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Design and Evaluation of Nystatin Emulgel For Superficial Skin Infection

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Abstract
Candida infection of skin, nails and vagina are very common worldwide. Cutaneous candidiasis is superficial mycotic infection of skin caused by the yeast candida albicans. Nystatin is a polyene antifungal characterized by broad spectrum of antifungal action including a wide range of pathogenic and non-pathogenic yeast & fungi. Nystatin is active against fungal pathogen including: candida, aspergillus, histoplasma, and coccidiosis's and has been used for yeast treat candida at the skin and those for mouth. Nystatin that interference with permeability of the cell membrane of sensitive fungi by binding to sterol, chiefly ergosterol. Its main action is against candida Albicans. Nystatin is come under BCS IV class drug having lower solubility & lower permeability made several challenges on topical formulation. Lower solubility & lower permeability can affect Bioavailability, permeability, penetration of drug as well as drug dissolution/diffusion study. The Aim of this study is improving Nystatin solubility, Dissolution rate & subcutaneous absorption to increase its efficacy for topical applications. This is achieved by gel-based emulsion-based gel. The emulsion-based gel has considered as one of the high advertence delivery system as it has binary release system which are gel and emulsion. The neem oil has an antibacterial and antifungal as well as antibacterial properties it has inhibitory action against candida albicans & aspergillus Niger.

Keywords
Antifungal, Candida, Emulgel, Fungi, Topical.

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INTRODUCTION:
Topical drug administration is localized drug delivery system anywhere in the body through ophthalmic rectal vaginal & skin as topical. Skin is one of the most accessible organs on the human body for topical administration and is main route of topical drug delivery system. This research work is concern with all detailed information regarding rational approach to topical formulation, basic components of topical drug delivery system over all clinical evidence indicated topical gel is safe and effective treatment option for use in management of skin related disease. Topical preparation is applied to skin or surface, local or systemic effects. Candida infection of skin, vagina & nails are very common worldwide the cutaneous candidiasis. Cutaneous candidiasis is a superficial mycotic infection of the skin usually caused by the yeast *Candida albicans*. The intertriginous skin folds are most frequently affected and patients who are obese or who have diabetes are at particular risk. Cutaneous candidiasis, usually caused by *Candida albicans*, may colonize occluded areas or folds of the skin, producing infection in areas such as the groin, axillae, and interdigital spaces. Clinical manifestations include erythema, scaling, maceration, vesicles, and pustules. The outcome of

treatment depends on the susceptibility of the pathogenic fungi to the antifungal agent. Little information is available, comparing the antifungal activity of commonly used agents against yeasts. 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. Nystatin exerts its antifungal activity by binding to sterols in the fungal cell membrane. As a result of this binding, the membrane is no longer able to function as a selective barrier, and potassium and other cellular constituents are lost. 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. The aim of this study is improving nystatin solubility, dissolution rate and subcutaneous absorption to increase its efficacy for topical application. This achieved by formulating nystatin in two different techniques, Nano emulsion and solid dispersion systems, then incorporating them into a gel base. Gel bases have gained more and more importance. This is because the gel bases are better percutaneous absorbed than cream and ointment. Topical drug delivery is a means for treatment of local infections via superficial application of a medicament to skin or mucous membrane. Its non-invasive nature, high patient compliance, and the absence of systemic toxicity, gastric irritation or exposure of secondary tissues to the relevant drug makes topical delivery a preferred administration route over other delivery systems. Topical delivery is associated with various barriers including the tight junction within the stratum corneum and high turnover rate of tissue fluids, such as in the eye, nose and vagina. These barriers limit the access of a drug/foreign moiety to its target site, reduce the residence period or result in rapid washout of a formulation from the application site. To improve therapeutic efficacy, various innovative formulations such as hydrogels, bio adhesive patches, rings, lenses, masks, lipid-based carriers and microneedles have been disclosed in published patents and continue to be explored. Modification of formulation excipients and the application of novel technologies can lead to the development of clinically effective, industrially viable and therapeutically safe topical drug delivery systems.

Theory:

Gel are defined as semi rigid system in which the movement of the dispersing medium is restricted by an interlacing three-dimensional network of particles or solved macromolecules of the dispersed phase. The gel word derived from “gelatin” and both gel and jelly can be drawn back to the Latin gelu for frost and gel are meaning “freeze” or “congeal”.

The USP defines gels as semisolid system containing either suspension made up of small in organic particles, or large organic molecules interpenetrated by a liquid. Where gel mass containing a network of small separate particles the gel is classified as a two-phase system in two phase system, if the particle size of dispersed phase is relatively large, the gel mass is sometime called magma. single phase gels consist of organic macromolecules uniformly circulated throughout a liquid in such way that no apparent boundaries occur between the dispersed macromolecules and the liquid.

Gels are generally considered to be more rigid than jellies because gels more covalent crosslinks, a higher density of physical bonds, or simply less liquid gel forming polymer produce material that span a range of rigidities beginning with a sol and increasing in rigidity to mucilage jelly gel and hydrogel. Some gel system is as clear as water and other are turbid because the ingredients may not be completely molecularly dispersed (soluble or insoluble), or they may form aggregates which disperse light. The concentration of the gelling agents is mostly less than 10% usually in 0.5% to 2.0% range with some exceptions.

CHARACTERISTICS OF GEL SWELLING:

Gels can swell absorbing liquid with an increase in volume this can be looked on as the initial phase of dissolution. Solvent penetrate the gel matrix so that gel-gel interaction is replaced by gel-solvent interactions. Limited swelling is usually the result of some degree of cross linking in the gel matrix that prevent total dissolution. Such gels swell considerably when the solvent mixture processes a solubility parameter comparable to that of the gallant.

Syneresis:

Many gel systems undergo concentration upon standing. The interstitial liquid is expressed, collecting at the surface of the gel this process is to referred as syneresis, is not limited to organic hydrogel but has been seen in organo gels and inorganic as well typically, syneresis becomes more pronounced as the concentration of polymer decreases. The mechanism of concentration has been related to relaxation of elastic stresses

developed during the setting of the gel. As these stresses is relieved, the interstitial space available for solvent is reduced, forcing the expression of fluid. Osmotic effects have been implicated as both pH and electrolyte concentration influence syneresis from gels composed of the ionic gel formers gelation or psyllium seed gum.

Ageing

Colloidal system usually exhibits slow spontaneous segregation. This process is referred to as ageing, in gels ageing results in the gradual formation of dense network of the gelling agent. The timer suggest that this process is similar to the original gelling process and continues after the initial gelation, since the fluid medium is lost from the newly formed gel.

Structure

The rigidity of a gel arises from the presence of a network formed by the interlinking of particles gelling agents. The nature of the particles and the type of force that is responsible for the linkage which determines the structures of the network and the properties of the gel.

Rheology

Solution of the gelling agents and dispersion of flocculated solid are pseudo plastic i.e. exhibiting non-Newtonian flow behaviour characterized by a decrease in viscosity with an increase in share rate. The tenuous structure of inorganic particles dispersed in water is disrupted by applied shear stress due to breaking down of interparticulate association, exhibiting a greater tendency to flow. Similarly, for macromolecules the applied shear stress aligns the molecules in the direction of stress straightening them out and lessening the resistance to flow.

MATERIAL AND METHOD:

Sr. No.	Material	Manufacturer
1.	Nystatin	Centurian Laboratory, Vadodara
2.	Neem Oil	Nagarjun Pharmaceutical PVT Limited, Gandhinagar
3.	HPMC	Analab fine chemicals, Mumbai
4.	CARBAPOL	Analab fine chemicals, Mumbai
5.	Teen 80	Research lab fine chem. industries, Mumbai
6.	Span 80	Research lab fine chem. industries, Mumbai
7.	Propyl Paraben	Ana lab fine chemicals, Mumbai
8.	Methyl Paraben	Analab fine chemicals, Mumbai
9.	Triethanolamine	Analab fine chemicals, Mumbai
10.	Propylene glycol	Loba chime Pvt. ltd. boiser, Palghar
11	Sodium Sulphite	Research lab fine chem. industries, Mumbai

Table No. 1 Material and their Manufacturer

Method of Preparation: -

Emulgel was prepared by Emulsification method. Following steps involved in formulation of Emulgel.

1) Preparation of Emulsion
A) Aqueous phase
B) Oleaginous phase
2) Preparation of Gel
3) Preparation of Emulgel

1) EMULSION

Emulsion prepared by an Aqueous & oleaginous phase.

A) Aqueous phase

The Aqueous phase as made by mixing of double distilled Water and Tween 80. The methyl paraben & propyl paraben dissolved in propylene glycol. And sodium sulphite added to the mixture of Tween 80 and double distilled Water. It was left for 24hrs.

B) Oleaginous phase

The oleaginous phase was Prepared by mixing of span 80, Nystatin, neem oil. The mixture was sonicated for 20 minutes. On the hot plate aqueous phase was heat up to 80°C, Oleaginous phase heat up to 70°C.

The aqueous phase was added to oleaginous phase with constant stirring by a magnetic stirrer at 2000 RPM and stirring was continued for 10-15 minutes until cooled at room temperature.

2) GEL

The gel was formulated by a dispersing a Carbopol in cold water and HPMC dissolved in hot water with continuously stirring at moderately speed of mechanical stirrer to get uniform solubility. The HPMC content added to Carbopol with constant stirring. Few drops of the triethanolamine was added to get acidic pH.

3) EMULGEL

The emulsion was mixed with gel in 1;1 ratio at 1500 RPM. To get uniform Emulgel.

Formulation	F1	F2	F3	F4	F5	F6	F7	F8	F9
Nystatin	01 gm	01gm	01 gm	01gm	01 gm	01gm	01gm	1 gm	1 gm
Neem Oil	10 ml	07ml	10 ml	07ml	10 ml	07ml	10 ml	07 ml	00 ml
HPMC	00	00	0.5gm	0.5gm	01gm	01gm	02gm	02gm	01gm
Carbopol	01gm	01gm	01gm	01gm	0.5gm	0.5gm	00gm	00gm	0.5gm
Propylene glycol	02 ml	20 ml	02ml	02 ml	02 ml	02 ml	02 ml	02 ml	00 ml
Tween 80	02 ml	02 ml	02 ml	02 ml	02 ml	02 ml	02 ml	02 ml	00 ml
Span 80	01 ml	01 ml	01 ml	01ml	01 ml	01 ml	01 ml	01 ml	00 ml
Propyl Paraben	0.02 gm	0.02 gm	0.02 gm	0.02 gm	0.02 gm	0.02 gm	0.02 gm	0.02 gm	0.02gm
Methyl Paraben	0.02 gm	0.02 gm	0.02 gm	0.02 gm	0.02 gm	0.02 gm	0.02 gm	0.02 gm	0.02gm
Sodium Sulphite	0.1 gm	0.1 gm	0.1 gm	0.1 gm	0.1 gm	0.1 gm	0.1 gm	0.1 gm	0.1 gm
Double Distilled Water	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml	100 ml	100ml	100 ml

Table No. 2 Formulation Ingredients

Preformulation Studies:

Preformulation is defined as an investigation of physical and chemical properties of a drug substance, alone and when in combined with excipients is called as Preformulation. Preformulation testing is the first in the rational development of dosage forms of a drug. The overall objective of developing the Pre-formulation testing is to generate information useful to the formulation developing stable and bioavailability of dosage forms which can be mass produced.

Description: The sample was evaluated visually for appearance, colour, odor.

Melting Point: The melting point of Nystatin was determined using the capillary tube method. It is observed value was compared with the reported value of Nystatin.

Solubility study:

The solubility study Nystatin and Neem oil was carried out in water as well as organic solvents. The 10mg of drug was taken in a three-test tube and recommended volume of respected solvent was added. Test tubes were shaken and observed for clarity of a solution.

IR spectrum:

The drug and potassium bromide (Kbr) disk were prepared manually by press method. About 1mg of drug was triturated with about 10mg of dry Kbr and the pressed into the pallet manually. Shimadzu iris 400 was used to obtain IR spectra of the prepared disc of ibuprofen. The scanning range was 400-4000cm. The spectrum was compared with that reported in literature. Potassium bromide was used as a blank while running spectrum.

Drug polymer compatibility study:

Fourier transformers infrared analysis:

The physical mixture of drug (Nystatin) with each of the excipient was prepared in1:1, The sample was kept in 38^0C for 15days and were analyzed for any interaction in between the drug and excipient. The analysis was done as per the procedure.

Evaluation of Emulgel:

Physical Appearance
pH
Viscosity
Drug content
Spread ability
In vitro dissolution Study
Stability Studies

Appearance:

In appearance prepared nystatin emulgel formulation were examined visually for their appearance such as a color, homogeneity, consistency. Consistency can visualize by phase separation of Emulgel.

Viscosity:

The viscosity of Emulgel was determined by Digital Brookfield viscometer at 10 RPM using spindle 6, At room temperature.

pH:

pH of topical dosage form is very important as it may cause irritation to the skin if varied from its normal pH Condition. The pH of Emulgel was measured by calibrated Digital PH meter using pH 4.0 & 7.0 standard buffer.

Drug content:

Weigh the Emulgel equivalent to 20 mg of Nystatin was taken in 100 ml volumetric flask containing 15 ml Methanol and stirred for 30 min. the remaining volume made up with Phosphate buffer and appropriates dilution were made. The resultant solution was filtered through 0.45 micro meter filter. The absorption of the solution was measured by a spectrophotometrically at 305nm.

In vitro drug release studies:

Apparatus: Diffusion Cell
Media: 25 ml of Phosphate Buffer.
Time intervals: 0, 0.5, 1, 2, 3, 6, 8 Hours.

Temperature: $37^0C \pm 0.5^0C$
The in vitro drug release from Emulgel was carried out using Diffusion cell at 50 rpm and $37^0C \pm 0.5^0C$. Phosphate Buffer was used as the dissolution medium. 01 ml of dissolution medium was withdrawn at predetermined time intervals and fresh dissolution medium was replaced. The samples were withdrawn at regular intervals and analyzed by UV spectrophotometer at 305 nm for the presence of the drug.

RESULTS AND DISCUSSION:

Evaluation of Emulgel:

1) Appearance
A) color
B) Homogeneity
C) Phase Separation
2. Viscosity.
3. pH
4. Drug Content
5. Spread ability
6) In vitro drug release studies

Appearance of Emulgel:

Color, Homogeneity, phase separations and Texture were found to be acceptable limit. The phase separations do not occur in any formulation and have a good Consistency & excellent homogeneity, which is indicates the good & very stable Formulation

Batch	Color	Homogeneity	Phase separations
F1	Yellow	Excellent	No separation
F2	Yellowish	Excellent	No separation
F3	Light Yellow	Excellent	No separation
F4	Yellow	Excellent	No separation
F5	Pale Yellow	Excellent	No separation
F6	Yellowish to slight brown	Excellent	No separation
F7	Yellow	Excellent	No separation
F8	Yellowish to brown	Excellent	No separation
F9	Light Yellow	Excellent	No separation

Table No 3: Color, Homogeneity and Phase separations of Emulgel.

Viscosity:

The viscosity of Nystatin Emulgel formulation is done as per standard procedure. It indicates increases the concentration of Carbopol 940 results in increase in the viscosity. The Carbopol and HPMC contributes pseudo plastic system. Where increment of polymer concentration results in the formulation weaker & impairment intermolecular bond between polymers molecule not covalent in nature. The viscosity of Emulgel was found as shown in table No. 4.

pH:

The Ph evaluations of Topical Dosage form is very important as it may cause irritation to skin if varied from the normal the pH of the skin condition. the pH of all batch formulation is done as per standard procedure. It indicates more polymer like Carbopol give consistency & having acidic nature. HPMC gel was found to be stable in Ph 3-11. The pH range of all the formulation was found to be within Acceptable range. The pH of all batches was found as shown in table no 4.

Drug content:

The drug content of all formulations was carried out as a per standard procedure. It also indicates the concentration of neem oil is increase results in increase solubility of Nystatin molecules lead to drug content. The Drug content was found to be shown in table no. 4.

Spread ability:

The Spread ability of Emulgel batches was done as per standard procedure. The Spread ability was shown in table.

Sr. No.	Viscosity	pH	Drug Content	Spread ability cm/sec
1	3772	6.86	100.4	1.50
2	3475	6.59	98.56	1.4
3	3752	7.49	97.81	1.35
4	3180	7.15	99.74	1.4
5	3265	7.25	98.14	1.76
6	3024	6.98	100.8	1.77
7	3251	7.58	99.28	1.38
8	2846	7.65	99.08	1.33
9	4800	7.87	93.68	1.01

Table no. 4 Viscosity, pH, Drug content of Formulation

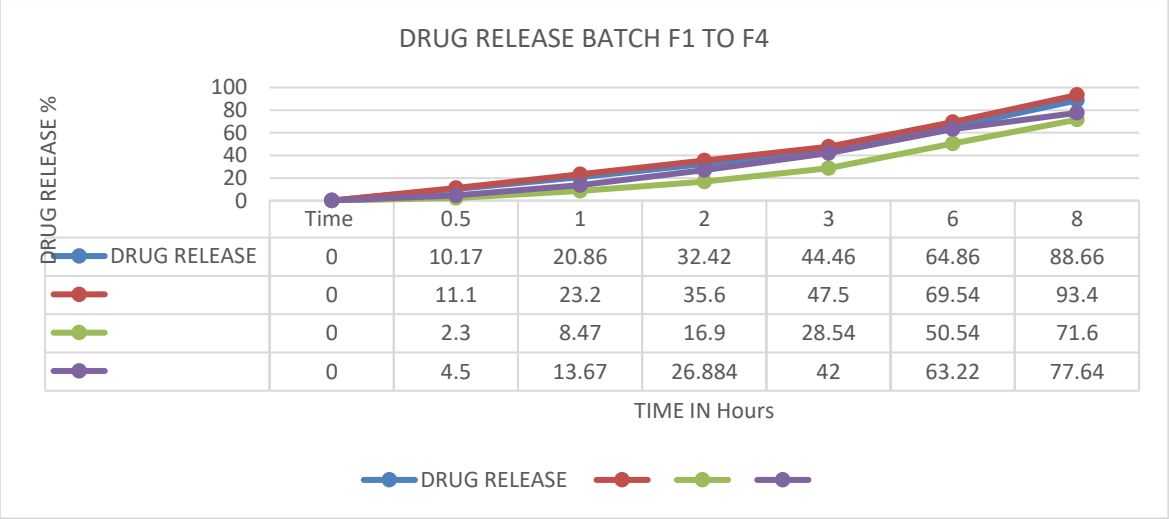
***In Vitro* Dissolution study Emulgel:**

The dissolution study was carried out using the Diffusion studies. The dissolution profile of Nystatin Emulgel shows total drug release within 8 Hrs. the drug release profile was significantly increase in formulation no 6 while drug release profile was significantly lowest dissolution rate. The drug release profile of Emulgel influenced by the concentration and types of Gelling agent, polymer, neem oil, surfactant, the Carbopol 940 provide viscosity to formulation and to form gel matrix than HPMC. The concentration of neem oil effects on drug release by greater hydrophobic matrix that decreases the dissolution media to penetrate the matrix and make a greater diffusion pathway for drug reaches to dissolution medium. The Emulgel dissolution profile show in following table.

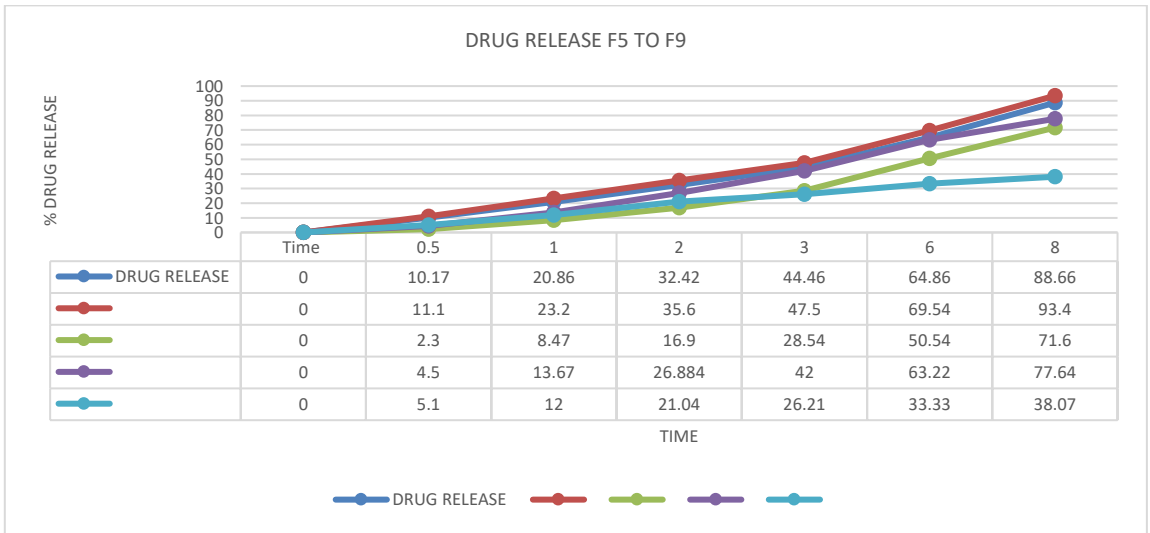
Drug Release Batch 01 to 09

TIME	F1	F2	F3	F4	F5	F6	F7	F8	F9
0.5	10.17	02.24	03.84	00.40	10.17	11.17	02.30	04.50	05.10
1	21.67	08.81	11.34	10.00	20.86	23.20	8.47	13.67	12.00
2	34.91	26.72	21.11	21.92	32.42	35.60	16.90	26.88	21.04
3	44.50	43.56	32.94	34.83	44.46	47.50	28.54	42.00	26.21
6	49.46	48.80	53.20	64.22	64.86	69.54	50.54	63.22	33.33
8	51.70	57.82	67.04	73.46	88.66	93.40	71.60	77.64	38.07

Table No. 5 % Drug Release Studies



Graph 1 Drug release Batch 0 1 to 04



Graph 2 Drug Release Batch 05 to 09

Stability Studies:

The nystatin emulgel formulation was packed & sealed in aluminum tubes (5 gm) and subjected to different temperature Which are 25,40, & 40 DC for 6 months. The specimen was collected after six months of storage and estimated for all parameter such as Appearance, phase separations, pH, drug content.

Sr No.	Colour	Phase Separation	homogeneity	pH	Viscosity	Drug content
01	Yellow	No separation	Excellent	6.81		100.4
02	Yellowish	No separation	Excellent	6.53		98.56
03	Light Yellow	No separation	Excellent	7.46		97.81
04	Yellow	No separation	Excellent	7.06		99.74
05	Pale Yellow	No separation	Excellent	7.12		98.14
06	Yellowish to slight brown	No separation	Excellent	6.84		100.8
07	Yellow	No separation	Excellent	7.72		99.28
08	Yellow	No separation	Excellent	7.49		99.08
09	Yellowish	No separation	Excellent	7.61		93.68

Table no. 6 Stability Data of Formulation

SUMMARY AND CONCLUSION:

Nystatin is a polyene anti-fungal used in the treatment of topical and transdermal fungal infection. The aim of the present study was to design and develop Nystatin emulsion-based gel for efficient delivery of drug to the skin with the help of Neem oil. Micro emulsion-based gel showed greater antifungal activity against Candida albicans as compared to control formulations.

The nystatin gel-based emulsion provides many advantages such as binary release systems which are gel and emulsion, easily spreadable, greaseless, emollient, can deliver both hydrophilic and hydrophobic drugs, easily removable non-staining, bio-friendly, water-soluble, higher stability, transparent and highly attractive appearance these characteristics enhance patient compliance.

The concomitant administration of the Neem oil that has antifungal activity with nystatin lead to modification of the therapeutic effect of nystatin and produce a synergistic effect that improve therapeutic efficacy.

From in vitro release analysis and stability study, the optimized formula (F6) which provide higher release rate in comparison with other nystatin gel-based emulsion formula and nystatin gel that make it promising formula in protection of topical surface from fungal infection.

The nystatin gel-based emulsion formulations with dual delivery system provide better release for nystatin in compare to nystatin gel that make gel-based emulsion superior on gel for delivery hydrophilic and hydrophobic drugs.

Nystatin is categorized as antifungal drug and is successfully used to treat Fungal disorders. Nystatin maximum wavelength is determined by UV-Visible spectrophotometer.

Nystatin Emulgels was formulated using Span 80 & Neem oil as oil phase and emulsifying agent's tween 80 for emulsion and incorporated into gel using HPMC and Carbopol 940 polymers in 1:1 ratio.

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