Development, Characterization, and Evaluation of Anti-fungal activity of Nystatin loaded Nanogel

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Abstract: Nystatin has been used as a fungal inhibitor. Nystatin is applied topically since the oral method of delivery irritates the gastrointestinal tract and can cause ulcers. The antifungal nanogel used in this study was designed to optimize in-vitro release and in-vivo release investigations while also reducing particle size. Using a homogenization process with carbopol 940 added as a gelling agent, Nystatin nanogels were created, yielding a smooth antifungal nanogel (F1-F6). Fourier transform infrared analysis of antifungal nanogels (F1–F6) revealed no interactions between the medication and excipients. The optimal formulation (F6) produced a high in-vitro release of 84.43% after 8 hours; the resulting values for particle size and zeta-potential were 230 nm and -27 mV, respectively. It was demonstrated by in-vitro release kinetic models that formulation-F6 adheres to high regression coefficient value, r 2 0.9967, and first-order kinetics. The optimal formulation (F6)'s scanning electron microscopy image shows that there is no nanogel breakup. Nystatin was reported to have a differential scanning calorimetry (DSC) thermogram of 166 oC. The physical mixture of carbopol 940 and Eudragit-S 100 was discovered to have a DSC thermogram of 129°C and 218°C. The medication and excipients did not interact, according to the DSC analysis of nanogel (F6). An in-vivo investigation using mice revealed that the optimal formulation, F6, had a superior therapeutic effect for dermatitis. The best formulation, F6, was determined to have improved dermatitis scoring and better in-vitro medication release.

Keywords: *Nystatin, Anti-fungal activity, Nanogel, Eudragit-S100, Glycerol, Dermatitis, etc.*

Introduction

The most prevalent skin condition is a fungal infection brought on by the environmental fungi Trichophyton rubrum and Trichophyton mentagrophytes. Fungi can be found on human skin, in soil, on interior surfaces, and in diabetic patients' bodies where they have become more complicated. a fungus that causes red rashes and persistently intense itching. A wide range of medications in liquid, semi-solid, and solid dose forms are prescribed by doctors. The majority of therapies call for a three-to nine-month oral dosage. The likelihood of negative effects would rise with prolonged usage of oral medications. To shorten the course of treatment, it is therefore preferable to concentrate on topical medication distribution. Topical medicines are the most often utilized treatment [1].

Fungi, not heat, is the cause of ringworm. Usually, the limbs and torso are affected. Other names for ringworm, including jock itch and athlete's foot, might refer to other parts of the body. A rash with a ring shape and slightly elevated borders is the primary sign of ringworm. Most of the time, the skin inside these round rashes appears healthy. It is frequently itchy and the rash can spread. A common and highly contagious fungal condition is ringworm. Topical medications can usually be used to treat it [2, 3]. The candida fungus is the source of cutaneous candidiasis. Our bodies naturally contain and are covered in this sort of fungus. Overgrowth may result in an infection. Warm, humid, and inadequately ventilated environments are conducive to Candida skin infections. Diaper rash is one instance of an area that may be impacted, as are the folds in the buttocks and beneath the breasts. Infection of nails by fungi is called onychomycosis. Although toenail infections are frequent, it can damage both finger and toenail nails. We are getting closer to the objective of developing safe and effective treatments for fungal skin diseases thanks to new developments in nanotechnology and a better knowledge of fungal infection [4].

Potential uses for nanotechnology include the delivery of therapeutics and analytical techniques. Compared to traditional dosage forms, the nanoparticulates drug delivery system has many benefits, such as less toxicity, increased biodistribution, and better patient compliance. The need for developing new topical drug delivery systems through nanotechnology is becoming more and more urgent. Nanogels have excellent transport properties, strong permeability, biodegradability, and biocompatibility. To increase the efficacy of their products, pharmaceutical manufacturing businesses are focusing on expanding their cutting-edge medication delivery technology.

Nystatin is a medication that is classified as antifungal. Nystatin was therefore selected as the best medication molecule for topical preparation in the current study project. The research project's objective was to create and assess an antifungal nanogel that would improve in-vitro release while lowering dosage frequency.

Material and Method

Materials

Manus Aktteva Biopharma LLP was the supplier of Nystatin. SDFC Pvt. Ltd. provided Cabopol-940. Yarrow Chemical Pvt. Ltd. sold Eudragit RS100. Sodium hydroxide, potassium di-hydrogen phosphate, and tween-80 were acquired from Sisco Laboratories Pvt. Ltd. HiMedia Lab Pvt. Ltd. supplied the glycerol on order. The remaining basic ingredients are all of pharmaceutical quality.

Methodology

Pre-formulation studies

Estimation of λ max of Nystatin

To determine the wavelength of maximum absorbance, a solution containing 10 µg/ml of Nystatin was produced and scanned over the wavelength range of 200–400 nm using an Ultraviolet (UV) 1700 spectrometer from Shimadzu Co., Japan [1].

Estimation of melting point

A tiny quantity of Nystatin was added to the capillary tube, which was then fastened to the thermometer twig. The thermometer was inserted into the liquid paraffin-filled Thiele's tube [2]. The Thiele's tube's side support was heated, and the temperature at which the medication starts to melt and finishes was noted.

Determination of saturation solubility of Nystatin

Four different solvents, including methanol, ethanol, water, and phosphate buffer pH 7.4, were used to test the saturation solubility. Each solvent was gradually infused with Nystatin and subjected to a 30-minute ultrasound until saturation was achieved. After centrifuging the

saturated solution for five minutes, the aliquots of supernatant solution were obtained, diluted to ten milliliters using the same solvents, and then UV quantification was performed using a UV-1700 spectrometer made by Shimadzu Co. in Japan.

Compatibility studies

Fourier transform infrared (FT-IR) was used to verify Nystatin compatibility with the polymer [3].

Standard curve of Nystatin

As the main solution, Nystatin was precisely weighed and dissolved to get concentration. A 10 ml volumetric flask was filled with 1 ml of the primary solution to create the secondary stock solution [5]. Using a UV-1700 spectrometer made by Shimadzu Co., Japan, and 0.2–1 ml of the secondary stock solution was transferred to a 10-ml volumetric flask and diluted with phosphate buffer (pH 7.4) to obtain a concentration of 2–10 µg/ml.

Formulation of Nystatin nanogel

A precise amount of stabilizers, namely Nystatin, Eudragit S-100, and Tween-80, were dissolved in glycerol while being stirred continuously. Meanwhile, an aqueous phase was created by dissolving Carbopol-940 in water and heating it continuously [3, 6]. The medication-containing phase was subjected to ultrasonication using a Wemsan brand of equipment. To create a homogenous o/w emulsion, the drug phase was poured drop by drop into an aqueous phase while stirring continuously. This homogenizer (Make-Remi-motors) was then used to turn the o/w emulsion into nano-droplets. At 8000 rpm, homogenization was maintained for one hour. The addition of Triethanolamine created nanogel. Several tests were performed on prepared Nystatin nanogels.

Evaluation of Nystatin nanogel

The following criteria were used to assess Nystatin nanogels: physical appearance, pH measurement, practical yield estimation, and homogeneity measurement, drug content uniformity estimation, spreadability measurement, and viscosity measurement. Particle size analysis, Zeta potential estimation, differential scanning calorimetry (DSC), scanning electron microscopy (SEM), in-vitro release data further subjected to statistical analysis using One-way ANOVA by GraphPad Prism5, FT-IR studies, and antifungal activity of nanogel are among the statistical analyses of experimental data performed using ANOVA using Excel.

Physical appearance

Clarity of formulation is one of the key characteristics of nanogel formulations [2, 7]. The produced anti-fungal nanogels were visually inspected against a black and white texture to determine their clarity.

Measurement of pH

Using a digital pH meter and the equipment manufacturer, Digisun Electronics Services, the pH of the prepared antifungal nanogels was determined [3].

Determination of practical yield

Percentage of practical yield calculated using the following formula.

% yield = the experimental yield divided by theoretical yield multiplied by 100.

Determination of homogeneity

All of the produced nanogel has yet to solidify in the beaker, demonstrating homogeneity by visual inspection [3, 8]. The physical characteristics and particle presence of nanogels were examined.

Estimation of drug content uniformity

One gram of nanogel was combined with fifty milliliters of phosphate buffer (pH 7.4), and the resulting liquid was filtered through a membrane filter in order to estimate the amount of drug

present in the nanogel. This mixture was pipetted out into two milliliters, and the resulting ten milliliters of sample were subjected to spectrophotometric analysis at 305 nm to determine its absorbance [3, 9]. The calibration curve was used to estimate the concentration of Nystatin in the test sample.

Determination of Nanogel spreadability

To achieve a consistent thickness and spreadability of a test sample, 1 kg of weight was placed on each of the two glass slides containing 1 gram of nanogel for ten minutes [3]. By retaining two slides, the test sample nanogels spreadability was tested in less than a minute.

Statistical data analysis using excel-sheet

One-way ANOVA was used for statistical analysis of the spreadability experiment results. Viscosity of anti-fungal nanogel measured Using a Brookfield viscometer (Dv-E, Brookfield), the viscosity of nanogels was determined. Using this technique, the spindle number-4 was nearly in contact with the nanogels surface [5, 11, 12]. Viscosity was measured and the Brookfield viscometer dial values were recorded at various rpms.

In-vitro release study

The conventional medication and the nanogel formulations (F1–F6) underwent in vitro release. The Franz diffusion cell (Make-Orchid Scientific) with a dialysis membrane made of cellophane was utilized. One gram of nanogel soaked in solutions of phosphate buffer pH 7.4 at ambient temperature with slow magnetic stirring was placed into the donor compartment [1, 14, 15]. One milliliter of an aliquot was taken out of the receptor chamber via the sampling port on a regular basis, and it was quickly replaced with a brand-new buffer solution of the same volume. Using a UV spectrophotometer set to 305 nm, the amount of medication released from the aliquot was diluted and measured.

In-vitro release kinetic study

The drug release kinetic for a dose form can be described by a variety of equations and kinetic models, in accordance with the pharmacokinetics principle [6, 16, 18].

Measurement of particle size and zeta potential

Using a Horiba sizer, the mean size and Zeta potential of the optimal nanogel formulation (F6) were determined.

DSC

The optimum formulation (F6) was subjected to DSC investigations utilizing Shimadzu equipment (DSC 60). The DSC thermogram revealed a prominent endothermic peak at 162°C, which is in line with Nystatin melting point and indicates the purity of the medication. Based on the DSC overlay thermogram of Eudragit-S 100 and pure medicine Nystatin, it can be concluded that there is no interaction between the drug and the excipients. Additionally, it shows that the medicine and excipients did not form a complex.

Imina was used to conduct EMSEM analysis, utilizing a high-energy electron beam with a magnification power of 600× and an electron voltage range of 10,000 electron volts [5, 19, 20].

FT-IR study

The interaction between Nystatin and polymer was examined using FT-IR antifungal nanogels [5]. The nanogels FT-IR spectrum, obtained using the FT-IR JASCO 460 Plus, demonstrates the absence of any interaction between the excipient and Nystatin.

Statistical data analysis using Graph Pad Prism 5

One-way ANOVA was used in GraphPad Prism5 to undertake statistical in-vitro data analysis [6, 21].

In-vivo studies of antifungal nanogel

Material and Method

Animal species

Balb/c mice, Gender: Female Age: grown-up Nystatin as the test sample Dose: Enough in amount to cover the affected region, Inducing agent: 3.124 mg/animal of imiquimod (IMQ), Administration route: topical, Duration: Following the induction, one week.

Treatment protocol

The balb/c mice's shaved backs were treated with the IMQ. Every day, 3.124 mg of the active ingredient or 62.5 mg of nanogel were applied. Beginning on the eighth day, the treatment was administered for one week [5, 22]. The skin affected area was treated with both formulations, which were then allowed to permeate into the skin. The dermatitis was scored after a one-week course of treatment.

Result and Discussion

Per-formulation studies

Standard curve of Nystatin

Nystatin λ max in phosphate buffer pH 7.4 revealed a maximum absorbance at 305 nm that is consistent with the value that was published. The amount of Nystatin in each sample was measured at 305 nm using a UV spectrophotometer. Nystatin calibration curve had a regression value of r^2=0.9992 and was linear throughout a concentration range of 2–10 µg/ml.

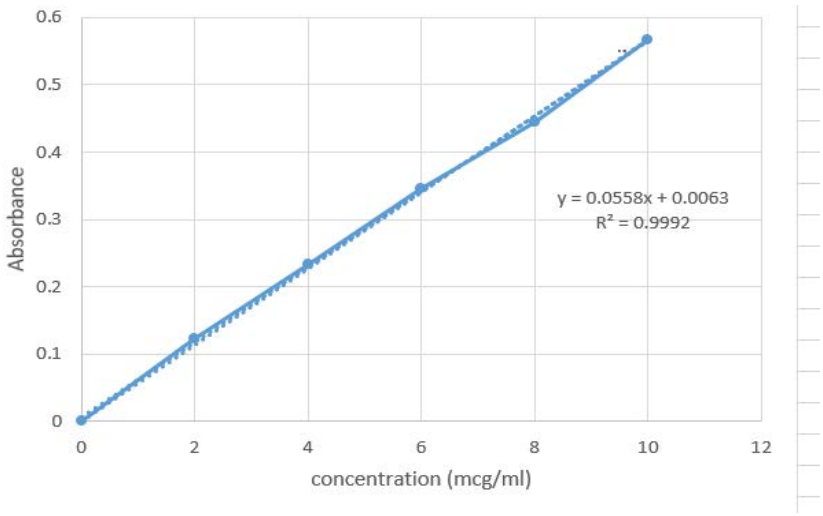

Fig. 1: Calibration curve of Nystatin

Determination of melting-point

According to published research, 165°C was determined to be the melting point of Nystatin.

Saturation solubility studies

Nystatin saturation solubility was calculated in a range of solvents. Nystatin solubility was determined to be 1.30 mg/ml, 100 mg/ml, and 80 mg/ml in ethanol, water, and phosphate buffer 7.4.

FT-IR analysis

FT-IR spectroscopy was used to record the physical mixture of Eudragit S-100 and pure Nystatin, as well as the polymer Eudragit S-100. When the FT-IR spectrophotometer was used to record the FT-IR spectrum of Nystatin and the excipients, the results were compared with standard functional frequencies. When FT-IR analysis was completed, it almost perfectly displayed the same distinctive peaks of Nystatin, suggesting that there was no interaction and that Nystatin was entirely compatible with Eudragit-S 100, which was utilized in the optimal formulation (F6).

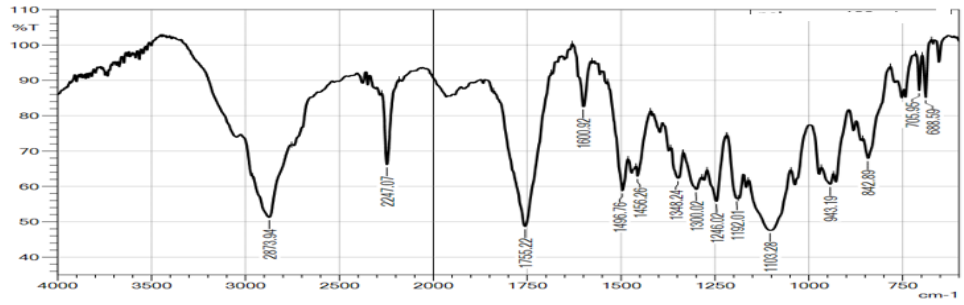

Fig. 2: FTIR Spectra of Nystatin

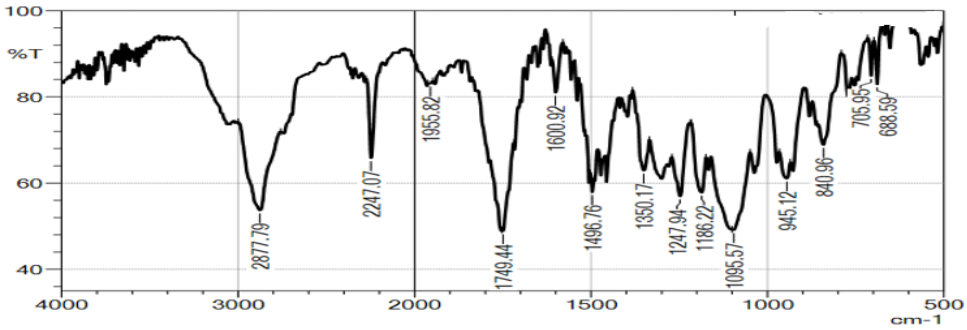

Fig. 3: FTIR Spectra of Eudragit S 100

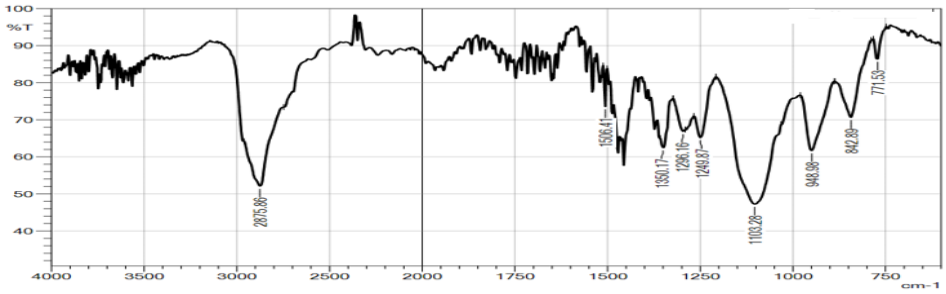

Fig. 4: FTIR Spectra of Carbopol- 940

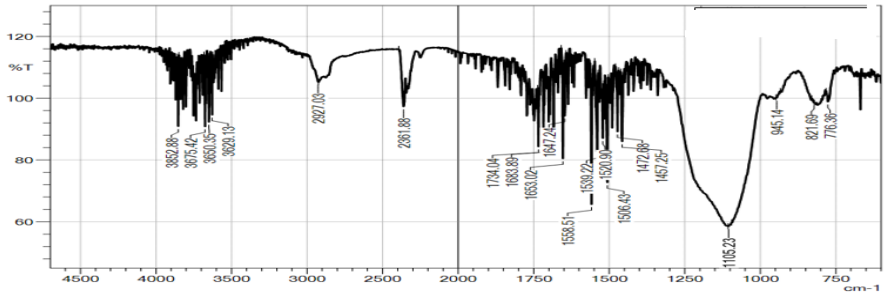

Fig. 5: FTIR Spectra of Physical mixture (Drug + Excipients)

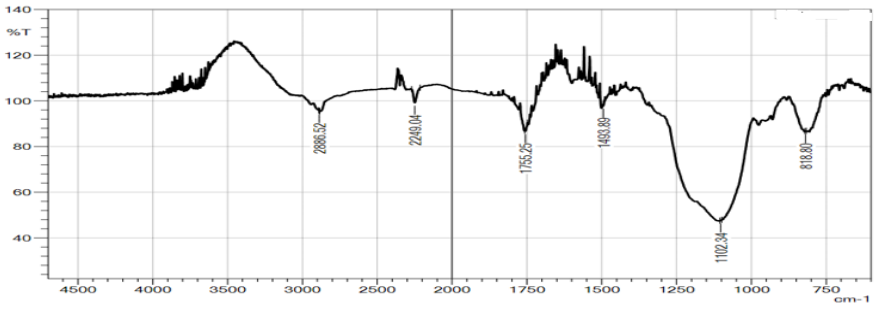

Fig. 6: FTIR Spectra of Nanogel formulation F6

Evaluation of Nystatin nanogel

A number of assessment criteria, including appearance, yield percentage, pH, drug content, spreadability, and viscosity, were applied to the prepared anti-fungal nanogels. It was discovered that the nanogels spreadability ranged from 6.3 to 8.0 cm. The range of viscosity for nanogel was determined to be 8617–9767 dyne s/cm. One-way ANOVA statistical analysis utilizing an Excel spreadsheet a one-way ANOVA was used for statistical analysis of the antifungal nanogel experimental results.

Table 1: A various assessment parameters for Nystatin nanogel

Formulations	Physical appearance	pH	% Yield	Homogeneity	Drug content	Spread ability	Viscosity (cps)

F1	Clear	6.6	73 %	Homogeneous	77 %	7.0 cm	8662
F2	Clear	6.9	78 %	Homogeneous	76 %	7.5 cm	8892
F3	Clear	6.7	81 %	Homogeneous	69 %	7.4 cm	9958
F4	Clear	6.8	71 %	Homogeneous	72 %	7.7 cm	9867
F5	Clear	6.3	80 %	Homogeneous	74 %	6.4 cm	9554
F6	Clear	7.0	83 %	Homogeneous	81 %	8.0 cm	9767

In-vitro release profile of Nystatin nanogel

The table and image present release data from the in vitro release of pure medication Nystatin utilizing nanogel formulations F1 to F6, using phosphate buffer pH 7.4.

Table 2: % cumulative release of formulation (F1-F6) and pure drug

Time (h)	Nystatin	F1	F2	F3	F4	F5	F6
1	7.00	13.9	20.8	13.9	25.3	28.5	27.0
2	15.7	19.0	29.0	20.8	37.0	37.0	39.3
3	24.4	24.2	39.3	24.8	42.5	42.5	43.9
4	36.6	29.0	42.2	39.0	49.3	48.5	52.2
5	47.7	35.9	49.3	46.5	54.5	57.9	61.3
6	57.2	42.8	51.3	48.5	57.6	61.9	71.3
7	70.9	48.8	53.9	51.3	60.5	67.9	77.6
8	72.3	52.3	54.8	56.8	64.72	74.0	84.5

In vitro release experiments with Nystatin and Eudragit-S 100 at varying ratios were carried out at 8 hours utilizing a Franz Diffusion Cell setup. The release outcomes of nanogels F1–F6 in vitro were contrasted with Nystatin. Nystatin Eudragit-S 100 nanogel, which was made by homogenizing the material.

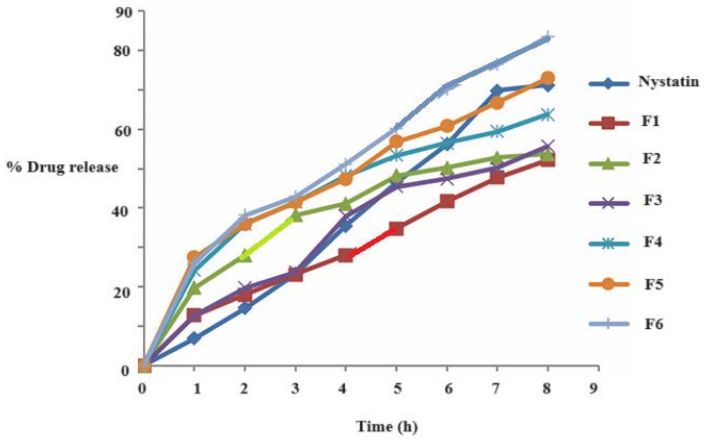

Fig. 7: In-vitro release profile of Nystatin nanogel formulations F1- F6

Zeta potential of nanogel formulation

The value of F6 was discovered to be -28 mV, suggesting that the particles inhibit aggregation and hence preserve the stability of the nanogel (F6). It was discovered that the nanogel formulation F6 had an average particle size of 231 nm.

Release kinetics-models

Several kinetic models, including the Higuchi-diffusion release kinetic model, the Zero-order release kinetics model, the First-order release kinetics model, and Peppa's kinetic model, were applied to in-vitro release data. The table for nanogel formulation F6 displays the regression coefficient (r) and "n" values for each kinetic model. In figure, plots are displayed. The above table's drug release kinetics can be followed by any kinetic model with a better degree of correlation coefficient; zero-order kinetics demonstrated the highest correlation coefficient

among the tested kinetic models. Particle size and Zeta potential were measured at 231 nm and -28 mV, respectively. The finalized nanogel formulation (F6) produced the highest in-vitro drug release of 84.43% at 8 hours. The in-vitro release kinetic models for nanogel formulation-F6 demonstrated that it follows First-order kinetics and had a high regression coefficient value of r2=0.9967.

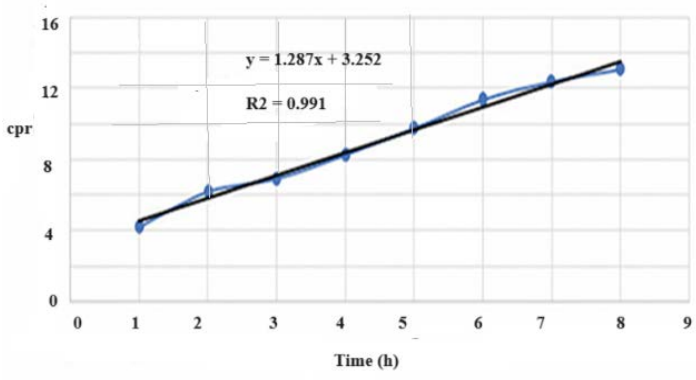

Fig. 8: Zero- order release-kinetic model

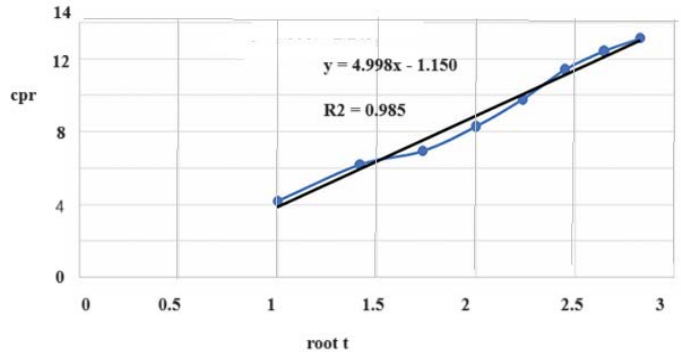

Fig. 9: First-order kinetic release model

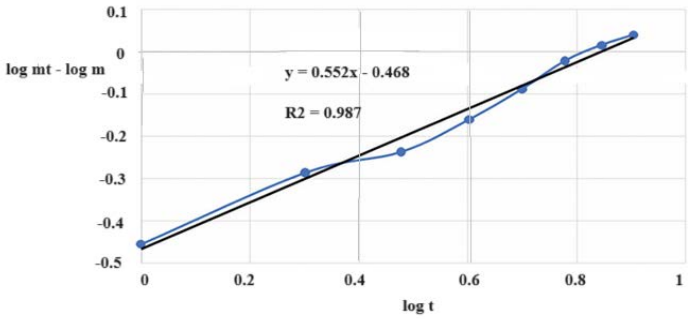

Fig. 10: Korsmeyer Peppa's kinetic model

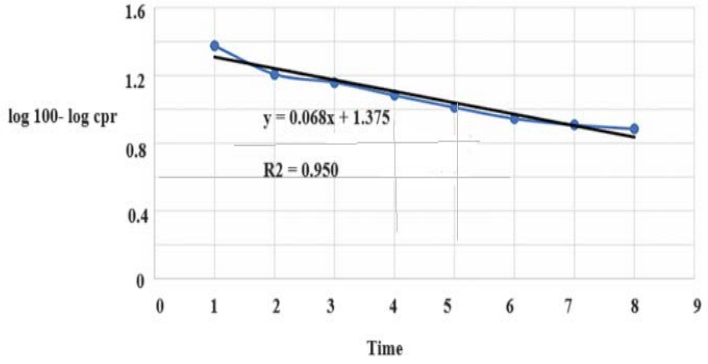

Fig. 11: Higuchi kinetic model

DSC analysis

DSC experiments were conducted on Nystatin and the optimal formulations to determine the drug's dispersion in the polymer Eudragit-S 100. The Perkin Elmer 4000 was the instrument used for the DSC investigation. Nystatin DSC thermogram displays a pronounced endotherm at 162°C, which is not far from the drug's real melting point. The Nystatin nanogel exhibited a pronounced endothermic peak at 171°C, which is quite close to Eudragit-S 100's real melting temperature.

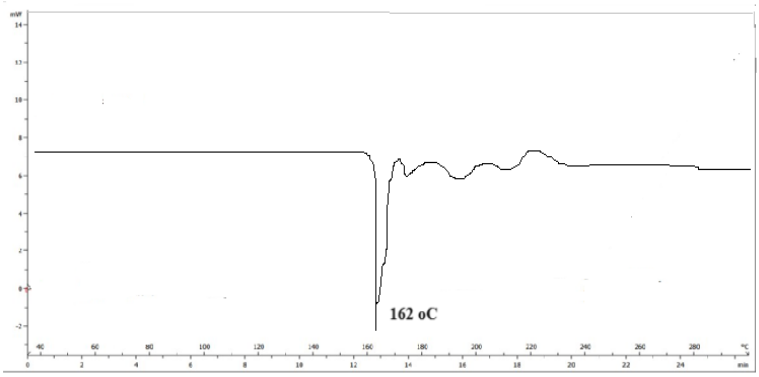

Fig. 12: Differential scanning calorimetry thermogram of Nystatin

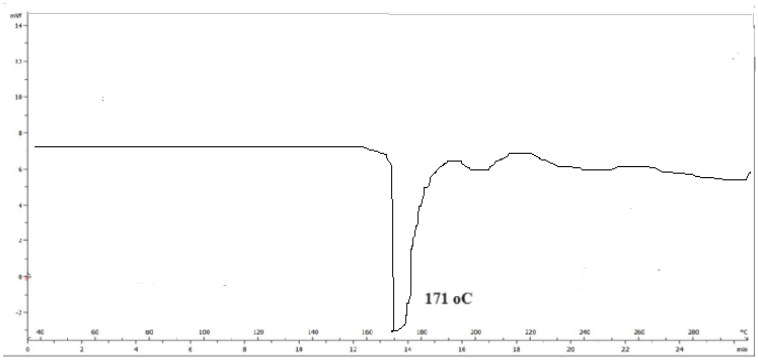

Fig. 13: Differential scanning calorimetry thermogram of optimized Nystatin nanogel

SEM

Using an instrument called IMINA, a scanning electron microscope (SEM) was used to examine the nanogel (F6) at a magnification of 600× and an energy range of 10,000 electron volts. The nanogel formulation F6 was found to have a size of 250 nm. The SEM scan demonstrates that the nanogel has not broken. Nystatin nanogel SEM pictures are displayed in the figure. Nystatin nanogel was shaped like a sphere in SEM images.

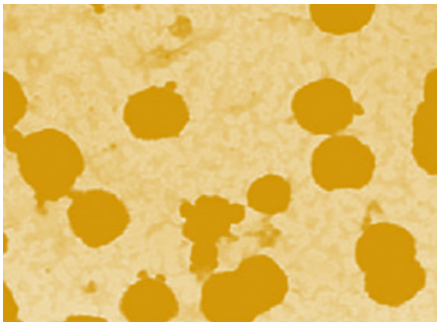

Fig. 14: Scanning electron microscopy of nanogel formulation (F6)

Statistical analysis using GraphPad Prism5

GraphPad Prism5 was used to undertake a statistical analysis of the in-vitro release of nanogels (Pure Drug as PD and nanogels F1-F6) using One-way ANOVA by Dunniet.

In-vivo study of antifungal nanogel (F6)

Severity scoring of dermatitis

Following the course of treatment, the dermatitis was evaluated on an individual basis using a scale of 0 to 4, with 0 denoting no dermatitis, 1 faint, 2 moderate, 3 marked, and 4 very marked (fig. 15-18).

Table 3: Dermatitis severity scoring data

Groups	Animal number	Erythema	Scaling	Thickening
IMQ control	1	3	3	4
	2	4	2	3
	3	3	2	3
	4	4	3	3
	5	4	2	3
	6	3	2	4
Mean		3.5	2.3	3.3
SEM		0.2	0.2	0.2
Nystatin marketed formulation	7	2	1	2
	8	3	2	2
	9	3	1	3
	10	2	2	1
	11	2	2	2
	12	3	2	3
Mean		2.5	1.7	2.2
SEM		0.2	0.2	0.3
Nystatin test sample	13	1	2	2
	14	2	1	2
	15	2	0	1

	16 17 18	1 2 2	1 0 1	2 1 1
Means		1.7	0.8	1.5
SEM		0.2	0.3	0.2

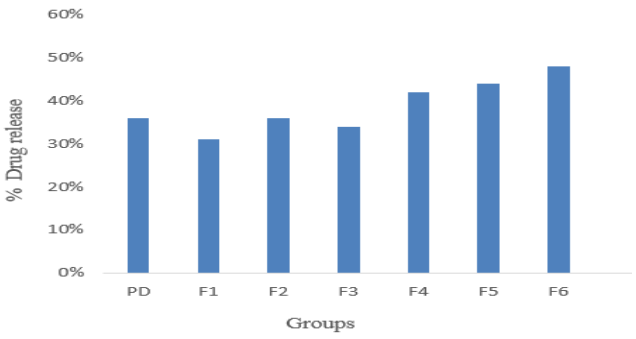

Fig. 15: Grouped data by Graphpad prism using one-way ANOVA

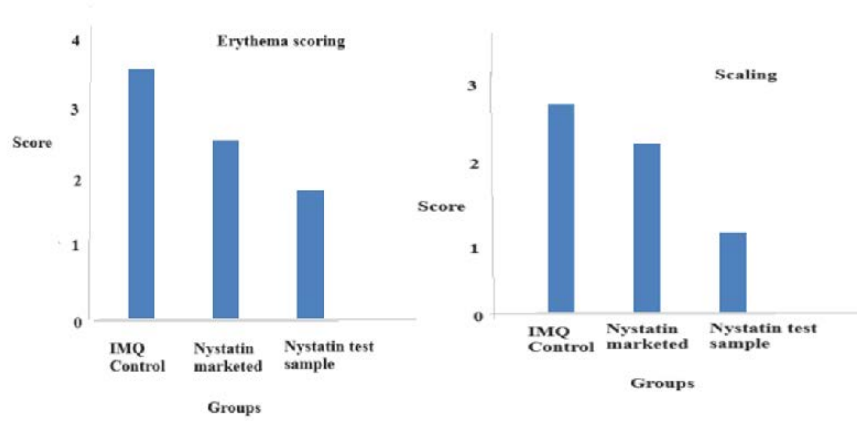

Fig. 16: Dermatitis characterized by scaling and erythema scoring

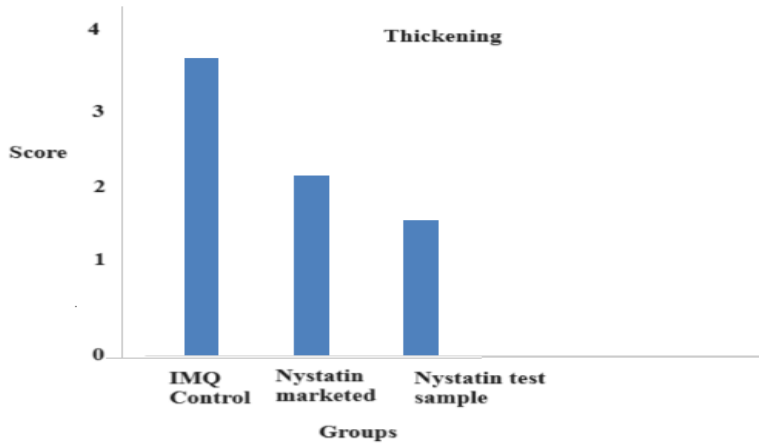

Fig. 17: Thickening dermatitis graph of antifungal nanogel-F6

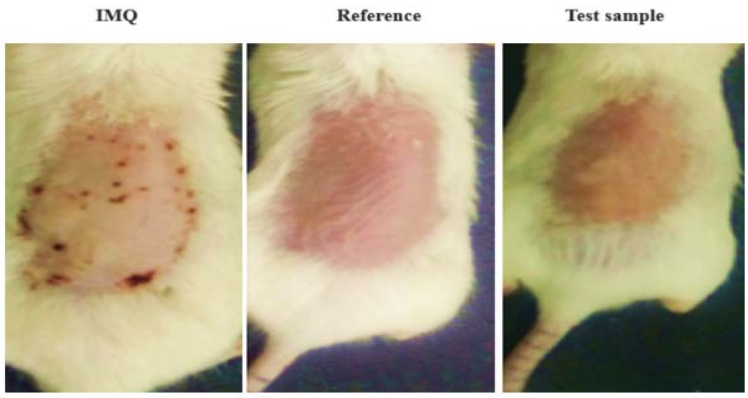

Fig. 18: In-vivo study-dermatitis model mice in Imiquimod (IMQ), reference and test sample (Nanogel formulation-F6)

Conclusion

The homogenization process was used to formulate the Nystatin nanogel. An inflection point was visible in the release profiles of the manufactured nanogels, indicating that the nanogels had formed on the diffusion membrane within the donor compartment of the diffusion cell. Drug release slowed down as the formulation transformed into the gel phase during nanogel production. The outcome shown that Nystatin could be released from a stable antifungal nanogel with a prolonged half-life that was skillfully produced. Actio mycosis treatment with nanogel (F6) has shown promise as it demonstrates reduced systemic absorption of potentially harmful

antifungal drugs with improved local absorption. However, because the test sample's dermatitis score was lower than that of the commercial formulation, it was discovered that the Nystatin test sample was more potent than the formulation.

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