Formulation and In-vitro Evaluation of Nystatin Nanoemulsion-Based Gel for Topical Delivery

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Abstract: The objective of the present study was to investigate the potential of a nanoemulsion formulation for topical delivery of nystatin. Labrafil M1944, Tween 80 and ethanol were selected for preparing nanoemulsion. Various oil-in-water nanoemulsions were prepared by the spontaneous emulsification method. The nanoemulsion area was identified by constructing pseudoternary phase diagrams. The prepared nanoemulsions were subjected to accelerated aging test. The nanoemulsion formulations that passed the accelerated aging test were characterized for its morphology and droplet size analysis. The optimized formulations were incorporated into polymeric gel of methylcellulose for convenient application and evaluated for pH, drug content and viscosity. The *in vitro* release was studied. A comparison between the nanoemulsion based gel formulations and a commercially available product, Nystatin® cream, was carried out to judge their efficacy. The drug release from the commercial preparation was lower than all the prepared nanoemulsion based gel formulations. F1 showed highest drug release percent (49.13%) followed by F8 (45.69%) in contrast, the marketed formulation released (22.88%) of the drug in 24 hrs**.** The *in vitro* nystatin release data were fitted to Korsmeyerpeppa's release model. The formulation exhibited non-fickian transport with zero order kinetics. Formulae F1 and F8, showed both small droplet size and highest extent of drug release, was microbiologically evaluated against *Candida albicans* (*C. albicans*) using agar dilution assay. The selected formulae showed superior antimycotic activity compared to the commercially available formulation.
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1. Introduction

Nystatin is a polyene antifungal characterized by a potent broad-spectrum antifungal action including a wide range of pathogenic and non-pathogenic yeasts and fungi. The Nystatin is active against a variety of fungal pathogens including: Candida, Aspergillus, Histoplasma, and Coccidioides and has been used for years to treat Candida at the skin and those for the mouth. This information, combined with the facts that the incidence of disseminated fungal infections has risen over the past decade, and that Candida is now the fourth most commonly encountered nosocomial bloodstream pathogen, shows that it is increasingly important to make available new products to fight these alarming trends [(1)]. One of the most promising techniques for enhancement of transdermal permeation of drugs is nanoemulsion. Nanoemulsions are thermodynamically stable transparent (translucent) dispersions of oil and water stabilized by an interfacial film of surfactant and cosurfactant molecules having a droplet size 5-200 nm [(2)]. Many studies have shown that nanoemulsion formulations possess improved transdermal and dermal delivery properties *in vitro* [(3, 4)], as well as *in vivo* [(5-7)]. Nanoemulsions have improved transdermal permeation of many drugs over the conventional topical formulations such as creams and gels [(5, 8)]. In The present study, different nanoemulsion formulations of nystatin were prepared in gel forms for topical application and evaluated for their physicochemical properties and their *in vitro* release. The *in vitro* antifungal activities of these formulations were also examined.

2.Materials And Methods

Materials

Nystatin was kindly donated as a gift from Delta Pharm company, Miglyol 812 (medium chain triglyceride oil from coconut oil), sorbitan sesquioleate and Labrafil M 1944 (polyoxyethylated kernel oil), were gift samples from GlaxoSmithkline Pharmaceutical company, (Egypt). Tween® 20 (polyoxyethylene 20 sorbitan monolaurate) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Tween® 80 (polyoxyethylene 20 sorbitan monooleate) was a gift sample from ADCO, (Egypt). Isopropyl myristate and Span® 20 (sorbitan monolaurate), were purchased from Fluka, (Switzerland). Sesame oil was purchased from Lab chemicals trading co. (Egypt). Methanol, Ethanol and n-Butanol were purchased from ADWIC, (Egypt). Nystatin® skin cream (Nystatin 1% w/w, manufactured by Pharaonia Pharmaceuticals) was purchased from a local pharmacy store. All other materials were of analytical grade.

2. Methods

Screening of components by solubility studies

An excess amount of Nystatin was added to different oily phases (Miglyol 812, Labrafil M1944, Isopropyl myristate and Sesame oil), surfactants (sorbitan sesquioleate, Tween 20, Tween 80 and Span 20) and cosurfactants (Ethanol and n-Butanol) and shaken in digital water bath shaker (lab. house) 25 ˚C for 48 hrs.[9]. At the end of test period, samples were centrifuged at 3000 rpm for 15 min., filtered the supernatant through membrane filter (0.45um) and the clear filtrate was diluted with methanol and was measured spectrophotometrically (Jenway Ltd, Model 6105 UV/V, United Kingdom) at respective λ max.

Pseudo-ternary phase diagram

On the basis of the solubility studies, Labrafil M1944 was selected as the oil phase. Tween® 80 and Ethanol were selected as surfactant and cosurfactant, respectively. Distilled water was used as an aqueous phase. Surfactant and cosurfactant (Smix) were mixed at different mass ratios (4:1, 5:1, and 6:1). These ratios were chosen in increasing concentration of surfactant with respect to cosurfactant for a detailed study of the phase diagrams. For each phase diagram, oil and Smix at a specific ratio was mixed thoroughly at different mass ratios from 1:9 to 9:1 in different glass vials. Different combinations of oil and Smix were made so that maximum ratios were covered for the study to delineate the boundaries of phases precisely formed in the phase diagrams. Pseudo ternary phase diagrams of oil, Smix and aqueous phase were developed using the aqueous titration method. Slow titration with aqueous phase was performed for each mass ratio of oil and Smix and visual observations were made for transparent/translucent and easily flowable o/w nanoemulsions. The physical state of the nanoemulsion was marked on a pseudo-three-component phase diagram with one axis representing the aqueous phase, the second one representing oil and the third representing a mixture of surfactant and cosurfactant at a fixed mass ratio [10].

Selection of nanoemulsion formulation

Considering the solubility and the amount of drug required to be incorporated in nanoemulsion, from each phase diagram, certain oil-Smix-water mixtures within the nanoemulsion region were selected and prepared.

Preparation of nanoemulsion formulation loaded with drug

The nanoemulsion formulations were prepared by spontaneous emulsification method as follow. Appropriate quantities of oil LabrafilM1944, surfactant Tween® 80 and co-surfactant ethanol were weighed and mixed well. The drug was accurately weighted to represent 1% of the total weight of the formulation and added to the previous mixture and stirred with a magnetic bar on magnetic stirrer, at room temperature until the drug completely dissolved. The weighed amount of water then added dropwise with continuous mixing.

Characterization of nanoemulsion formulation

Stability of nanoemulsion (accelerated aging)

The stability of nanoemulsion was evaluated by exposure to a total of three complete freeze-thaw cycles, each cycle consisting of 24 hrs. at 25 °C followed by 24 hrs. at -5 °C [5].

Droplet size measurements

Size analysis of nanoemulsion was carried out by dynamic light scattering with Zetasizer Nano ZS (Malvern instruments ltd., Malvern, U.K). Samples were placed in square glass cuvettes and droplet size analysis was carried out at Temperature 25°C.

Transmission electron microscopy analysis

Morphology and size of the nanoemulsion was studied using Transmission electron microscope (TEM), Jeol, JEM 1010, Japan. It is capable of point to point resolution using negative staining technique. Combination of bright field imaging at increasing magnification and of diffraction modes was used to reveal the form and size of the nanoemulsion. A drop of nanoemulsion was placed on a carbon coated copper grid, stained by 2% uranyl acetate and examined by TEM.

Formulation of nanoemulsion-based gel

The formulations that showed stability and proper droplet size were selected for further development of nanoemulsion-based gel. As nanoemulsions have low viscosity and are difficult to apply on the skin as such, they should be gelled with suitable gelling agents. The nanoemulsion-based gel was prepared by sprinkling the weighed amounts of the methyl cellulose powder (5%) gently in sufficient quantity of warm distilled water, and magnetically stirred at high speed. Stirring was continued until a thin hazy dispersion, without lumps, was formed. For complete gel dispersion it was necessary to leave samples overnight in the refrigerator [11]. Then the nystatin loaded nanoemulsion was slowly added to the viscous solution of methyl cellulose under magnetic stirring.

Evaluation of nanoemulsion-based gel

Determination of pH

The pH of nanoemulsion-based gel was measured on digital pH meter standardized using pH 4.0 and 7.0 standard buffers before use.

Drug content studies

Nanoemulsion-based gel equivalent to 20mg of nystatin was taken in 100 mL volumetric flask containing 15 mL methanol and stirred for 30 min. The volume was made up to 100 mL with phosphate-citrate buffer (pH 5.5) and appropriate dilutions

were made. The resultant solution was filtered through 0.45 µm membrane filter. The absorbance of the solution was measured spectrophotometrically at 305 nm.

Viscosity measurement

A Cole parmer viscometer was used to measure the viscosity of the prepared gel bases. The spindle was rotated at 10 rpm. Samples of the bases were allowed to settle over 30 min at room temperature before the measurements were taken [11].

***In-vitro* release studies**

The *in vitro* release studies of Nystatin nanoemulsion-based gel was investigated through Semipermeable membrane obtained from Sigma which has molecular wt cut off 12,000 Daltons, was used in this study using a modified USP 17 dissolution apparatus I. A glass cylindrical tube (2.5 cm in diameter and 6 cm in length) was attached instead of the basket and was tightly covered with the semipermeable membrane. Nystatin loaded nanoemulsion gel bases were placed in the cylindrical tube at the semipermeable membrane surface. The cylindrical tube was dipped in 100 ml methanolic citrate-phosphate buffer (30%:70%) at pH 5.5 to allow the establishment of the sink conditions and to sustain permanent solubilization. The release study was carried out for 24 hrs. at 32 ^{o}c. the stirring shaft was rotated at speed of 100 r.p.m[12].

At predetermined time intervals (1, 2, 4, 6, 8, 12, 20, 24 hrs.) aliquots of one milliliter of the release medium were withdrawn and diluted then filtered for analysis and replaced with equal volume of the buffer solution to maintain a constant volume. The absorbance of the collected samples was measured by UV at λmax of 305 nm.

Release kinetic studies of nanoemulsion-based gels

To study the drug release mechanism of each formulation, the release data were fitted to the general exponentional function: $M_t / M_0 = kt^n$; where M_t / M_0 represents the fractional uptake of solvent (or release of a solute) normalized with respect to the equilibrium conditions; n is a diffusion exponent characteristics of the release mechanism, and k denotes properties of the polymer and the drug. This equation has been used frequently, due to its utility in describing the relative importance of Fickian (n = 0.5) and Case II ($n \leq 1.0$) transport in anomalous diffusion. Kinetic studies were performed by adjusting the release profiles to Higuchi and zero-order equations [13].

Microbiological assay of nystatin

Antifungal activity of different formulations was determined by agar dilution assay. Different formulations were added to the Sabaurod's agar medium separately. The different formulations were weighed and dissolved directly in agar for the concentrations 20%. Standard Petri dishes (92 × 16 mm (diameter × height) were used and the agar volume in each plate was 25 ml. After the agar was poured, plates were allowed to cool and were then used immediately. There were triplicate plates for each formula. The medium without the agents was used as a growth control and the blank control used contained only the medium.

C. albicans (RCMB 005002) colonies were grown on Sabaurod's agar medium. Colonies were diluted in phosphate-buffered saline (PBS) and the suspension was adjusted to approximately 2 × 10^6 colony forming units (CFU)/ml for *C. albicans* using a turbidimeter (DEN-1 McFarland Densitometer, Biosan). The surface of the agar plates were inoculated with a 20 µl of *C. albicans* suspension and incubated at 37 ^{o}c for 24 hrs. The populations (CFU) of *C. albicans* recovered after incubation period were determined [14].

This test is carried out by The Regional Center for Mycology and Biotechnology.

3. Results and Discussion

Screening of components by solubility studies

The excipients selected needed to be pharmaceutically acceptable, nonirritating, and non-sensitizing to the skin and to fall into the GRAS (generally regarded as safe) category. Among the selected oils that were screened, maximum solubility of nystatin was found in labrafil M1944 as compared to the other oils as shown in (Table 1). Its solubility in labrafil M1944 is 5 folds higher than miglyol 812 and 19 folds higher than isopropyl myristate. Safety is a major determining factor in choosing a surfactant, as a large amount of surfactants may cause skin irritation. Non-ionic surfactants are less toxic than ionic surfactants. An important criterion for selection of the surfactants is that the required hydrophilic lipophilic balance (HLB) value to form the o/w nanoemulsion be greater than 10. In this study, high drug solubility was found in Tween 80, so it is selected as a surfactant with an HLB value of 15. Transient negative interfacial tension and fluid interfacial film are rarely achieved by the use of single surfactant; usually, addition of a cosurfactant is necessary. The presence of cosurfactant decreases the bending stress of interface and allows the interfacial film sufficient flexibility to take up different curvatures required to form nanoemulsions over a wide range of composition [15]. Thus, the cosurfactant selected for the study was ethanol which shows higher drug solubility and has an HLB value of 4.2 [16]. Propylene glycol and propane-diol 1,2 when mixed with nystatin showed brown colour indicating nystatin instability, so they are excluded from this study.

Table (1): Solubility of nystatin in various components.

Component	Solubility (mg/gm)
Labrafil M 1944	3.53
Miglyol 812	0.68
Isopropyl myristate	0.19
Sesame oil	0
Sorbitan sesquioleate	8.85
Tween® 20	11.23
Tween® 80	26.94
Span® 20	11.14
Propylene glycol	brown colour
Propane-diol 1,2	brown colour
Ethanol	4.97
n-Butanol	0.58

Pseudo-ternary phase diagram

The construction of pseudoternary phase diagrams is used to determine the concentration range of components in the existence range of nanoemulsion. The pseudoternary phase diagrams with various weight ratios of tween 80 to ethanol are depicted in Figure 1. The translucent nanoemulsion region is presented in phase diagrams with no distinct conversion from water-in-oil (W/O) to oil-in-water (O/W) nanoemulsions were observed. The rest of the region on the phase diagram represents the turbid and conventional emulsions based on visual observation. The area of nanoemulsion isotropic region changed in size with the increasing ratio of surfactant to co-surfactant. Pseudo-ternary phase system with Tween80:ethanol (6:1) exhibited maximum area for nanoemulsion formation.

The wider region indicated better nanoemulsifying efficiency of the developed formulation and better interaction among oil phase, Smix, and aqueous phase. In Figure 1, the Smix ratio 4:1 (Figure 1A) has a low nanoemulsion area. The maximum concentration of oil that could be solubilized in the phase diagram was only 15.9 % wt/wt using 67.6 % wt/wt of Smix. As the surfactant concentration was increased in the Smix ratio 5:1 (Figure 1B), a slight higher nanoemulsion region was observed, perhaps because of further reduction of the interfacial tension, increasing the fluidity of the interface, thereby increasing the entropy of the system. There may be greater penetration of the oil phase in the hydrophobic region of the surfactant monomers. The maximum concentration of oil that could be solubilized in the phase diagram was 17.2 % wt/wt using 73.8 % wt/wt of Smix. As we further increased surfactant concentration, Smix 6:1 (Figure 1C), the nanoemulsion region increased as compared with the region in 3:1 and 4:1, The maximum concentration of oil that could be solubilized by this ratio was 18.9% wt/wt using 71.3% wt/wt of Smix.

Selection and preparation of nanoemulsion formulation

Depending on the solubility and the amount of drug required to be incorporated in nanoemulsion, from each pseudoternary phase diagram, the formulations in which the visual observations were made for transparent/translucent and easily flowable o/w nanoemulsion were selected and prepared for the further studies.

Characterization of nanoemulsion formulation

Stability of nanoemulsion (Accelerated aging)

The stability study of 1% w/w nystatin-loaded nanoemulsion formulations were done by exposing them to freeze-thaw cycles for 72 hours. The only nanoemulsion formulations found to be stable and showed no physical changes were selected for further study. Composition of selected nanoemulsion formulations are given in Table 2.

Droplet size measurements

The mean droplet size of F1-F9 was in the range of 176.2–309.4 nm (Table 3) which was slightly higher than the usual nanoemulsion droplet size range of 5-200 nm [(2)]. An increase in Smix ratio from 4:1 to 5:1 and from 4:1 to 6:1 resulted in a decrease in droplet size in the formulations. This result is in accordance with the report that the addition of surfactant to nanoemulsion systems causes the interfacial film to condense and stabilize, while the cosurfactant causes the film to expand [(6)].

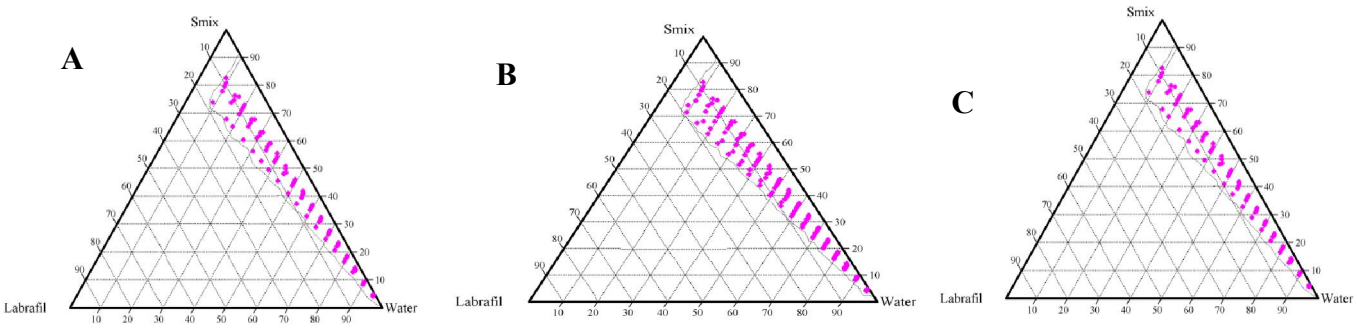
Transmission electron microscopy analysis

TEM determination is one of the studies conducted in order to confirm the particle size obtained by the laser scattering spectroscopy. Because recently people started to have doubts about measurements made by using laser scattering spectroscopy method which usually need significant dilution of samples. In the TEM image, the nanoemulsion appeared bright and the surroundings were dark (Figure. 2). The micrograph exhibits, the droplets size of the sample were in the range of nanoemulsion and in agreement with results obtained from droplet size analysis using Zetasizer.

Evaluation of microemulsion-based gel

Determination of pH

The pH of nanoemulsion based gel systems was found to be in the range of 5.6 to 6.29 (Table 3) which was within the acceptable limits for topical application.



Figure(1): Pseudoternary phase diagrams indicating oil-in-water nanoemulsion (shaded area) region of Labrafil (oil), Tween 80 (surfactant), and Ethanol (cosurfactant) at different Smix ratios indicated in parts A (Smix 4:1), B (Smix 5:1), C (Smix 6:1).

Table 2. Composition of selected nanoemulsion formulations prepared by spontaneous emulsification method.

Formulation Code	Smix (ratio)	Oil/ Smix (ratio)	% w/w of Components in Nanoemulsion formulation			Drug % w/w
			Oil	Smix	Water	
F_1	4:1	1:6	8.6	51.5	40	1
F_2	4:1	1:7	7.5	52.4	40	1
F_3	4:1	1:9	6.1	54.5	39.4	1
F_4	5:1	1:6	8.6	51.5	40	1
F_5	5:1	1:7	7.5	52.4	40	1
F_6	5:1	1:9	6.1	54.5	39.4	1
F_7	6:1	1:6	8.6	51.5	40	1
F_8	6:1	1:7	7.5	52.4	40	1
F_9	6:1	1:9	6.1	54.5	39.4	1

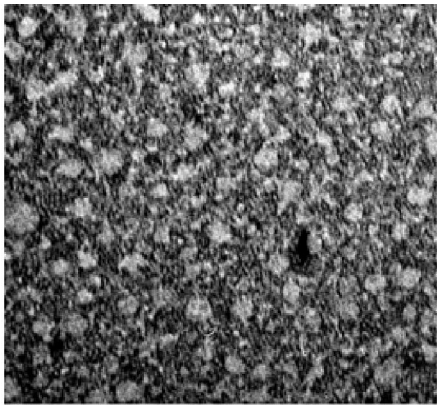


Figure 2.TEM photograph of F1 formulation.

Drug content studies

Drug content of all formulations was in the range (95.13-101.25%) as shown in Table 3.

Viscosity measurement

The viscosity of the nanoemulsion based gel systems could be observed from Table 3. The most viscous formulation was formulation F4 which has 845.1 Ps. followed by formulation F5 which has 797.1 Ps. F9 has the lowest viscosity compared to other formulations which was 281.2 Ps.

Table (3): Characterization of Nystatin nanoemulsion system

Code	Smix (ratio)	Oil/ Smix (ratio)	Droplet diameter (nm)	pH	% Drug content	Viscosity (Ps)
F_1	4:1	1:6	207.8±6.01	6.16	99.25±1.2	348.1±10.2
F_2	4:1	1:7	290.3±6.36	6.25	95.12±0.4	428.4±7.5
F_3	4:1	1:9	309.4±27.22	6.09	99.5±0.56	360.9±4.2

F_4	5:1	1:6	181.9±13.72	6.29	98.75±2.07	845.1±8.7
F_5	5:1	1:7	176.2±10.75	6.28	99.375±1.4	797.1±3.9
F_6	5:1	1:9	259.4±21.78	6.14	97.25±0.37	296.2±6.3
F_7	6:1	1:6	184.0±8.91	6.03	98.5±0.51	327.8±10.4
F_8	6:1	1:7	188.8±1.20	5.6	97.13±0.76	323.8±1.6
F_9	6:1	1:9	214.9±19.70	5.88	101.25±0.81	281.2±2.9

***In-vitro* release studies**

Dissolution studies by using semipermeable membrane were performed to compare the release of drug from nine different nanoemulsion based gel formulations (F1−F9) against marketed formulation having same quantity (1%) of nystatin (Figure 4). The release of drug from all nanoemulsion based gel formulations was much faster and higher in methanolic citrate-phosphate buffer (30%:70%) pH 5.5 than the marketed formulation. F1 showed highest drug release percent (49.13%) followed by F8 (45.69%) in contrast, the marketed formulation released (22.88 %) of the drug in 24 hrs due to low aqueous solubility . The comparative drug release profile is depicted in Figure 3. Reduction in the droplet size leads to higher surface area and higher dissolution of nystatin in oily phase of nanoemulsion based gel formulations eventually permitted drug release at faster rate from nanoemulsion based gel formulations showing the significance of the nanosizing of the oils globules [15]. By the study of *in vitro* drug release we can conclude that formulations F1 and F8 which having Smix ratio of (4:1 and 6:1 respectively) show better drug release.

The primary objective of a topical formulation for the treatment of cutaneous disease is that the drug reaches the target site at the required concentration and achieves its therapeutic action. Thus, the clinical efficacy of the formulation depends on the ability of the vehicle to release the drug which must then penetrate the stratum corneum. Therefore, this efficiency could be somehow evaluated by measuring the *in vitro* release of nystatin from different nanoemulsion based gel formulations.

Drugs can permeate the stratum corneum through 2 micropathways, one is the intercellular route and the other is the transcellular way. Of these routes, the intercellular route plays a major role in the percutaneous uptake of drugs. It is known that a complex mixture of essentially neutral lipids that are arranged as bilayers with their hydrophobic chains facing each other, form a hydrophobic bimolecular leaflet. Most of the lipophilic drugs pass through this region, and it is called the lipid pathway. Polar head groups of lipids face an aqueous region forming a polar route that hydrophilic drugs generally prefer.

A dermally applied nanoemulsion is expected to penetrate the stratum corneum and to exist intact in the whole horny layer, altering both the lipid and the polar path-ways [17]. The lipophilic domain of the microemulsion can interact with the stratum corneum in many ways. The drug dissolved in the lipid domain of the microemulsion can directly partition into the lipids of the stratum corneum, or the lipid vesicles themselves can intercalate between the lipid chains of the stratum corneum, thereby destabilizing its bilayer structure. In effect, these interactions will lead to increased permeability of the lipid path-way to the drugs.

On the other hand, the hydrophilic domain of the nanoemulsion can hydrate the stratum corneum to a greater extent, and plays an important role in the percutaneous uptake of drugs. When the aqueous fluid of the nanoemulsion enters the polar pathway, it will increase the interlamellar volume of the stratum corneum lipid bi-layers, resulting in the disruption of its interfacial structure. Since some lipid chains are covalently attached to corneocytes, hydration of these proteins will also lead to the disorder of lipid bilayers. Similarly, swelling of the intercellular proteins may also disturb the lipid bilayers; a lipophilic penetrant like nystatin can then permeate more easily through the lipid pathway of the stratum corneum [18].

In order to understand the complex mechanism of drug release from the nanoemulsion based gel formulations, the in-vitro nystatin release data were fitted to Korsmeyerpeppa's release model and interpretation of release exponent values (n) enlightens us in understanding the release mechanism from the dosage form (Table 4). The release exponent values thus obtained were from 0.7068 to 0.8109. Based on these values we can say that the formulation exhibited non-fickian transport. The formulations showed higher (r) values for zero order plots indicating that drug release followed zero order kinetics

From all of the previous studies, formula F1 and F8 showed both small droplet size and highest extent of drug release, so they have been chosen for further microbiological evaluation.

Table (4): Kinetic Profile of various formulations

Code	(n) value	r	Zero-order kinetic		Higuchi model		Possible mechanism Of drug release
			R	K	R	K	
F_1	0.7233	0.9803	0.9946	0.0313	0.9634	1.4261	Zero-order, Non-fickian
F_2	0.7068	0.9820	0.9962	0.0220	0.9723	1.0125	Zero-order, Non- fickian
F_3	0.7280	0.9897	0.9907	0.0184	0.9730	0.8525	Zero-order, Non- fickian
F_4	0.7400	0.9796	0.9838	0.0253	0.9498	1.1486	Zero-order, Non- fickian
F5	0.7930	0.9872	0.9941	0.0272	0.9619	1.2393	Zero-order, Non- fickian
F6	0.7635	0.9808	0.9789	0.0196	0.9732	0.9199	Zero-order, Non- fickian
F7	0.8052	0.9812	0.9900	0.0303	0.9770	1.4077	Zero-order, Non- fickian
F8	0.8109	0.9720	0.9874	0.0301	0.9604	1.3778	Zero-order, Non- fickian
F9	0.7585	0.9691	0.9953	0.0287	0.9645	1.3091	Zero-order, Non- fickian

Microbiological assay of nystatin

The effect of base composition on antimycotic activity of nystatin against *C. albicans* using agar dilution assay was investigated and the result showed in Table 5. F1 and F8 showed 58% and 54% reduction in Log_{10} CFU/gm respectively compared to the control value which was significantly greater than the percent reduction exhibited by the commercial preparation Nystatin® cream (35% reduction in Log_{10} CFU/gm). This may be explained by the higher release of nystatin from F1 and F8 formulations as well as the greater solubility of nystatin in the nanoemulsion based gel in comparison to the commercial preparation and hence greater partitioning of nystatin at the boundary between the diffusion medium and the preparation.

Clinical studies are in progress to get clearer idea on what happen when nystatin nanoemulsion based gel is applied topically.

Table (5): Colony count of *C. albicans* (RCMB 005002) after 24 hours.

Code	CFU/gm
Control	$2.7 \times 10^6 \pm 0.45$
Commercial	$1.4 \times 10^4 \pm 0.24$
F1 [a]	$9.4 \times 10^2 \pm 0.83$
F8 [a]	$5.1 \times 10^2 \pm 0.14$

N.B.: a is significant different from commercial cream using one way ANOVA followed by Turkey Kramer as post ANOVA test for multiple comparison at $P < 0.05$.

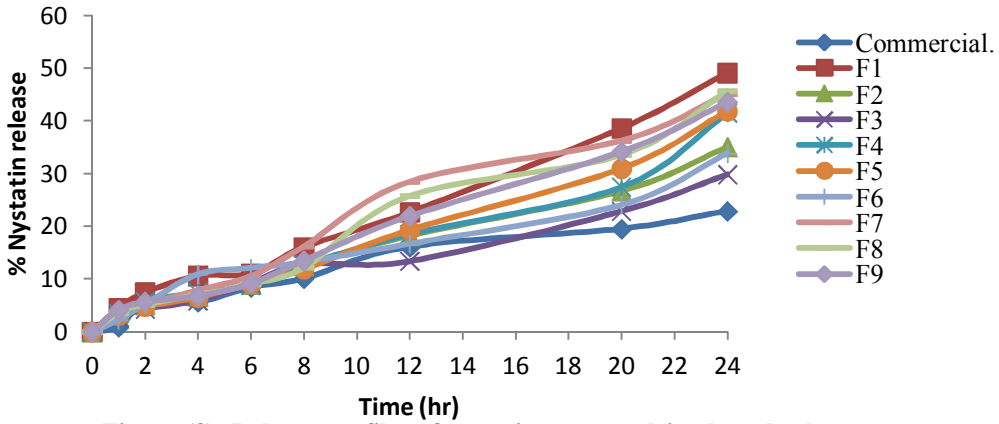


Figure (3): Release profiles of nystatin nanoemulsion based gel

Conclusion

In this work, nanoemulsion base gel with suitable viscosity was constructed to deliver nystattin for topical administration. The nanoemulsion base gel formulation of nystatin containing 8.6% of oil phase (Labrafil), 51.5% of Smix (4:1) (Tween 80 and ethanol) and 40% of distilled water has been optimized. From in-vitro data it can be concluded that the developed nanoemulsion-based gel have great potential for topical drug delivery.

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