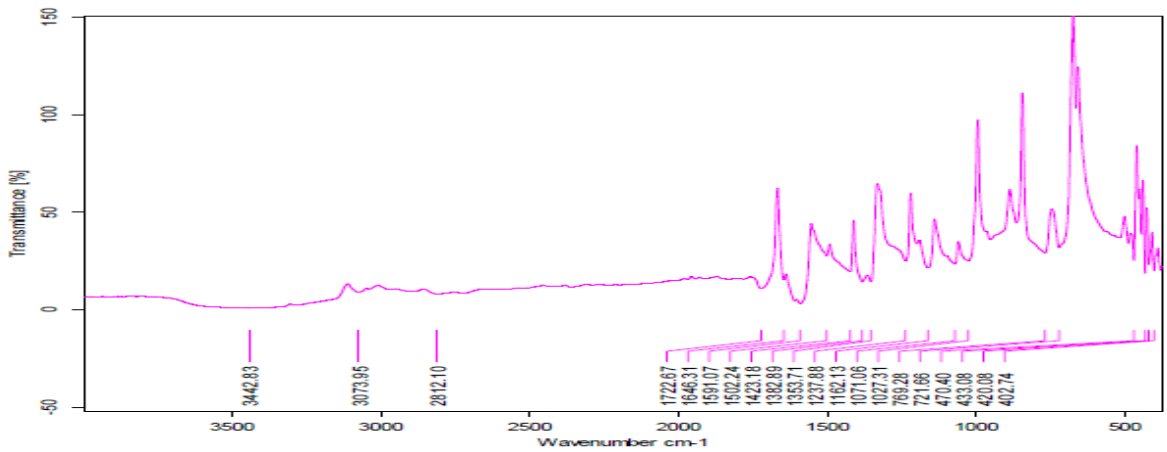

Figure 2: FTIR Spectrum of Chitosan

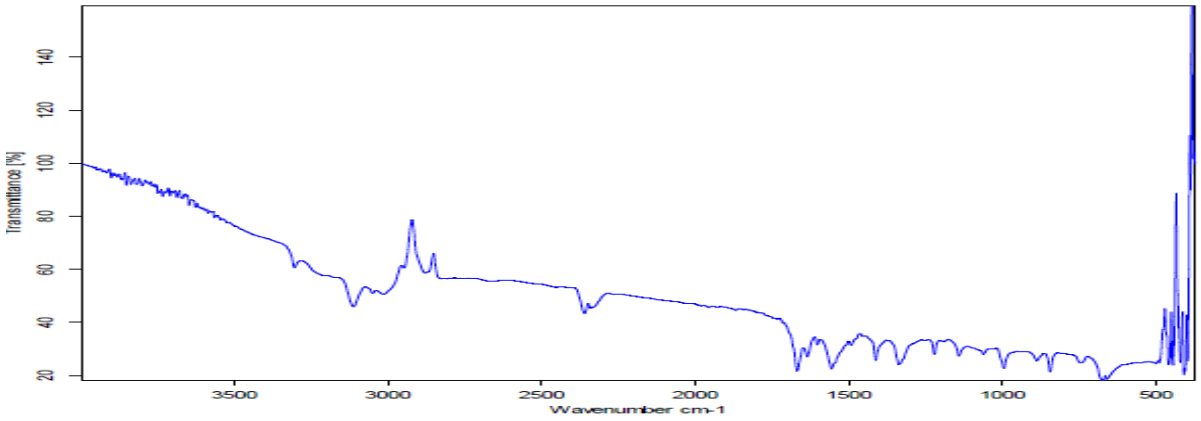

Figure 3: FTIR Spectrum of Isoniazid-Chitosan

The major characteristics peaks of the drug remain unaffected in the IR spectra for drug + excipient mixture, which clearly indicates that no interaction occurred between the drug and excipient.

3.2 PERCENTAGE YIELD:

Percentage yield of isoniazid nanoparticles was calculated by drug and polymer are collected after formulation. The % yields for formulations are given in Table 3:

Table 3: Percentage yield of Isoniazid nanoparticles

FORMULATION	PRECENTAGE YIELD (%)
F1	53.77
F2	57.20
F3	69.09
F4	68.00
F5	66.28

According to the obtained result, the % yield of F3 formulation is more than from all the formulations i.e. 69.09 % and this result is shown in Figure 4:

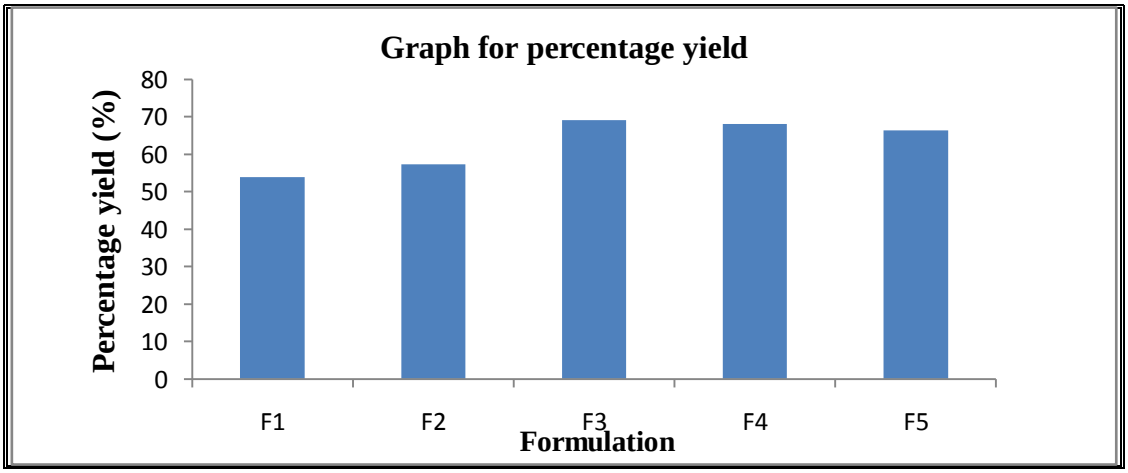

Figure 4: Percentage yield graph of Isoniazid nanoparticle

3.3 ENTRAPMENT EFFICIENCY (E %):

Entrapment efficiency gives the amount of drug entrapped in the nanoparticles. Entrapment efficiency (E %) of the prepared formulations influence by chitosan concentration and ionizing agent on the isoniazid entrapment in nanoparticles was evaluated. This calculation is shown in Table 4. According to the obtained result, the entrapment efficiency of F3 formulation is more than from the entire formulations i.e. 69.09 % and this result is shown in Figure 5.

Table 4: Entrapment efficiency of Isoniazid nanoparticles

FORMULATION	ENTRAPMENT EFFICIENCY (%)
F1	53.75
F2	57.20
F3	69.05
F4	68.00
F5	66.25

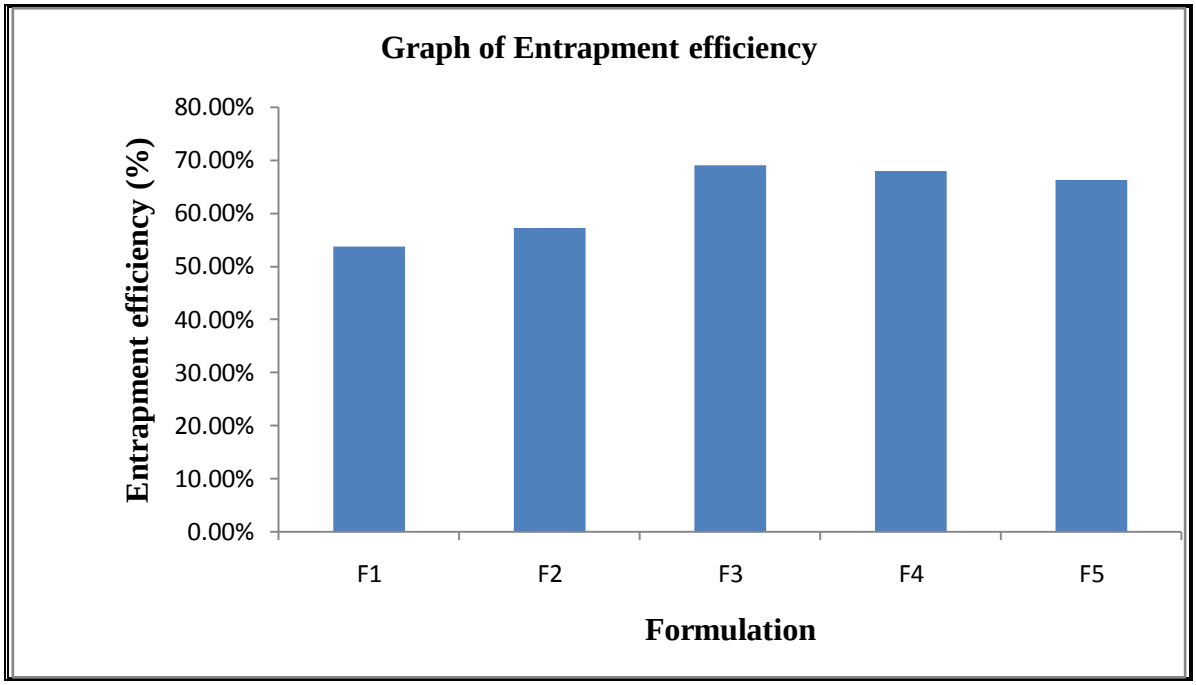

Figure 5: Entrapment efficiency graph of Isoniazid nanoparticles

The highest E% was found in formulations prepared with the lesser amount of chitosan i.e. F1. This is because the increase in chitosan concentration leads to increased solution viscosity that decreased the loading capacity of the nanoparticles as a consequence of the reduced drug solubilisation. Part of the drug was not dissolved during preparation process and/or it was probably lost from the nanoparticles during the washing steps.

3.4 MEAN SIZE AND ZETA POTENTIAL FOR OPTIMIZED FORMULATION:

The particle size (Malvern particle size analyzer) and zeta potential (Malvern zetasizer) were done for optimized F3 formulation in the size ranging from 1000 nm to 4000nm. The size of nanoparticles was affected by the stirring time and the concentration of polymer. The mean particle size of the Isoniazid loaded nanoparticles were found to be 1.81 nm and Zeta potential of isoniazid loaded formulations were found to be 52.4 mV that shows that F3 formulation having high potential to conduct the surface charges with good stability. It can be observed in the Table 5.

Table 5: F3 formulation with their particle size and Zeta Potential

Formulation	Mean Particle Size	Zeta potential (mV)
F3	1.81 nm	52.4

According to this result, particle size and zeta potential are shown in Figure 6,7:

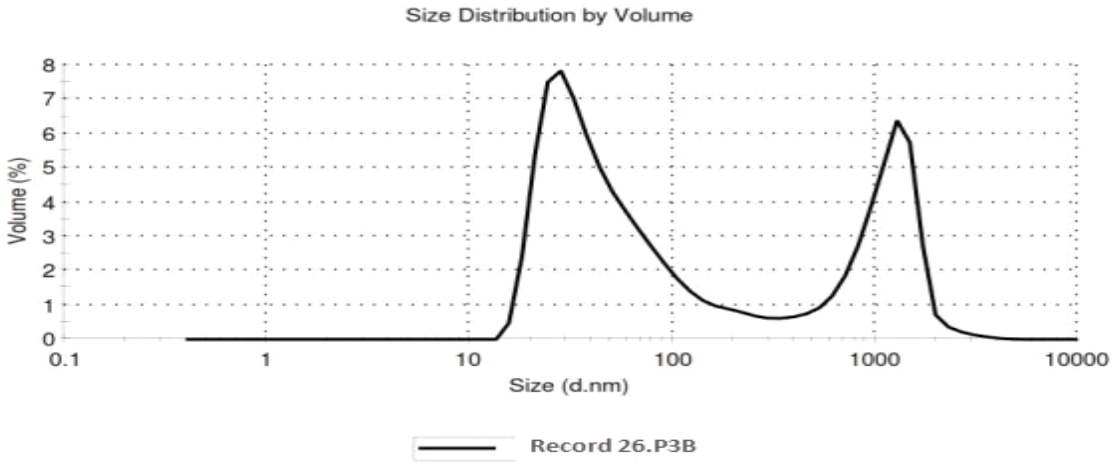

Figure 6: Measurement of particle size

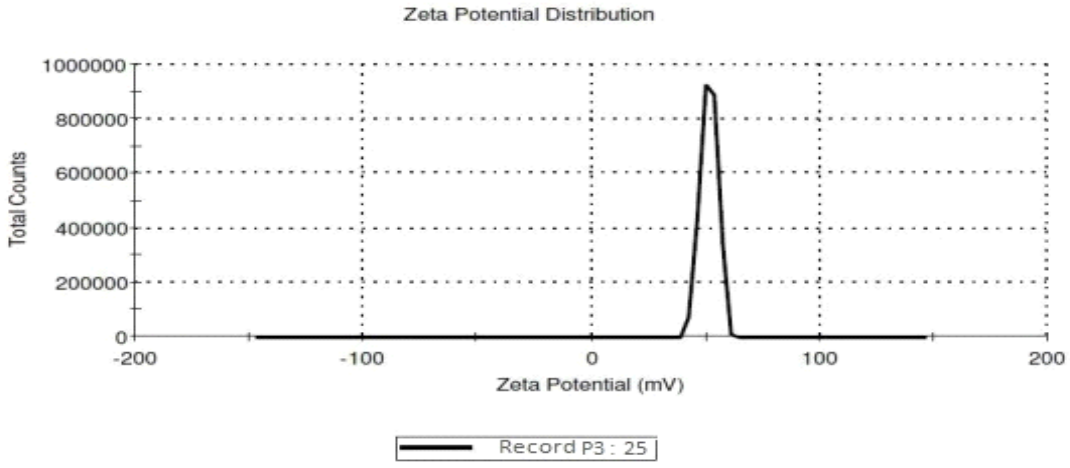

Figure 7: Distribution of zeta potential

The F3 formulation of isoniazid nanoparticle parameter shows that, particle size of nanoparticle increases when the concentration of the chitosan increases. The result of Zeta potential in formulation F3 shows that it having high potential to conduct the surface charges with good stability.

3.5 SCANNING ELECTRON MICROSCOPY:

Shape and surface morphology of the isoniazid nanoparticles studied with optimized F1 and F3 formulation was observed by scanning electron microscopy; SEM micrographs showed that nanoparticles were spherical and more regular in shape, the surface of the particle showed a characteristic smoothness, and that the particle size was in the nanometric range, as depicted by SEM. This was essential for pulmonary delivery of the drugs and was in the range of 1000 nm to 5000 nm in different formulation different chitosan concentration. These

Scanning electron microscopy reports are shown in Figure 8, 9:

Scanning electron microscopy report:

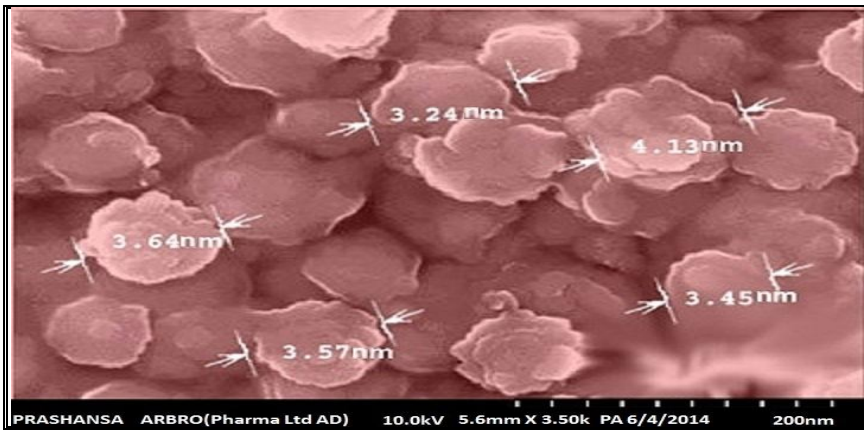

Figure 8: SEM result for F1 formulation
(Observe nanoparticles in the range of 1000nm to 5000)

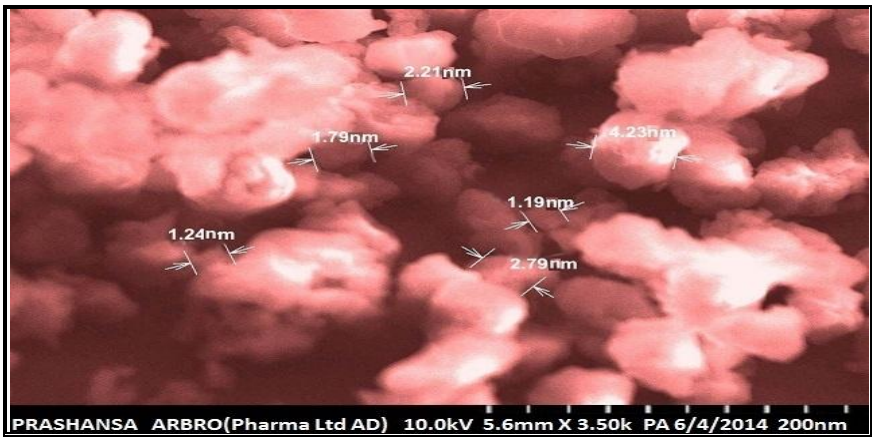

Figure 9: SEM result for F3 formulation
(Observe nanoparticles in the range of 1000 to 5000nm)

The result of SEM clearly shows that the F3 formulation of isoniazid nanoparticles was nearly spherical in nature, this is essential for pulmonary delivery (1-3 µm) of drug and the surface of the particle was smooth and that the particle size was in the nanometric range.

3.6 *IN-VITRO* STUDIES OF ISONIAZID NANOPARTICLES:

In-vitro release of Isoniazid Nanoparticles was determined using, as the release media, phosphate buffer (pH 7.4) in order to simulate the condition in the lungs. The *in-vitro* drug release studies for all five formulations of Isoniazid loaded nanoparticles were carried out in pH 7.4 phosphate for 24 hrs. And these represented in Table 6:

Table 6: *In-vitro* drug release profile of Isoniazid nanoparticle

S. NO	TIME (HOURS)	C.D.R. (%)				
		F1	F2	F3	F4	F5
1	0	0	0	0	0	0
2	1	3.01	3.27	2.93	3.22	3.30
3	2	10.25	11.97	9.45	12.20	12.23
4	4	17.63	22.21	15.45	21.52	21.19
5	6	24.66	31.11	24.43	30.43	32.56
6	8	33.26	40.12	32.22	39.40	41.20
7	10	42.56	49.11	43.52	48.34	51.12
8	12	48.62	57.15	52.24	57.28	60.17
9	14	59.61	66.13	62.34	65.62	70.15
10	16	68.20	74.46	67.21	74.67	79.13
11	18	76.65	81.23	72.33	80.32	80.01
12	20	84.55	87.31	74.23	86.02	87.43
13	22	90.67	92.32	81.24	91.05	92.12
14	24	96.26	96.64	87.52	96.03	96.24

The results of *in-vitro* drug release for the various formulations are represented in Figure 10:

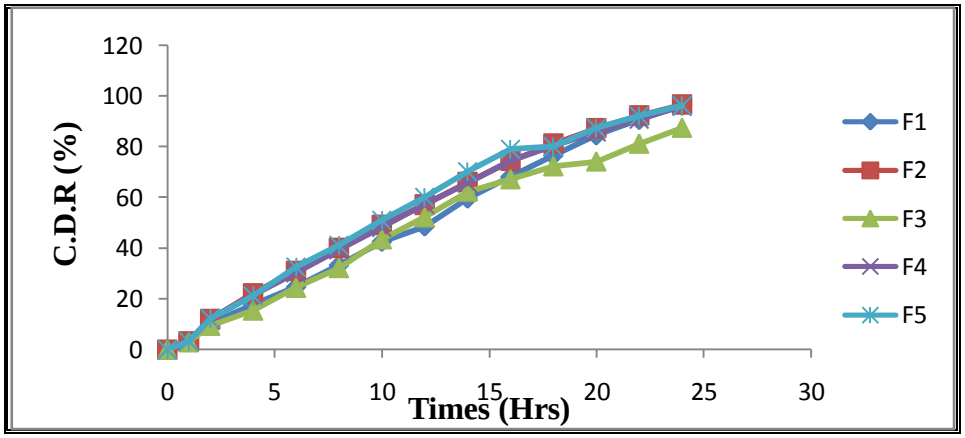

Figure 10: *In-vitro* drug release profile graph of Isoniazid nanoparticles

From the above results it was observed that, all the formulations show the ability to control drug release from the formulated nanoparticles. But F3 formulation shows best control release due to its smaller particle size which shows the suitability of the nanoparticles for achieving the goals of sustained drug release. And better shape, which shows that the formulation has been formed correctly, hence it's has the ability to control drug release. In general the drug release from all formulation followed a steady pattern.

3.7 *IN-VITRO* DRUG RELEASE KINETICS:

The release kinetics should apply on best F3 formulation. Isoniazid nanoparticles are evaluated by fitted the obtained *in-vitro* release data into various kinetic models like zero order, first order, korsmeyer peppas and Higuchi equations. The drug release kinetic data of F3 Isoniazid nanoparticles was shown in Table 7:

Table 7: Kinetics of F3 formulation

RELEASE MODEL		F3
K- MODELS	R^2	RESULT
Zero order	R^2	0.9819
First order	R^2	0.7888
Korsmeyer peppas	R^2	0.9864
Higuchi	R^2	0.984

The results of *in-vitro* drug release kinetics for the optimized F3 formulation are represented in Figures 11, 12, 13, 14:

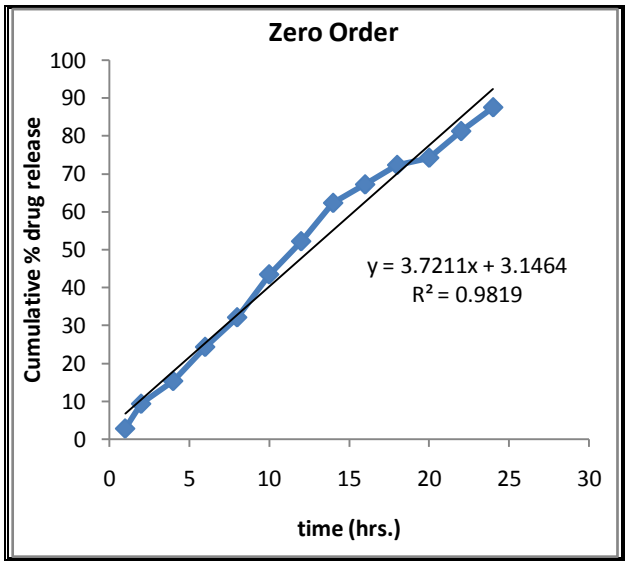

Figure 11: Zero order kinetics of F3 formulation.

Zero order kinetics was applied on F3 formulation and R^2 value is 0.9819.

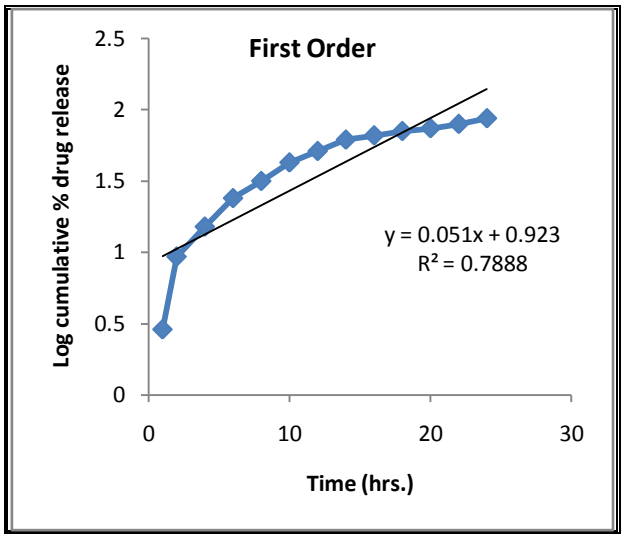

Figure 12: First order kinetics of F3 formulation

First order kinetics was applied on F3 formulation and R^2 value is 0.7888

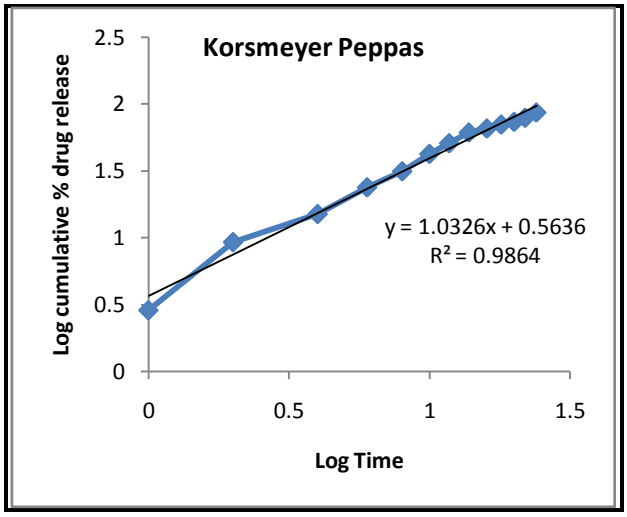

Figure 13: Korsmeyer peppas kinetics of F3 formulation

Korsmeyer peppas kinetics was applied on F3 formulation and R^2 value is 0.9864.

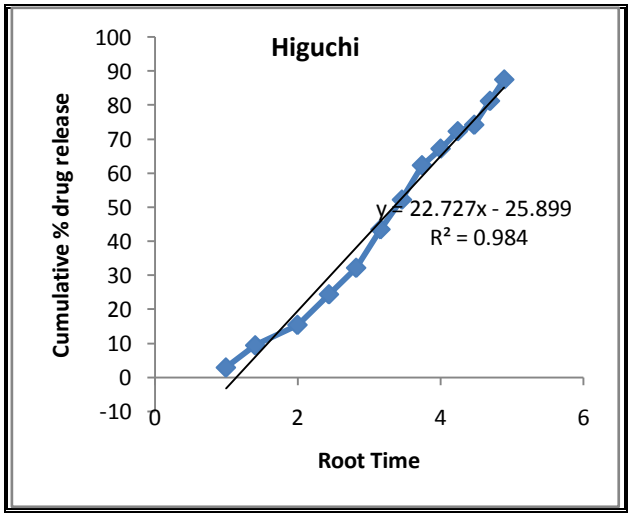

Figure 14: Higuchi kinetics of F3 formulation

Higuchi kinetics was applied on F3 formulation and R^2 value is 0.984.

It was observed that F3 follows the Korsmeyer peppas model R^2 value were 0.9864 respectively which are higher than other models. So, it was concluded that this formulation follows Korsmeyer peppas kinetics, which releases drug in control manner and it is the ideal method of drug release to achieve pharmacological prolonged action. The values of release exponent (n) of F3 lies within of n = 0.5 to 1 have been observed, which are regarded as non-fickian diffusion mechanism. When a glassy hydrogel (film) is brought into contact with water or water-solvent mixture, the water diffuses into the film and the film expands resulting in swelling of the hydrogel. Diffusion involves migration of water into pre-existing or dynamically formed species between hydrogel chains. Swelling of hydrogel involves larger scale segmental motion resulting ultimately increase in separation between film chains. Analysis of the mechanism of water or water-solvent mixtures diffusion into swellable polymeric system for chitosan films

was found to be non-Fickian and could be described as anomalous which correlates with our studies as the calculated diffusion exponent is greater than 0.5 indicating that the diffusion of solvent into the film was non-Fickian.[14] Hence it is concluded that the mechanism of drug release from the prepared F3 formulation is non fickian diffusion.

CONCLUSION

Isoniazid nanoparticles were prepared by ionic gelation method and evaluation was done. The compatibility studies of Isoniazid and other excipients were carried out by FTIR studies to rule out the possibility of any interactions between the drug and the polymer. The peaks for major functional groups in drug and excipients spectra are reproducible when compared to the pure drug spectra. And this shows there is no interaction between the Isoniazid and other excipients used in the formulation.

A total of five formulations were prepared by varying the concentration of chitosan. Particle size parameter shows that, the particle size of prepared Isoniazid nanoparticles increased with increase in the concentration of chitosan which can be attributed to increased solution viscosity. The entrapment efficiency for the formulations F1-F5 was found to be in the range of 50-70% depending upon the polymer concentrations. It was observed that with an initial increase in polymer i.e. chitosan concentration entrapment efficiency increases, however it dips as concentration of chitosan is increased further because of increased solution viscosity which decreases the loading capacity of nanoparticles. And the result of mean particle size of the best F3 formulation is 181 nm and zeta potential of F3 formulation is 52.4 mV this result shows that F3 formulation having high potential to conduct the surface charges with good stability. SEM results clearly represents that the prepared Isoniazid nanoparticles were nearly spherical in nature, the surface of the particle was smooth and particles are in nanometric range.

Prepared Isoniazid nanoparticles are evaluated for the total drug content, percentage yield and entrapment efficiency. All the formulations were able to control the release of drug. But while considering the zeta potential, particle size, SEM, *in-vitro* drug release and kinetic studies, it was concluded that F3 formulation is the best formulation among all the formulations.

It was concluded that this method of preparation Isoniazid nanoparticles is best which lead to better results like high entrapment efficiency, good percentage yield, high drug content and chitosan was a better choice of polymer for loading Isoniazid in it to prepare nanoparticles.

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CONFLICT OF INTEREST-Authors have no conflict of interest.

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