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Formulation and Evaluation of Chitosan Nanoparticles for Sustained Release of Clotrimazole

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Abstract

The main goal of the study was to develop and assess Chitosan nanoparticles for use in nanoparticle drug delivery systems to increase the therapeutic efficacy of Clotrimazole. Chitosan is employed as the polymer in this approach to create nanoparticles by using ionic gelation. Scanner electron microscopy (SEM), X-ray diffraction (XRD), entrapment efficiency, in vitro drug release investigations, and infrared (FTIR) spectral analysis are used to assess the prepared nanoparticles for external morphological analyses. Since there was no contact between the medication and the polymers, according to the Fourier transform IR spectra, they are compatible. For the F3 formulation, the percentages of entrapment and yield were higher. The nanoparticles' SEM analysis reveals that all formulations had perfect surface shape and were spherical and smooth. The XRD study of clotrimazole loaded nano formulation it was reported that nanoparticles showed broaden peak at (2θ=18.21° and 18.64°) which indicates the presence of clotrimazole in the formulation and all formulations were tested for in-vitro dissolution in phosphate buffer PBS (pH 5.8) at 37°C using a UV spectrophotometer at different time intervals, according to USP Dissolution Apparatus Type 2 the dissolving profile for all formulations in the pH 5.8 range. Over the course of 15 hours, it led to a sustained release of medication at a constant rate, with release rates of 88.6 ± 1.719, 93.6 ± 1.907, and 97.9 ±1.685. It was discovered that formulation F3 displayed the biggest release among the other formulations over a 15-hour timeframe. As a result, F3 was selected as the best formulation overall.

Keywords

Clotrimazole, Nanoparticles, Chitosan, Ion gelation method.

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1. INTRODUCTION

Nanotechnology is a branch of science that naturally spans a wide range of disciplines, including surface science, organic chemistry, molecular biology, semiconductor physics, energy storage, microfabrication, molecular engineering, etc. Utilising matter at the atomic, molecular, and supramolecular levels for industrial purposes is known as nanotechnology. The first and most popular definition of nanotechnology, currently

known as molecular nanotechnology, focused on the specific technological objective of accurately manipulating atoms and molecules for the creation of macroscale objects. **[1-2]**

A more generalized description of nanotechnology was subsequently established by the National Nanotechnology Initiative, which defined nanotechnology as the manipulation of matter with at least one dimension sized from 1 to 100 nanometres (nm). This definition reflects the fact that quantum mechanical effects are important at this quantum-realm scale, and so the definition shifted from a particular technological goal to a research category inclusive of all types of research and technologies that deal with the special properties of matter which occur below the given size threshold. It is therefore common to see the plural form "nanotechnologies" as well as "nanoscale technologies" to refer to the broad range of research and applications whose common trait is size. **[3]**

The potential effects of nanotechnology are currently up for discussion among scientists. Nanotechnology may be able to develop a wide variety of new items, including consumer goods, nanomedicine, nanoelectronics, biomaterials, and energy generation. On the other hand, nanotechnology poses a lot of the same problems as any new technology, such as worries about the toxicity and environmental impact of nanomaterials, as well as its potential to have an impact on the world economy and a variety of apocalyptic scenarios. **[4]**

The usage of nanomaterials in cosmetics, insecticides, food packaging, water treatment, and other industries has grown in recent years. For instance, silver nanoparticles are used as a pesticide because they can stop the growth of hazardous organisms. The possibility that these particles could endanger both human and environmental health is an increasing worry. In a recent study, scientists at the University of Missouri created a trustworthy technique for finding silver nanoparticles in fresh vegetables and other food goods. **[5]**

1.1 CONSIDERATIONS OF NANOPARTICLES

i. By binding to specific ligands on their surfaces, nanoparticles can be utilized to drive the drug to the precise target cells.
ii. Nanoparticles can be delivered by parenteral, oral, nasal, and ocular routes.
iii. Enhances therapeutic index, stability, and reduces adverse effects.
iv. By adjusting the particle size and surface characteristics of nanoparticles, drug targeting can be accomplished both actively and passively.

1.2 FORMULATION ASPECT OF NANOPARTICLES

1.2.1 Active pharmaceutical agent

Clotrimazole is an antifungal medicine used as an active pharmaceutical agent, mainly used in the treatment of fungal infection such as vaginal yeast infection, oral thrush and ringworm. It is also used to treat athlete's foot and jock itch.

1.2.2 Polymer

Deacetylated chitin, or Chitosan, is derived commercially from the exoskeletons of crustaceans such as shrimp, crab, and other shellfish. Chitosan occurs as odourless, white or creamy white powder or flakes. Chitosan is used as a biopolymer. It contains more than 5,000 glucosamine units, respectively, and has a molecular weight of over one million Daltons.

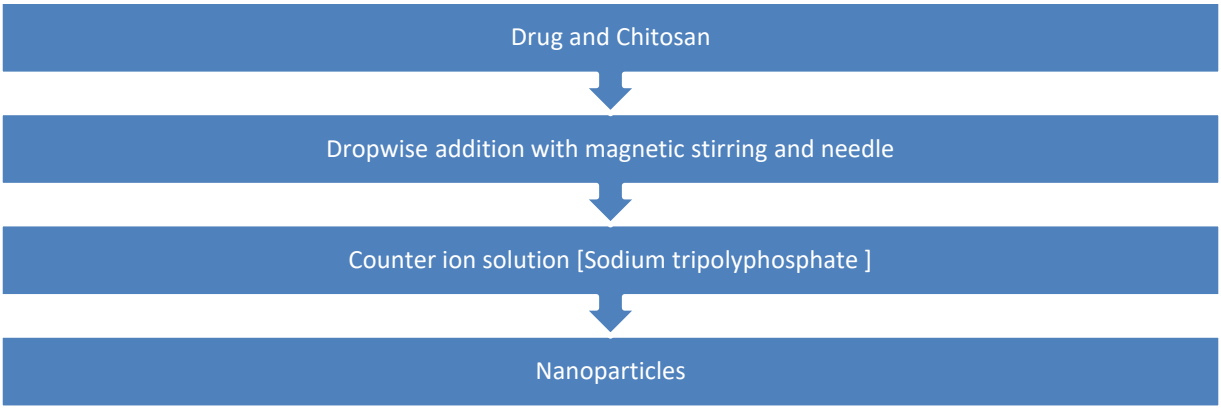
1.2.3 Cross linking agent

The chemical substance sodium tripolyphosphate has the formula Na5P3O10. It is the sodium salt of the conjugate base of triphosphoric acid, the polyphosphate penta-anion. As a cross-linking agent, it is utilized in nano formulation.

1.3 SYNTHESIS OF NANOPARTICLES

1.3.1 Ionotropic gelation method

The ion gelation approach is used in the current study to create blank chitosan nanoparticles. A solution of STP (sodium tripolyphosphate) in distilled water was gradually added to the homogeneous Chitosan solution while being continuously stirred. A white-colored suspension (Chitosan nanoparticles) has gradually formed during addition. The solution was agitated for roughly two hours after addition was finished. The generated chitosan nanoparticles were kept for subsequent investigation after being centrifuged for approximately 15 minutes at 5,000 rpm **[6].** Polyelectrolytes' capacity to cross links in the presence of opposing ions to produce nanoparticles is the basis for ionotropic gelation. The drug-loaded polymeric solution is dropped into the polyvalent cations' aqueous solution to create the nanoparticles. A three-dimensional lattice of ionically cross-linked molecules is formed as the cations diffuse into the drug-loaded polymeric droplets. To maintain their three-dimensional structure, biomolecules can also be loaded into these nanoparticles under benign circumstances.



Flow chart of ionotropic gelation method

Ionotropic gelation process influences by:

a) **Polymer and cross-linking electrolyte concentration:** The concentration of the polymer and electrolyte plays a significant role in the ionotropic gelation method's formulation of beads. Both should be concentrated in a ratio determined by the quantity of cross-linking units. The type and concentration of electrolytes have an impact on the percentage of entrapment efficiency.

b) **pH of cross-linking solution:** The pH of the cross-linking solution is an important consideration during formulation since it has an impact on the reaction rate, bead shape, and size.

c) **Drug concentration:** The ratio of the drug to the polymer in the nano formulation should be correct since the drug concentration has a significant impact on the effectiveness of entrapment. If the ratio of the drug to the polymer is out of range, bursting of the drug may be conceivable. **[7]**

2. DRUG AND POLYMER PROFILE

Clotrimazole: Clotrimazole is a broad spectrum antimycotic or antifungal agent. Clotrimazole's antimycotic properties were discovered in the late 1960[s]. Clotrimazole falls under the *imidazole* category of *azole* antifungals, possessing broad-spectrum antimycotic activity. It is available in various preparations, including creams, pessaries, and troche formulations (slowly dissolving tablets). As well as its antifungal activity, clotrimazole has become a drug of interest in treating several other diseases such as sickle cell disease, malaria, and some cancers. The minimal side effect profile of this drug and its uncomplicated metabolic profile have led it to gain widespread acceptance for the treatment of mycotic outbreaks such as vaginal yeast infections as well as athlete's foot **[8-9].**

Chitosan: Chitosan and chitin are polysaccharide polymers with molecular weights of over one million Daltons and more than 5,000 glucosamine and acetyl-glucosamine units, respectively, in each. Arthropods, fungus, and marine invertebrates all contain chitin. Chitin is a material that is produced commercially from the exoskeletons of crustaceans including prawns, crab and other shellfish. Chitin, a cellulose-like polysaccharide polymer made primarily of unbranched chains of N-acetyl-D-glucosamine, is deacetylated to produce chitosan. Chitosan, also known as deacetylated chitin, is made up of chains of D-glucosamine. Chitosan is found as an odourless, creamy white, or white powder or flake. During precipitation, fibre production is prevalent, and the chitosan may resemble cotton **[10].**

3. EXPERIMENTATION

3.1 MATERIAL AND EQUIPEMENTS

Clotrimazole as a pure drug was obtained from Yarrow chem Pvt Ltd, Mumbai, Maharashtra, and Chitosan as a biopolymer (Degree of deacetylation 89%) was obtained from CIFT Cochin, Kerala, sodium tripolyphosphate, glacial acetic acid and methanol was obtained from the College Laboratory.

UV VIS spectrophotometer (specord210 plus, Analytic Jena, Germany) was used for the development of the calibration curve and dissolution sample. The dissolution apparatus (EDT08LX, Electro Lab) was applied to conduct in vitro experiments on dissolving. Electronic Digital Balance (model ME204, Mettler-Toledo) was used for accurate weighing of samples and chemicals. Magnetic stirrer (REMI 2-MLH) was used to dissolve the solutions. Ultrasonic bath sonicator (Navyug, India), micro refrigerated centrifuge (Navyug, India) was used. Dialysis membrane was used for *in vitro* dissolution of the nanoparticles formulations (HIMEDIA, Dialysis Membrane-70). Perkin Elmer 1600 FTIR stands for Fourier Transform Infrared Spectrophotometer., USA, digital analytical an electron microscope for

scanning (JSM 6100(JEOL) USA), X-ray Diffractometer (Panalytical Xpert Pro, Japan) from Punjab University, Chandigarh were used for characterization of sample on paid basis.

3.2 COMPUTATIONAL STUDIES

Preformulation studies are the first phase in the logical development of a drug substance's dosage form, and they concentrate on the physicochemical characteristics of a new drug candidate that may influence its effectiveness and the development of a dosage form. This can support the necessity for molecular change or offer crucial information for formulation design. Every medicine has a natural chemical and physical characteristic that has been considered before the creation of a pharmaceutical formulation. When creating dosage forms, this feature offers the basis for the mixing of medications and pharmaceutical substances. Preformulation testing's main goal is to produce data that the formulator can use to create stable, bioavailable dosage forms.

3.2.1 DRUG CHARACTERIZATION

3.2.1.1 Organoleptic Features

By using visual inspection, organoleptic examinations of things like appearance, colour, and smell were carried out. Colour: A small amount of the medication was taken in butter paper and examined in a well-lit area. Very little smell was observed in order to detect the aroma. Taste: Only a very small amount was tasted to determine the flavour.

3.2.1.2 Melting Point

The USP technique was used to determine the melting point. A sealed capillary tube was filled with a little amount of the medication. The melting point device was used with the tube. The apparatus's temperature was steadily raised while it was documented at what temperature the drug first began to melt and what temperature it reached when it had completely melted.

3.2.1.3 FTIR Analysis

For the purpose of identifying clotrimazole, Fourier transform infrared spectroscopy was used. Using the KBr disc approach, FTIR spectroscopy of clotrimazole and a drug and polymer mixture was performed. The samples were adequately diluted with dry KBr before being compacted with pressure between 7 and 10 Newtons into discs. Infrared spectra were captured in the 4000-400/cm scanning range.

3.2.1.4 Selection of λ_{max}

The dilutions were made with phosphate buffer, which has a pH of 5.8, and were then examined in a UV spectrophotometer. For the quantitative examination of the drug and dissolution sample, the appropriate wavelength (max) was chosen.

3.2.1.5 Preparation of calibration curve of clotrimazole in phosphate buffer 5.8 pH

Drug 25 mg was dissolved in 50 ml of methanol to create a stock solution with a concentration of 500 g/ml. Then, using phosphate buffer 5.8 pH, dilutions in the range of 100 g/ml to 500 g/ml were made from the stock solution. Utilising a UV spectrophotometer set to the appropriate 261(max), dilutions were examined.

3.3 PREPERATION OF BLANK CHITOSAN NANOPARTICLES

Chitosan nanoparticle blanks were created using the ion gelation process. With constant stirring, 200 mg of STPP (sodium tripolyphosphate) in 25 ml of distilled water was gradually added to the homogenous Chitosan solution (150 mg in 30 mL of 4% acetic acid). A white suspension (Chitosan nanoparticles) has gradually formed during addition. The solution was agitated for roughly two hours after addition was finished. The resulting chitosan nanoparticles were centrifuged at 5,000 rpm for approximately 15 minutes.

The solution from the supernatant was decanted. To get rid of the unreacted sodium tripolyphosphate, the precipitate was centrifuged, resuspended in distilled water, and washed two to three times in total. This supernatant is appropriate **[11]**.

3.4 PREPERATION OF CLOTRIMAZOLE LOADED NANOPARTICLES

With constant stirring, a solution containing 100 mg of the medication in 5 ml of methanol was added to the homogenous Chitosan solution (150 mg in 30 mL of 4% acetic acid). The STPP solution was then gradually added to this combination for around two hours with adequate stirring (200 mg TPP in 25 ml distilled water). The resulting chitosan nanoparticles were centrifuged at 5,000 rpm for approximately 15 minutes.

Additionally, the supernatant was taken for additional examination. To remove the unloaded medication, the precipitate was centrifuged after being resuspended in distilled water and washed two to three times. Using UV spectroscopy, the corresponding supernatant solution was collected and examined for drug encapsulation.

3.5 CHARACTERIZATION OF CLOTRIMAZOLE LOADED NANOPARTICLES

3.5.1 Percentage yield

The percent yield measures the nanoparticles' actual yield in relation to their useful yield.

It is calculated as the theoretical yield multiplied by 100 divided by the practical yield.

Equation used to calculate the percentage yield:

% yield is calculated as: (nanoparticle practical yield)/ (theoretical yield) ×100. (Eq.1)

3.5.2 Scanning electron microscopy (SEM)

Analytical SEM with 15 kV voltages for secondary electron imaging was used to examine the surface and interior morphology of the nanoparticles. The liquid nitrogen snap freezing process was used to create the sprayed nanoparticles, which were then coated in gold using an ion sputter coater (Hitachi S3400N, US) and lyophilized.

3.5.3 X-ray diffraction (XRD)

XPERT- PRO diffraction metre with k filter created at 45kv voltage and 40mA current across a diffraction angle 2 was used to record the drug's X-ray diffraction pattern.

3.5.4 Entrapment Efficiency (EE)

In order to achieve the intended therapeutic efficacy, EE is essential in delivering bioactive to the targeted region at the correct therapeutic dose. The nano formulation was spun at 5,000 rpm for 15 minutes to obtain the pellets needed to evaluate the EE. The recovered supernatant was carefully diluted with PBS to a pH of 5.8, and the entrapment efficiency was assessed using a UV spectrophotometer (instrument name) at 261 nm in comparison to a blank solvent **[12].**

As indicated in equation 2, the following formula can be used to evaluate the EE:

EE is calculated as:

(weight of drug in nano formulation)/(weight of drug taken initially) x 100.(Eq.2)

3.5.5 *In vitro* drug release studies

Utilising a paddle apparatus, the in-vitro release of clotrimazole from the formulation was investigated. The dissolving medium was freshly made PBS (pH 5.8). The dissolving medium was used to soak the cellulose membrane over the night. A precise amount that matched the medication dose in the formulation was added to this assembly. The membrane was suspended in 900 ml of dissolution medium that was kept at 37±2°C with the paddle attached and the membrane barely touching the surface of the receptor liquid. Paddles were used to agitate the dissolving medium at a speed of 100 rpm. At regular intervals, 5ml aliquots of each volume were removed and replaced with an equal volume of the fresh media. A UV spectrophotometer was used to analyse the samples to determine the drug release at 261 nm **[11].**

4. RESULTS AND DISCUSSION

4.1 Drug Characterization

4.1.1 Organoleptic properties

Clotrimazole was odourless and tasteless white crystalline powder. Organoleptic properties results were shown in Table No.1:

Table 1: Drug's organoleptic characteristics.

Drug	Test	Specification	Observation
Clotrimazole	Color	White powder	White powder
Clotrimazole	Odour	Odourless	Odourless
Clotrimazole	Taste	Tasteless	Tasteless

4.1.2 Melting point

According to table 2, the capillary fusion method was used to estimate the drug's melting point, which ranges from 147 to 149^0 C. The substance was identified because the melting point determined by this method was near to values, i.e., 147-149^0 C, published in literature.

Table:2 Drug melting point.

Drug	Test	Specification	Observation
Clotrimazole	Melting point	147-148˚C	147-149 ˚C

4.1.3 FTIR Analysis

FTIR of clotrimazole drug,chitosan polymer and physical mixture of drug and polymer shown in fig 1,2 and 3.The drug's identity was established by the FTIR spectrum in Fig.1, which revealed several characteristic peak assignments at 3063 cm-1 for the

C-H stretch (aromatic stretch), 1490 cm-1 for the C=C aromatic stretch, 763 cm-1 for the C-Cl stretch, and 1210 cm-1 for the C-N stretch. The FTIR spectrum in Fig.2 was used to demonstrate the polymer's identity. It revealed many typical peak assignments at 2918 cm-1, 3084 cm-1, and 2918 cm-1, which correspond to C-H stretch (aromatic stretch), O-H stretch, and N-H bending, respectively. The FTIR spectrum in Fig.3 demonstrated numerous peaks assigned at 3424 cm-1 according to O-H stretch and at 3063 cm-1 relating to various polymer and drug peaks.

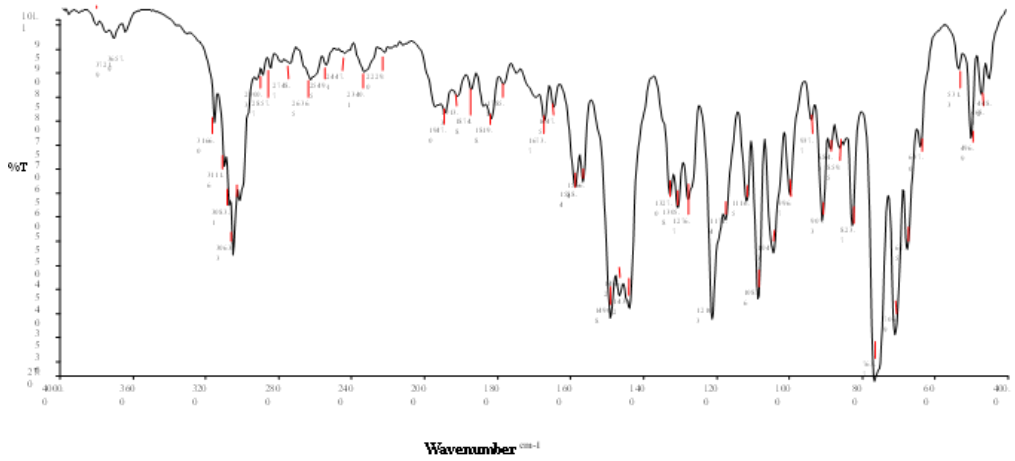


Fig.1 FTIR of pure drug clotrimazole

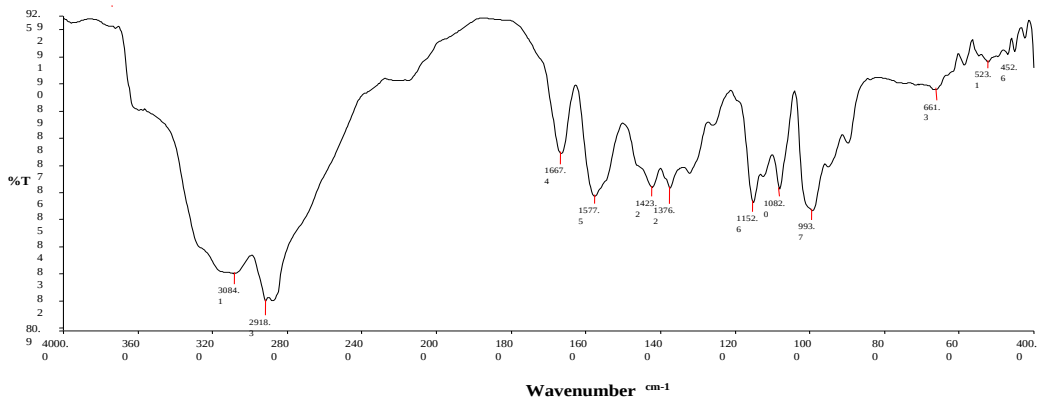


Fig.2 FTIR of Chitosan

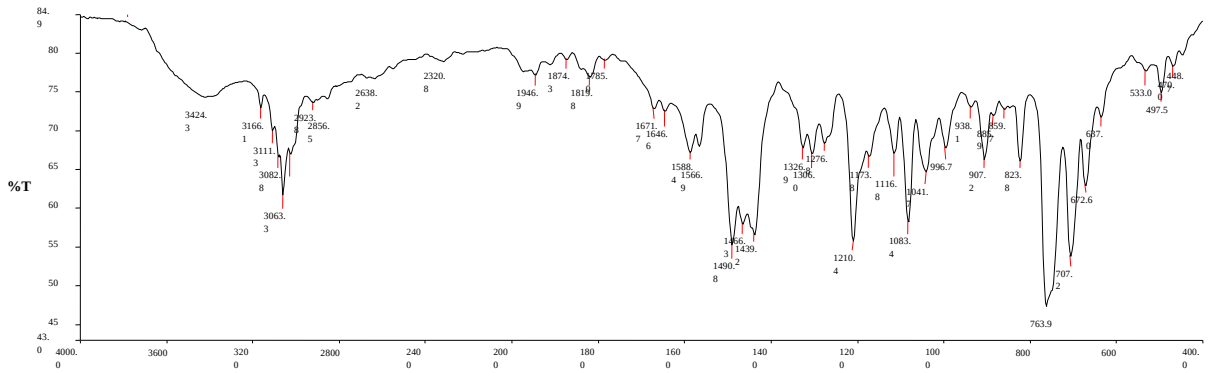


Fig.3 FTIR of physical mixture of drug and polymer

4.1.4 Selection of λmax

Clotrimazole displayed a highest peak at 261 nm, which was ultimately chosen for the quantitative analysis of the drug and sample of drug dissolution.

4.1.5 Calibration plot of clotrimazole in phosphate buffer 5.8 pH

The medication was dissolved in phosphate buffer, 5.8 pH, at a concentration of 100–500 ug/ml to produce the calibration curve for clotrimazole. Table.3 includes the absorbance values. Figure.4 shows a graph of absorbance against concentration. The clotrimazole standard curve revealed the regression equation Y = 0.002x-0.021 with a R value of 0.997, demonstrating high linearity.

Table-3 Different drug dilutions at 261 nm in phosphate buffer at pH 5.8.

S. No	Concentration (µg/ml)	Absorbance
1	100	0.2418 ± 0.012
2	150	0.3600 ± 0.017
3	200	0.5308 ± 0.021
4	250	0.6828 ± 0.020
5	300	0.8077 ± 0.009
6	350	0.9153 ± 0.014
7	400	1.1046 ± 0.016
8	450	1.1967 ± 0.039

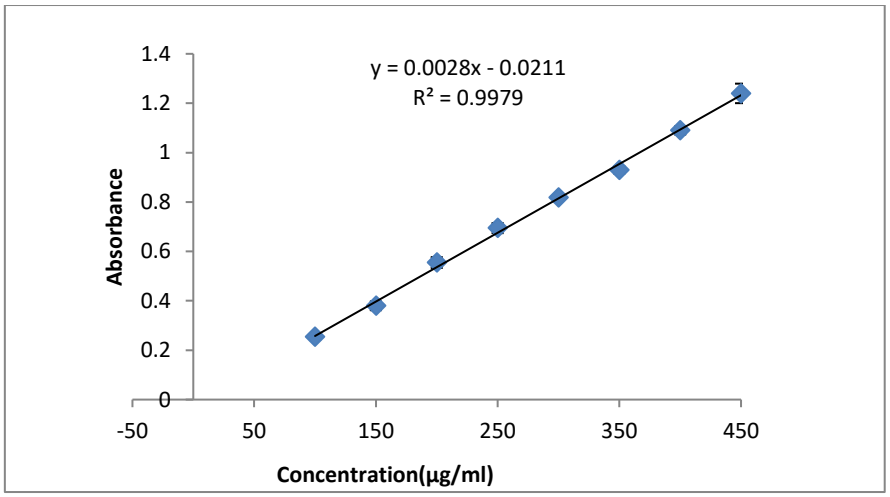


Fig.4 Calibration curve for clotrimazole in methanol.

4.1.6 XRD Analysis

XRD of clotrimazole was shown in Fig.5, The XRD analysis showed that clotrimazole shows stronger reflection (at 2θ= 9.28° and 12.47°) which indicate its crystalline structure.

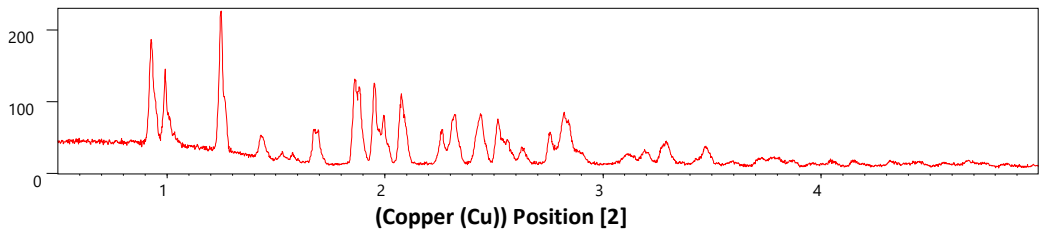
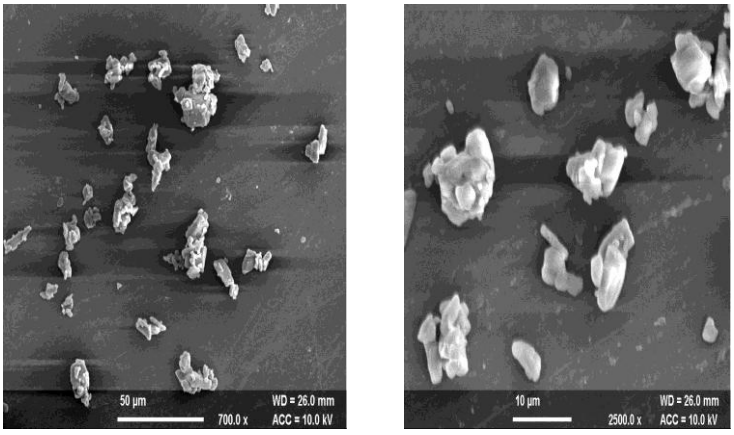


Fig.5 XRD of clotrimazole.

4.1.7 SEM Analysis

Clotrimazole of SEM analysis was depicted in Fig. 6A and 6 B. The clotrimazole particles were discovered to be uniformly disseminated, and an interfacial adhesion between the particles was demonstrated. The rhombic form and aggregated behaviour of the clotrimazole particles further demonstrated the drug's crystalline origin.



(a) **(b)**

Fig.6: SEM images of Clotrimazole at magnifications (a) 700X (b) 2500X

4.4 CHARACTERIZATION OF NANOPARTICLES

4.4.1 Percentage yield

Ion gelation technique was used to formulate nanoparticles of Chitosan and drug. Formulation F3 was best with respect to its % yield and hence it was used for further experimental studies**.** The percent yield of different formulations was found to be, as shown in Table 4:

Table-4 Percent yield for different batches

S. No	Formulations	Percentage yield (%)
1	Blank Chitosan nanoparticles	72.8
2	F1	68.7
3	F2	72.2
4	F3	77.2

4.4.2 Entrapment efficiency

Clotrimazole was found to have an entrapment efficiency of 64.2% for formulation F1, 76.6% for formulation F2, and 82.4% for formulation F3. The drug doses for the three separate batches were 50 mg, 100 mg, and 200 mg; as a result, the entrapment efficiency varied. **[50]** According to research, the addition of drug concentration, stirring speed, stirring time, and sonication period all play important roles in the effectiveness of drug entrapment. High stirring rates and sonication amplitudes may cause early bursts of drug nanoparticles, which could cause drug to leach out.

4.4.3 FTIR analysis

FTIR analysis of drug-free Chitosan nanoparticles and drug-loaded-nanoparticle is shown in Fig.7 and Fig.8,

In Fig.7 the identity of Chitosan nanoparticles was proved by FTIR spectrum which showed various characteristic peak assignment at 3418 cm^{-1} corresponding to O-H stretch, 1636 cm^{-1} corresponding to N-H bending and 1074 cm^{-1} corresponding to C-N stretching.

In Fig.8 the drug loaded nanoparticles was proved by FTIR spectrum which showed various characteristic peak assignment at 3420 cm^{-1} corresponding to O-H stretch, 3062 cm^{-1} showed as a sharp peak of clotrimazole, 1635 cm^{-1} corresponding to N-H bending ,1073cm^{-1} corresponding to C-N stretching, 767 cm^{-1} and 709cm^{-1} corresponding to C-Cl stretching.

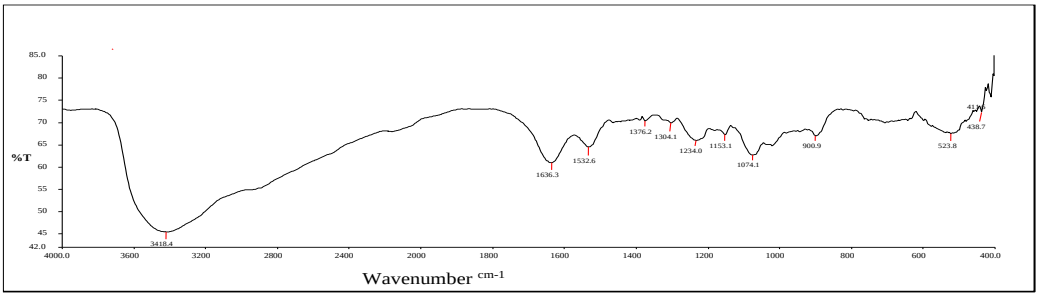


Fig.7 FTIR of Chitosan nanoparticles.

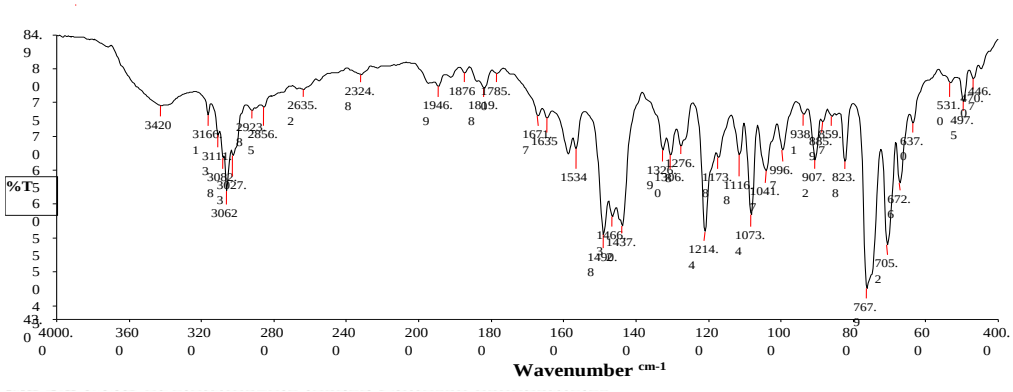


Fig .8 FTIR of clotrimazole loaded Chitosan nanoparticles.

4.4.4 XRD Analysis

Results of the XRD analysis of drug-loaded nanoparticles and chitosan nanoparticles was shown in fig.9 and 10:

In Fig.9, Chitosan nanoparticles showed broaden peak at (2θ=23.58°) which indicates the amorphous structure of the formulation.

In Fig.10, the XRD study of clotimazole loaded nano formulation it was reported that nanoparticles showed broaden peak at (2θ=18.21° and 18.64°) which indicates the presence of clotrimazole in the formulation.

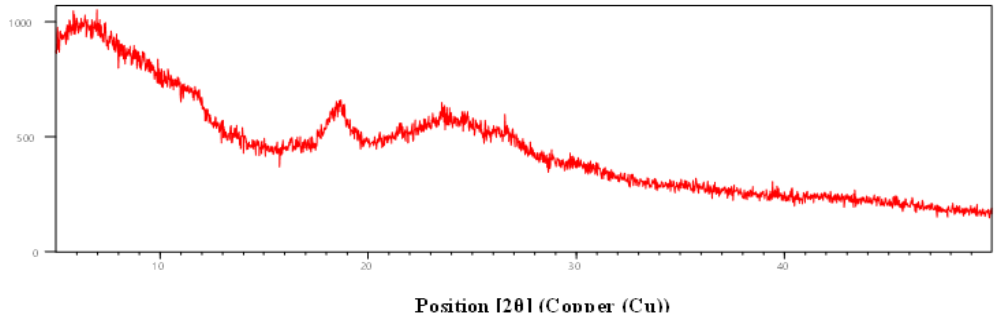


Fig.9 XRD of Chitosan nanoparticles

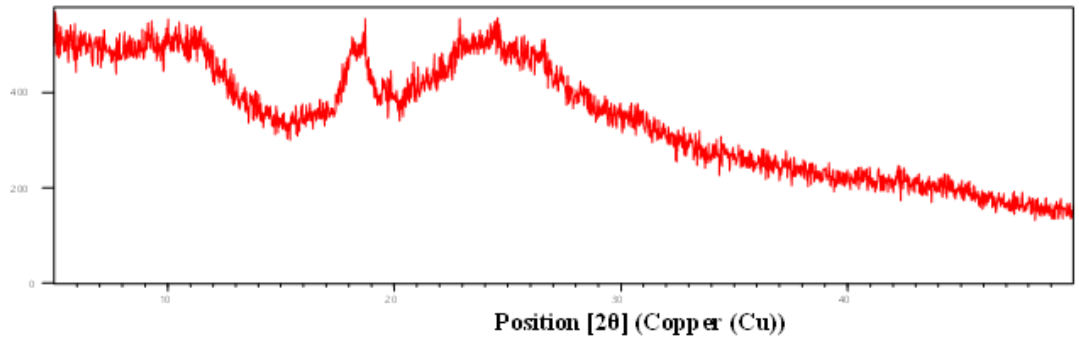


Fig.10 XRD of clotrimazole loaded nanoparticles.

4.4.5 SEM Analysis

The SEM analysis of chitosan nanoparticles and drug loaded nanoparticles shown in Fig.11 and 12. The micrograph (Fig.11) demonstrates that the Chitosan nanoparticles were rather coarse in the absence of Clotrimazole. Chitosan nanoparticles may not have crystallised in this configuration, which indicates their amorphous nature. Even though the clotrimazole nanoparticles in the chitosan nanoparticles in Fig.12 were seen to be uneven and rough, it was discovered that they were equally distributed throughout the chitosan matrix. Additionally, there is evidence of an interfacial adhesion between Chitosan nanoparticles and drug-loaded nanoparticles. An earlier study using Chitosan nanoparticles and drug-loaded nanoparticles revealed a similar effect.

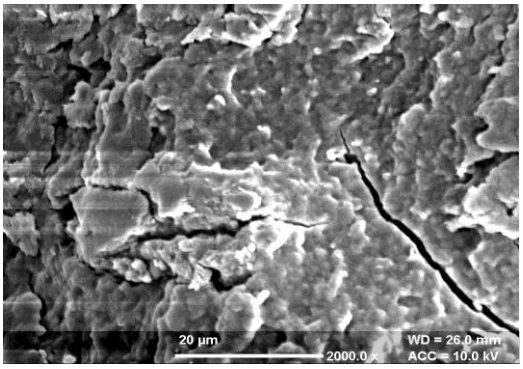


Fig.11 SEM images of blank Chitosan at 2000X

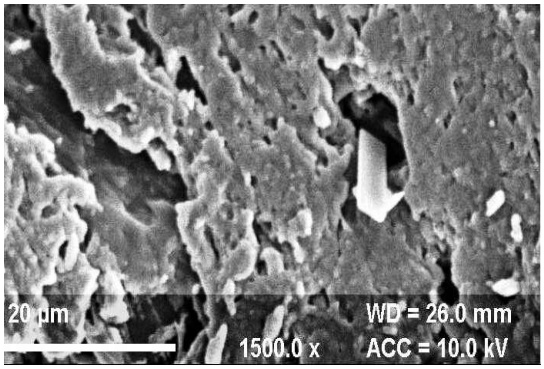


Fig.12 SEM drug loaded nanoparticles at 1500X.

4.4.6 *In vitro* drug release studies for pure drug

Using a UV spectrophotometer at 261 nm at various time intervals, the USP dissolution equipment Type 2 was used to measure the *in-vitro* dissolution of pure drug in phosphate buffer PBS (pH 5.8) at 37±2°C. Clotrimazole dissolution profile shows in Fig.13 at pH 5.8. It demonstrated a notably high rate of drug release 98.4%±1.664 in 9 hours. Clotrimazole percentage release shown in Table no.5:

Table 5: Percent drug release of clotrimazole.

Time (h)	Percent drug release (%)
0.01	20.6 ± 0.086
0.03	30.8 ± 0.208
0.08	39. 3 ± 2.815
0.16	48.0 ± 3.025
0.25	56.8 ± 0.596
0.5	58. 3 ± 0.785
1	63.0 ± 1.741
2	64.9 ± 1.012
3	69.3 ± 2.398
4	74.2 ± 1.129
5	78.2 ± 0.714
6	82.6 ± 0.503
7	87.5 ± 3.957
8	92.3 ± 2.788
9	98.4 ± 1.664

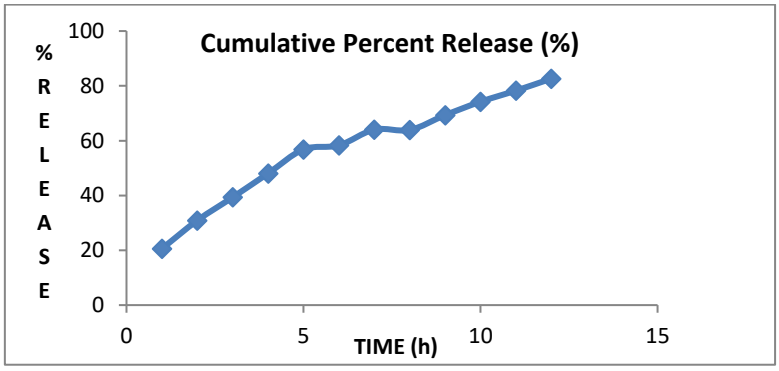


Fig.13 Dissolution profile of pure drug.

4.4.7 Drug release *in vitro* for various nano formulations

All formulations were tested for *in-vitro* dissolution in phosphate buffer PBS (pH 5.8) at 37± 2°C using a UV spectrophotometer at different time intervals, according to USP Dissolution Type 2 Apparatus was used. Figure.14 shows the dissolving profile for all formulations in the pH 5.8 range. Percent drug

release of different nano formulation shows in Table no .6. It resulted in a sustained release of medication at a steady rate with release rates of 88.6 ± 1.719, 93.6 ±1.907, and 97.9 ±1.685 over the course of 15 hours. In comparison to other formulations, formulation F3 demonstrated the largest release during a 15-hour period, it was determined. F3 was therefore chosen as the top formulation overall.

Table-6: Percent drug release of different Nano formulation.

Time (h)	Percentage release of drug from nano formulation		
	F1	F2	F3
0.08	11.1 ± 0.05	13.3 ± 0.225	14.2 ± 0.164
0.25	13.1 ± 0.1	15.3 ± 0.125	17.3 ± 0.565
0.5	15.5± 0.125	18.9 ± 0.368	22.0 ± 0.259
1	20.2 ± 0.229	21.2 ± 0.850	26.9 ± 0.262
1.5	22.0 ± 0.5	24.5 ± 3.025	32.3 ± 0.271
2	29.1 ± 1.116	26.2 ± 1.693	38.2 ± 2.392
3	32.3 ± 1.105	28.7 ± 0.625	43.8 ± 1.980
4	34.5 ± 1.177	35.4 ± 0.510	49.4 ± 0.919
5	36.8 ± 0.621	40.9 ± 0.340	54.2 ± 1.630
6	39.3 ± 0.175	45.5 ± 0.507	57.5 ± 0.735
7	42.5 ± 0.785	50.1 ± 0.653	63.2 ± 1.573
8	47.2 ± 0.846	56.3 ± 0.305	65.6 ± 1.111
9	52.3 ± 0.808	63.3 ± 2.930	68.8 ± 2.446
10	58.8 ± 0.492	64.9 ± 2.973	73.3 ± 0.899
11	68.4 ± 2.204	72.5 ± 1.271	76.8 ± 1.887
12	73.4 ± 1.697	77.5 ± 2.557	81.7 ± 1.557
13	78.5 ± 1.776	82.8 ± 2.220	87.5 ± 2.255
14	83.3 ± 1.125	88.0 ± 1.742	93.8 ± 1.203
15	88.6 ± 1.719	93.6 ± 1.907	97.9 ± 1.685

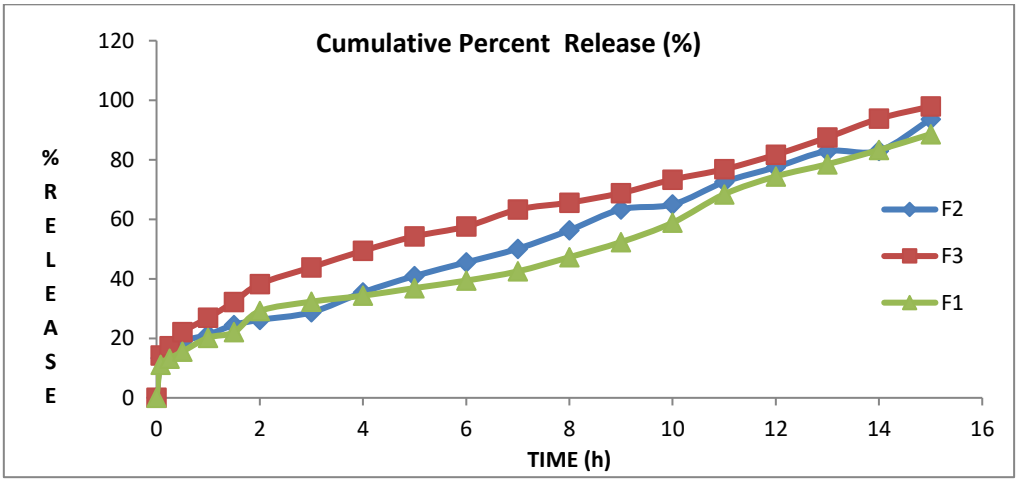


Fig.14 Dissolution profile for percentage release of all Nano formulation.

5. CONCLUSION

Preformulation and Post formulation studies, which were carried out of drug, polymer and nano formulation resulted in following manner. The study of the drug's melting point showed that the given sample was clotrimazole. Drug and polymer did not interact, according to the FTIR examination of the physical mixture of the two substances. The XRD examination made clear that Chitosan nanoparticles are amorphous and Clotrimazole is crystalline in nature. The amorphous nature of Chitosan nanoparticles and drug-loaded nanoparticles was made clear by SEM experiment. The ion gelation procedure was used to formulate the nanoparticles. Due to its simple biodegradability and biocompatibility, chitosan was chosen as the biopolymer in the formulation. The produced nanoparticles were evaluated based on their efficacy by entrapment of drugs and *in-vitro* drug release experimental method. In-*vitro* research

supported that F3 formulation has maximum release of drug, and it gives sustained release action as compared to all formulations.

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