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Research Article

Formulation And Evaluation of Gel Containing Zinc Oxide Nanoparticle of Ketoconazole With Aqueous Honey Solution

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ABSTRACT

The aim and objective of the present study was to formulate and evaluate Ketoconazole Nanoparticle. Prepared Ketoconazole loaded zinc oxide nanoparticle. The prepared Nanoparticle formulation was evaluated for particle size and Polydispersity index. The prepared Nanoparticle further formulation gel was prepared with Carbopol 934 and Carbopol 940 Polymers. The honey used as antimicrobial and antifungal agent. The prepared Formulation was evaluated Drug content, viscosity. Spreadability, pH, In vitro Drug release study and Stability studies, antifungal activity. Particle size analysis showed that the average particle size of Ketoconazole Nanoparticle using ethyl cellulose was found to be 369.2 nm with Poly Dispersity Index (PDI) value 0.291. The drug content of all formulations was in the range of 86% to 96%. The in-vitro drug release of optimized batch F3 showed the maximum drug release of 96% in 12 hr. Viscosity was 3567 cps. The Antifungal activity result Showed more zone of inhibition in honey containing gel. Optimized Batch F3 formulation was subjected to stability studies for 3 months, it could be concluded that formulation was stable after 3-months stability study.

INTRODUCTION

The therapeutic agents based on nanoparticles open the door to substituting the conventional anti-microbial, fungicidal, and wound healing agents, and they have been used as a key ingredient in a wide range of consumer, pharmaceutical, and industrial products. The design of multifunctional agents in a single unit with different properties was made possible by nanoparticles. (1)

The nanoparticles based therapeutic agents have the way to replace the traditional anti-microbial, fungicidal and wound healing agents and it has been used as a major component in a wide variety of consumer, pharmaceutical and industrial products. The ZnO nanoparticles exhibit large of –OH group on the surface and easily modify with drug or other bio-molecules and slowly dissolve in acidic and strong basic conditions. Previous

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studies also indicated that double-layer polymer shell coating makes the ZnO nanoparticles as water soluble. The ZnO nanoparticles highly suitable for dermal applications due to easily imaged through a microscope in the skin and it mostly stayed in stratum corneum without any toxic effect. [2]

To maximize bioavailability and lessen adverse effects, topical drug delivery refers to the administration of a formulation to a particular body part, such as the skin, ophthalmic, rectal, nasal, or vaginal area. Topical drug delivery is the localized administration of medication for the treatment of dermatological disorders when it is not designed for systemic distribution. Examples include the topical treatment of eczema and psoriasis. Examples of drugs applied topically include corticosteroids, antivirals, antifungals, antibiotics, and local anaesthetics. [3][4]

Ketoconazole is a Cis-1-acetyl-4-[4-[2-(2,4-dichlorophenyl)-2H imidazolyl methyl]-1,3-dioxolan-4-yl compound. phenyl] methoxy]- The antifungal substance piperazine works both systemically and topically. Ketoconazole is only weakly soluble in strong acids and bases, and completely insoluble in water. It is a derivative of imidazole with a molecular weight of 531.44. Ergosterol is produced by the enzyme cytochrome P450-dependent lanosterol C14 demethylase, which is inhibited by this substance production of fungal cell walls. [5]

Due to their high efficiency, strong surface reactivity, broad use for several purposes, and environmental friendliness, nanomaterials have become one of the most well-liked alternatives to adsorbents in recent years. Due to their effective adsorptive and magnetic properties, metallic nanoparticles are recognized to be promising. [6][7]

The gel is a high to low viscous semisolid formulation consisting of dispersion formulated of either a large organic molecule or small inorganic particles or enclosing and interpenetrated by the liquid phase. The gels exhibit no flow when in a steady state due to a dilute cross-linked polymer system. The gel is a system that is rich in liquids. The presence of a continuous structure provides solid-like properties. A natural or artificial polymer creates a three-dimensional matrix inside of a dispersion medium or hydrophilic liquid to produce a gel. On application of gel formulation on the skin, the liquid gets evaporated and a thin film of gel formulation is formed in which the drug is entrapped. Gel formulation forms a thin matrix covering the skin physically. [8]

One of the most well-known types of bees, honey bees produce the sweet and viscous fluid known as honey. Bee colonies are fed via the production and storage of honey. The sugary plant fluids, predominantly flower nectar, or the secretions of other insects, such as aphid honeydew, are collected and refined by bees to create honey. This process of purification involves both enzymatic activity and regurgitation within individual bees, as well as water evaporation during storage in the hive, which concentrates the honey's sugars into a thick, viscous substance. [9]

MATERIAL AND METHODS

Materials-

Ketoconazole, Dextrose, Zinc Nitrate, NaoH, Carbopol 934, Carbopol 940, Methyl paraben, Propyl paraben, Propylene glycol, honey.

Methodology-

Preparations of ketoconazole-loaded zinc oxide nanoparticles-

- 0.3 g of dextrose and Zinc nitrate added into 100ml of water. The continue stirrer on magnetic stirrer for the synthesis.
- Then, add 10ml of 0.2 M potassium hydroxide solution was added.
- The mixture is agitated for 2 hours.
- Then add 2 gm of the ketoconazole drug was added. The solution was centrifuged 4000rpm for 10 min after 2 hrs.

- The resulting pellets was wash by methanol washes.
- The last pellet was kept in hot air oven at 100°C for calcification.
- The dry pellets were put into mortar and pestle.

Preparation of Gel-

- For the semi-solid gel preparations.
- Take water in the beaker and put on the magnetic stirrer. Propylene glycol and some amount of water.
- The formulation was prepared by dispersing weighed amount of polymers carbopol 934 and carbopol 940, adding prepared zinc oxide nanoparticles (20mg) and honey.
- The pH gel was adjusted using TEA.
- Finally, preservatives methyl and propyl paraben were added slowly with continuous stirrer.
- The prepared gels were packed in wide mouth glass containers covered with srew capped plastic lid.

Table 1: Composition of Gel

Ingredient	F1	F2	F3	F4	F5	F6
Carbopol 934	0.5 g	0.5g	1.25g	0.5g	1.25g	2g
Carbopol 940	0.5g	2g	0.5gm	1.25g	1.25g	0.5g
Methyl paraben	0.1g	0.1g	0.1g	0.1g	0.1g	0.1g
Propyl paraben	0.1g	0.1g	0.1g	0.1g	0.1g	0.1g
Propylene Glycol	5ml	5ml	5ml	5ml	5ml	5ml
Honey	2g	2g	2g	2g	2g	2g
Triethanolamine	Q. S	Q. S	Q. S	Q. S	Q. S	Q. S
Water	Q. S	Q. S	Q. S	Q. S	Q. S	Q. S

Compatibility studies:

Fourier Transform Infra-Red Spectroscopy:

The compatibility between Ketoconazole and the excipients used was examined using FTIR spectroscopy. In the FTIR spectroscopy technique, significant changes in the shape and position of the absorbance bands are analyzed. It analyses significant changes in the shape and position of the absorbance bands to show the assumption of different functional groups of present and subsequent molecules.

Differential Scanning Calorimetry Analysis:

DSC spectrum of drug and mixture of drugs with polymers were obtained. The heat flow into or out of a sample is measured as a function of temperature or time, while a sample is exposed to a controlled temperature program.

Determination of λmax By UV spectrophotometer:

Ketoconazole 10 mg was dissolved in 40 ml of methanol in a 100 ml volumetric flask to create a standard stock solution. To obtain a clear solution, the solution underwent a 15- minute sonication process. The volume was marked up with methanol to get the final 100 g/ml concentration for the stock solution. Using a UV-visible Spectrophotometer (Jasco, Japan model V), the solution was pipetted out in the amount of 1 ml, put into a 10 ml volumetric flask, and diluted with methanol to the appropriate concentration to create 10 g/ml.

Evaluation Parameter of Gel

Organoleptic Properties:

The drug sample was evaluated for its Organoleptic Properties like colour, odour, taste and appearance and the result was recorded.

Determination of pH:

The pH of the gel was evaluated as per standard protocol with the help of a digital pH meter. Glass electrode of the pH meter was immersed in optimized zinc oxide nanoparticle gel formulation and revolved to determine gel ph.

Determination of viscosity:

The viscosity of the gel was evaluated as per standard protocol with some modifications as follows. In brief, obtained ZONPS gel was evaluated based on physical appearance, and then the viscosity of ZONPs gel was evaluated through Brookfield Viscometer

Spreadability:

Spreadability was measured by a parallel plate process typically used to assess and measure the spreadability of semi-solid preparations. One Gel was pressed between two horizontal plates of dimension 20× 20 cm, the upper of which weighed 125 g. The spread diameter was measured after 1 min.

Drug content:

1 g gel was dissolved in 100 ml of phosphate buffer pH 7.4. the volumetric flask containing gel solution was shake for 2hr on mechanical shaker in order to get complete solubility of drug. Absorbance was measured at 275 λmax nm using UV spectrophotometer.

In Vitro Diffusion Study:

100 ml of pH 7.4 buffer solution was taken in 250 ml beaker. 1 ml of gel was taken into dialysis membrane and hung inside the beaker containing buffer solution. The content of beaker was rotated at 300 rpm with a magnetic bead at 37°C temp. 1 ml of sample was withdrawn at specified time interval with replenish of same volume of buffer solution. Conc. of drug was measured spectrophotometrically at 275 nm

In vitro anti-fungal activity:

Ketoconazole-coated ZnO nanoparticles, Gel and Gel with honey dissolved in in sterile dimethyl sulphoxide at a concentration of 10 mg/ml. The anti-fungal studies, the inoculants of Malassezia (density of 0.5 in McFarland scale) were prepared in the sterile sabouraud liquid medium. Then, the strains were suspended in sabouraud's liquid medium (final density of 1 × 104 CFU/ml) and put into 96 well plates. Solutions of free drug, ketoconazole–ZnO and suspensions were inoculated into 96 well plates. The medium containing fungi and sterile medium was used as growth control and sterility control, respectively. The plates were cultivated under normal atmospheric conditions at 25°C for 48 h and the crystal violet solution was added to study the viability. The absorbance of crystal violet of each plate was measured in a microplate reader to calculate minimum inhibitory concentration values.

Stability Study:

The stability studies were carried out to determine the physical and chemical stabilities of prepared formulations. The optimized formulation was kept in air tight container for a period of 3 months. The formulation was evaluated visually and for its pH, viscosity, drug content and in vitro drug release.

Results And Discussions:

Organoleptic Properties:

The prepared gel formulae were inspected visually for their colour. The developed preparations were much clear and transparent. All developed gel formulations showed good homogeneity with absence of lumps.

Compatibility Study:

FTIR:

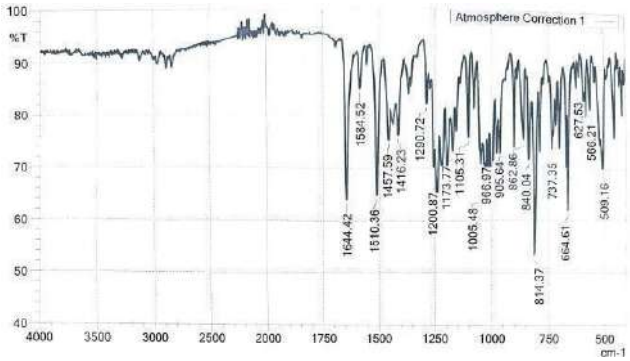


Figure 1: FTIR of Ketoconazole

The FTIR spectra of the drug, carrier, and mixtures are shown in fig. The pure ketoconazole shows intense absorption bands at 1644 cm-1 (C=O stretch), 1510 cm-1 (C=C aromatic stretch) and 1105 cm-1 (C–O–C stretching vibrations). All

characteristic absorption bands of drug and polymer were also observed on the obtained mixtures' spectra, which show only a reduction in the sharpness of peaks as compared to the spectra of pure component.

Table 2: Functional Group

Functional Group	Observed Peak (cm^{-1})
C=O Stretching	1644
C=C aromatic stretching	1510
C-O-C stretching	1105

FTIR Drug with Excipients:

The compatibility between Ketoconazole and the excipients used was examined using FTIR spectroscopy. In the FTIR spectroscopy technique, significant changes in the shape and position of the absorbance bands are analyzed. It analyses significant changes in the shape and position of the absorbance bands to show the assumption of different functional groups of present and subsequent molecules.

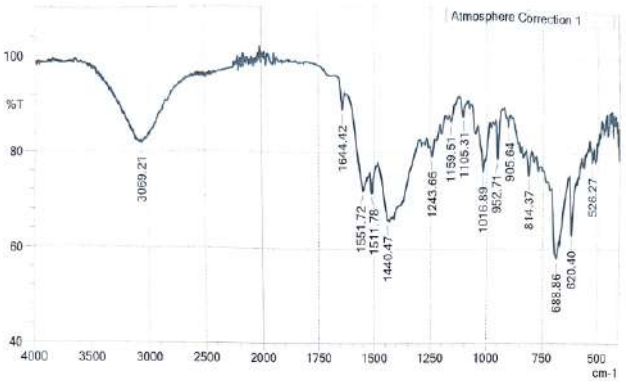


Figure 2: FTIR of Drug + Nanoparticle Excipients

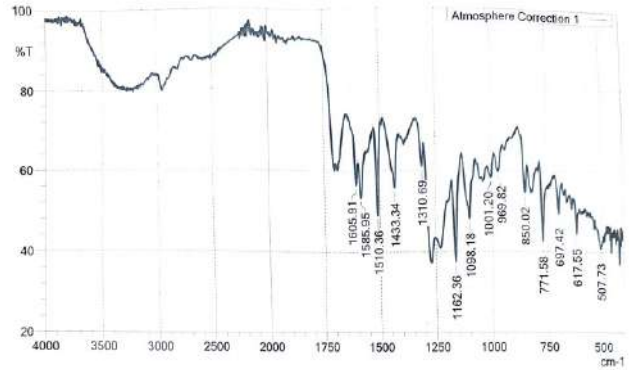


Figure 3: FTIR Drug + Gel Excipients

DSC:

DSC spectrum of drug and mixture of drugs with polymers were obtained. The heat flow into or out of a sample is measured as a function of temperature or time, while a sample is exposed to a controlled temperature program.

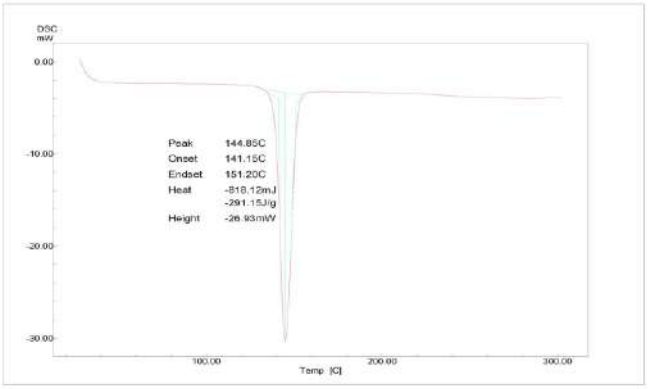


Figure 4: DSC of Ketoconazole

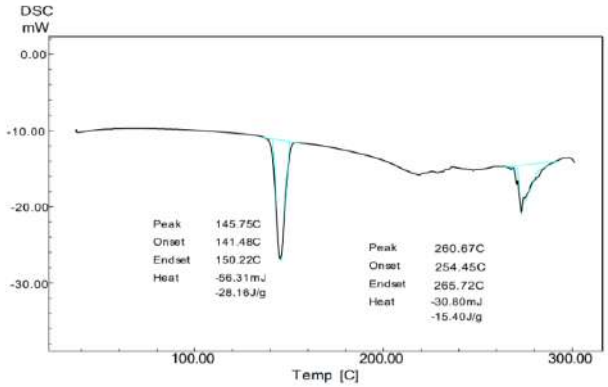


Figure 5: DSC of Drug + Excipients

UV spectroscopy:

On the basis of preliminary identification test it was concluded that the drug complied the preliminary identification. From the UV spectrum of the drug showed the maximum absorbance at 275 nm, which was same as reported. Hence drug was identified as Ketoconazole and further confirmed by FT- IR.

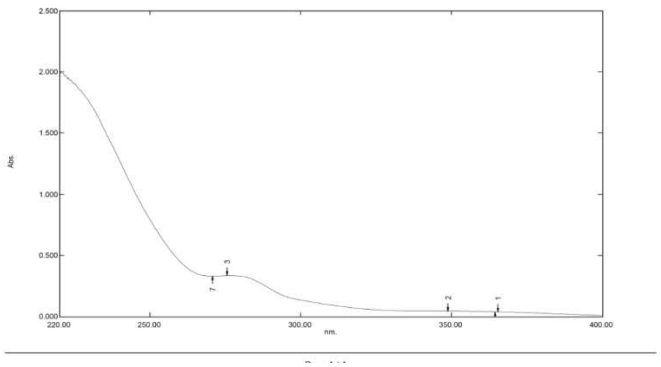


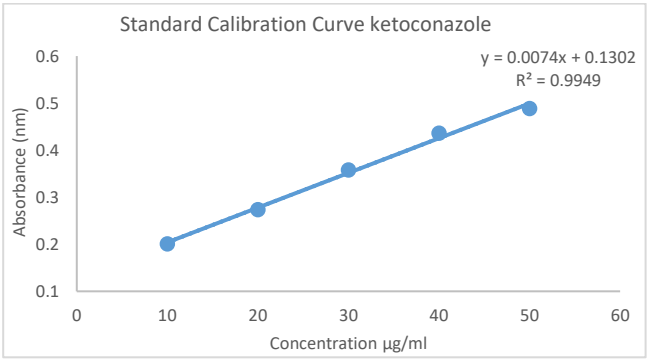
Figure 6: Absorbance Maximum of Ketoconazole

Calibration Curve of Ketoconazole:

As shown in Table no 2 the calibration curve follows a linear ketoconazole relationship and the curve obeys Beer-Lambert law within a concentration range of 10-50 µgm/ml. The correlation coefficient value (R2) was found to be 0.9949

Table 3: Calibration Curve of Ketoconazole

Sr.No	Concentration	Absorbance (nm)
1	10	0.201
2	20	0.274
3	30	0.358
4	40	0.436
5	50	0.489



Particle size determination:

Optimized batch were evaluated for Particle Size and Poly Dispersity Index. The particle size and Poly Dispersity Index of the prepared Nanoparticle was measured using Beckman Coulter Counter (Delsa t Nano) Particle size analyser and depicted in Figure No.6 Particle size analysis showed that the average particle size of Nanoparticle using was found to be 369.2 nm with Poly Dispersity Index (PDI) value 0.291

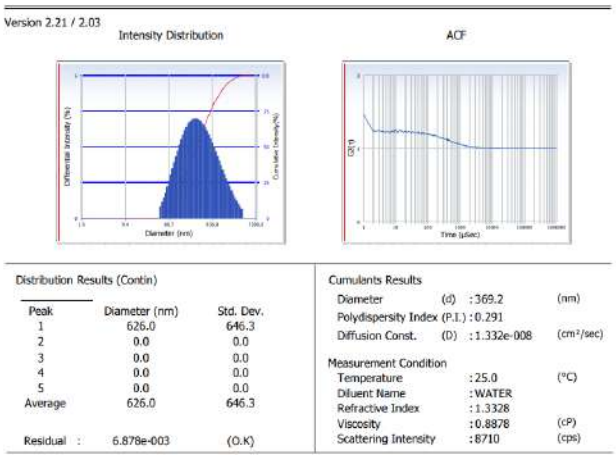


Figure 7: Particle Size and PDI

Visual examination:

The prepared gel formulae were inspected visually for their colour. The developed preparations were much clear and transparent. All developed gel formulations showed good homogeneity with absence of lumps.

Spreadability:

Spreadability was measured by a parallel plate process, The All Batches Spreadability is Good and Under Values.

pH Determination:

The pH values of all developed batches were found in the range of 5-6 which is considered acceptable to avoid the risk of irritation upon application to the skin. Results are tabulated in table no.3

Viscosity:

According to rheological studies, the gel viscosity was tested using a Brookfield viscometer. At 100 rpm, the viscosity of gel shows in table no. 3

Table 4: Evaluation of Gel

Topical Gel	color	Spreadability	pH	% Drug Content	Viscosity
F1	Shiny Transparent	5.55	5.5	92%	2556 cps
F2	Shiny Transparent	5.33	5.7	91%	2834 cps
F3	Transparent	5.80	6	96%	3567 cps

F4	Transparent	5.45	5.9	93%	3378 cps
F5	Translucent Yellowish	5.5	5.8	89%	3123 cps
F6	Transparent	5.25	5.5	94%	3089 cps

In-vitro Drug Diffusion Study:

The results of in vitro diffusion studies of all batches of topical gel across cellulose membranes are shown in Table 8. Batch F3 showed the most of drug release in average 12 hr. of time period.

Table 4: Drug release

Time (hr)	F1	F2	F3	F4	F5	F6
0	0	0	0	0	0	0
1	6.9	5.6	7.66	6.34	4.45	5.78
2	13.3	16.4	16.54	14.67	11.23	15.56
3	27.5	24.88	29.1	27.1	24.76	27.65
4	38.9	36.89	39.45	36.76	33.23	36.67
5	51.11	56.98	60.1	50.34	48.54	50.54
6	63.44	65.23	69.65	65.23	55.34	61.78
7	72.13	72.34	76.79	71.67	65.56	70.78
8	81.23	79.8	85.95	82.89	78.05	78.54
10	88.59	85.67	91.57	88.67	85.34	88.08
12	91.67	90.12	96.89	92.78	88.9	94.01

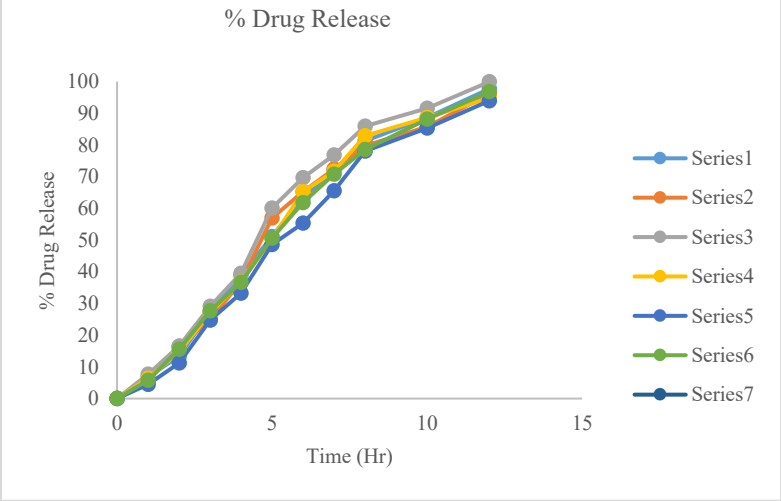


Figure 7: % of Drug release

Antifungal Result:

Zone of Inhibition: The zone inhibition is a circular area around the spot of antimicrobial in which the microbial colonies do not grow. The zone of inhibition can be used to measure the susceptibility of the microbes towards the antibiotic. In figure, Plate A was blank, Plate B with zinc oxide nanoparticle, Plate C with antifungal gel formulation and Plate D with antifungal gel formulation with addition of honey. In plate D the more zone of inhibition was observed as compared to plate C because of the presence of honey in plate D. As a result the gel formulation with honey has more zone of inhibition than the only gel formulation.

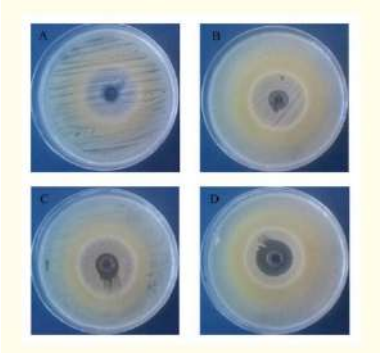


Figure 9: Antifungal activity

Stability Studies:

Formulation F3 was selected for stability studies. Results revealed the storage has no significant effect on the release pattern of the formulation. A slight increase in viscosity was observe changes in pH and drug content. Formulation F3 showed promising results regarding its physical properties, stability over storage period.

Table 6: Stability Study

Evaluation	After 1 Month	After 2 month	After 3 month
Viscosity	3567	3566	3566
pH	6	6	5.9
% drug release	96.80	96.70	96.50
Drug content	95.96	95.90	95.81

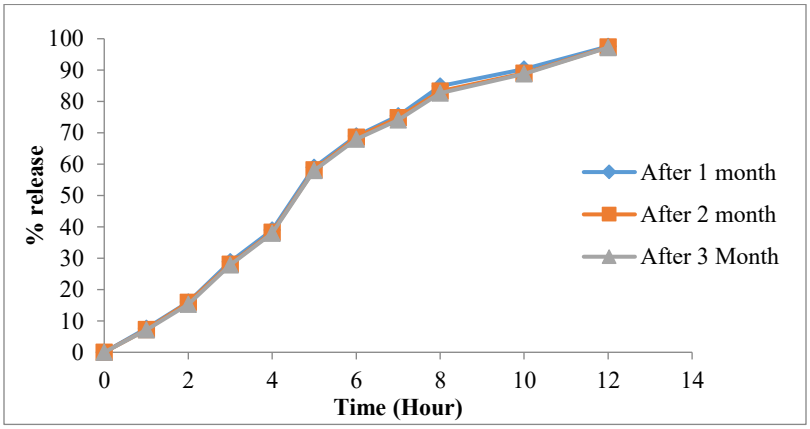


Figure 8: Graph of In Vitro Drug Release After Stability Study

CONCLUSION

Preformulation parameters for identification of drug such as UV spectrophotometry, melting point, and solubility were evaluated and the result found to be satisfactory and all the values obtained to be satisfactory and all the values obtained comply with pharmacopoeia limits.

- FT-IR Spectrum of drugs was carried out. In that, all the characteristic peaks of Ketoconazole were present at their respective wavelength.
- Compatibility studies were performed using FT-IR and DSC, and there is no interactions were found between drugs and excipients.
- Prepared nanoparticle measured in particle size determination.
- The particle size determination results 369 nm.
- Prepared zinc oxide nanoparticle in the antifungal gel was prepared.
- Carbopol 934 and carbopol 940 were used as polymers.
- The antifungal activity of Gel with honey shows good zone of inhibition.
- Nanoparticles with antifungal gel were evaluated for particle size distribution, viscosity, gelation, Drug release, and In-vitro

release study. And results found to be satisfactory.

- Batch F3 gave good results as expected.
- Optimized formulation was then subjected to stability studies for 3 month.

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