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FORMULATION DEVELOPMENT AND CHARACTERIZATION OF ISONIAZID NANOPARTICLES

Nurukurthi Sushmitha Shainey*[1], Mrs. Raj Khiani[1], Dr T.K.V. Kesav Rao[1]
[1]Department of Pharmaceutics, Pydah College of Pharmacy Patavala, Andhra University, Kakinada, Andhra Pradesh.

Abstract:
Nanoparticles represent a promising drug delivery system of controlled and targeted drug release. They are specially designed to release the drug in the vicinity of target tissue. The aim of this study was to prepare and evaluate nanoparticles containing Isoniazid in different drug to polymer ratio by solvent evaporation technique. Prepared Nanoparticle was evaluated for its Particle Size, scanning electron microscopy, Percentage practical yield, Drug Entrapment Efficiency, and in-vitro drug release studies. FT-IR studies indicated that there was no chemical interaction between drug and polymer and stability of drug.
The optimized Nanoparticles were found with particle size of 95.41 nm, percentage practical yield was 96.72 %. Entrapment efficiency (% EE) of 93.20 %, scanning electron microscopy irregular shape. The in-vitro release profile was found to be 98.19% sustained up to 12 h.
The developed formulation overcome and alleviates the drawbacks and limitations of Isoniazid sustained release formulations and could possibility be advantageous in terms of increased bioavailability of Isoniazid.
Keywords : *Nanoparticles, Isoniazid and solvent evaporation technique*

Corresponding author:
Nurukurthi Sushmitha Shainey,
Department of Pharmaceutics,
Pydah College of Pharmacy
Kakinada, Andhra Pradesh
Email Id- shaineysandy6@gmail.com

QR code

INTRODUCTION:

The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body to achieve promptly and then to maintain the desired drug concentration. That is, the drug delivery system should deliver drug at a rate dictated by the needs of the body over a specified period of treatment. This idealized objective points to the two aspects most important to drug delivery namely spatial placement and temporal delivery of a drug. Spatial placement relates to targeting of drug to a specific organ or tissue, while temporal delivery refers to controlling the rate of drug delivery to the target tissue. An appropriately designed controlled release drug-delivery system can be a major advance towards solving these two problems. It is for this reason that the science and technology responsible for development of controlled-release pharmaceuticals has been, and continues to be the focus of a great deal of attention in both industrial and academic laboratories.

Conventional drug therapy [1]:

To gain appreciation for the value of controlled drug therapy, it is useful to review some fundamental aspects of conventional drug delivery. Consider single dosing of a hypothetical drug that follows a simple one-compartment pharmacokinetic model for disposition. Depending on the route of administration, a conventional dosage form of the drug e.g.: A solution, suspension, capsule tablet etc. can produce a drug blood level versus time profile. The term drug blood levels refer to the concentration of drug in blood or plasma, but the concentration in any tissue could be plotted on the ordinate. Administration of a drug by either intravenous injection or an extra vascular route, e.g., orally, intramuscularly or rectally does not maintain drug blood levels within the therapeutic range for extended periods of time. The short-duration of action is due to the inability of conventional dosage forms to control temporal delivery. If an attempt is made to maintain drug blood levels in the therapeutic range for longer periods by for e.g., increasing the initial dose of an intravenous injection, toxic levels can be produced at early times. This approach obviously is undesirable and unsuitable. An alternative approach is to administer the drug repetitively using a constant dosing interval, as in multiple-dose therapy. In this case the drug blood level reached and the time required to reach that level depend on the dose and the dosing interval. There are several potential problems inherent in multiple dose therapy.

1. If the dosing interval is appropriate for the biological half-life of the drug, large peaks and valleys in the drug blood level may result. For e.g., drugs with short half-lives require frequent designs to maintain constant therapeutic levels.
2. The drug blood level may not be within the therapeutic range at sufficiently early times, an important consideration for certain disease states.
3. Patient non-compliance with the multiple-dosing regimens can result in failure of this approach.

In many instances, potential problems associated with conventional drug therapy can be overcome. When this is the case, drugs given in conventional dosage forms by multiple dosing can produce the desired drug blood level for extended period of time. Frequently, however these problems are significant enough to make drug therapy with conventional dosage forms less desirable than controlled-release drug therapy. This fact, coupled with the intrinsic inability of conventional dosage forms to achieve spatial placement, is a compelling motive for investigation of controlled-release drug delivery systems.

Terminology [2,3]:

Modified-release delivery systems may be divided conveniently into four categories:

1. Delayed release
2. Sustained release
3. Site-specific targeting
4. Receptor targeting.

Delayed-release systems are those that use repetitive, intermittent dosing of a drug from one or more immediate-release units incorporated into a single dosage form. Examples of delayed release systems include repeat-action tablets and capsules and enteric-coated tablets where timed release is achieved by a barrier coating.

Sustained-release systems include any drug delivery system that achieves slow release of drug over an extended period of time. If the systems can provide some control, whether this is of a temporal or spatial nature, or both, of drug release in the body, or in other words, the systems is successful at maintaining constant drug levels in target tissue or cells, it is considered controlled-release systems.

Site-specific and receptor targeting refer to targeting of a drug directly to a certain biological location. In the case of site-specific release, the target is adjacent to or in the diseased organ or tissues, for receptor release, the target are the particular receptor for a drug within an organ or tissue. Both of these systems satisfy the spatial aspect of drug delivery and are also considered to be controlled drug-delivery systems.

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MATERIALS:

Isoniazid   Procured from Aurobindo pharma Ltd, Hyderabad, India. Provided by Sura Labs, Dilsukhnagar, Hyderabad ,Eudragit RSPO   Lactel, Durect corporation Birmingham Division ,Poloxomer 188   Eastman company, UK ,Acetone   SRL

METHODOLOGY:

Preparations of buffer :

Preparation of 0.2M Potassium Dihydrogen Orthophosphate Solution: Accurately weighed 27.218 gm of monobasic potassium dihydrogen orthophosphate was dissolved in 1000 mL of distilled water and mixed.

Preparation of 0.2M sodium hydroxide solution: Accurately weighed 8 gm of sodium hydroxide pellets were dissolved in 1000 mL of distilled water and mixed

Preparation of pH 7.4 phosphate buffer : Accurately measured 250 mL of 0.2M potassium dihydrogen ortho phosphate and 195.5 mL of 0.2M NaOH was taken into the 1000 mL volumetric flask. Volume was made up to 1000 mL with distilled water.

Preparation of Standard Graph:

100mg of Isoniazid pure drug was dissolved in 15ml of Methanol and volume make up to 100ml with 0.1N HCL (stock solution-1). 10ml of above solution was taken and make up with100ml by using 0.1 N HCL (stock solution-2 i.e 100µg/ml). From this take 0.2, 0.4, 0.6, 0.8 and 1 ml of solution and make up to 10ml with 7.4 phosphate buffer to obtain 2, 4, 6, 8 and 10 µg/ml of Isoniazid solution. The absorbance of the above dilutions was measured at 260 nm by using UV-Spectrophotometer taking 7.4 phosphate buffer as blank. Then a graph was plotted by taking Concentration on X-Axis and Absorbance on Y-Axis which gives a straight line Linearity of standard curve was assessed from the square of correlation coefficient (R^2) which determined by least-square linear regression analysis.

Method of preparation of isoniazid loaded nanoparticles:

Isoniazid loaded nanoparticles were prepared by using double emulsion solvent evaporation technique. Various formulations were prepared to know the effect of polymer concentration and were assigned formulation code F1 to F8 as shown in Table. Firstly polymeric solution is prepared by dissolving specific amount of Eudragit RSPO in 5 ml of Acetone dissolved. Above obtained solution is emulsified by the drop wise addition of aqueous drug solution under magnetic stirring at 1000-1200 rpm for 15 min to get primary W/O emulsion. Further this is added to 20 ml distilled water containing poloxamer188 under stirring for 10 min to achieve a stable W/O/W double emulsion. The W/O/W emulsion is separated by ultracentrifugation at 11000 rpm for 40 min. Finally they obtained product is freeze dried or lyophilized which leads to the formation of nanoparticles.

Table 1: Composition of the Nanoparticles

INGREDIENTS	FORMULATION CODES							
	F1	F2	F3	F4	F5	F6	F7	F8
Isoniazid	100	100	100	100	100	100	100	100
Eudragit RSPO (mg)	100	150	200	250	100	150	200	250
Poloxomer 188 (mg)	10	10	10	10	10	10	10	10
Acetone (ml)	5	5	5	5	5	5	5	5
Water (ml)	20	20	20	20	20	20	20	20

RESULTS AND DISCUSSION:

Preparation of Standard Graph :

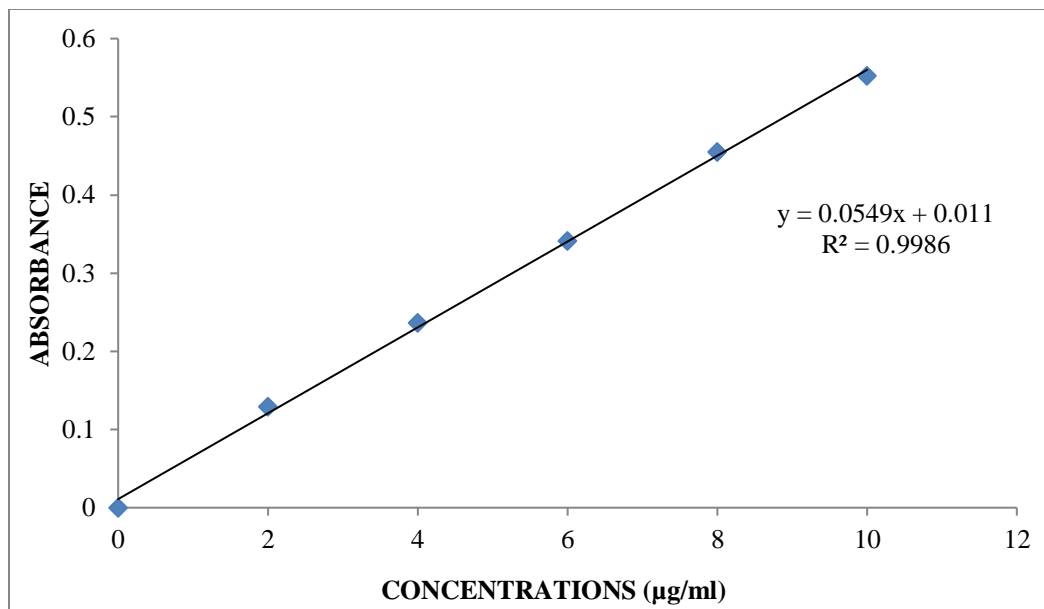**a. Determination of absorption maxima**

The standard curve is based on the spectrophotometry. The maximum absorption was observed at 260 nm.

b. Calibration curve

Graphs of Isoniazid was taken in 7.4 Phosphate buffer

Table 2 : Calibration curve data for Isoniazid at 260 nm

Concentrations [µg/mL]	Absorbance
0	0
2	0.129
4	0.236
6	0.341
8	0.455
10	0.455

$y = 0.0549x + 0.011$
$R^2 = 0.9986$

Fig 1 : Standard graph of Isoniazid in 7.4 Phosphate buffer

Evaluation of isoniazid loaded nanoparticles :

Table 3 : Evaluation of Nanoparticles

Batch No	Mean Particle size (nm)	%Yield	Drug encapsulation efficiency
F1	91.42	58.15	81.96
F2	93.14	61.48	76.33
F3	94.52	67.31	80.12
F4	95.41	96.72	93.20
F5	90.92	86.13	69.52
F6	93.86	51.25	72.60
F7	93.40	59.14	82.46
F8	91.82	63.95	70.58

Quality control parameters for tablets:
Table: *In vitro* Drug release studies:

Table 4 : *In vitro* Drug release studies of Isoniazid F1, F2, F3, F4

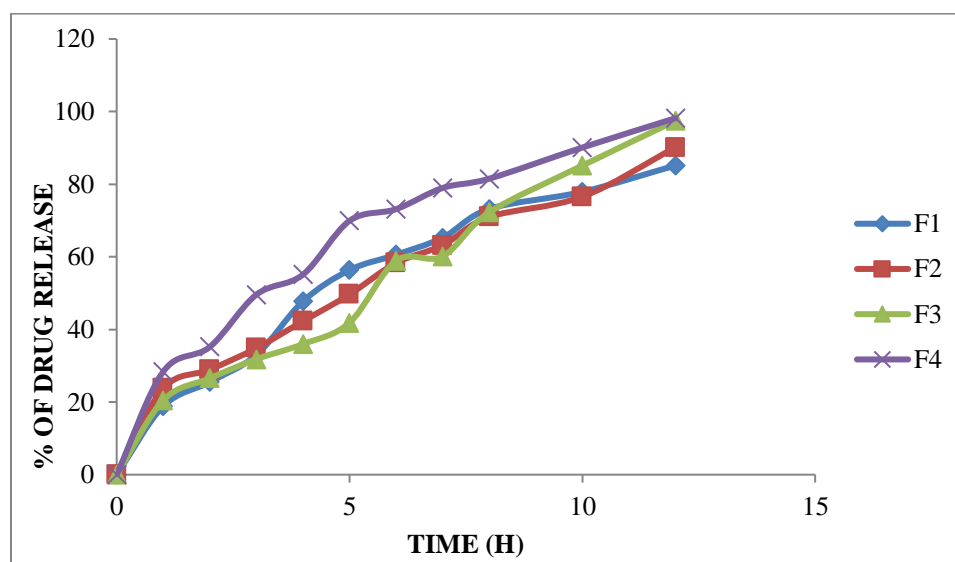| TIME (hr)	CUMULATIVE PERCENT OF DRUG RELEASED			
	F1	**F2**	**F3**	**F4**
0	0	0	0	0
1	18.86	23.74	20.49	28.36
2	25.52	28.91	26.54	35.19
3	32.98	34.85	31.82	49.58
4	47.72	42.26	35.96	55.10
5	56.35	49.64	41.85	69.91
6	60.58	58.32	58.90	73.13
7	65.27	63.19	60.15	78.97
8	73.18	70.98	72.24	81.52
10	77.90	76.56	85.12	90.10
12	85.15	90.14	97.45	98.19

Figure No 2 : Dissolution study of Isoniazid Nanoparticles

Table 5 : *In vitro* Drug release studies of Isoniazid F5, F6, F7, F8

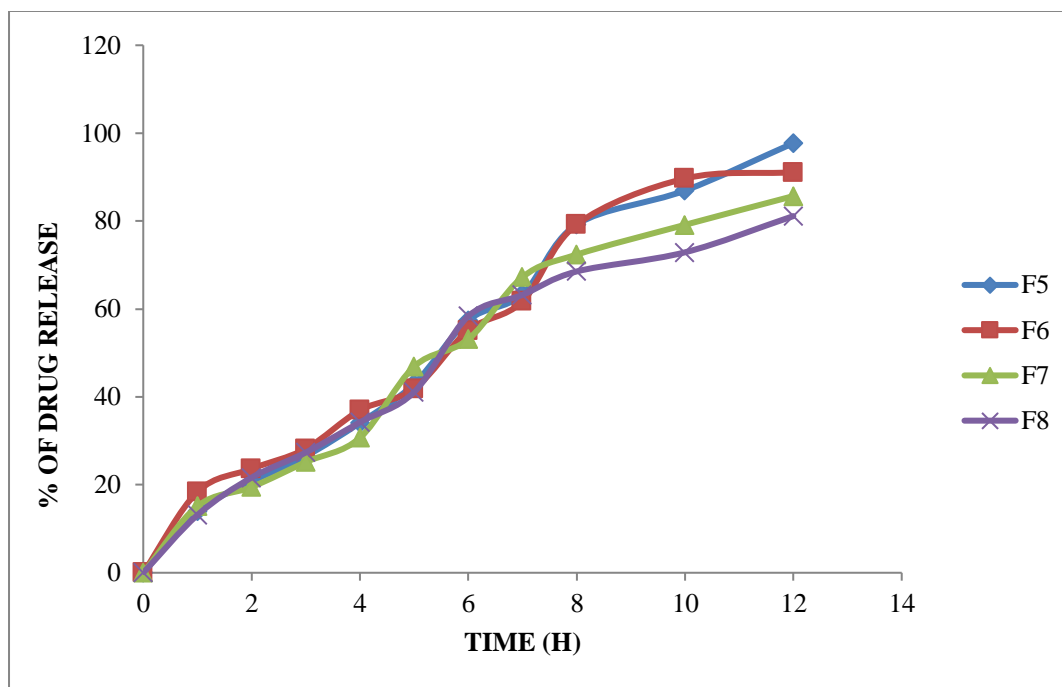| TIME (hr)	CUMULATIVE PERCENT OF DRUG RELEASED			
	F5	**F6**	**F7**	**F8**
0	0	0	0	0
1	13.95	18.39	15.14	13.14
2	20.71	23.65	19.50	21.65
3	26.53	28.20	25.18	27.35
4	34.12	36.97	30.70	34.24
5	42.97	41.83	46.91	41.10
6	57.44	55.14	53.11	58.35
7	63.36	61.75	67.25	63.21
8	79.21	79.22	72.38	68.54
10	86.95	89.68	79.15	72.90
12	97.73	91.10	85.69	81.14

Figure No 3 : Dissolution study of Isoniazid Nanoparticles

Table 6: Release Kinetics:

CUMULATIVE (%) RELEASE Q	TIME (T)	ROOT (T)	LOG(%) RELEASE	LOG (T)	LOG (%) REMAIN	RELEASE RATE (CUMULATIVE % RELEASE / t)	1/CUM% RELEASE	PEPPAS log Q/100	% Drug Remaining	Q01/3	Qt1/3	Q01/3-Qt1/3
0	0	0			2.000				100	4.642	4.642	0.000
10.13	0.5	0.707	1.006	-0.301	1.954	20.260	0.0987	-0.994	89.87	4.642	4.479	0.162
20.11	1	1.000	1.303	0.000	1.902	20.110	0.0497	-0.697	79.89	4.642	4.307	0.335
26.58	2	1.414	1.425	0.301	1.866	13.290	0.0376	-0.575	73.42	4.642	4.187	0.454
34.81	3	1.732	1.542	0.477	1.814	11.603	0.0287	-0.458	65.19	4.642	4.025	0.617
46.67	4	2.000	1.669	0.602	1.727	11.668	0.0214	-0.331	53.33	4.642	3.764	0.878
49.89	5	2.236	1.698	0.699	1.700	9.978	0.0200	-0.302	50.11	4.642	3.687	0.955
54.15	6	2.449	1.734	0.778	1.661	9.025	0.0185	-0.266	45.85	4.642	3.579	1.062
61.21	7	2.646	1.787	0.845	1.589	8.744	0.0163	-0.213	38.79	4.642	3.385	1.256
69.72	8	2.828	1.843	0.903	1.481	8.715	0.0143	-0.157	30.28	4.642	3.117	1.525
74.59	9	3.000	1.873	0.954	1.405	8.288	0.0134	-0.127	25.41	4.642	2.940	1.702
87.64	10	3.162	1.943	1.000	1.092	8.764	0.0114	-0.057	12.36	4.642	2.312	2.329
92.54	11		1.966	1.041	0.873	8.413	0.0108	-0.034	7.46	4.642	1.954	2.688
99.61	12	3.317	1.998	1.079	-0.409	8.301	0.0100	-0.002	0.39	4.642	0.731	3.911

Zero

Cumulative % drug relase

$y = 7.4565x + 20.689$
$R^2 = 0.8976$

time
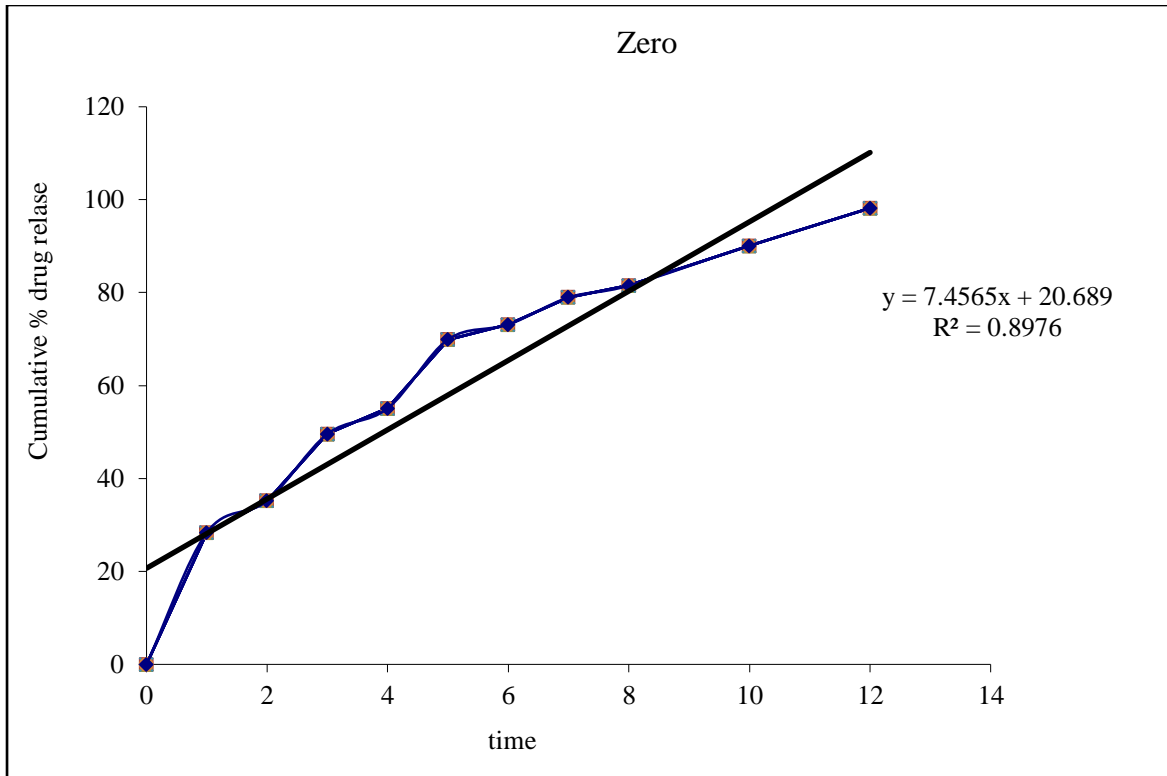
Figure 4 : Graph of zero order kinetics

Higuchi

Cumulative % drug release
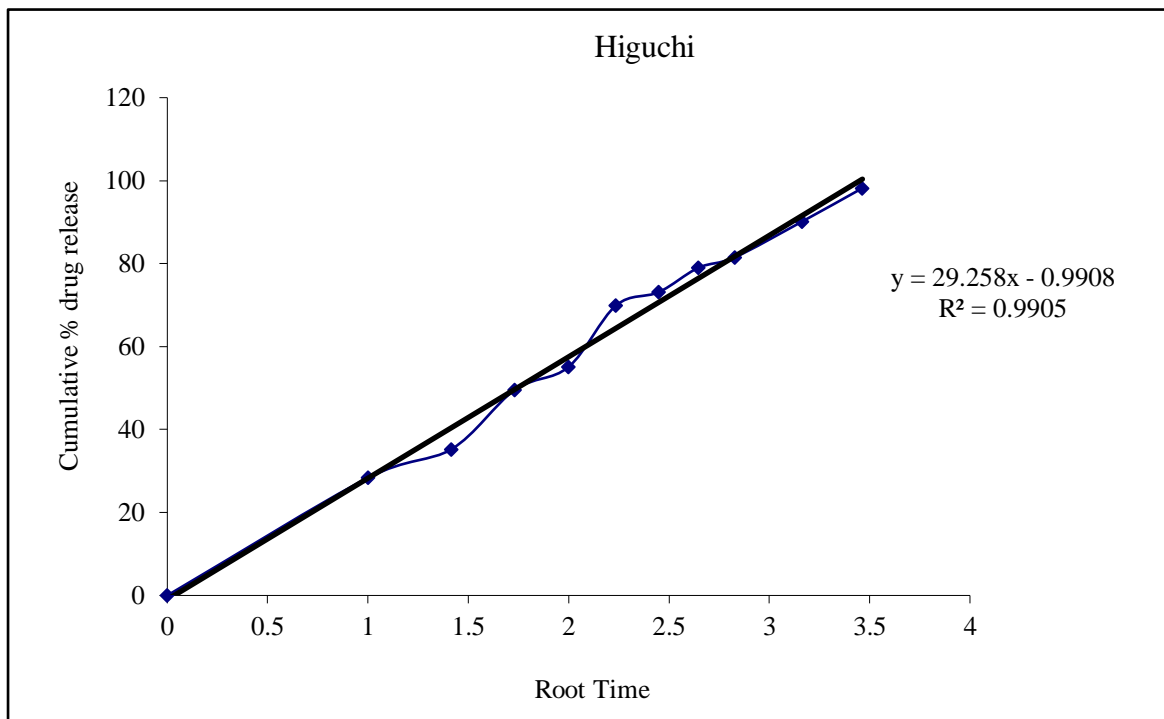
$y = 29.258x - 0.9908$
$R^2 = 0.9905$

Root Time

Figure 5 : Graph of higuchi release kinetics
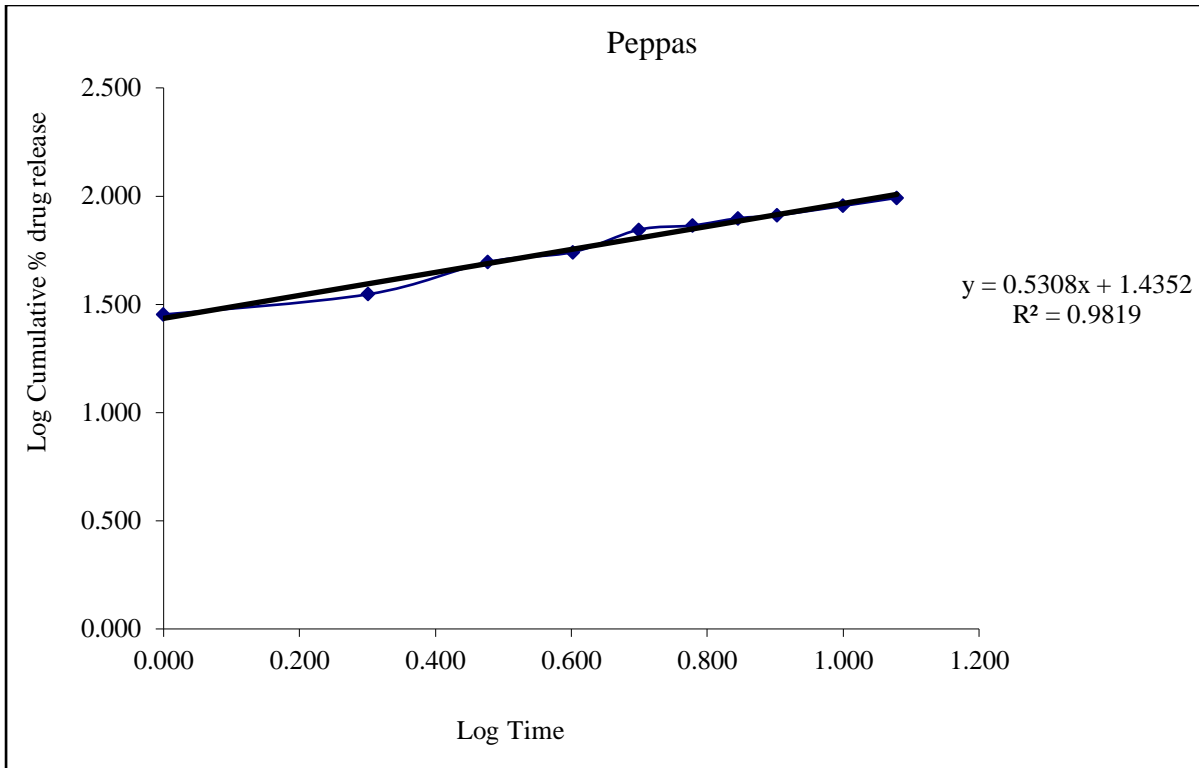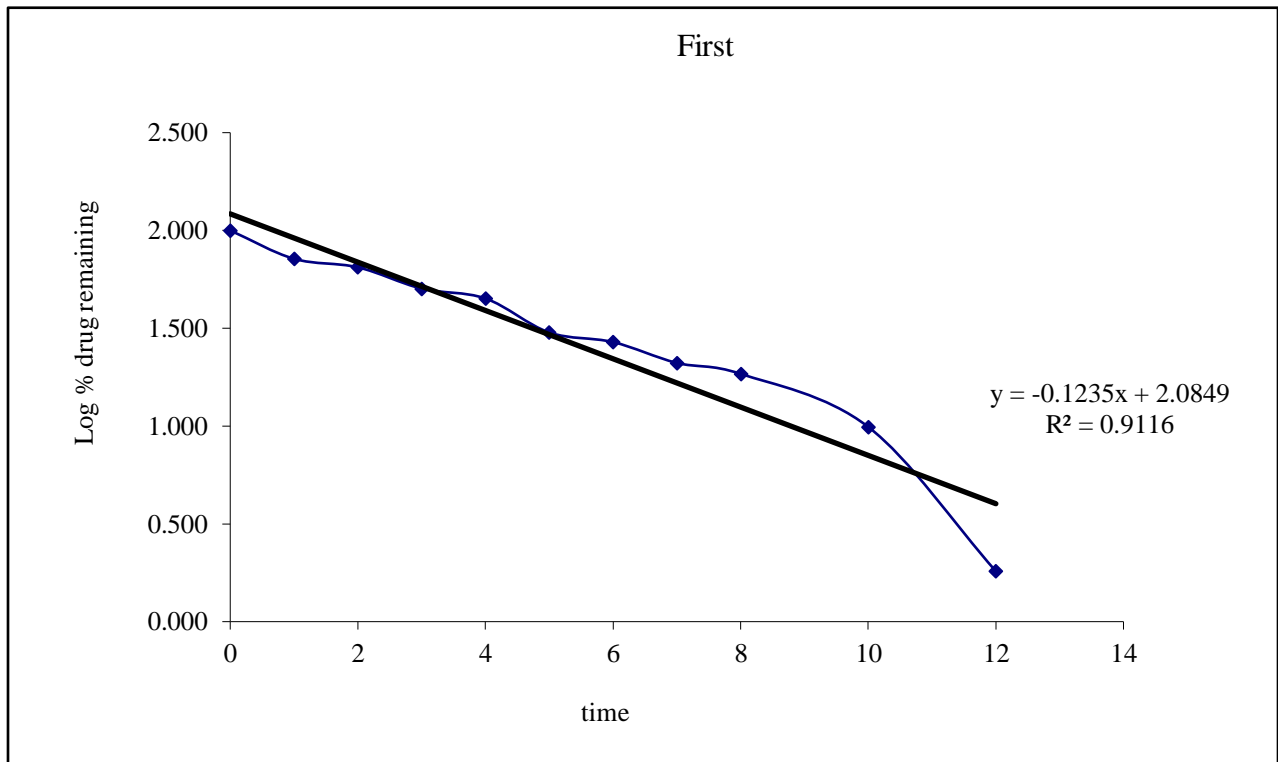
Figure 6 : Graph of peppas release kinetics

Figure 7 : graph of first order release kinetics

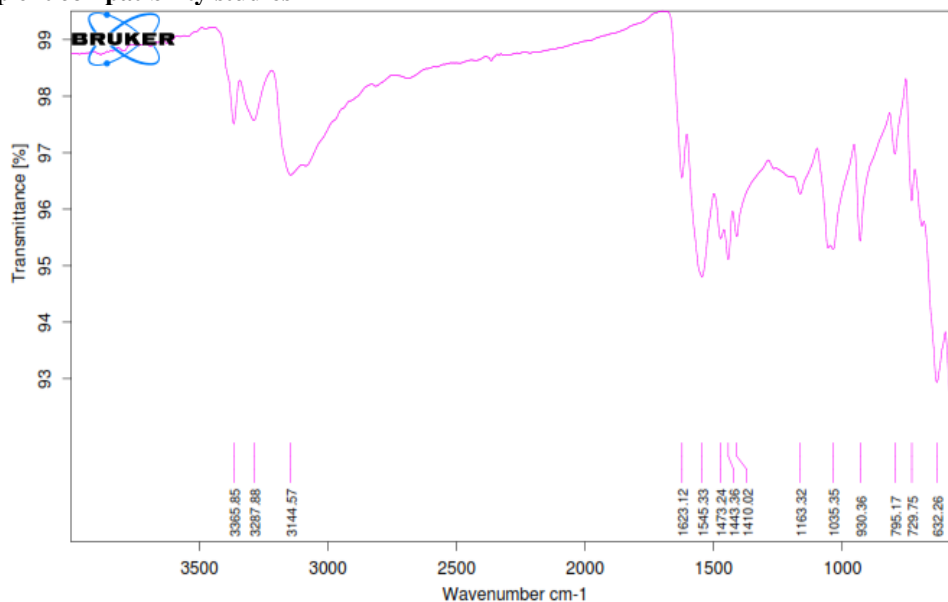**Drug – Excipient compatibility studies**

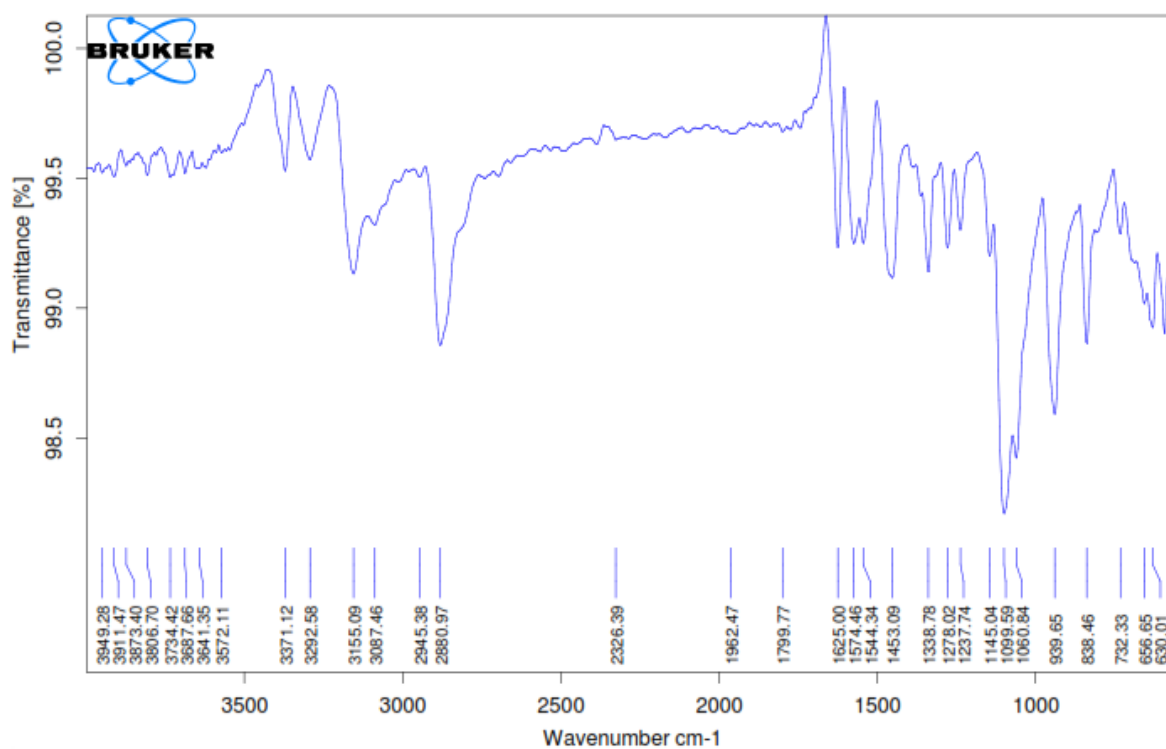**Figure 8 : FT-TR Spectrum of Isoniazid pure drug.**

Figure 9 : FT-IR Spectrum of Optimised Formulation

CONCLUSION:

The method of preparation of nanoparticles of Isoniazid was found to be simple and reproducible. Nanoparticles prepared by solvent evaporation technique. The prepared formulations were evaluated for Mean Particle size, %Yield, Drug encapsulation efficiency and *In vitro* drug release. Formulation F4 registered highest entrapment of 93.20 % and practical

yield of 96.72 % The incompatibility studies between the drug and polymer was evaluated using FTIR spectrophotometry. There was no significance difference in the IR spectra of pure drug & excipients. The *in-vitro* drug release of formulation F4 is found to be 98.19 % over 12 h in controlled manner hence the present study was a successful attempt to formulate and extend the drug release of Isoniazid by nanoparticulate system.

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