Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v30i18.3252

FORMULATION AND EVALUATION OF CHITOSAN MICROSPHERES OF COMBINED DRUG (ISONIAZID AND PYRIDOXINE) RELEASE PROFILE BY IONOTROPIC GELATION METHOD.

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ABSTRACT

Tuberculosis is an infectious disease that mainly affects the lungs. For the treatment of tuberculosis conventional drug delivery system fails to achieve efficient drug delivery at the target site. Now a days Chitosan microspheres become a topic of great interest as a novel drug delivery system. Chitosan microspheres for improving therapeutic action of drug increasing prolongs action,lowering dose frequency of dosage form and to improve patient complies. The aim of this combinedstudy was to overcome the side effect of antitubercular drug (Isoniazid) which is neuropathy and influence of the molar mass and deacetylation degree of chitosan microspheres at the different concentration of three different cross-linking agents viz, tripolyphosphate (TPP), formaldehyde (FA) and Gluteraldehyde (GA) has been prepare by Ionotropic gelation method.The influence of these cross-linking agent on the properties of chitosan microsphers was extensively investigated. The the partical size and encapsulation efficiencies of thus preparation chitosan microspheres rangedmainly between

Areas covered This research article Isoniazid/Pyridoxin combination therapy for the treatment of tuberculosis. The reduction of side effect of Isoniazid (neuropathy) and patients compliance of Isoniazid/pyridoxine in tubercular patients are discussed based on the available literature.

Expert opinion: It was found that Isoniazid/pyridoxine combination therapy for the treatment of tuberculosis combination therapy could significantly improve bioequivalence studies. It also reduces side effect of isoniazid nuropathy.

Keywords: Microspheres, Gluteraldehyde, tripolyphosphate (TPP), formaldehyde (FA) Neuropathy.

INTRODUCTION

Noval drug delivery system means of improving the therapeutic effectiveness of incorporated drugs

by providing controlled delivery targeting and sustained delivery. The drugs in to dosage for with the aim of sustaining drug level and hence drug action is optained for as prolongperiod of time in body. Microspheres are small spherical particles, with diameters in the micrometerrange (typically 1 μm to 1000 μm. Microspheres are carrier drug delivery system which plays an important role in micro-particulate novel drug delivery system. Microspheres are spherical, free flowing, monolithic matrix type. The main goal of the microspheres drug delivery system is to provide therapeutic amount of drug to the target site in the body. Microspheres are designed to release the drug in sustained and controlled manner, improving bioavailability, entrapment efficiency and lowering dose frequency of drug in the dosage form.

Tuberculosis is a cronic infection disease caused by the tubercle bacillus. typically attacks the lungs but can also affect other part of the body, spread through air. About 1/3 of the world's population have infected by Mycobacterium tuberculosis. Due to TB about 2 million people will die yearly. WHO introduce the modern standard short course therapy for tuberculosis treatment based on four drug regimen of isoniazid, rifampicin, pyrazinamide and ethambutol for 2 months followed by treatment with combination of isoniazid and rifampicin for 4 months 2 Isoniazid is a first line antitubercular drug. It has broad spectrum antimicrobial activity. They act as both bacteriostatics for resting bacilli and bacteriocidal for dividing microorganisms.Isoniazid is a prodrug i.e. converted by enzyme known as mycobacterial catalase peroxidase into an active metabolite. Mycolic acid is a unique fatty acid component of mycobacterial cell wall. Isoniazid is inhibits the biosynthesis of mycolic acid in bacterial cell wall. Isoniazid acts on enoyl-ACP reductase of fatty acid synthase-II, cause saturation of fatty acid in mycolic acid biosynthesis Isoniazid is absorbed by oral and parental administration.Peripheral neutritis, neurological manifestations, hepatotoxicity, rashes, fever, acne and arthralgia etc. are common side effects.

Isoniazid (INH) is a widely used anti micro bacterial agent for first line therapy of tuberculosis . the druge is characterized by a short half life ranging from 1 h to 4 h, depending on the rate of metabolism. INH is in actionvated in the liver, mainly by acetylation and dehydrazination; the rate of acetylation is genetically determined and subject to individual variation . long –term continuous therapy with INH leads to hepatotoxicity and peripheral neuritis. So reduced the side effect of INH and improve the bioavailability of drug it is important to have the combined drug (INH and Pyridoxine) formulation with chitosan microspheres especially in the small intestine.

Chitosan, a natural cationic polysaccharide, finds many applications in the pharmaceutical and biomedical fields, that have been extensively reviewed in the literature (Akbuga, 1995), due to its favourable characteristics such as non-toxicity, biocompatibility, biodegradability and properties such as bio adhesion. The pharmaceutical application of chitosan microspheres as controlled drug delivery systems for conventional drugs, protein drugs and DNA has attracted increasing attention since the beginning of the past decade (He et al., 1999). Numerous controlled release delivery systems either for implantation or for oral delivery have been described in the literature. Also chitosan has been applied in delivery systems for offering mucoadhesive characteristics. This is due to its unique polymeric cationic character and its gel and film forming properties. The positively charged microparticles enhanced the mucoadhesive properties and made these suitable for delivery of drugs via the nasal or gastrointestinal routes (He et al. 1999).

Processing techniques for the preparation of chitosan microspheres have been extensively developed since the 1980s. Four main approaches have been proposed: ionotropic gelation with an oppositely charged, simple or complex coacervation, emulsification/solvent evaporation and, more recently, spray-drying (Huanget al. 2003). The investigation of chitosan microspheres formation by the Ionotropic gelation method is justified by interesting results presented in the literature. Chitosan microspheres obtained by this method arecharacterized by high sphericity and specific surface area (Rege, 2003), important parameters for application in the pharmaceutical field (drug delivery systems). Concerning the release of drugs from chitosan microspheres, various release profiles may

be possible depending on the relative magnitude of the rate of polymer swelling to the rate of drug diffusion. Frequently, the rate of drug release from hydrogels can be regulated by controlling the crosslinking density and the extent of water swelling (Kim and Lee, 1992). In many studies, chitosan has been crosslinked with aldehydes, such as, glutaraldehyde and formaldehyde to make it a more rigid polymer to be used as a core material in controlled release research. However, the biological acceptance of such crosslinked products depends upon the amount of crosslinking agent present in the final products and the toxicity of aldehydes have been enormously limited the exploitation of the crosslinked chitosan microparticles in the pharmaceutical field. To overcome the restriction in using toxic crosslinkers, d,l-glyceraldehyde has been proposed as a biocompatible crosslinker reagent for protein microspheres (Vandelli et al, 1995), which use does not introduce toxicity problems, as it is present in the human organism as a metabolic product of fructose. In this work, the chemical crosslinking of d,l-glyceraldehyde with spray-dried chitosan microspheres was investigated. The method consists of the exposure of spray- dried microspheres to the cross-linking agent in liquid phase and under mild conditions. The effect of the preparation variables (crosslinker concentration and crosslinking duration) was evaluated with regard to the morphological aspect, the particle size, the zeta potential and the swelling behavior of the microspheres.

MATERIALS AND METHODSMATERIALS

Isoniazid was a kind gift sample---------- from Pyridoxine was a kind gift sample from SDFCL s d
fime-Chem Ltd. Mumbai. Chitosan (Hi Media Laboratories Pvt. Ltd. Mumbai.) polymer 1%w/v Aqueous acetic acid solution were used as solvent for the chitosan polymers and 1%w/v sodium tri poly phosphate (Yarrow Chem Products Mumbai),Glutaraldehyde 25%, Formaldehyde 37-41%w/v LR was used as the cross-linker. All chemical were of analytical grade and no further purification was required.

METHODS

IONIC GELATION METHOD:

Chitosan solution (0.5% w/v) were prepared by dissolving chitosan in an aqueous acetic acid solution the polymer solution were continuously stirred using magnetic stirrer for 2 h at 25^0C to ensure complete dissolution. Than in another beaker 10%w/v of drug (INH+VIT B6) solution is formed using distilled water. Now using 22 gauze syringe sodium Tripolyphosphate solution (1.0%w/v) or Gluteraldehyde (25%) or Formaldehyde ware slowly added to the chitosan-drug solution under mechanical stirring at 1,500 rpm for 30 min. The microspheres ware fomed are leftin chitosan-drug solution for overnight. Which are then washed with n-hexane to remove excess chitosan and filtered and drid at room temperature.

Table 1: Formulation design for the preparation of combined drug loaded chitosan microspheres with different conc. of different cross-linking agents.

Formulation code	Chitosan concentration (%w/v)g Polymer	DrugINH (mg)	Drug VitB6 (mg)	Cross-linking agent	Cross-linking agent concentration (%w/v)
F1	1	100	50	Na-TTP	0.5
F2	1	100	50	Na-TTP	1
F3	1	100	50	Glutaraldehyde 25%	0.5
F4	1	100	50	Glutaraldehyde 25%	1
F5	1	100	50	Formaldehyde 37%	0.5
F6	1	100	50	Formaldehyde 37%	1

EVALUATION OF DRUGE CHITOSAN MICROSPHERESPARTICAL SIZE ANALYSIS

Size of drugs loaded chitosan microspheres was measured by optical microscopy method. A standard stage micrometer was used to calibrate the eye micrometer. Size of 100 microspheres from each batch was measured and average

SWELLING INDEX:

Swelling index is determined using graduated measuring cylinder. Microspheres are transferred in the cylinder such that they occupy only 1ml space and simulated intestinal fluid is then introduced to the 10 ml .the system is kept at 37^0C fir about 24 hrs and then final volume of microspheres are noted and swelling index is determined using the following formula:

Swelling index =final volume-initial volume / initial volume × 100

Scanning Electron Microscopy: A high resolution scanning electron microscope SEM (JSM-6510, Tescan) chitosan partical were frozen through immersion in liquid nitrogen and then freeze dried for 48 h. The dried particles ware examined using scanning electron microscopy samples were covered by a thin gold layer using a sputter coater and observed on SEM.

FOURIER TRANSFORMED INFRARED SPECTROSCOPY

The solid chitosan and dried chitosan cross –linking sodium tripolyphosphate ,formaldehyde amd Gluteraldehyde particles were crushed in 100mg potassium bromide (KBr), separately . this mixture was transferred to a vial of stainless steel and pressed for the formation of a disk . spectroscopic structural study of solid bulk chitosan particles was carried out by fourier transformed infrared spectroscopy (FTIR MB, Bomem B100, Canada) for reading in the range of 4.000-400cm^{-1} .Results were obtained by software .

DETERMINATION OF ENTRAPMENT EFFICIENCY:

An accurately weighed sample (10 mg) of the Isoniazid and pyridoxine loaded chitosan microspheres was placed in 25 ml of a solvent system consisting of methanol and phosphate-buffer saline at PH 7.0 in 2:1 ratio at room temperature for 24 h. The solution was then filtered using a Whatmann No.1 Qualitative filter paper. The filtrate was assayed spectrophotometrically for drug content at 265 nm and 322 nm (Genesys 10 UV Spectrophotometer, Thermo, USA). The same method was utilized confirm the non-interference of unloaded microspheres in the Spectrophotometric determination of drug content prior to the Drug Loading studies. All experiments were performed in triplicate.

Drug Loading was calculated using the formula in Equation-1Drug Loading in % = W/Wt x 100 Eq 1

where,

W = drug content of the microspheres

Wt = weight of the microspheres Entrapment Efficiency was calculated using the formula in Equation-2

Entrapment Efficiency in % = Wc /Wo x 100..... Eq 2

where,

Wc = total drug present in the microsphere batch

Wo = theoretical drug loading Theoretical drug loading was determined by calculation assuming that the entire drug present in the polymer solution gets entrapped in microspheres and no loss occursat any stage of preparation of the microspheres.

Drug entrapment efficiency = experimental drug content / initial drug content × 100

calculated drug concentration × 100

Loading efficiency (%) = Theoretical drug concentration

***IN VITRO* DRUG RELEASE**

The *in vitro* drug release study was carried out in USP dissolution apparatus 1. The chitosan microspheres were placed in basket and immersed in the dissolution medium PH 7.0 Distilled water contained in 900 ml flask. The flask was maintained at $37^0C \pm 0.5^0C$ by a constant temperature bath and rpm was set at 100. The sample were then withdrawn at an interval of half an hours and replenished with an equal volume of fresh dissolution media then absorption was measured using UV Spectroscopy to determine amount of druge in solution.

STATISTICAL ANALYSIS:

The in vitro release profile was compared with zero order, first order, and higuchi's matrix models.

3. RESULT AND DISCUSSION

The purpose of research work was to prepare combined drug chitosan based microsphere with different cross linkers like TTP, Glutaraldehyde and formaldehyde can be used to formulate microspheres. INH and Vitamin B6 by Ionotropic gelation method and examined the effect of various factor like concentration of cross linkers on drug release rate

Micromeritic Properties:

The results of all formulations F1 to F6 of drugs (INH,Vit B6) microspheres are shown in Table 6.19, which were evaluated for variable parameters such as bulk density, tapped density, % Compressibility index, Hausner's ratio and angle of repose. The % Compressibility index was in the range of 11-18 for all the formulations F1 to F6 indicating good flow property.

The values of angle of repose for formulations F1,F2, F5 and F6 was found to be in the range of 20-25 which indicated the good flow potential.

Table 6.15 Different Micromeritic properties of formulation batches of chitosan microspheres

Formulation	Bulk density(g/cm^2)	Tapped density(g/cm^2)	CompressibilityIndex (%)	Hausner'sratio
F1	0.433±0.005	0.464±0.009	11.681±1.12	1.071±0.002
F2	0.523±0.004	0.506±0.004	13.54±1.32	1.088±0.02
F3	0.572±0.008	0.516±0.008	14.05±1.27	1.031±0.011
F4	0.505±0.013	0.539±0.001	11.13±1.04	1.021±0.02
F5	0.520±0.014	0.539±0.011	12.52±1.27	1.036±0.08
F6	0.590±0.016	0.636±0.014	13.23±1.21	1.077±0.026

Size analysis of microspheres

The main particle size of the drug loaded chitosan microspheres were determined by optical microscopy using a calibrated micrometer. About 300 (three hundred) microspheres were analyzed for each preparation and the mean diameter was calculated. Triplicates were performed for each of the experiments

Fig 5.8 (b) Determined by optical microscopy

Percentage yield:

Percentage yield of different formulation F1 to F6were calculated and the yield was found to be 79.7%, 75.21%, 67.8%, 61.12%, 70.19%,and 62.18% respectively. The percentage practical yield slightly decreased as the polymer ratio increased. The results of all formulation F1 to F6of microspheres are shown in table 6.18 and fig 6.17.

Entrapment efficiency

The values of %drugs loading and %entrapment efficiency of various batches (F1-F6) were determined. The result revealed that increase in polymer concentration lead to increase in particle size entrapment efficiency and loading capacity to certain concentration but after that there was no significant increase in entrapment efficiency and drug loading capacity as shown in Table .As the cross linker concentration was increased the %drug loading decreased and %entrapment efficiency was increased due to increase in the viscosity of the solution. This can be attributed to the permeation characteristics of each crosslinkerd used, that could facilitate the diffusion of part of entrapped drug to the surrounding medium during preparation of microspheres Comparison of %drug loading and %entrapment efficiency.

Swelling index:

It was seen that as the concentration of cross linkers were increased in the formulation [F1(0.5%),F2(1%)],[F3(0.5%),F4(1%)] and [F5(0.5%),F6(1%)] so the swelling ability of the polymer is decreases . So the swelling index (%) is good in these formulations (F1, F3 & F5) as shown in

Table: Result of % yield, partical size ,% entrapment efficiency and swelling index

Formulation Code	% yield	Practical size(µ m)	Entrapment (efficiency)		Swelling index (%)
			INH	Vit B6	
F1	79.71	532 ±	88.1 ± 0.48	78.1 ± 0.08	70.1 ±11.3
F2	75.21	510 ±	77.2 ± 0.56	67.2 ± 0.16	65.12 ± 5.2
F3	67.8	500 ±	86.1 ± 0.12	76.1 ± 0.32	69.13 ± 4.12
F4	61.12	403 ±	60.1 ± 0.61	60.1 ± 0.78	56.45 ± 7.2
F5	70.19	542 ±	70.2 ± 0.56	70.2 ± 0.50	67.12 ± 4.2
F6	62.18	256 ±	50.1 ± 0.45	50.1 ± 0.12	56.12 ± 8.12

Fourier transform infrared (FTIR) spectroscopy

The drug INH and Vit B6 , mixture of raw materials and the microspheres to detect interaction between The drugs and the excipients. The FT-IR spectrum of INH and Vit B6 showed a strong C=O stretch band (AmideI around 1650cm-1 and an Amide II due to N-H bend at 1620cm^{-1} and -C=O stretch band is 1663cm^{-1}, -C- OH 3574cm^{-} of VIt B6 . These peaks were, however ,completely masked in the FT-IR spectrum of the drug loaded microspheres (fIg.)

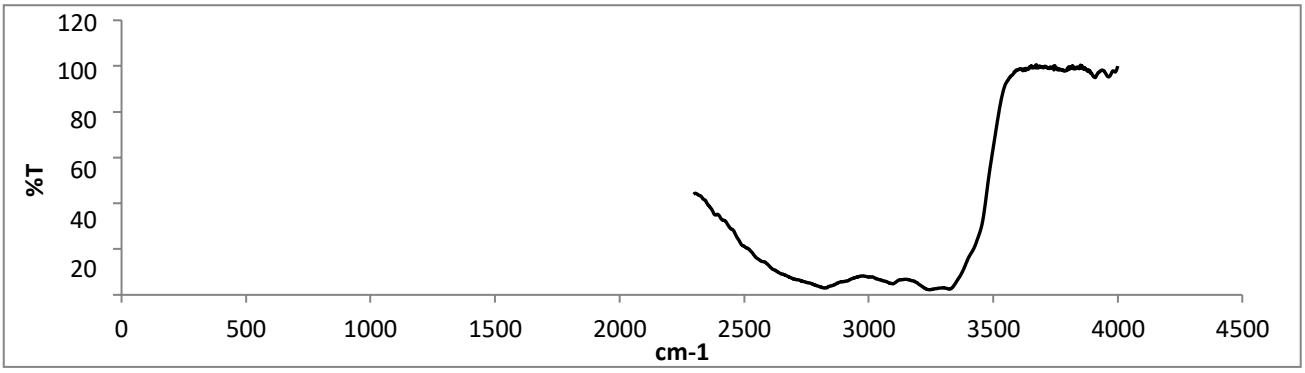


Fig 6.8(b) IR spectrum of Pyridoxine (Sample)

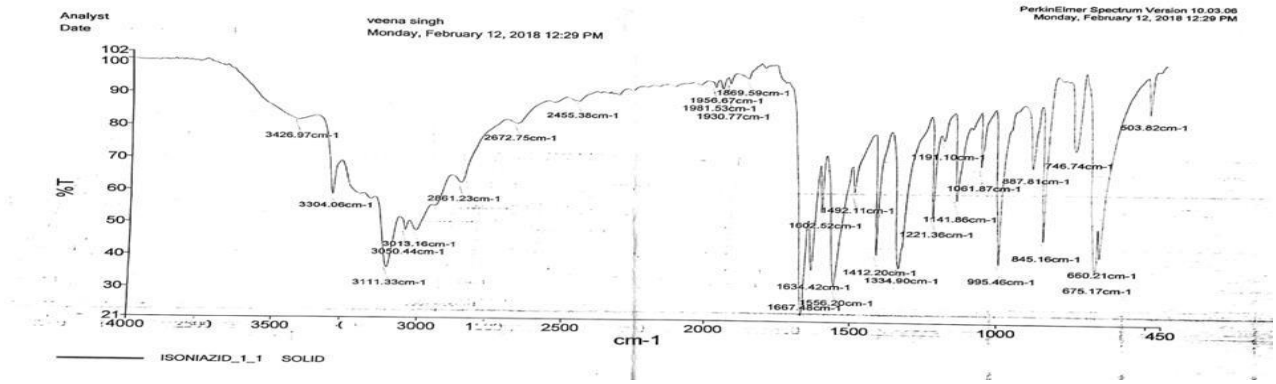


Fig 6.1.3(b) IR spectrum of Isoniazid (Sample)

FT-IR Spectrum of drug + chitosan and polymer (F1)

The characterization absorption peaks of Isoniazid, pyridoxine were observed in recorded IR spectra of physical mixture containing druge (INH and Vit B6) and excipients (chitosan sodium Tripolyphosphate) usedin F1 showed a strong C-N stretch band around 1412cm$^{-1,}$–C=O stretch band around 1663cm$^{-1,}$ C=O(amide) showed stretch band around 1667cm^{-1}and NH_2 showed stretch band is around 3426cm-^{1}.

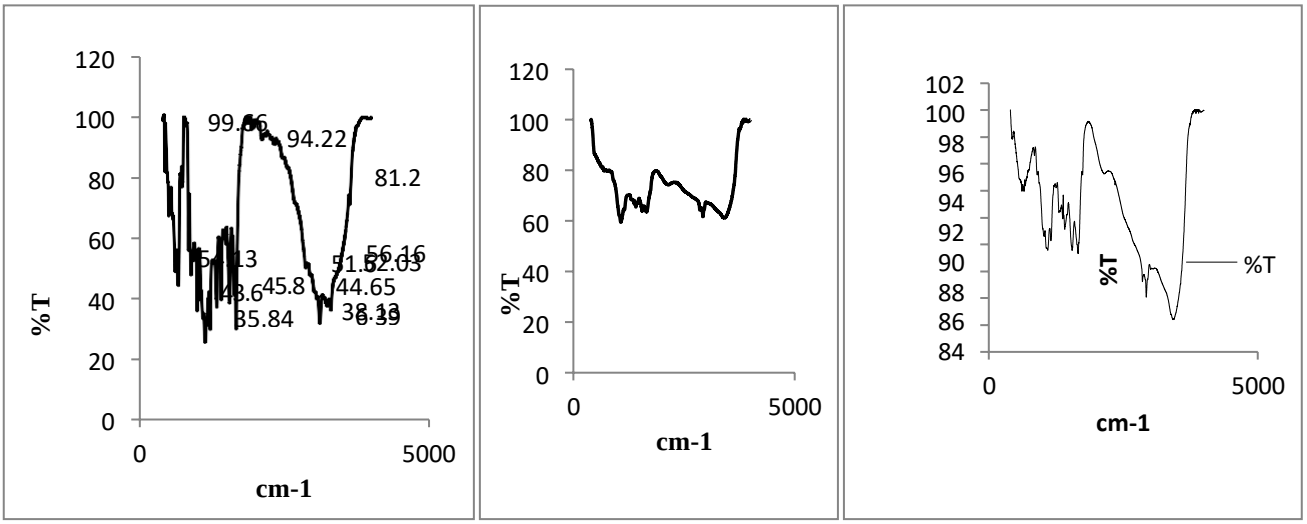
FT-IR Spectrum of drug + chitosan and polymer (F3)

The characterization absorption peaks of Isoniazid, pyridoxine were observed in recorded IR spectra of physical mixture containing druge (INH and Vit B6) and excipients (chitosan sodium Tripolyphosphate) usedin F1 showed a strong C-N stretch band around 1412cm$^{-1,}$–C=O stretch band around 1663cm$^{-1,}$ C=O(amide) showed stretch band around 1667cm^{-1}and NH_2 showed stretch band is around 3426cm-^{1}

FT-IR Spectrum of drug + chitosan and polymer (F5)

The characterization absorption peaks of Isoniazid, pyridoxine were observed in recorded IR spectra of physical mixture containing druge (INH and Vit B6) and excipients (chitosan sodium Tripolyphosphate) usedin F1 showed a strong C-N stretch band around 1412cm$^{-1,}$–C=O stretch band around 1663cm$^{-1,}$ C=O(amide) showed stretch band around 1667cm^{-1}and NH_2 showed stretch band is around 3426cm-^{1}

F3 The FTIR result revealed that ther was no interaction between drug (INH and Vit B6) and excipients (chitosan, sodium Tripolyphosphate) used in formulation IR Spectra is shown in fig 6.9



F1 **F3** **F5**

. In vitro drug release study:

Dissolution studies on all the six formulations of Drugs microspheres were carried out using a USP dissolution apparatus Type II. Distilled water was used as the dissolution medium. The in- vitro drug release data of different formulations are shown in Table. No.6.20 and Figure.No.13.The cumulative percent drug release after 12 hours was found to be in the range of 81.723, 79.038,76.389 and 71.558% for the formulations F1, F2, F3 and F4 respectively where as cumulative percent drug release after 12 hours was 82.14,80.57,74.474,69.093,63.568% for formulations F5 to F6 respectively. The cumulative drug release significantly decreased with increase in crosslinkerd concentration. The increased density of the crosslinker matrix at higher concentrations results in an increased diffusional path length. This may decrease the overall drug release from the polymer matrix. Furthermore, smaller microspheres are formed at a lower crosslinker concentration and havea larger surface area exposed to dissolution medium, giving rise to faster drug release.

Table 6.18 In–vitro drug release for Drugs (Isoniazid and pyridoxine) Chitosan Microspheres

MODELS	FORMULATION					
	F1	F2	F3	F4	F5	F6
Zero order model	0.9400	0.9386	0.8911	0.9350	0.9183	0.7250
First order model	0.9845	0.9589	0.9814	0.9565	0.9874	0.8761
Higuchi model	0.9400	0.9421	0.9354	0.8911	0.6963	0.7950
Hixson–crowell modal	0.9569	0.9462	0.9524	0.8532	0.7433	0.8852

Table 6.12.1 Drug kinetics for Isoniazid

Drug kinetics for pyridoxine

MODELS	FORMULATION					
	F1	F2	F3	F4	F5	F6
Zero order model	0.9890	0.9123	0.8356	0.92354	0.9712	0.8231
First order model	0.9999	0.9589	0.9818	0.956	0.9874	0.8741
Higuchi model	0.9527	0.8569	0.9912	0.9214	0.9945	0.9948
Hixson–crowell modal	0.9560	0.9423	0.9524	0.9532	0.9433	0.9452

6.13.2 Zero order release

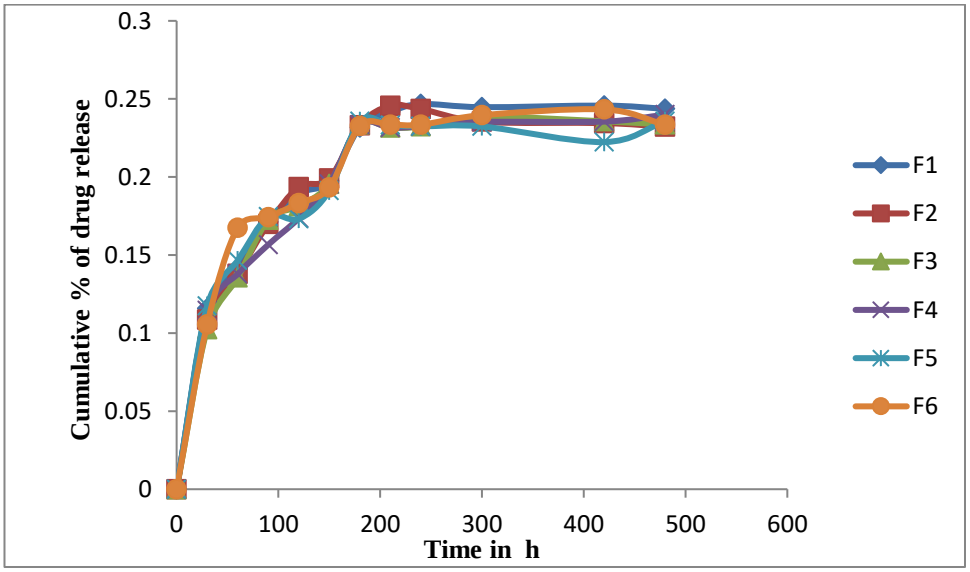


Fig 6.13.2 zero order release kinetics for prepared microspheres

6.13.3 First order release

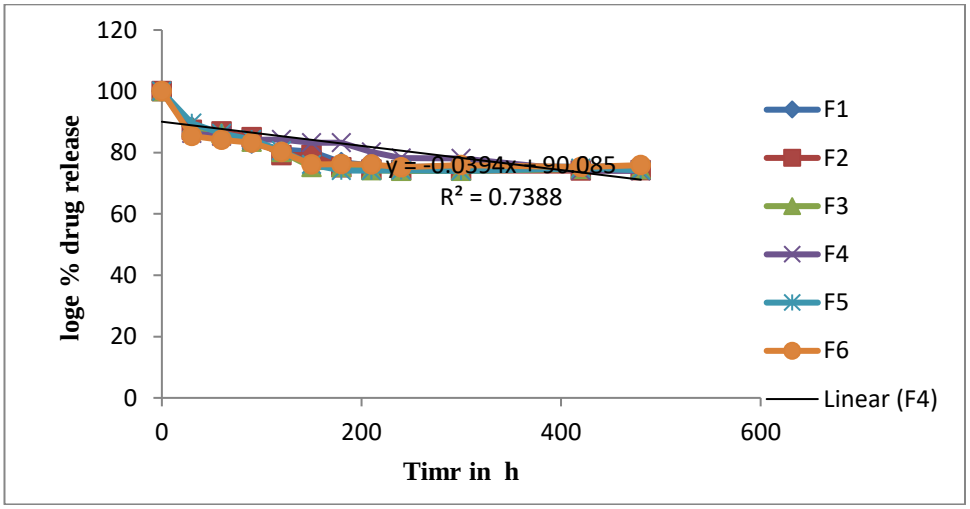


Fig 6.13.3 First order release kinetics for prepared microspheres

6.13.4 Higuchi release kinetics

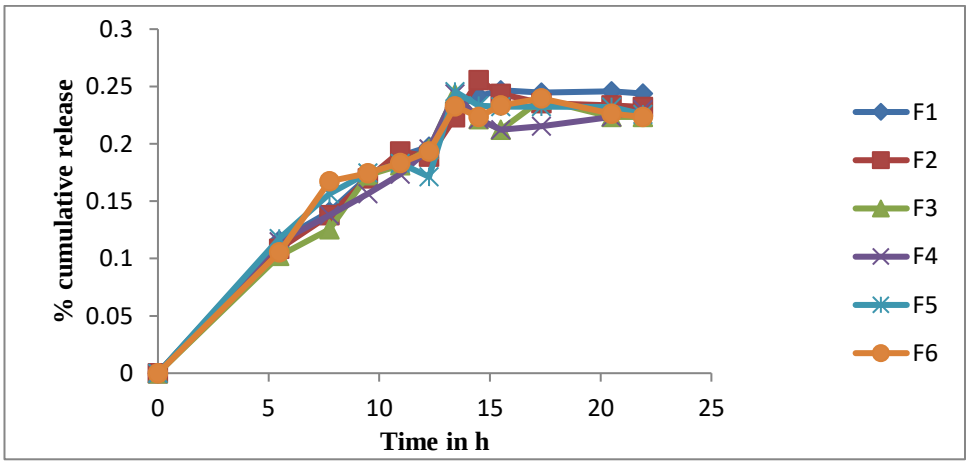


Fig 6.13.4 Higuchi release kinetics for prepared microspheres

6.13.5 Hixson – Crowell release kinetics

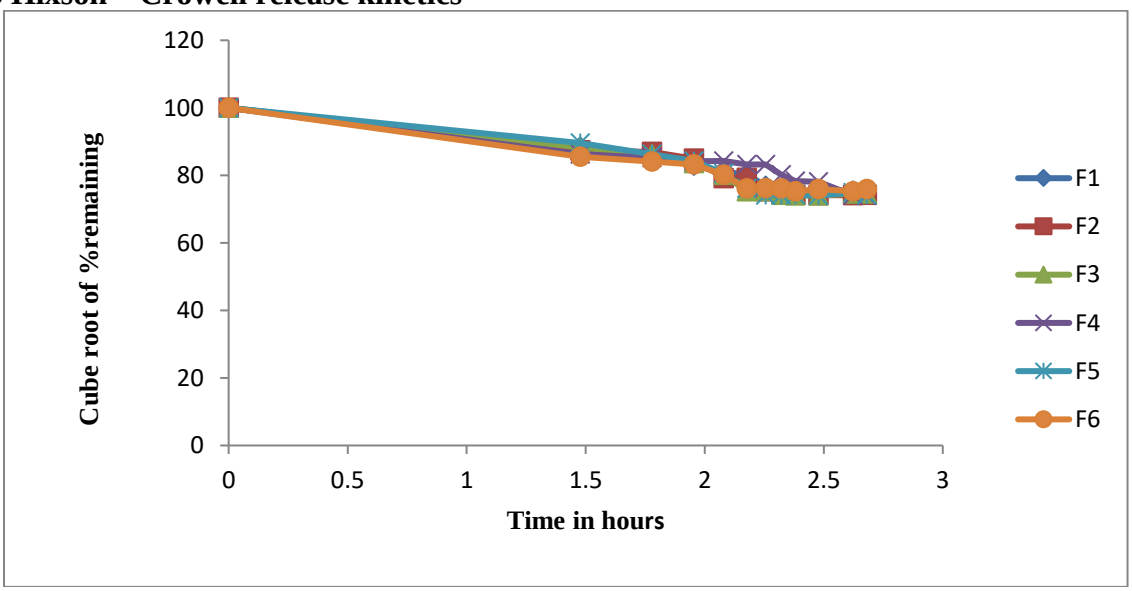


Fig 6.13.5 Hixson - Crowell release kinetics for prepared microspheres

6.14 Release Kinetics:

The results obtained from in-vitro drug release were plotted adopting 4 different mathematical models of data treatment as follows: % Cum. Drug Release Vs. Time (Zero order rate kinetics). Log % Cum. Drug Retained Vs. Time (First order rate kinetics). % Cum. Drug release was plotted against √T (root time). (Higuchi model) and cube root of %remaining vs time in hours (Hixson – crowell release modal) The curve fitting results of the release rate profile of the designed formulation. Which gave an idea on the release rate and the mechanism of release? The values were compared with each other for model and drug equation as shown in Table 15 based on the highest regression values (r2), fitting of the release rate data to various models revealed that all the formulations (F1 to F6) follow first order release kinetics with regression values ranging from0.9400, 0.999, 0.9400 and 0.9569 for Isoniazid and 0.9845 to 0.8451 and 0.9999 to 0.8741 for pyridoxine.

CONCLUSION

The present study reports a novel attempt to formulate microspheres of the Drugs Isoniazid and pyridoxine by using chitosan and different cross linkers like sodium Tripolyphosphate, Gluteraldehyde and formaldehyde as carrier for better treatment of TB and Reduced the side effect of Isoniazid. Chitosan based Microspheres of ant tubercular drug (INH,Vit B6)were prepared by Ionotropic gelation method. Various evaluation parameters were assessed, with a view to obtain controlled release of drugs. Details regarding preparation and evaluation of formulations have been discussed in previous chapters. From the study following conclusions could be drawn,

FTIR study indicated that the drug is compatible with all the excipients.

Chitosan and different crosslinkers like TTP, Glutaraldehyde and formaldehyde can be used to formulate microspheres. Micromeritic studies revealed that the mean angle of repose is excitant.

SEM analysis of the microspheres revealed that chitosan containing microspheres were smooth, spherical and slightly aggregated particles when compared with the microspheres of which were porous, rough, grossly, discrete spherical.

Good percentage of drug entrapment and practical yields were obtained with all the polymers. As the polymer concentration was increased the % drug loading decreased and % entrapment efficiency was increased due to increase in the viscosity of the solution.

Cumulative percentage drug release significantly decreased with increase in polymer concentration. The overall curve fitting into various mathematical models was found to be on an average. The formulations F1 to F6 were best fitted to first order kinetic model.

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