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FORMULATION DEVELOPMENT AND CHARACTERIZATION OF VORICONAZOLE NANOPARTICLES

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Abstract:

When it comes to developing new medications, nanoparticles offer hope since they allow for the regulated release of doses. They work wonderfully for localizing medications to the target tissue. Developing and evaluating voriconazole-containing Carbopol p934 nanoparticles in various polymer solutions was the objective of this work. Scanning electron microscopy revealed a distinct spherical shape for the nanoparticles. Evidence from the FT-IR facility disproves any chemical link between the medicine, the polymer, and the drug's shelf life. All of the drug loading groups' in vitro release lines were thought of as initial release, and they gave 12 hours of sustained release. The created remedy satisfies and lessens the negative aspects of the voriconazole sustained release definition, and it has the potential to increase the drug's bioavailability in the future.

Keywords: *Nanoparticles, PLGA, Carbopol p934, Eudragit RL and Voriconazole.*

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INTRODUCTION:

In recent years, nanotechnology has emerged as a crucial field. Nanoparticles are the fundamental units of nanotechnology. Particles composed of carbon, metals, metal oxides, or organic compounds that range in size from one nanometer to one hundred nanometers are known as nanoparticles. Nanoparticles differ from bigger particles in a number of important ways, including their physical, structural, and natural characteristics. The higher mechanical strength, enhanced reactivity or stability in chemical processes, somewhat bigger surface area to volume, etc., are the primary causes of this distinctiveness. 2. The versatile nature of nanoparticles is due to their unique features. No matter the substance, nanoparticles are distinct in size, shape, and number of points. Thirdly, nanoparticles can have a single constant point for their length, extent, and density, making them zero-layer particles. B. Nanodots only have one limiting value and are single-layered. B. Graphene has a length and breadth that divide it into two layers. There are limiting values for B. carbon nanotubes, which are threelayered. B. Dimensions, including breadth, depth, and density, for instance.

B. Mesoporous gold particles:

There is a wide range in the size, shape, and structure of nanoparticles. Their sizes vary from 1 nm to 100 nm, and their forms can be anything from spherical to flat, conical to hollow, spiraling, or otherwise strange. It is possible for surfaces to be both smooth and rough, or to have a mix of the two. Nanoparticles can be glassy or poorly defined, and some include primary rock solids either alone or in aggregates. In order to enhance qualities while decreasing manufacturing costs, several mixing procedures are created or improved upon. Producing nanoparticles with enhanced optical, mechanical, physical, and chemical characteristics requires tweaks to two or three methods. Nanoparticles have been better characterized and used thanks to major advancements in instrumentation. Now you may find nanoparticles in your electrical grid, your appliances, and even your airplane. Future generations will be healthier and smarter thanks to nanotechnology.

Nanoparticle Benefits:

When it comes to drug discovery methods, nanoparticles provide a number of benefits. The following benefits are only a few of many:

When compared to more conventional methods of drug discovery, nanoparticles provide a number of crucial functions.

Nanoparticles alter the movement of drug molecules within organs and regulate and improve medication release at the border. Additionally, they improve the absorption, circulation, effectiveness, and symptom alleviation of drugs in the blood.

Many methods exist for administering nanoparticles, such as orally, nasally, parenterally, and intravenously. Currently, pharmaceutical delivery in the body can be enhanced by isolating nanoparticles using different measuring technologies and then binding to particular cell types or receptors.

Because of their minuscule molecular size, nanoparticles are able to traverse the body's physiological barriers and enter cells, blood vessels, the stomach lining, and the blood-brain barrier with relative ease.

Nanoparticles enhance the bioavailability of medications by making them more watersoluble. In their role as dispersed drug transporters, nanoparticles lessen the harmful effects of drugs and increase their efficient distribution.

Polymeric nanoparticles are a great tool for developing drugs that treat infectious diseases, prevent birth defects, seal deadly wounds, and more, since they can have their drug release structures altered using polymers.

Aids in the differentiation of various disorders o Improved implant safety.

Longer shelf life Even used in dentistry to patch minor gaps in teeth.

Adjustments to the methods used to create new drugs in an effort to attract more customers or lower production costs.

Nano Particles Restrictions :

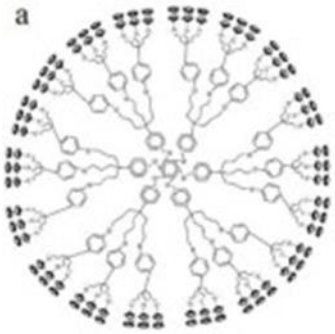
a) Nanoparticles are notoriously difficult to treat in both liquid and dry solutions due to the interparticle mixing that can result from their small size and huge surface area.

b) The buildup and abrupt release of drugs are limited by the small particle size and vast surface area. Prior to their commercial availability or usage in therapeutic settings, many practical concerns involving nanoparticles must be addressed. nine, ten, Nanoparticle categorization The three main categories of nanoparticles are carbon-based, inorganic, and natural.

Exemplary nanoparticles:

Certain natural polymers and nanoparticles go under several names, such as dendrimers, micelles, liposomes, ferritin, and so on. Nanocapsules, which contain biodegradable and nontoxic nanoparticles like micelles and liposomes, are vulnerable to heat, electricity, and light, as well as other forms of electromagnetic radiation. Their exceptional qualities provide them the perfect option for the creation of pharmaceuticals. Their size, shape, and surface

morphology are standard attributes, but their strength, capacity to transport drugs, and delivery mechanism (bound drug vs. adsorbed drug system) round out their usefulness and use. The medical industry makes extensive use of conventional nanoparticles. They find application in fields like medicine delivery systems due to their long-lasting properties and their ability to be blended into certain locations of the body. Targeted drug development is the name given to this process.



Organic nanoparticles

Inorganic nanoparticles:

Nanoparticles that are not composed of carbon are known as inorganic nanoparticles. Inorganic nanoparticles are those that are composed of metals or metal oxides.

A metal-based nanoparticle is one that is nanoscale sized and constructed from metals using bombardment or functional techniques. Nanoparticles may theoretically contain any metal. Among the many metals that may be used to attach nanoparticles, the most popular ones are aluminum, cadmium, cobalt, copper, gold, iron, lead, silver, and zinc. Among the most noticeable characteristics of nanoparticles are their extremely small size (ranging from 10 to 100 nm), high surface-to-volume ratio, large pore size, surface properties (including surface charge and density), and the wide range of reactions and aversions to environmental variables (including air, humidity, electricity, and sunlight).

Formulated using metal oxides. To alter the characteristics of other metal nanoparticles, oxide-based nanoparticles are combined with them. For instance, when exposed to oxygen at ambient temperature, iron nanoparticles oxidize instantly to copper oxide (Fe_2O_3), which is a different kind of reactivity than iron nanoparticles. Due of their enhanced reactivity and endurance, metal oxide nanoparticles are mostly used in mixed media. Al_2O_3, CeO_2, Fe_2O_3, Fe_3O_4, SiO_2, TiO_2, and ZnO are common oxide combinations. Other oxides that are commonly mixed include magnetite, cerium oxide, iron oxide, and silicon dioxide. Nanoparticles like this outperform their metallic counterparts in every way.

Using Carbon:

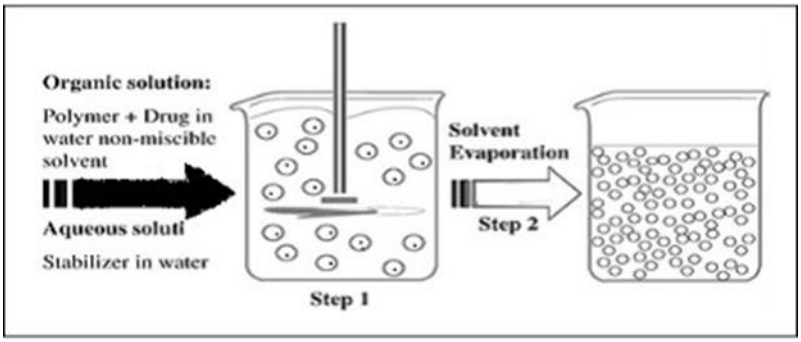
The term "carbon-based" describes nanoparticles that are completely composed of carbon. Different names for these carbon-based materials include fullerenes, graphene, CNTs, nanofibers, mats, and even nanosized carbons. The fullerene molecules. Carbon fullerenes (C60) are sp2 hybridized carbon molecules with a spherical form. A circular plane as wide as 8.2 nm for single layers and 4 to 36 nm for complex fullerenes is approached by around 28–1500 carbon particles. Carbon nanotubes. One of carbon's allotropes is graphene. A two-dimensional honeycomb structure made of carbon particles, graphene looks like a honeycomb. The typical thickness of a graphene layer is around 1 nm. Long, thin tubes made of carbon (SNCs). Nanotubes made of graphene with a honeycomb structure of carbon atoms twisted into hollow chambers are called carbon nanotubes (CNTs). These tubes may have widths of as little as 0.7 nm for single-walled CNTs and 100 nm for multi-walled CNTs, and lengths that vary between 2 to 3 micrometers and millimeters. Both open and closed ends containing half fullerene molecules are possible. Fibres made of carbon. Carbon nanofibers are made from the same graphene nanosheets as CNTs, but instead of the typical round empty chambers, they are twisted into a cone or cup form. Fiber made of carbon. An amorphous, carbonaceous substance with a 20- 70 nm pitch that is rounded. Aggregates of around 500 nm in size are formed when the particle bonds are sufficiently strong.

Approach to manufacturing:

Dispersion of solutes:

A new way to make nanoparticles is by using a soluble dispersion technique. A crucial component of

this approach is the creation of nanoemulsions. A typical solution (ethyl acid, dichloromethane, or chloroform) is used to separate the polymer. The medication is dispersed during this procedure. Mechanical mixing, sonication, or microfluidization (high pressure homogenization through a narrow channel) are used to process the mixture into an emulsion in water. The surfactants used in the emulsion may include polysorbates, poloxamers, sodium dodecyl sulfate, polyvinyl alcohol, or gelatin. Once an emulsion has formed, the naturally soluble ingredients are mixed continuously while the temperature and pressure are raised and lowered.



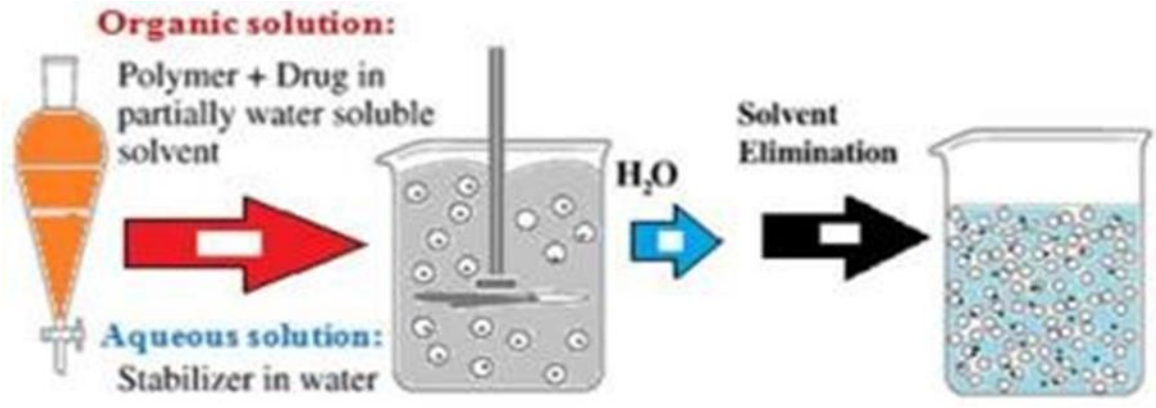
Representation of the solvent-evaporation technique

Double Emulsification method:

A two-step emulsification process is required because hydrophilic medications are notoriously difficult to disperse by conventional emulsification and distribution frameworks. Change of aqueous sedate response for standard polymer activity with strong mixing allows for a few noteworthy issues without emulsion. The final w/o/w emulsion was generated by arranging this aqueous arrangement with strengthened mixing thereafter ordinary dissolved killed by high centrifugation. Method for Dispersing Emulsions Leroux et al. pointed out that this tactic is a modified version of the salting out approach. This arrangement is saturated with water, and the polymer dissolves in a water-miscible dissolvable (such as propylene carbonate or benzyl alcohol).

Method for Dispersing Emulsions:

Leroux et al. pointed out that this tactic is a modified version of the salting out approach. This arrangement is saturated with water, and the polymer dissolves in a water-miscible dissolvable (such as propylene carbonate or benzyl alcohol). An emulsified polymer-water dissolvable organ is prepared using a liquid process that incorporates a stabilizer. Afterwards, soluble substances were killed by dispersion or filtering. High representation efficiency (by a massive 70%), lack of homogenization need, high collecting-to-bunch repeatability, ease of scaleup, narrow estimate course, and simplicity are key advantages of this technology. A few problems with this method include the large amounts of water that need to be removed from the suspension and the fact that during emulsification, the water-soluble arrangement leaks into the saturated aqueous external organ, which reduces the efficiency of the process.



Representation of the emulsification-diffusion technique

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.

Procedure:
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 252 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 Voriconazole was prepared in Methanol 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 Voriconazole 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 R2 value are reported.

Preparation of nanoparticles:
Preparation of Voriconazole loaded nanoparticles:
Voriconazole loaded Nanoparticle was prepared by previously reported emulsification sonication method. Voriconazole was dissolved in organic solvent (20 ml, methanol and DCM 30ml). Polymers in different concentrations were dissolved in water. The organic phase was added drop wise into the polymeric solution for emulsification. Then the dispersion was sonicated (20 min) with the application of ultra-probe sonication (60 W/cm3, Hielscher, Ultra- sonics, Germany). The formulation was stirred at 1500 rpm for 6 h using a magnetic stirrer to evaporate the organic solvent. The prepared NPs were centrifuged at 15,000 rpm for 20 min at 25 °C (Remi, Mumbai, India). NPs were separated and lyophilized using cryoprotectant (Mannitol 0.2%) and stored for further evaluation.

Table : COMPOSITION OF NANOPARTICLES (F1-F9)

Excipients	F1	F2	F3	F4	F5	F6	F7	F8	F9
Voriconazole	200	200	200	200	200	200	200	200	200
PLGA	100	200	300	-	-	-	-	-	-
Carbopol p934	-	-	-	100	200	300	-	-	-
Eudragit RL	-	-	-	-	-	-	100	200	300
Span 60 (mL)	2	4	6	2	4	6	2	4	6
Distilled water (ml)	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s	q.s
Dichloromethane (ml)	30	30	30	30	30	30	30	30	30
Methanol	20	20	20	20	20	20	20	20	20

Powder X-ray Diffraction (PXRD) Studies:
The prepared mixtures were also analyzed using X-ray powder diffractometer (PXRD) which confirms the formation of the new solid phases. The difference in the 2 theta lines confirms the formation of the new solid phases as no two solids have same 2 theta lines,

thus revealing the formation of new solid phases. It also reveals the information about the crystal structure, chemical composition, and physical properties of the material and also helps in structural characterization. This technique detects changes in the crystal lattice and is therefore a powerful tool for studying polymorphism, pharmaceutical salts, and co crystalline phases. Spectra of PXRD were taken on a sample stage Spinner PW3064. The samples were exposed to nickel filtrate Cukœ radiations (40 KV, 30 mA) and were scanned from 10° to 40°, 2Θ at a step size of 0.045° and step time of 0.5 s.

***In Vitro* Release Studies:**

Drug release was determined by dialysis method; two ml of each formulation (test and control) were poured into dialysis bags and put into 25 ml phosphate buffer (pH 6.8) and stirred (100 rpm, room temperature). At predetermined time intervals, 2 ml of phosphate buffer was taken and then substituted by fresh phosphate buffer. Finally, the amounts of released Simvastatin in phosphate buffer were measured by spectrophotometer at 235 nm. Aliquots withdrawn were assayed at each time interval for the drug released at λ max of 235 nm using UV-Visible spectrophotometer by keeping phosphate buffer pH 6.8 as blank and the amount of released drug was estimated by the standard curve.

Fourier Transform Infrared (FTIR) spectroscopy:

The formulations were subjected to FTIR studies to find out the possible interaction between the drug and the excipients during the time of preparation. FT IR analysis of the pure drug and optimized formulation were carried out using an FT IR spectrophotometer (Bruker FT-IR - GERMANY).

Differential Scanning Calorimetry:

The possibility of any interaction between the drug and the Excipients during preparation of SLN was assessed by carrying out thermal analysis of optimised formulation using DSC. DSC analysis was performed using Hitachi DSC 7020, on 5 to 15 mg samples. Samples were heated in sealed aluminum pan at a rate of 10°C/min conducted over a temperature range of 30 to 350°C under a nitrogen flow of 50 mL/min.

SEM (Scanning Electron microscope) studies:

The surface morphology of the layered sample was examined by using SEM (Hitachi, Japan). The small amount of powder was manually dispersed onto a carbon tab (double adhesive carbon coated tape) adhered to an aluminum stubs. These sample stubs were coated with a thin layer (30Å) of gold by employing POLARON-E 3000 sputter coater. The samples were examined by SEM and photographed under various magnifications with direct data capture of the images onto a computer.

RESULTS AND DISCUSSION:

Preparation of Standard Graph:

a. Determination of absorption maxima

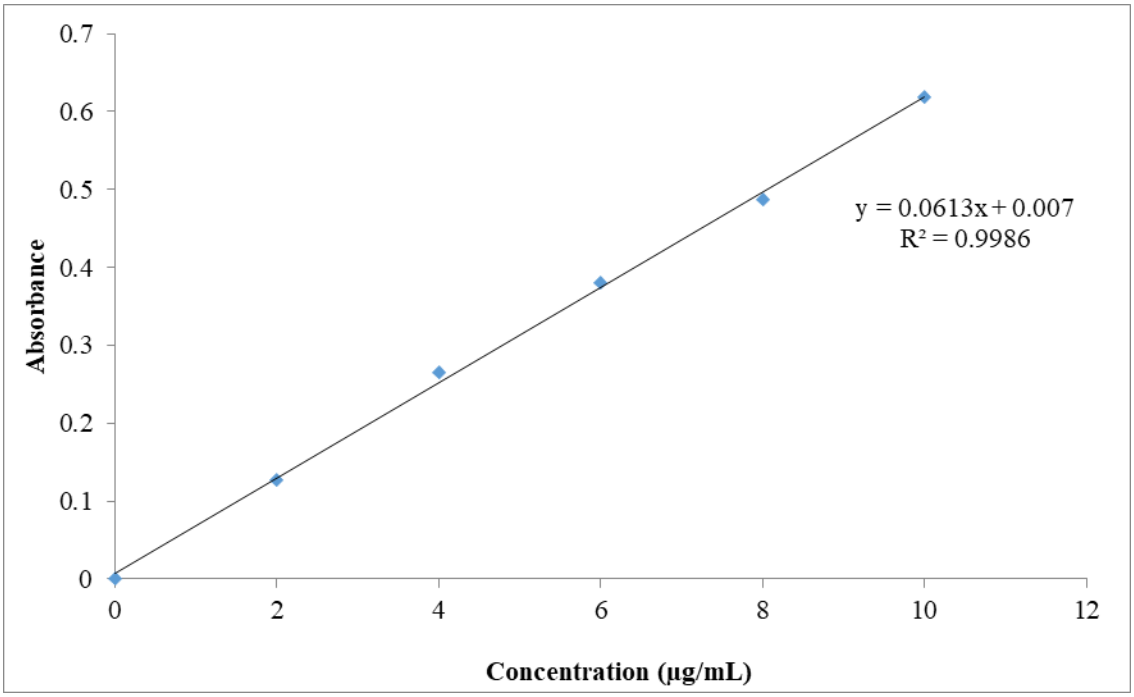
The standard curve is based on the spectrophotometry. The maximum absorption was observed at 252 nm.

b. Calibration curve

Graphs of Voriconazole was taken in 7.4 Phosphate buffer.

Calibration curve data for voriconazole at 252 nm

Concentrations [µg/mL]	Absorbance
0	0
2	0.128
4	0.265
6	0.381
8	0.487
10	0.619



Standard graph of Voriconazole in 7.4 Phosphate buffer

Standard graph of Voriconazole was plotted as per the procedure in experimental method and its linearity is shown in Table 8.1 and Fig 8.1. The standard graph of Voriconazole showed good linearity with R^2 of 0.998, which indicates that it obeys "Beer- Lamberts" law.

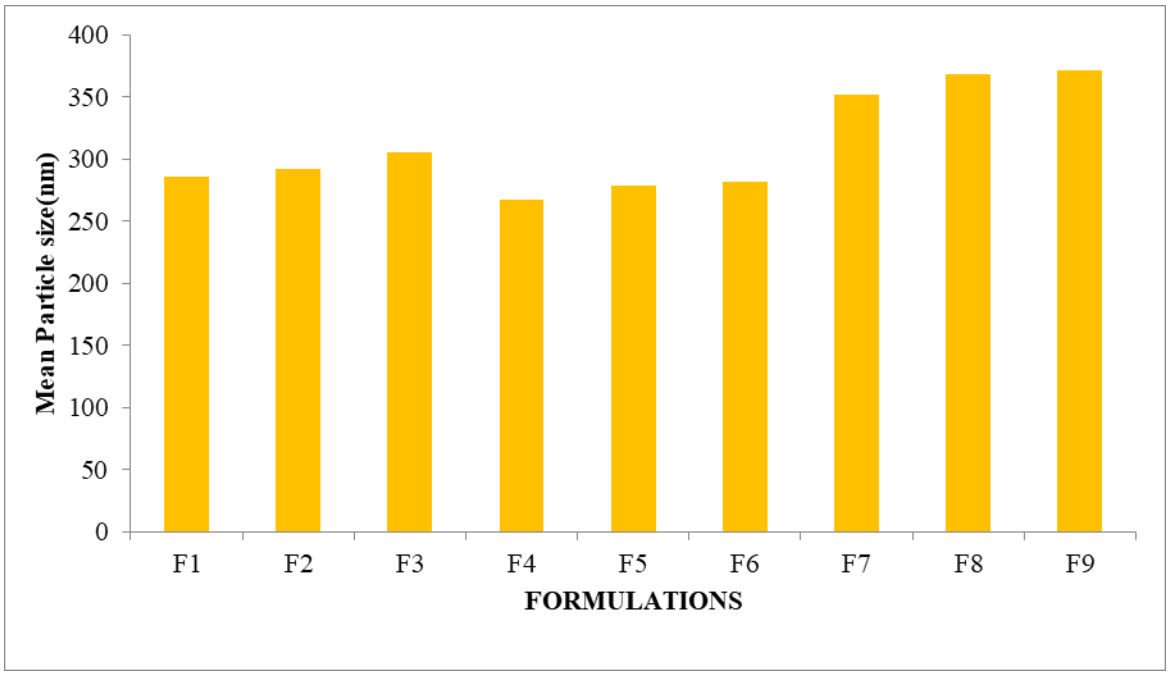
Evaluation of voriconazole loaded nanoparticles:

Table :Evaluation of Nanoparticles

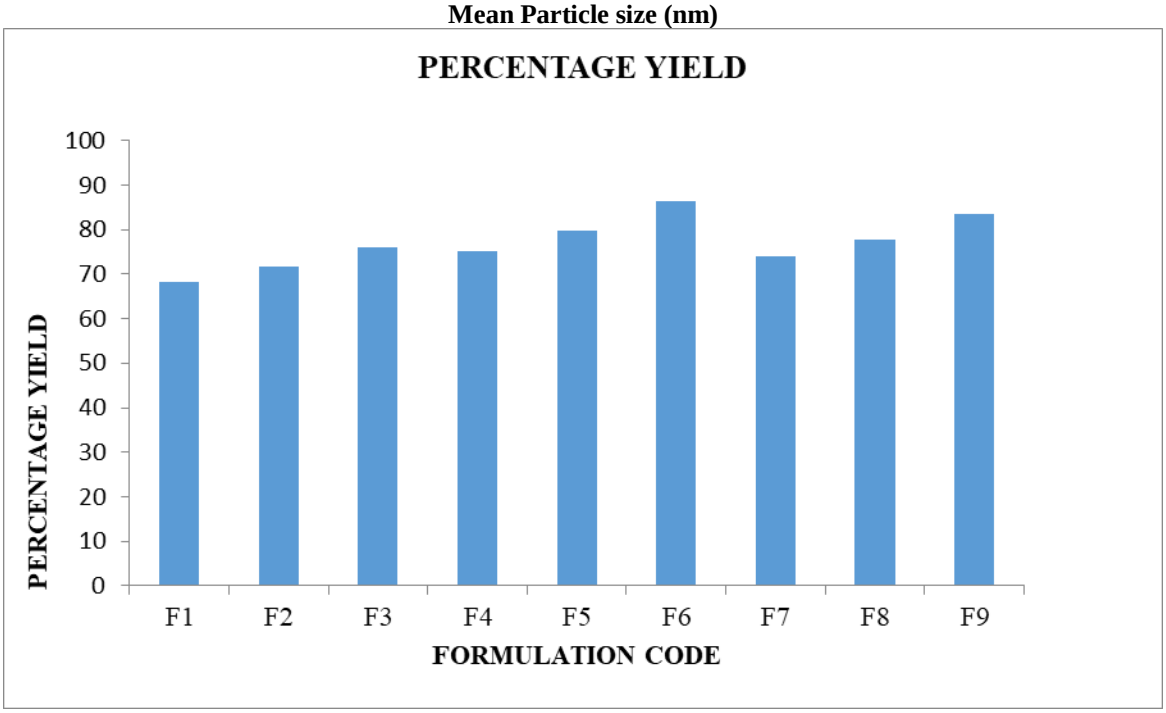
Batch No	Mean Particle size(nm)	%Yield	Drug Content	Drug encapsulation efficiency	PDI	Zeta Potential (mV)
F1	286.12 ± 18	68.14	93.51	63.92	0.668	-26.12 ± 1.8
F2	292.22 ± 19	71.54	95.81	72.29	1.268	-28.22 ± 1.9
F3	305.19 ± 16	75.92	97.65	80.41	1.153	-30.19 ± 1.6
F4	267.22 ± 20	75.20	91.54	76.91	0.868	-27.22 ± 2.0
F5	278.56± 18	79.81	94.82	82.83	0.577	-28.56± 1.8
F6	281.72± 23	86.34	98.84	87.92	0.309	-32.61± 2.3
F7	351.72± 23	73.92	95.14	62.79	0.498	-25.72± 2.3
F8	368.32± 42	77.69	97.14	70.30	0.385	-26.32± 2.2
F9	371.52± 32	83.44	97.82	76.98	0.325	-27.52± 2.4

Percentage yield of formulations F1 to F9 by varying drug was determined and is presented in Table. Highest drug content, Highest Entrapment efficiency observed for F6 formulation.

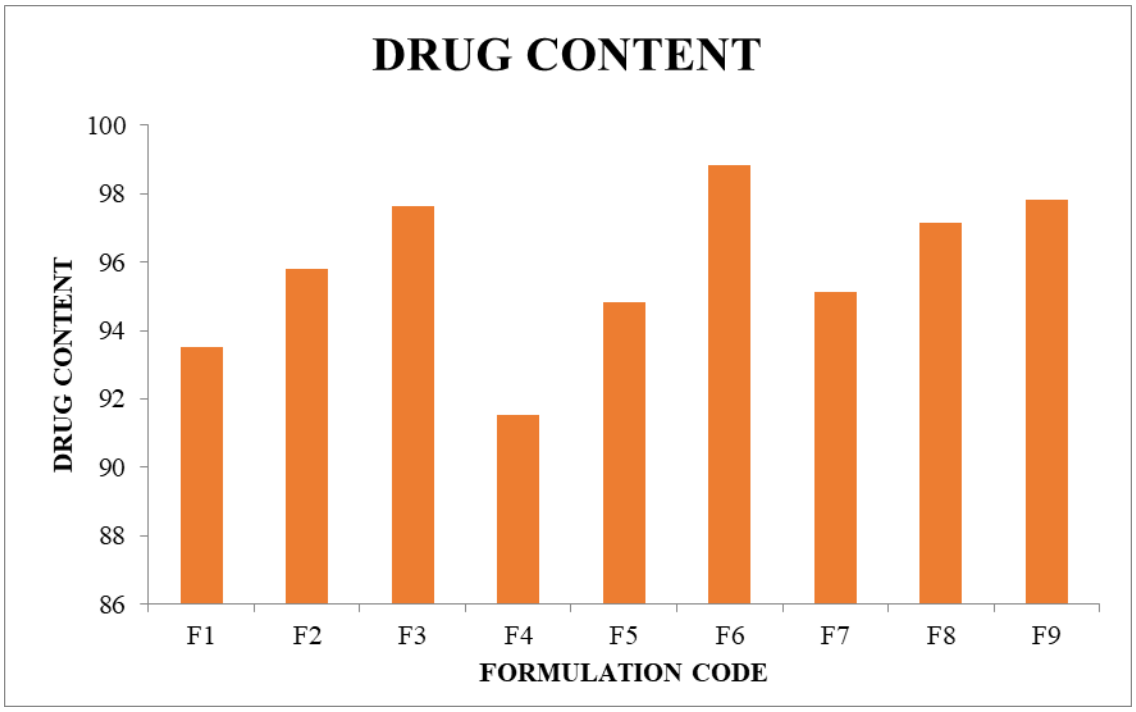
PDI observed in the F6 formulation i.e., 0.309 respectively. The Zeta potential range from -25.72 mV to -32.61 mV to all the formulations.



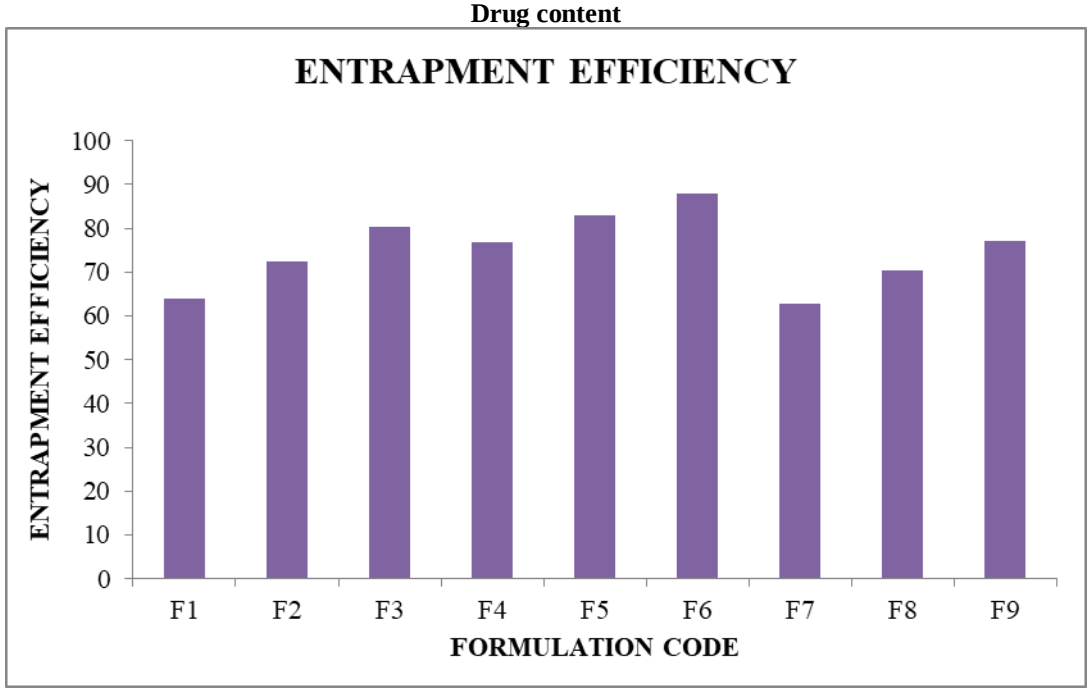
Mean Particle size (nm)



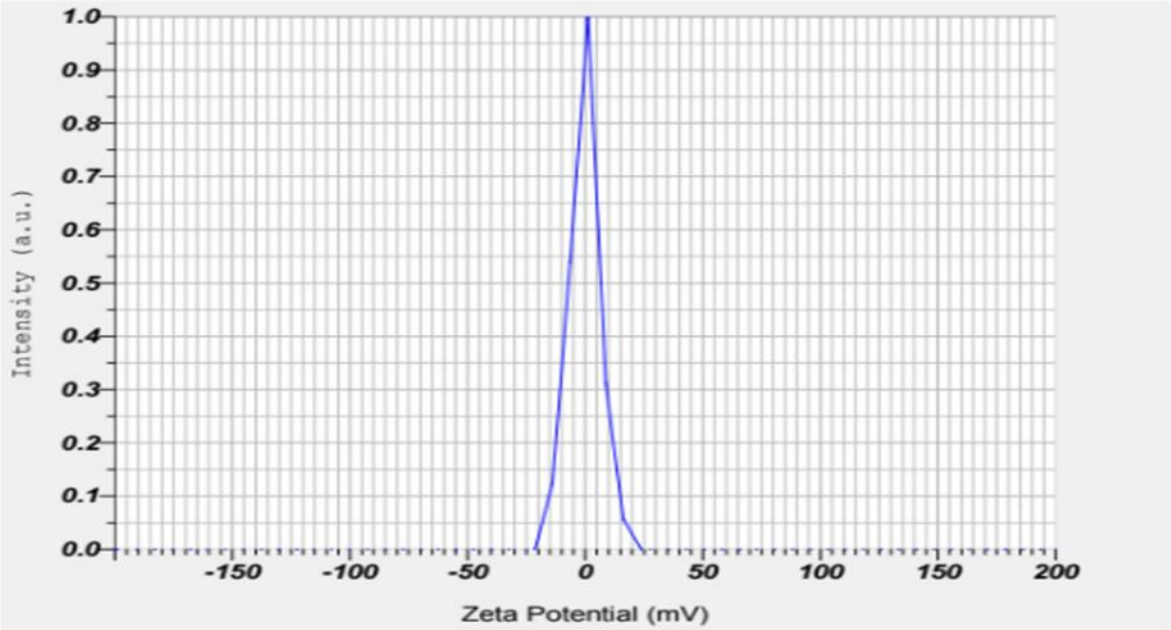
%Yield



Drug content



Drug encapsulation efficiency

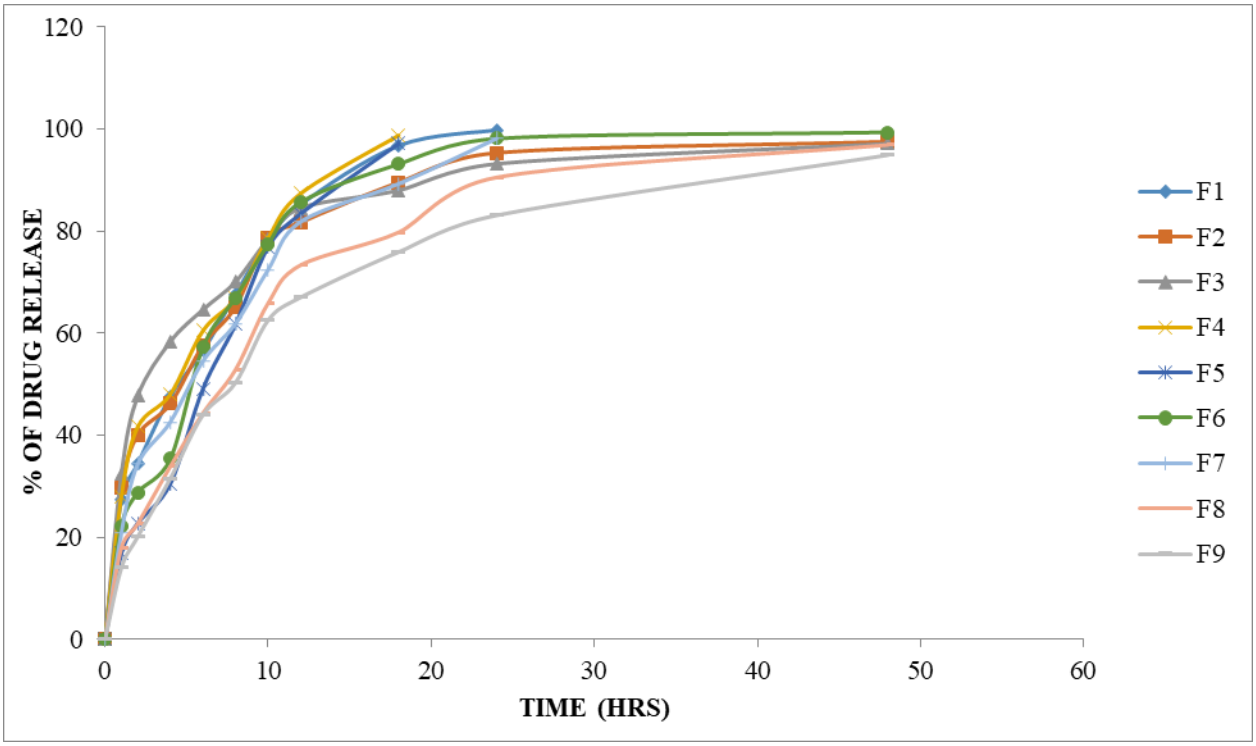


Zeta Potential of F6 Formulation

***In vitro* Drug release studies:**

***In vitro* Drug release studies of Voriconazole**

TIME (hr)	CUMULATIVE percent of drug released								
	F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**
0	0	0	0	0	0	0	0	0	0
1	27.42	29.69	32.41	27.93	16.85	22.26	20.92	17.92	14.01
2	34.39	40.09	47.69	41.62	22.76	28.78	34.36	22.65	20.08
3	47.60	46.16	58.34	48.02	30.50	35.36	42.61	33.89	31.51
4	56.51	57.65	64.61	60.47	49.11	57.23	54.53	44.32	43.98
5	67.62	65.19	70.08	66.85	61.78	66.98	61.88	52.87	50.31
6	78.37	78.67	78.39	78.68	76.89	77.46	72.46	65.90	62.57
7	85.26	81.76	84.56	87.39	83.43	85.68	81.87	73.36	67.04
8	96.78	89.54	87.98	98.77	97.14	93.14	89.29	79.77	75.91
10	99.82	95.34	93.18			98.13	98.14	90.53	83.09
12		97.54	97.14			99.37		96.91	94.91



Dissolution study of Voriconazole Nanoparticles

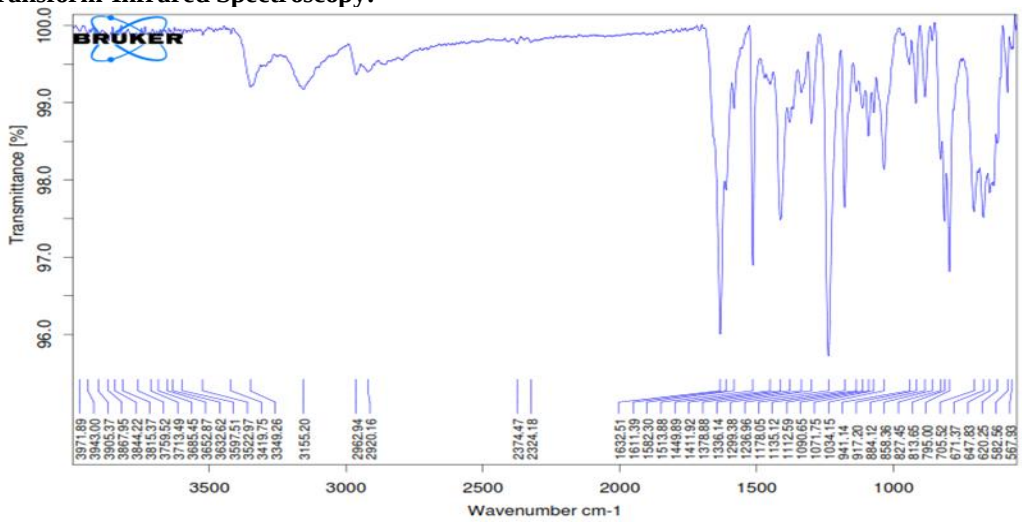
Hence based on dissolution data of 9 formulations, F6 Carbopol p934 (1:3) (300mg) formulation showed better release (99.37%) up to 12 hours. So F6 formulation is optimised formulation.

Application of Release Rate Kinetics to Dissolution Data:

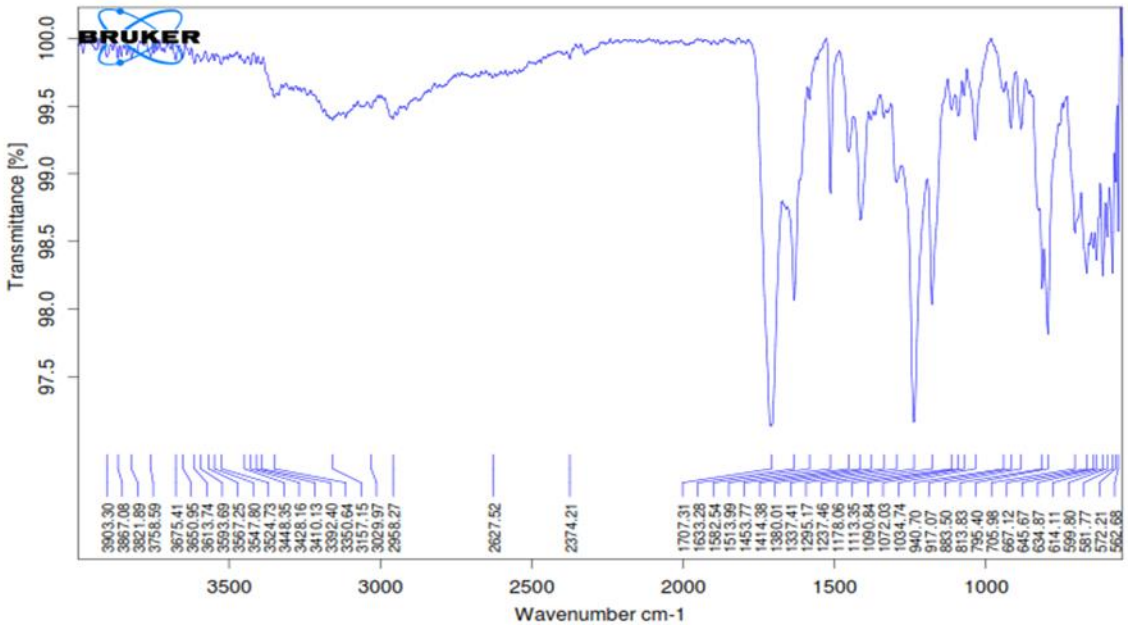
Data of *in vitro* release studies of formulations which were showing better drug release were fit into different equations to explain the release kinetics of drug release from Nanoparticles. The data was fitted into various kinetic models such as zero, first order kinetics; higuchi and korsmeyer peppas mechanisms and the results were shown in below table it follows the zero order kinetics.

Drug – Excipient compatibility studies

Fourier Transform-Infrared Spectroscopy:



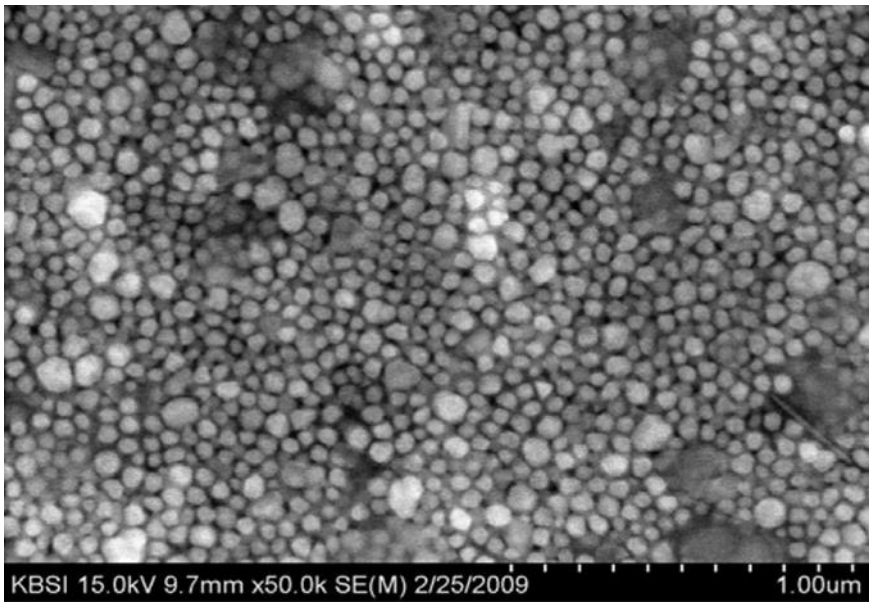
FT-IR Spectrum of Voriconazole pure drug.



FT-IR Spectrum of Optimised Formulation

There is no incompatibility of pure drug and excipients. There is no disappearance of peaks of pure drug and in optimised formulation.

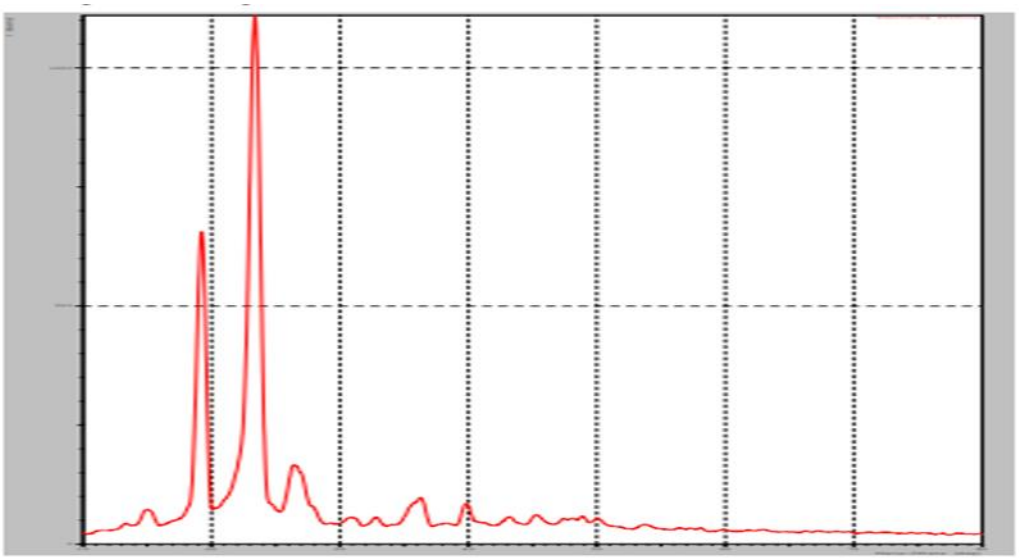
SEM



SEM graph of optimized formulation

SEM studies showed that the Voriconazole - loaded nanoparticles had a spherical shape with a smooth surface as shown in Figure.

XRD



Voriconazole F6 optimised formulation

CONCLUSION:

Nanoparticles have a special place in nanoscience and nanotechnology, not only because of their particular properties resulting from their reduced dimensions, but also because they are promising building blocks for more complex nanostructures.

In our current work, we have prepared Voriconazole nanoparticles. emulsification sonication method is a simple, fast and reproducible method which is widely used for the preparation of both nanospheres and nanocapsules and its superior advantage is obtaining small particles size and narrow size distribution. The optimized Voriconazole loaded Carbopol p934 nanoparticles formulations (F6) were in nano size range (281.72±23nm) with high drug release (99.37%) adequate encapsulating efficiency exhibiting a homogenous, stable and effective.

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