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Research Article

Nystatin Nanosponge Gel: Formulation and Evaluation for the Treatment of Candidiasis

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Abstract

Nystatin is a polyene antimycotic obtained from *Streptomyces noursei* used in the treatment of topical and transdermal fungal infection. Nystatin is nearly insoluble in water (<0.1) and it is amphoteric in nature. The purpose of this research was to prepare nystatin loaded nanosponge gel for Sustained release of drug, increase the drug solubility, increases the drug permeability, to reduce the dosing frequency and side effects. Homogenization technique followed by centrifugation was employed to prepare 10 Nanosponge formulations using various polymers. The formulations were characterized for drug entrapment efficiency, drug content, particle size and SEM analysis. The entrapment efficiency and drug content of all formulations was found to be 97.85 to 99.21%, and 82.90 to 95.71. The average particle size of nystatin loaded nanosponge F3 and F7 was found to be 231.1 nm and 370.3 nm respectively. The SEM analysis of nanosponge shows the spherical surface of the particles. *In-vitro* release of nystatin nanosponge optimized formulation F3wasfound to be 36.28% and F7 was 45.66 % at the end of 24 hours. The prepared nanosponges were formulated to gels using carbopol 934 as a gelling agent and studied for pH, assay, viscosity and in vitro drug release. The drug content, pH and viscosity of the gel G1 and G2 was found to be 25.15, 28.88 %, 4.89, 4.92 and 2.939x 10^6 cps, 2.853x 10^6 cps respectively. The *in-vitro* release of nystatin nanosponge gel formulation G1wasfound to be 23.15% andG2 was 28.88 % at the end of 24 hours. Anti-fungal activity of nystatin nanosponge gel G2 showed highest zone of inhibition followed by gel G1 and marketed sample. It was concluded that the nystatin loaded nanosponge gel may have increased the solubility, drug release and antifungal activity (Increase in Zone of Inhibition) and provide Sustained effect.

Keyword : Nystatin, Nanosponge gel, Homogenization technique, Anti-fungal activity

Introduction : The drug delivery technology has certainly a new interest for drugs by providing them new life through their therapeutic targets. Nowadays, targeting drug delivery is the major problem which is being faced by the researchers. Target oriented drug administration with improvements in therapeutic efficacy reduction in side effects and optimized dosing regimen, shall be the leading trends in the area of therapeutics. Targeted drug delivery implies for selective and effective localization of pharmacologically active moiety at pre identified target in therapeutic concentration, while restricting its access to non-target normal cellular linings and thus minimizing toxic effects and maximizing therapeutic index of the drug [1]

Nanosponges are novel class of hyper-cross linked polymer based colloidal structures consisting of solid nanoparticles with colloidal sizes and nanosized cavities.

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The outer surface is typically porous, allowing sustain release of drug and use for topical drug delivery. Size range of nanosponge is 50nm-100nm with an average diameter below 4µm. This technology is being used in cosmetics, over-the counter skin care, sunscreens and prescribed drugs. Conventional formulation of topical drugs accumulates excessively in epidermis and dermis. Nanosponges prevent the accumulation of active ingredient in dermis and epidermis. Nanosponge system reduce the irritation of effective drug without reduce their efficacy [2]. Nanosponge technology offers entrapment of ingredients and is believed to contribute towards reduced side effects, improved stability, increased elegance and enhanced formulation flexibility. Nanosponges are non-irritating, non-mutagenic, non allergenic and non-toxic. Nanosponges are tiny mesh-like structures that used for the treatment of many diseases and this technology is five times more effective at delivering drugs for breast cancer than conventional methods. Nanosponges are more like a three dimensional network or scaffold. The backbone is a long length of polyester which is mixed in solution with small molecules called cross linkers that act like tiny grappling hooks to fasten different parts of the polymer together [3].The nanosponges are solid in nature and can be formulated as oral, parenteral, topical or inhalational dosage forms. For oral administration, these may be dispersed in a matrix of excipients, diluents, lubricants and anti-caking agents which is suitable for the preparation of tablets or capsules. For parenteral administrations, these can be simply mixed with sterile water, saline or other aqueous solutions. For topical administration, they can be effectively incorporated into topical hydrogel. Topical nanosponge can be more patient compliant and provide sufficient patient benefits by reducing repeated doses and side effects [4]. Oral candidiasis is one of the most common pathological conditions affecting the oral mucosa. Local delivery of drugs to the tissues of the oral cavity has a number of applications including the treatment of toothache, periodontal diseases, dental caries, bacterial and fungal infections. The conventional formulations for the local delivery of drugs to the oral cavity are the mouth paints, rinses, troches, creams and suspensions [5, 6]. The reason for incomplete eradication of candidiasis in most cases may be due to the short residence time of antifungal agents in the oral cavity. The other reason may be degradation of antifungal agents in salivary fluid. One way to improve the efficacy in eradicating the infection is to deliver the antifungal locally in the oral cavity. Better stability and longer residence time will allow more of the antifungal to penetrate through the oral mucous layer to act on Candida species for longer duration of time. Therefore some researchers had prepared and reported new formulation such as gels, mucoadhesive tablets, pH sensitive excipients composition mucoadhesive microspheres, which were able to reside in oral cavity for an extended period for more effective candidiasis eradication [7, 8]. Nystatin is a polyene antifungal antibiotic characterized by a potent broad-spectrum antifungal action against a variety of fungal pathogens including Candida, Aspergillus, Histoplasma, and Coccidioides

[9]. Successful eradication of cutaneous fungal infections requires 2-4 applications of all available topical dosage forms of nystatin [10]. The purpose of this research was to prepare nystatin loaded nanosponge gel for sustained release of drug, increase the drug solubility, increase the drug permeability, to reduce the dosing frequency and side effects.

Materials and Methods

Preparation of extracts:

Materials

Nystatin was obtained as a gift sample from Caplin point Laboratories Pvt Ltd. Ethyl cellulose; polyvinyl alcohol and Polymethyl methacrylate were purchased from Qualigens Fine chemicals, New Delhi. All other chemical used were purchased from S.D Fine Chemical Limited, Mumbai. Double distilled water was prepared freshly and used whenever required. All other chemicals used in this study including those stated were of analytical reagent (A.R.) grade.

Preformulation Studies

Physical Compatibility

Physical compatibility of the drug and excipients were carried at Room temperature and at 40˚C±2˚C/75±5%RH (in days) with the physical admixture of drug and excipients.

Fourier Transforms Infrared (FT-IR) Spectroscopic studies

The spectroscopic studies were carried out to find out the interaction between pure drug, excipients and its physical mixture by KBr pellet technique using FT-IR spectrophotometer. The IR spectrum of the physical mixture is then compared with the spectrum of pure drug (Nystatin) to assess the compatibility of the excipients and drug. Thescanning range is 450-4000 $cm^{-1}$and the resolution is $4cm^{-1}$.

Standard Curve of Nystatin

100mg of drug was accurately weighed and dissolved in 30 ml methanol and made up to 100ml with phosphate buffer pH 5.5. Calibration curve was prepared in a mixture of phosphate buffer and methanol (7:3) at λ max 305 nm.

Formulation of nystatin loaded nanosponge

Nystatin nanosponges were prepared by Emulsion solvent evaporation method. Two different polymers were used in the formulation. Ethyl cellulose (EC) and Polymethyl methacrylate (PMMA) were the Polymers used. Polyvinyl alcohol in Distilled water is used as the aqueous phase. The Drug is dissolved in the required solvent (Dimethylsulphoxide) and the Polymers (1:1, 1:2, 1:3, 1:4, and 1:5) were dissolved in Dichloromethane. The Drug solution was poured into the polymer solution and the mixture was shaken well. Then the Drug polymer mixture was poured into the aqueous phase and the mixer is subjected to homogenization using High speed homogenizer in 1500 rpm for 2 hours at 35˚C.The formed Nanosponges were centrifuged by high speed cooling centrifuge and the residue was freeze dried [11].

Characterization of Nystatin Loaded Nanosponge

Determination of drug content

The total drug content of Nanosponge was determined by spectrophotometric analysis.10milligramequivalentof Nystatin loaded Nanosponge taken in a beaker(closed to avoid evaporation) containing (10 ml)of Methanol and stirred for 30 minutes in magnetic stirrer, 3ml of that solution is pipetted out and that volume was made upto10ml by using pH5.5phosphatebuffer to make1µg/ml concentration. The absorbance was measured at 305nm **λ_{max}** using UV spectrophotometer. From the absorbance drug content was calculated.

Determination of drug entrapment efficiency

The entrapment efficiency was determined by measuring the concentration of the drug in the supernatant after centrifugation. The unentrapped Nystatin were determined by adding 10 mg Nystatin loaded Nanosponge in 10 ml of methanol and then the dispersion were centrifuged at 9,000rpm for 30 minutes at 4ºC using a cooling centrifuge in order to separate entrapped from the unentrapped drug. The free drug concentration in supernatant layer after centrifugation is determined at λ_{max}(305nm) using UV Spectrophotometer

***in- vitro* release studies**

The *in vitro* release of Nystatin from Nanosponge was evaluated by the Dialysis Bag diffusion technique. The release studies of Nystatin from Nanosponge were performed in Phosphate buffer of pH5.5 and methanol (70:30) 18mg equivalent Nystatin Nanosponge were suspended in 10 ml of buffer pH 5.5 mixture and placed in the dialysis bag(donor compartment) and sealed at both ends. The dialysis bag was immersed in receptor compartment containing 100ml of buffer mixture, which was stirred at 100 rpm and maintain 32 ± 2˚C. The receptor compartment was covered to prevent evaporation of the diffusion medium. Samples were taken from the receptor compartment and the same amount was replaced with the diffusion medium. Samples are taken upto 24hrs. Nystatin in the samples was measured spectrophotometrically at λ 305nm. Same procedure was carried out for pure drug and *in vitro* release was measured spectrophotometrically at λ 305nm [12, 13].

Selection and Evaluation of Optimized Formulation

The best formulation selection based on the results obtained from particle size, entrapment efficiency, *in-vitro* release studies and kinetics of drug release.

FT-IR spectroscopic studies

The compatibility study was carried out for optimized formulations (F3 and F7) using FTIR at wavelength range of 4000to400cm^{-1}. Spectrum for optimized formulation were taken and compared.

Morphology of Nanosponge by scanning electronmicroscopy (SEM) technique

The surface morphology of the nanosponge can be measured by SEM. The formulations are poured in a circular aluminum stubs using double adhesive tape, and coatedwith gold in HUS– 5GB vaccum evaporator and observed in Hitachi S– 3000N SEM at an acceleration voltage of 10 Kv and a magnification of5000X.

Particle size distribution

Particle size (z-average diameter), and polydispersity index (as a measure of the widthof the particle size distribution)of Nystatin loaded Nanosponge dispersion is performed by dynamic light scattering also known as photon correlation spectroscopy(PCS) using a Malvern Zeta sizer 3000NanoS(Malvern instruments, UK) at 25°C. Prior to measurements all samples were diluted using ultra–purified water to yield a suitable scattering intensity. The diluted nanosponge dispersion was poured into disposable sizing cuvette which is then placed in the cuvette holder of the instrument and analyzed. Air bubbles were removed from the capillary before measurement [13].

Mathematical treatment of *in-vitro* release data

The quantitative analysis of the values obtained in dissolution/release tests is easier when mathematical formulas

that express the dissolution results as a function of some of the dosage forms characteristics are used.

1. Zero-order kinetics: The pharmaceutical dosage forms following this profile release the same amount of drug by unit of time and it is the ideal method of drug release in order to achieve a pharmacological prolonged action. The following relation can, in a simple way, express this model:

$$Q_t = Q_o + K_o t$$

Where Q_t is the amount of drug dissolved in time t, Q_o is the initial amount of drug in the solution (most times, Q_o=0) and K_o is the zero order release constant.

2. First-order kinetics: The following relation expresses this model:

$$\log Q_t = \log Q_o + \frac{K_1 t}{2.303}$$

Where Q_t is the amount of drug dissolved in time t, Q_o is the initial amount of drug in the solution and K_1 is the zero order release constant.

In this way a graphic of the decimal logarithm of the released amount of drug versus time will be linear. The pharmaceutical dosage forms following this dissolution profile, such as those containing water-soluble drugs in porous matrices, release drug in a way that is proportional to the amount of drug remaining in its interior, in such way, that the amount of drug released by unit of time diminish.

3. Higuchi model: Higuchi developed several theoretical models to study the release of water-soluble and low soluble drugs in semi-solid and/or solid matrixes. Mathematical expressions were obtained for drug particles dispersed in a uniform matrix behaving as the diffusion media.

The simplified Higuchi model is expressed as:

$$Q = K_H . t^{1/2}$$

Where Q is the amount of drug released in time t and K_H is the Higuchi dissolution constant. Higuchi model describes drug release as a diffusion process based in the Fick's law, square root time dependent. This relation can be used to describe the drug dissolution from several types of modified release pharmaceutical dosage forms such as transdermal systems and matrix tablets with water-soluble drug.

4. Korsmeyer-Peppas model: Korsmeyer *et al.* used a simple empirical equation to describe general solute release behaviour from controlled release polymer matrices:

$$\frac{M_t}{M_\infty} = a\, t^n$$

Where M_t/M_∞ is fraction of drug released, a is kinetic constant, t is release time and n is the diffusional exponent for drug release. 'n' is the slope value of log M_t/M_∞ versus log time curve. Peppas stated that the above equation could adequately describe the release of solutes from slabs, spheres, cylinders and discs, regardless of the release mechanism. Peppas used this n value in order to characterize different release mechanisms, concluding for values for a slab, of *n* =0.5 for fickian diffusion and higher values of *n*, between 0.5 and 1.0, or *n* =1.0, for mass transfer following a non-fickian model. In case of a cylinder *n* =0.45 instead of 0.5, and 0.89 instead of 1.0. This equation can only be used in systems with a drug diffusion coefficient fairly concentration independent. To the determination of the exponent *n* the portion of the release curve where $M_t/M_\infty < 0.6$ should only be used. To use this equation it is also necessary that release occurs in a one-dimensional way and that the system width-thickness or length-thickness relation be at least 10. A modified form of this equation was developed to accommodate the lag time (*l*) in the beginning of the drug release from the pharmaceutical dosage form:

$$\frac{M_{t-l}}{M_\infty} = a\,(t-l)^n$$

When there is the possibility of a burst effect, b, this equation becomes:

$$\frac{M_t}{M_\infty} = at^n + b$$

In the absence of lag time or burst effect, l and b value would be zero and only at^n is used. This mathematical model, also known as Power Law, has been used very frequently to describe release from several different pharmaceutical modified release dosage forms [14-16]

Stability studies

The stability studies of the optimized Nanosponge were performed at different conditions of temperature and the effect on physical characteristic, entrapment efficiency and drug content was noted. The Nanosponge were kept in the airtight container and stored at 40±2˚C and in Relative humidity 75±5% for 45 days. The samples were analyzed for the above parameter in 15days, 30 days and 45days.

Formulation of Nystatin Loaded Nanosponge Gel

The formulation of Nanosponge prepared using the optimized ratio of Polymer containing Nystatin equivalent to18mg was incorporated into the gel base composed of Carbopol 934 (1%), Glycerol (15%), Triethanolamine (q.s.) and distilled water up to 1g.

Evaluation of Nanosponge Gel

Physical Appearance

The prepared gel was examined for clarity, color, homogeneity and the presence of foreign particles.

pH

2.5g of gel was accurately weighed and dispersed in 25 ml of distilled water. The pH of the dispersion was measured by using a digital pH meter.

Viscosity Measurement

Viscosity was determined by Brookfield viscometer. In the present study, spindle no. S64 with an optimum speed of 0.6 rpm was used to measure the viscosity of the preparation.

Content Uniformity

The drug content of the prepared gel was carried out by dissolving accurately weighed quantity of gel equivalent to 9mg of the drug in a beaker containing 10 ml of methanol, stir the solution for 30 minutes and centrifuged in High speed cooling centrifuge and 3ml of the solution is made upto 10 ml with phosphate buffer pH 5.5. The samples were analyzed spectrophotometrically at λmax 305 nm against blank using UV- Visible spectrophotometer.

***In-vitro* Drug Diffusion study**

In- vitro drug diffusion study was studied using dialysis bag. The Nanosponge gel equivalent to 18mg of the drug was placed in a Dialysis Bag having 8 cm length and 3 cmbreath, both the sides are tied with thread. This acted as the donor compartment. Then the bag was placed in a beaker containing 100 ml

phosphate buffered methanol pH 5.5, which acted as receptor compartment. The temperature of the receptor medium was maintained at 37°±2°C and the medium was stirred at a speed of 100 rpm using a magnetic stirrer. 5 ml of the samples were collected at a predetermined time and replenished immediately with the same volume of fresh buffer PB mixture pH5.5. The sink condition was maintained throughout the experiment. The collected samples were analyzed spectrophotometrically at 305 nm using UV- Visible spectrophotometer [17, 18].

Microbiological Study

Antifungal activity for the prepared Nanosponge gel was carried out. Candida albicans was grown in Malt Extract Agar,(MAE) with medium composition: malt extract 17 g/L and agar 20g/L. The assay was done using the agar well diffusion method. Sterile agar plates were prepared by pouring the sterilized media in sterile Petri dishes under aseptic conditions.1 mm of the test organism was spread on agar plates. Using a sterile tube with a diameter of 6mm, the wells were made according to the number of samples. The wells were inoculated with 80µL of sample. The antimicrobial activity was interpreted based on the size of inhibition zone (IZ) diameter, which was measured in mm from observation of clear zones surrounding the wells [19].

Result and Discussion :

The drug and the excipients of the formulation are physically compatible with each other. They were evaluated for 10, 20 and 30 days at room temperature and at 40˚C±2˚C/75±5% Relative Humidity Table 3. The compatibility between drug and Polymer was confirmed using FT-IR Spectroscopy. Infrared spectroscopic analysis for drug (Nystatin), Polymer, Drug-Polymer admixture was carried out. The peak shows the presence of NH, OH, aromatic CH, aliphatic CH and CarboxylicC=O groups in the Nystatin sample. The peak shoes that the Ethyl cellulose has Characteristics Aliphatic CH and Methyl CH- streching. The peak shows that the Characteristics C=O, Aliphatic CH and Methyl CH – stretching. The peak observed in the FTIR Spectrum of Nystatin pure drug with Polymer(PMMA) showed no shift and no disappearance of characteristic peaks of pure drug suggesting no interaction between the drug and Polymer. The peak observed in the FTIR Spectrum of Nystatin pure drug with Polymer (Ethyl cellulose) showed no shift and no disappearance of characteristic peaks of pure drug suggesting no interaction between the drug and Polymer Figure 1-5. The UV spectrometric method was used to analyze Nystatin. The absorbance of the drug in phosphate buffered methanol pH 5.5. (70:30) was measured at a wavelength of 305 nm Figure 6. The drug content of the formulations was observed to be between 82.90 to 95.71 and the entrapment efficiency was found to be 97.85 to 99.21. The highest entrapment efficiency was observed with 99.21 and 98.94 for the formulations F3 and F7 Table 4. Surface morphology of nanospongewas measured by Scanning Electron Microscopy (SEM). SEM picture shows the formation of spherical nanoparticles Figure 7 & 8. The average particle size of Nystatin loaded Nanosponge (F3) is 231.1 nm and the poly dispersity index was found to be 1.000 and the average particle size of Nystatin Nanosponge (F7) was 370.3nm and the polydispersity index was found to be 1.000 Figure 9 & 10. The *in-vitro* release of the formulations was observed to be between15.53% to 45.66 %. The results shown that the increase in polymer concentration increase the drug release and then it is decreased as described by Prathima Srinivas et al[11]. From the result it was observed that the formulations F3 and F7 Showed sustained release of drug Figure 11& 12. The *in–vitro* release of the Gel formulation of F3 and F7 was compared with marketed sample of Nystatin cream, and the release of Nanosponge gel (G1 and G2) was 23.15% and 28.88% respectively. The release of gel was increased due to the decreased particle size and increased surface area Figure 13. All the formulations followed first- order kinetics and their R^2 value lied between 0.896 to 0.944 indicating the release to be dose dependent. The drug release was proportional to the square root of time indicating that Nystatin release from Nanosponge and Gel was diffusion controlled. The n value for the korsmeyer- peppas for formulation F3, F7,G1 and G2 was found to be lied between 0.768 to 0.857.. So the formulation F3, F7,G1 and G2 follows non- Fickian type mechanism. The Drug release pattern from Nystatin loaded Nanosponge follows First order release, Higuchi model and Non-fickian transport. The release of Nystatin Nanosponge gel occurs through the First order, Higuchi model and non - fickian diffusion mechanism Table 5.

The antifungal activity for nystatin nanosponge gel G2 zone of inhibition was 19.33mm, for G1 zone of inhibition was 18mm and the zone of inhibition obtained for marketed sample was 11.667mm. The antifungal activity of the nanosponge gel showed enhancement of nystatin antifungal effect Table 7.

Conclusion

It was concluded that the Nystatin loaded Nanosponge Gel may have increased the solubility, drug release and Antifungal activity (Increase in Zone of Inhibition), and provide Sustained effect.

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Table 1: Formulation of nanosponge

S.No	INGREDIENTS	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
1.	Drug (mg)	100	100	100	100	100	100	100	100	100	100
2.	EC(mg)	100	200	300	400	500	-	-	-	-	-
3.	PMMA (mg)	-	-	-	-	-	100	200	300	400	500
4.	PVA (g)	0.2	0.2	0.2	0.2	0.2	0.3	0.3	0.3	0.3	0.3
5.	DCM (ml)	20	20	20	20	20	20	20	20	20	20
6.	Distilled water(ml)	100	100	100	100	100	100	100	100	100	100

Table 2: Preparation of gel

S. No.	INGREDIENTS	GEL BASE
1.	Carbapol 934	1 %
2.	Glycerol	15 %
3.	Triethanolamine	Quantity Sufficient
4.	Distilled water	Up to 15g

Table 3: Physical compatibility

S. No.	Description and condition							
	Drug (D) and excipients	Initial	Room temperature (in days)			40˚C±2˚C/75±5% RH(in days)		
			10	20	30	10	20	30
1.	Drug	NC	NC	NC	NC	NC	NC	NC
2.	D+EC	NC	NC	NC	NC	NC	NC	NC
3.	D+PMMA	NC	NC	NC	NC	NC	NC	NC
4.	D+PVA	NC	NC	NC	NC	NC	NC	NC

NC – No Change

Table 4: Drug content and entrapment efficiency of nystatin nanosponge

Formulation Code	%Drug Content (%w/v)	%Entrapment Efficiency (% w/v)
F1	94.52±0.109	98.79
F2	91.31±0.633	98.14
F3	**90.26±0.266**	**98.94**
F4	92.54±1.173	98.86
F5	82.90±1.239	97.85
F6	90.36±0.366	96.57
F7	**95.714±0.231**	**99.21**
F8	85.44±0.555	85.44
F9	88.95±0.524	88.95
F10	86.70±0.708	86.70

Table 5: Drug release kinetics for optimized formulation and their gel

FORMULATIONCODE	ZERO ORDER	FIRST ORDER	HIGUCHI MODEL	HIXSON CROWELL	KORSMEYER PEPPAS	
	R^2	R^2	R^2	R^2	R^2	n
F3	0.914	0.944	0.973	0.935	0.975	0.793
F7	0.892	0.929	0.958	0.918	0.975	0.768
G1	0.871	0.896	0.981	0.888	0.882	0.812
G2	0.908	0.934	0.980	0.926	0.89	0.857

Table 6: Evaluation of gel

S.No.	Code	Physical appearance	%Drug content	Viscosity (cps)	pH	%Drug release
1.	G1	Good	91.10	2.939×10^6	4.89	25.15
2.	G2	Good	94.55	2.853×10^6	4.92	28.88

Table 7: Antifungal activity of nystatin nanosponge gel

S. No.	Formulation code	Anti fungal activity(Zone of inhibition) inmm
1.	Control	0
2.	G1	18±0.8164
3.	G2	19.33±1.2472
4.	Marketed sample	11.667±0.4714

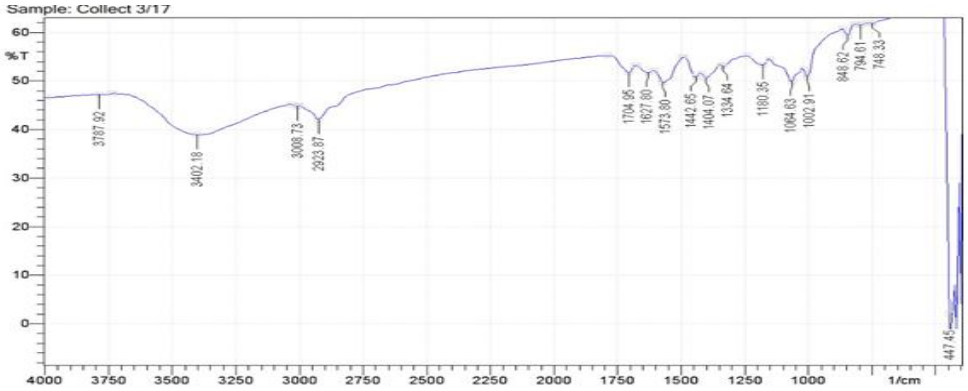

Figure 1: FT-IR spectrum of nystatin

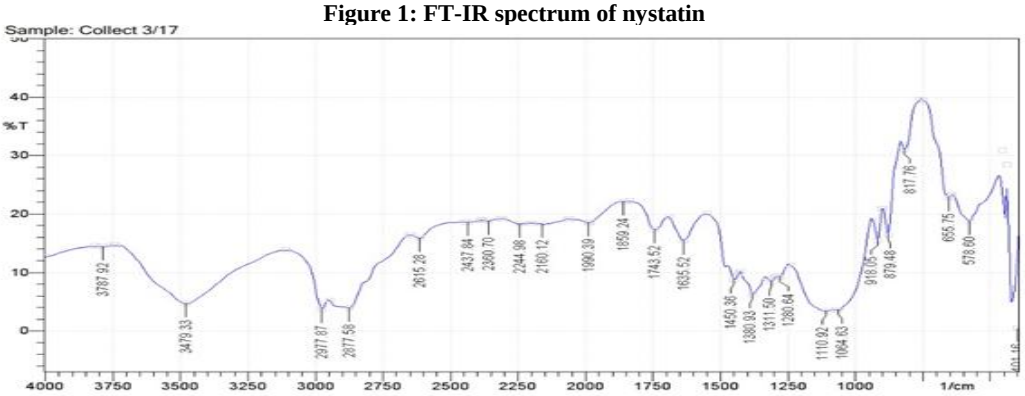

Figure 2: FT-IR spectrum of ethyl cellulose

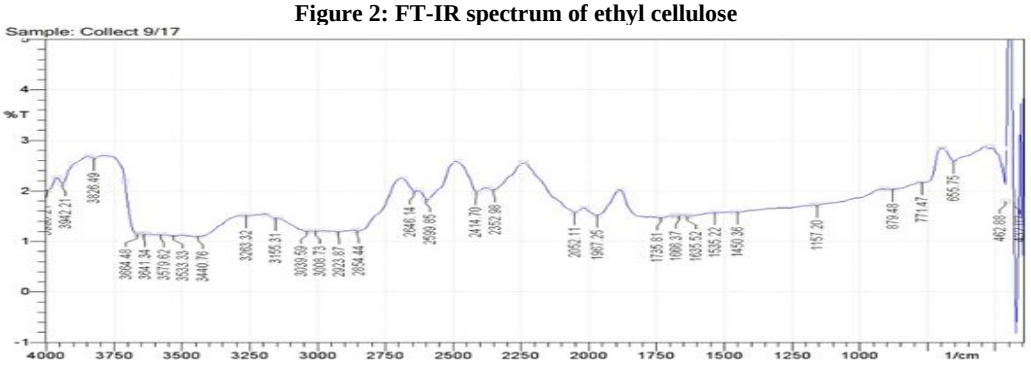

Figure 3: FT-IR spectrum of polymethyl methacrylate

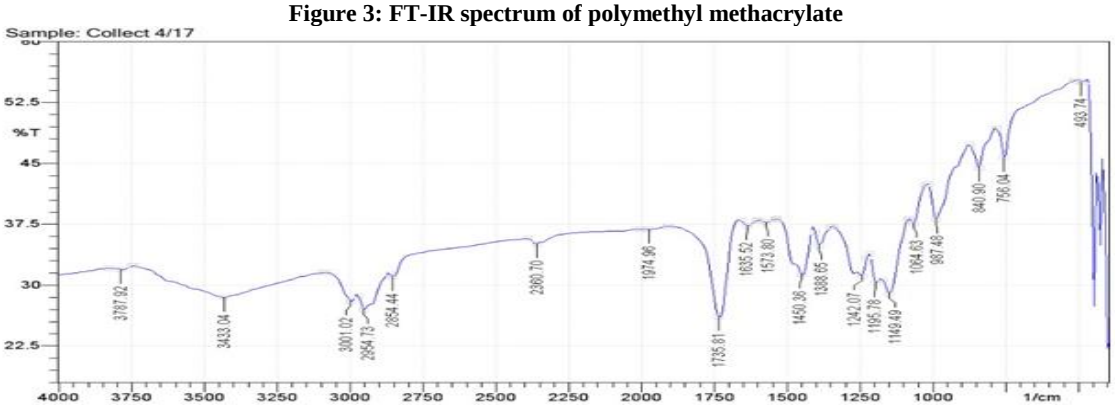

Figure 4: FT-IR spectrum of nystatin with PMMA

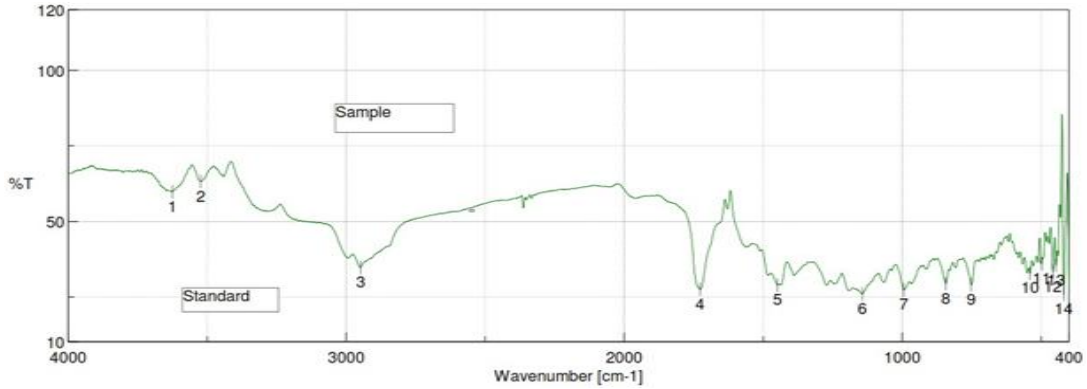

Figure 5: FT-IR spectrum of nystatin with ethyl cellulose

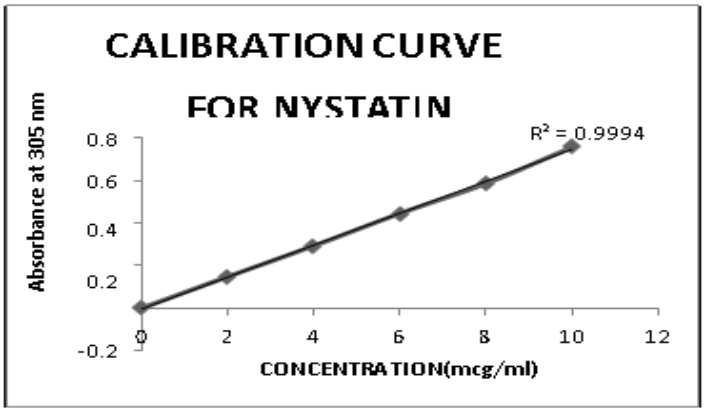

Figure 6: Calibration curve for nystatin

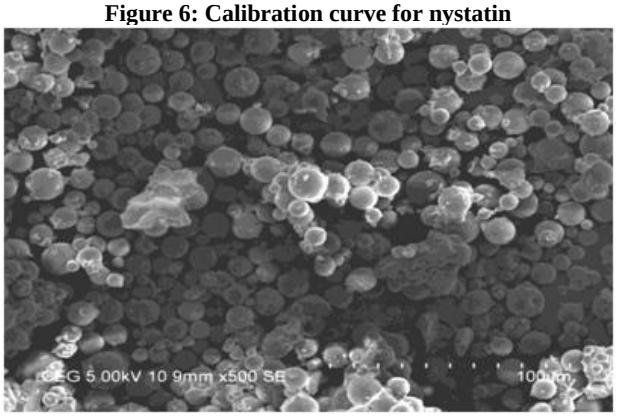

Figure 7: SEM image of optimized formulation (F3)

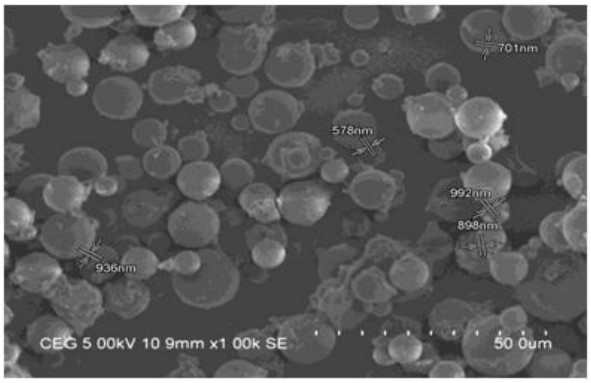

Figure 8: SEM image of optimized formulation (F7)

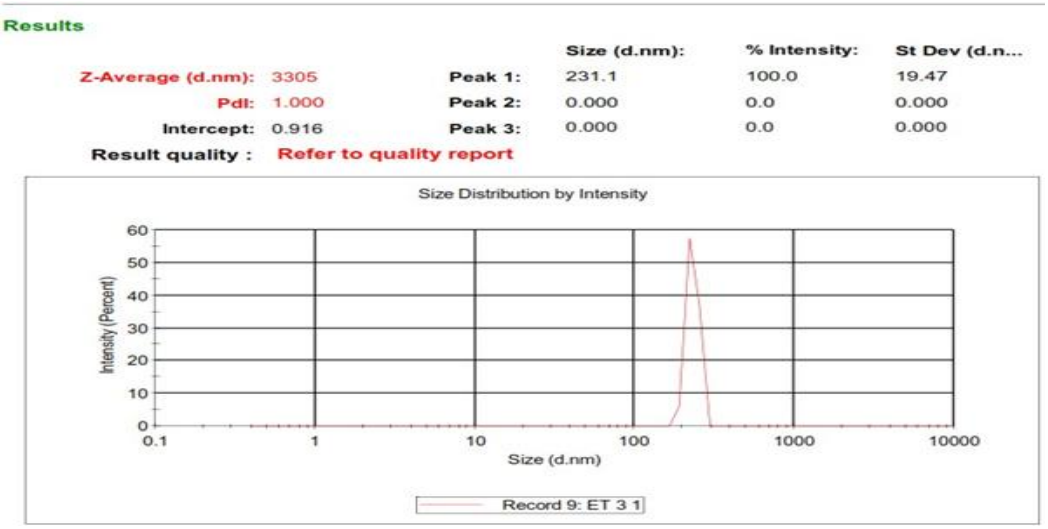

Figure 9: Size distribution of optimized formulation F3

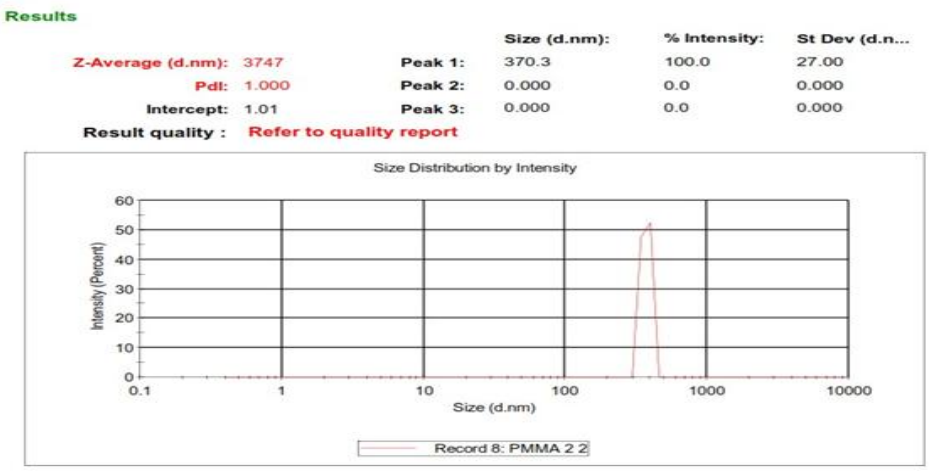

Figure 10: Size distribution of optimized formulation F7

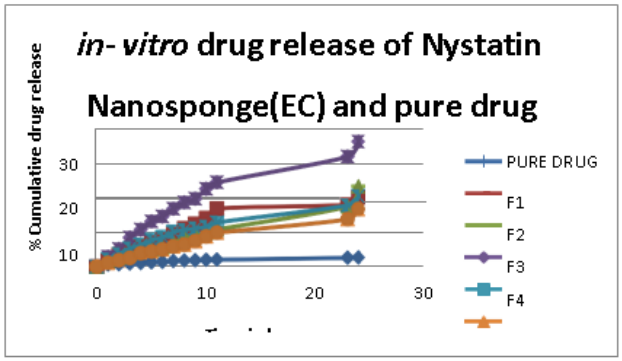

Figure 11: *in- vitro* drug release of nystatin nanosponge (EC) and pure drug

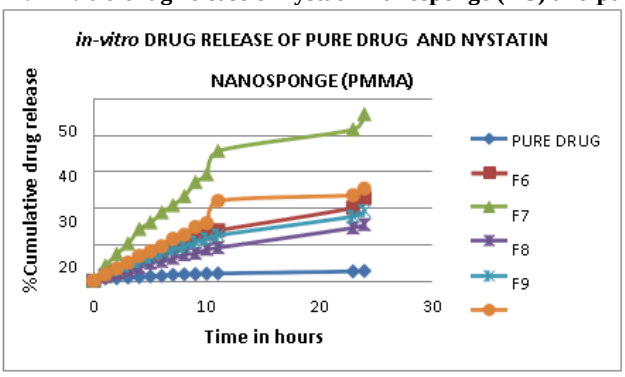

Figure 12: *in -vitro* drug release of nystatin nanosponge (PMMA) with pure drug

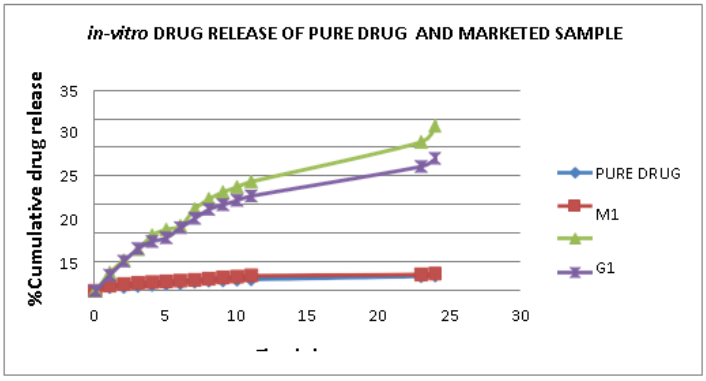

Figure 13: *in-vitro* drug release of pure drug, marketed sample (Nistatina cream) and nystatin nanosponge gel G1 and G2