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Formulation and evaluation of polymeric nanoparticles of itraconazole for antifungal therapy

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ABSTRACT

The formulation of only moderately water-soluble pharmaceuticals can benefit from the incorporation of nanoparticles, which increases the drugs' bioavailability. The primary objective of this work was to create and evaluate Itraconazole-loaded nanoparticles using the ionic gelation process to improve their solubility and bioavailability. Ionic gelation was used to prepare Itraconazole nanoparticles, which are classified as a BCS class II drug. These particles were then characterized using techniques such as Fourier transform infrared spectroscopy, differential scanning Calorimetry, powder X-ray diffraction, scanning electron microscopy, zeta potential, and *in-vitro* drug release studies.There was no evidence of contact between the drug and the polymers based on the differential scanning Calorimetry results, powder X-ray diffractometry, and Fourier transforms infrared spectroscopy. Images obtained by scanning electron microscopy revealed that the nanoparticles had a spherical form.Nanoparticulate formulation prepared with Chitosan in 1:6 ratio showed satisfactory results i.e. average particle size 201.67 nm, polydispersity index 0.111, zeta potential -46.2 mV, and entrapment efficiency 89.04%. FTIR study concluded that no major interaction occurred between the drug and polymers used in the present study.This technology on a laboratory scale and this strategy could be used to improve the solubility and bioavailability of BCS class II medications.

Keywords: Itraconazole, ionic gelationmethod, Chitosan,BCS class II drug, Bioavailability, Dissolution and Nanoparticles.

INTRODUCTION

Nanotechnology is the field of investigation since last century, Nanotechnology was first introduced by Nobel laureate RicharardP.Feynmen in his lecture of "There's plenty of room at the bottom". Then, there have been different advancements within the field of nanotechnology is made. The development of Nanotechnologies is important in terms of diagnosis, treatment, and prevention of disease. The word nanoparticles come from the Greek word nanus which means dwarf or very small. Nanoparticles (NPs) are the novel invention of modern science in which drug is surrounded by a polymeric membrane where the drugs are dissolved, entrapped, adsorbed, attached and/or encapsulated into or onto a Nano-particulate matrix. The drug delivery vehicles are generally < 100 nm in size with at least one dimension and consist of different biodegradable materials such as natural or synthetic polymers, lipids, or metals. It is composed of three layers i.e. (a) the surface layer, which can be functionalized with a variety of little molecules, metal ions, surfactants, and polymers. (b) The shell layer, which is with chemicals completely different material from the core in all aspects, and (c) The core, that is the central portion of the NPs[1].

Several polymers are being utilized for the fabrication of nanoparticles. Macromolecules include peptides and protein can be easily and effectively deliver through the Nano delivery system. Nanoparticles deliver the drug at a controlled and sustained rate to the site of action[2].

Nowadays, nanoparticles are more attractive due to some of their unique features such as surface to mass ratio, ablity to adsorb and carry compounds such as drugs, probe, and proteins[3]. Nanoparticles also represent a promising carrier system for the targeting of anti-cancer agents to tumors. They are also reported successfully employed in Brain Drug Targeting. HexapeptideDalargin (Tyr-D-Ala-Gly-Phe-Leu-Arg), is the first drug nanoparticle drug that was delivered to the brain as the other medicine that was successfully transported into the brain in a form of nanoparticles are loperamide, phytotoxin, and antibiotic drug[3]. So, where conventional techniques reach their limits, nanotechnology provides opportunities for medical applications.

Advantages Of Nanoparticles

- Site-specific targeting is often achieved by attaching targeting ligands to the surface of particles.
- Drug release can be controlled or sustained which will increase the therapeutic efficacy of a drug.
- Side effects and toxicity shall be reduced.
- Passive and active drug targeting can be easily achieved by manipulating surface and particle size characteristics.
- Both hydrophilic and hydrophobic drug can be easily delivered.
- It can be administered through different routes like oral, nasal, parenteral.

Disadvantages

- The cost of manufacture is high and encapsulation efficiency is less.
- The solvent system used during the preparation process may produce toxicity.
- Particle aggregation and physical handling of nanoparticles in dry and liquid form are difficult.
- Leakage and sudden release of the drug may be one of the critical problems.
- The higher surface to volume ratio makes the particles more reactive or catalytic.

Types of Nanoparticles

Nanoparticles can be classified in various types based on their structures, sizes or physical and chemical properties. A few of them are carbon-based nanoparticles, lipid-based nanoparticles, and polymeric nanoparticles.

Carbon-based Nanoparticles

These nanoparticles contain carbons. It includes two main materials: carbon nanotubes (CNTs) and fullerenes. CNTs are graphene sheets that are rolled into a tube. These materials are mainly used for structural strengthening as they are 100 times stronger than steel. CNTs are classified into single-walled carbon nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs). CNTs are one of a kind in a way as they are thermally conductive along the length and non-conductive over the tube.

Ceramic Nanoparticles

They are inorganic solids made up of oxides, carbides, carbonates, and phosphates. These types of nanoparticles are having chemical inertness and high heat resistance. These are useful in drug delivery for many diseases like bacterial infections, glaucoma, and cancer.

Metal Nanoparticles

These nanoparticles can synthesize by chemical, electrochemical, or photochemical strategies. By chemical methods, we can get metal nanoparticles by reducing the metal-ion precursors in solution by chemical reducing agents. These can adsorb small molecules and have high surface energy. They are widely used in research areas, detection and imaging of biomolecules, environmental and bioanalytical applications.

Semiconductor Nanoparticles

They are having properties like those of metals and non-metals. They are found in the periodic table in groups II-VI, III-V or IV-VI. Some examples are InP, InAs GaP, GaN, germanium and silicon etc. These are used in electronics devices, photo-optics, photocatalysis, and water splitting applications.

Lipid-Based Nanoparticles

They are generally spherical in shape and having a diameter ranging from 10 to 100nm. It comprises of a strong center made of lipid and a network containing soluble lipophilic particles. The outside layer of these nanoparticles is stabilized by surfactants and emulsifiers. These nanoparticles have applications in the biomedical field as a drug carrier and delivery and RNA release in cancer therapy.

MATERIALS

ItraconazoleProvided by SURA LABS, Dilsukhnagar, Hyderabad, ChitosanProcured from Gattefosse Pvt. Ltd., Mumbai, Tween 80Purchased from Merck Limited, Mumbai (India), Acetic AcidPurchased from Merck Limited, Mumbai (India), MethanolPurchased from Merck Limited, Mumbai (India), WaterPurified

METHODOLOGY

Analytical Method Development

Determination of absorption maxima

Absorption maxima are the wavelength at which maximum absorption takes place. For accurate analytical work, it is important to determine the absorption maxima of the substance under study.

For the preparation of calibration curve stock solution was prepared by dissolving 100 mg of accurately weighed drug in 100ml of methanol(1mg/ml). Further 1ml of the stock solution was pipette out into a 100 ml volumetric flask and volume was made up with phosphate buffer (5.5pH). From this stock solution pipette out 1ml and dilute to 10 ml with phosphate buffer and subject for UV scanning in the range of 200-400 nm

using double beam UV spectrophotometer. The absorption maxima were obtained at 260 nm with a characteristic peak.

Preparation of calibration curve

It is soluble in Methanol; hence Methanol was used for solubilizing the drug. Stock solution (1 mg/mL) of Itraconazole was prepared in Dichloromethane and subsequent working standards (2, 4, 6, 8 and 10 µg/mL) were prepared by dilution with phosphate buffer of pH-5.5. These solutions were used for the estimation Itraconazole by UV method. The whole procedure was repeated three times and average peak area was calculated. Calibration plot was drawn between concentrations and peak area. Calibration equation and R^2 value are reported.

Preparation of nanoparticles

Preparation of Itraconazole loaded nanoparticles

Chitosan was dissolved in aqueous solution of acetic acid (2%) in 50 ml of distilled water. Under magnetic stirring at room temperature, tween 80 was add above solution and 10 ml of (w/v) solution was added drop wise using syringe needle into 100 ml Chitosan solution containing respective mg of Itraconazole. The stirring was continued for about 2.30 hrs. The resultant nanoparticles suspensions were centrifuged at 12000 × g for 15 minutes using C24 centrifuge. Samples were washed with water and dried. The formation of the particles was a result of the interaction between the negative groups of the TPP and the positively charged amino groups of chitosan (ionic gelation).

Table 1: Composition of nanoparticles formulations (F1 to F12)

Formulation	Itraconazole (mg)	Chitosan (mg)	Tween 80 (mL)	Acetic Acid (%)	Methanol	Water (mL)
F1	200	100	0.2	2	25	50
F2	200	150	0.3	2	25	50
F3	200	200	0.4	2	25	50
F4	200	250	0.5	2	25	50
F5	200	300	0.6	2	25	50
F6	200	350	0.7	2	25	50
F7	200	400	0.8	2	25	50
F8	200	450	0.9	2	25	50
F9	200	500	1	2	25	50
F10	200	550	2	2	25	50
F11	200	600	3	2	25	50
F12	200	650	4	2	25	50

All the quantities were in mg

RESULTS AND DISCUSSION

Calibration Plot OfItraconazolePhosphate Buffer Of Ph -5.5

A standard graph of Itraconazole in phosphate buffer of pH-5.5 was plotted using Absorbance and concentration as shown in Table and Fig. Equation for linearity curve and R^2 were calculated as Y=0.076X+0.007 and R^2 =0.999. Itraconazole showed maximum absorbance in phosphate buffer (pH 5.5) at 260 nm. The solution obeyed Beer-Lambert's law for concentration range of 2 to 10µg/mL with regression coefficient of 0.999. Standard curve of prepared Itraconazole in phosphate buffer pH 5.5 is shown below.

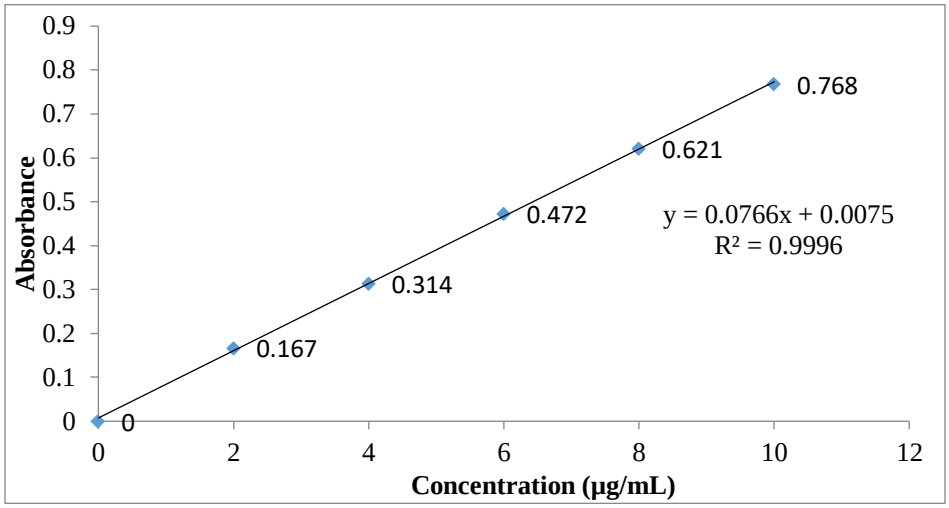

Fig 1: Calibration curve of Itraconazole in phosphate buffer pH 5.5

Characterization of nanoparticles

Table 2: Percentage yield, Drug Content, Entrapment Efficiency of all nanoparticle's formulations

FORMULATION	Percentage yield	Drug Content	Entrapment Efficiency
F1	87.03	97.11	78.92
F2	92.78	98.31	83.60
F3	93.14	97.92	89.03
F4	96.21	98.34	82.14
F5	96.80	97.08	73.98
F6	97.14	98.14	89.04
F7	95.89	96.32	76.92
F8	93.67	95.14	81.02
F9	81.28	97.36	83.01
F10	76.79	94.01	75.00
F11	73.10	97.55	76.31
F12	70.05	97.90	72.95

Table 3: Particle Sizes, PDI, Zeta Potential of all nanoparticles formulations

FORMULATION	Particle Size (nm)	PDI	Zeta Potential (mV)
F1	298.34	0.147	-29.3
F2	272.61	0.124	-32.6
F3	263.87	0.120	-36.1
F4	231.18	0.118	-40.9
F5	214.82	0.114	-43.8
F6	201.97	0.111	-46.2
F7	363.81	0.168	-28.1
F8	284.98	0.147	-34.7
F9	269.62	0.139	-31.5
F10	241.80	0.127	-29.0
F11	236.72	0.121	-26.4
F12	226.97	0.119	-24.9

DSC

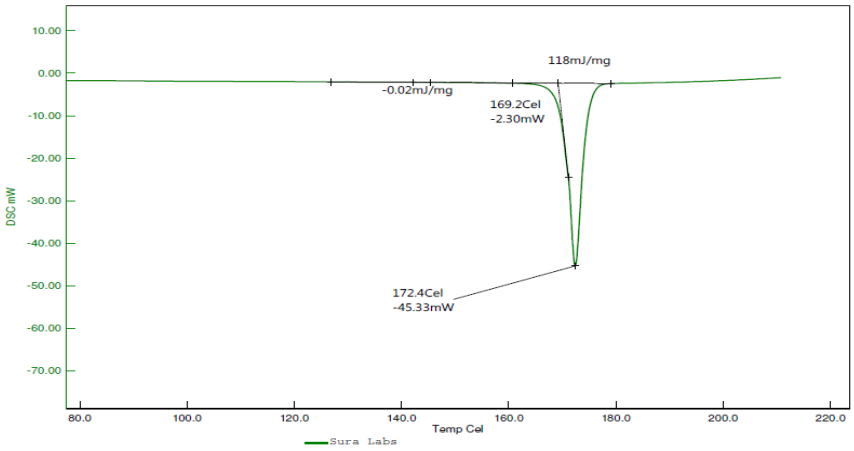

Fig 2: ItraconazolePure

SEM

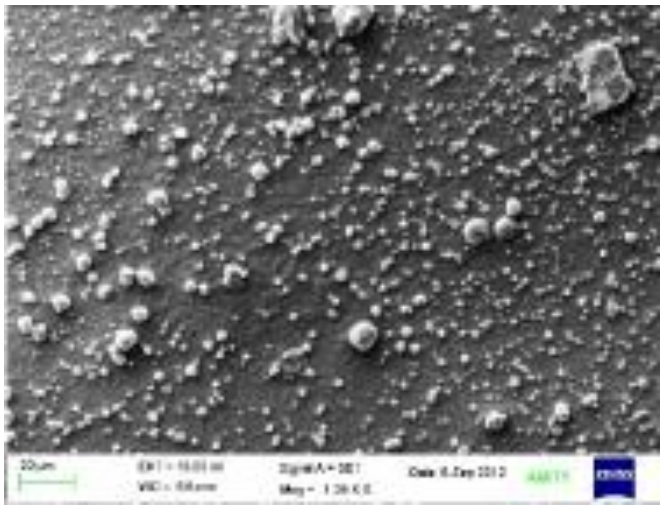

Fig 3: ItraconazoleF6 optimisednanoparticles

XRD

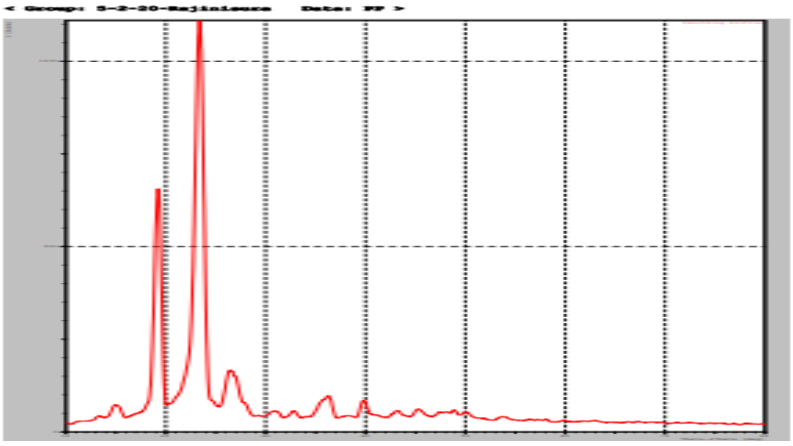

Fig 4: XRD ItraconazoleF6 nanoparticles

Table 4: *In vitro* dissolution studies of F1-F6nanoparticles formulations in percentage

Time (hour)	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
1	52.62	48.87	45.62	41.38	36.23	34.97
2	69.25	63.17	58.81	56.14	50.38	47.65
4	76.82	72.35	62.20	60.63	58.79	54.16
6	80.71	76.38	71.39	66.82	62.88	60.98
8	95.82	85.38	83.85	70.40	67.54	64.29
10		92.39	90.34	85.09	72.17	71.73
12			97.13	91.46	80.62	76.22
18				94.02	87.93	82.73
24					98.87	88.40
48						99.01

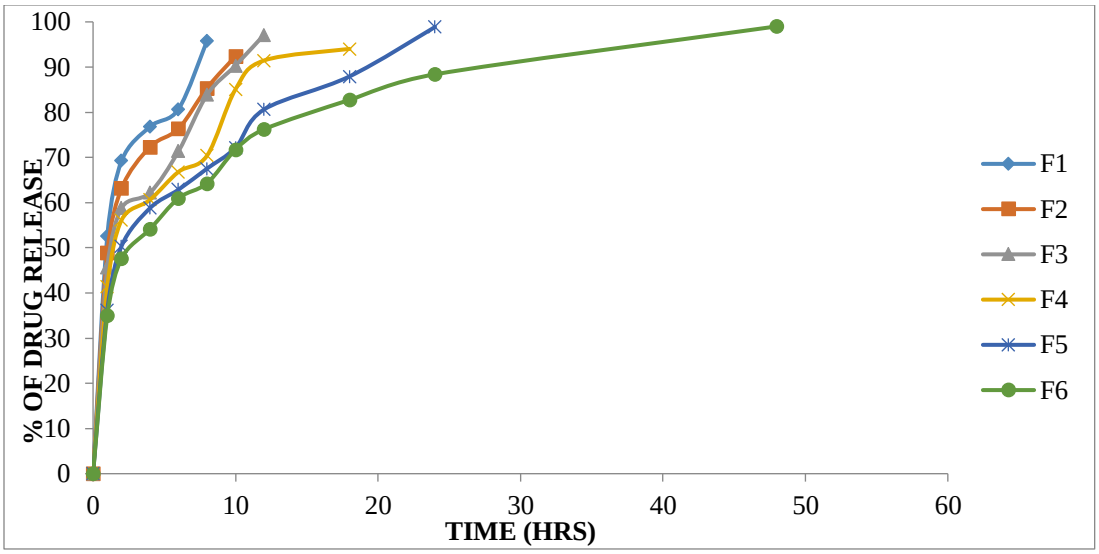

Fig5:*In vitro* dissolution studies of F1-F6nanoparticles formulations in percentage

Table5:*In vitro* dissolution studies of F7-F12nanoparticles formulations in percentage

Time (hour)	F7	F8	F9	F10	F11	F12
0	0	0	0	0	0	0
1	31.97	28.31	25.22	23.49	19.07	15.59
2	44.65	40.38	37.38	35.21	30.31	27.75
4	51.08	47.43	42.45	40.07	38.03	31.22
6	58.12	54.86	50.59	46.17	44.12	38.76
8	60.39	59.75	57.83	55.56	51.13	42.91
10	67.81	64.46	63.26	62.58	60.09	58.85
12	74.37	72.13	70.15	68.27	65.17	62.24
18	80.95	76.16	76.29	74.68	71.24	67.83
24	85.20	81.77	78.76	77.37	75.36	71.76
48	96.01	91.85	85.27	81.77	78.81	76.70

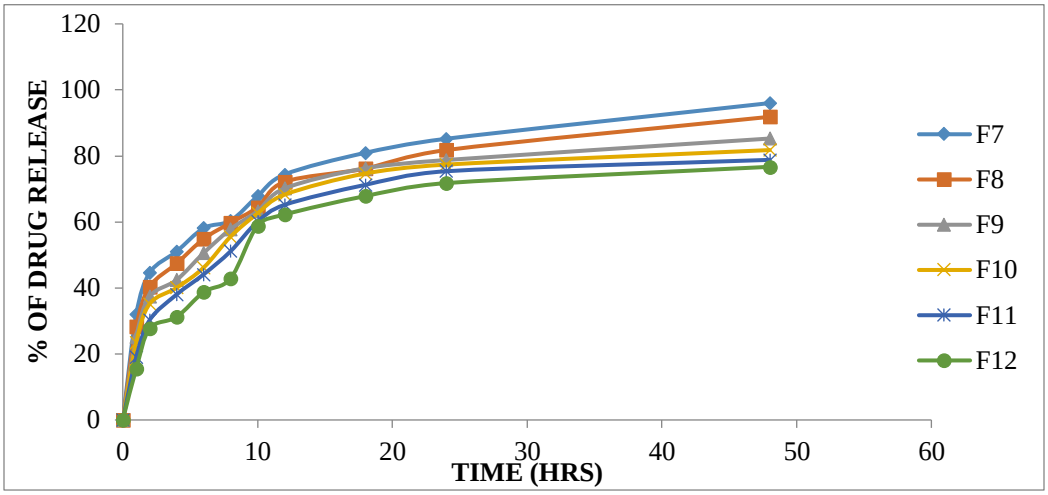

Fig6:*In vitro* dissolution studies of F7-F12nanoparticles formulations in percentage

Table6: Release kinetics of optimised formulation

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
34.97	1	1.000	1.544	0.000	1.813	34.970	0.0286	-0.456	65.03	4.642	4.021	0.620
47.65	2	1.414	1.678	0.301	1.719	23.825	0.0210	-0.322	52.35	4.642	3.741	0.901
54.16	4	2.000	1.734	0.602	1.661	13.540	0.0185	-0.266	45.84	4.642	3.579	1.063
60.98	6	2.449	1.785	0.778	1.591	10.163	0.0164	-0.215	39.02	4.642	3.392	1.250
64.29	8	2.828	1.808	0.903	1.553	8.036	0.0156	-0.192	35.71	4.642	3.293	1.349
71.73	10	3.162	1.856	1.000	1.451	7.173	0.0139	-0.144	28.27	4.642	3.046	1.595
76.22	12	3.464	1.882	1.079	1.376	6.352	0.0131	-0.118	23.78	4.642	2.876	1.766
82.73	18	4.243	1.918	1.255	1.237	4.596	0.0121	-0.082	17.27	4.642	2.585	2.057
88.4	24	4.899	1.946	1.380	1.064	3.683	0.0113	-0.054	11.6	4.642	2.264	2.378
99.01	48	6.928	1.996	1.681	-0.004	2.063	0.0101	-0.004	0.99	4.642	0.997	3.645

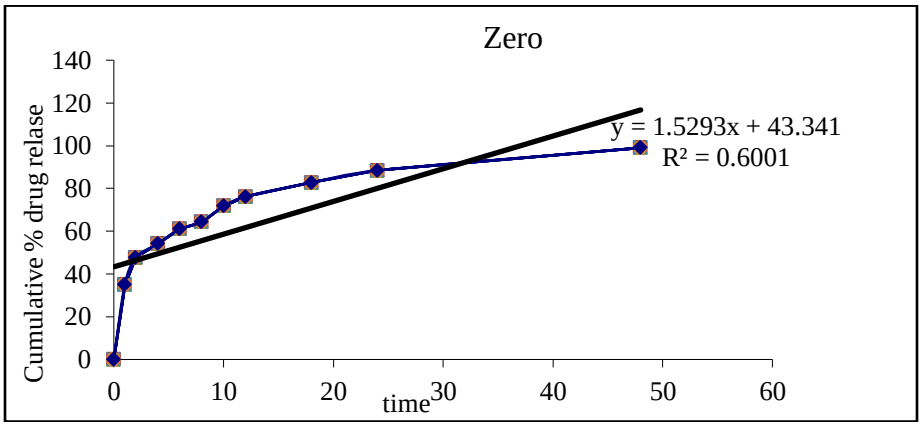

Fig7:Zero order release kinetics graph

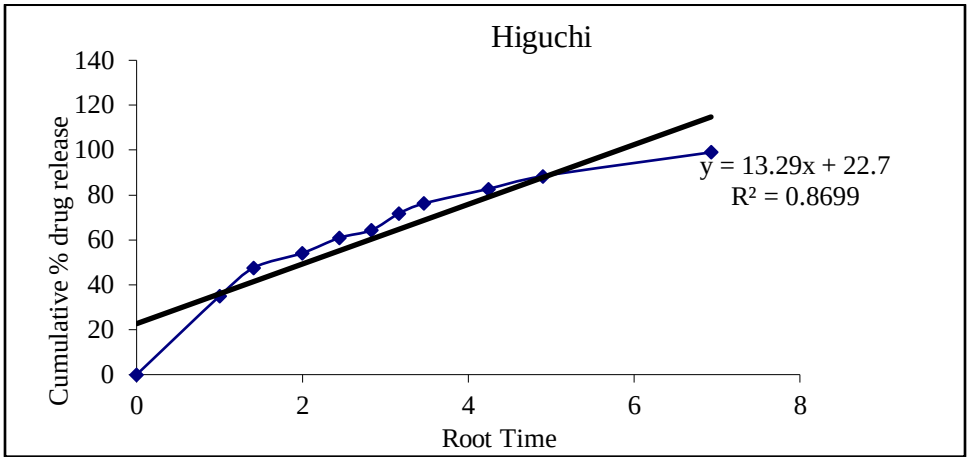

Fig8:Higuchi release kinetics graph

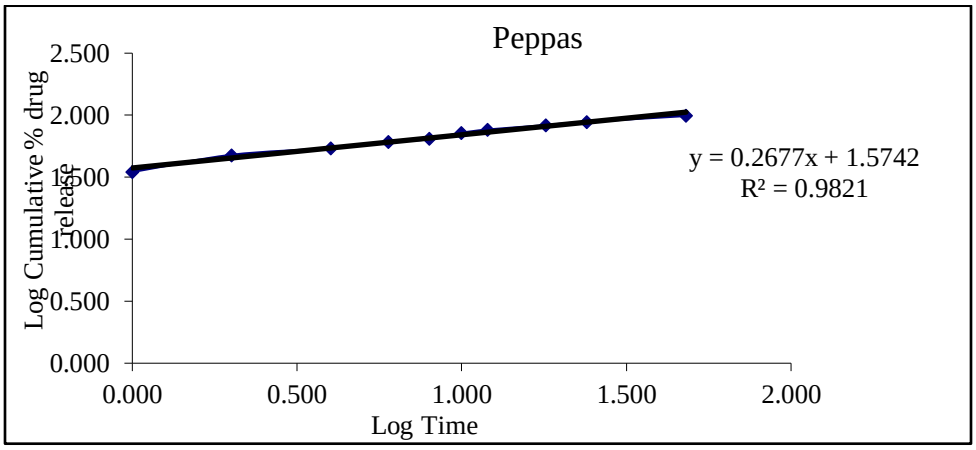

Fig 9:Peppas release kinetics graph

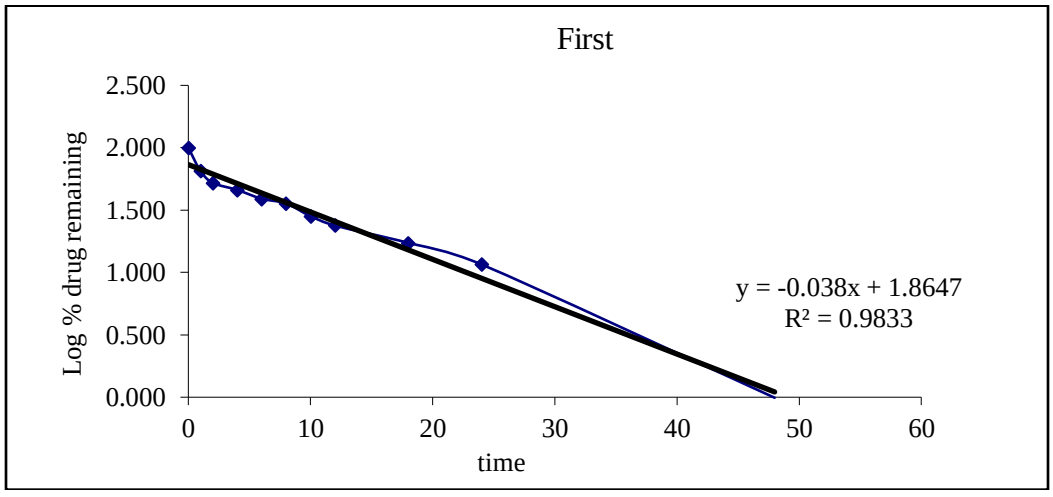

Fig10:First order release kinetics graph

Drug – Excipient compatibility studies

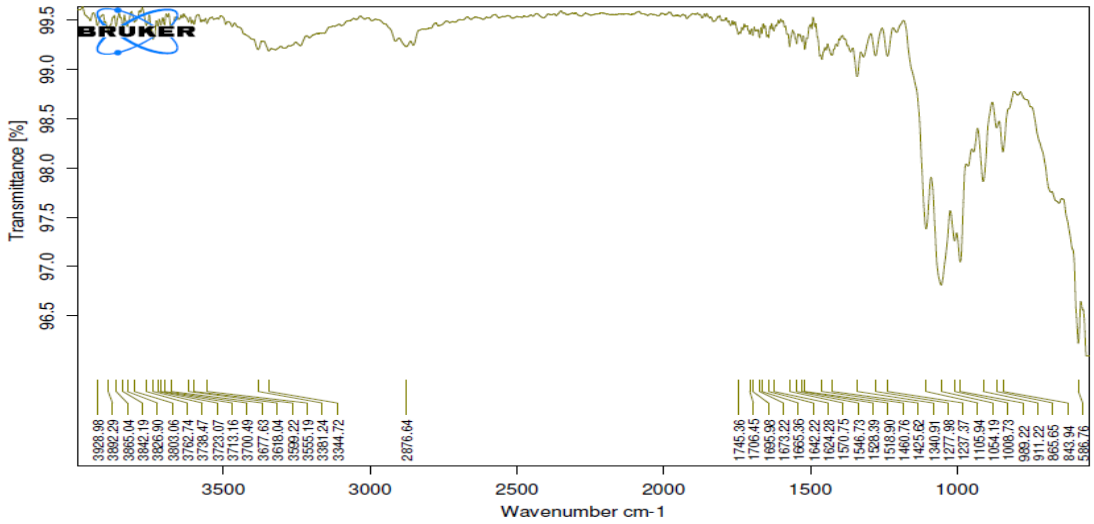

Fig 11: ItraconazolePure drug FTIR

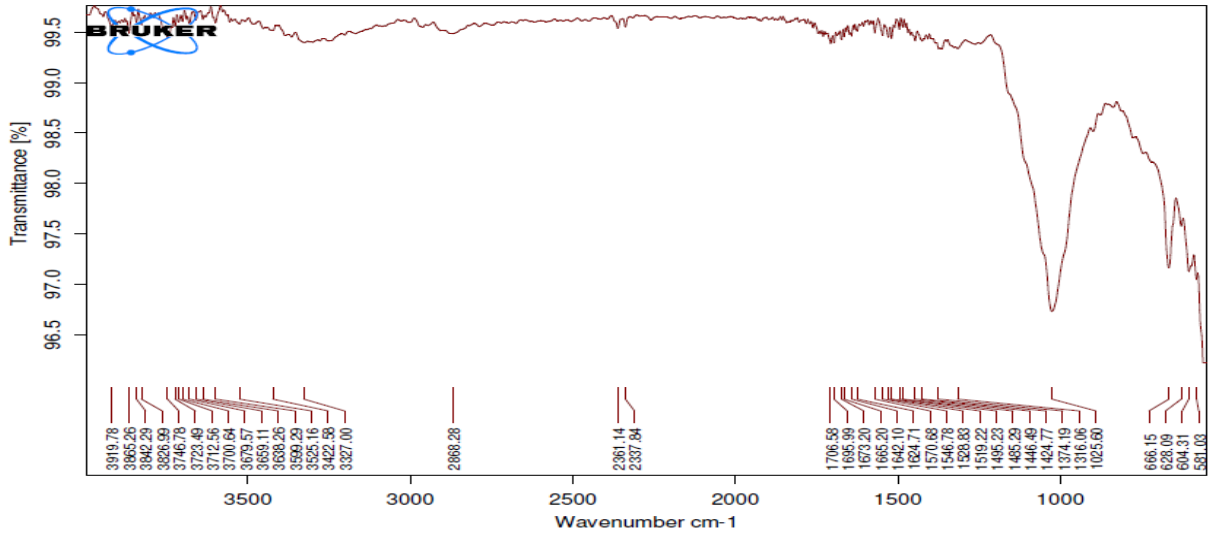

Fig 12: ItraconazoleF6 optimised

CONCLUSION

The nanoparticles were prepared by ionic gelation method by varying concentration of polymer such as Chitosan and surfactant (Tween 80). The nanoparticles were characterized by particle size, entrapment efficiency, drug loading and *in-vitro* drug release studies. The effect of variables like polymer concentration (Chitosan) and surfactant concentration (Tween 80) on particle size, entrapment efficiency and drug loading were investigated.The particle size was found to be in the range of 201.97 to 298.34nm.The entrapment efficiency and drug loadingwere found to be in the range of 72.95- 89.04% and 94.01- 98.34 % respectively. Formulation F6, which contained Chitosan in a ratio of 1:6drug to polymer, demonstrated good results among the several nanoparticulate formulations that were created using the ionic gelationprocess. The FT-IR investigation concluded that there was no significant interaction between the medication and the polymers that were used in this study. Therefore, the approach used to address the poor solubility and bioavailability of the medicine Itraconazole nanoparticles was successful.

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