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FORMULATION AND EVALUATION OF SOLID LIPID NANOPARTICLES BASED GEL CONTAINING AN ANTIFUNGAL AGENT

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Abstract

The objective of present investigation was to prepare & evaluate the solid lipid nanoparticle (SLN) based gel using an antifungal agent Miconazole nitrate (MN). Compatibility of drug and excipients were confirmed by IR studies. The formulation was prepared by Micro emulsion and homogenization method using stearic acid and tween 80. All the Solid lipid nanoparticle formulations were subjected to particle size, drug entrapment efficiency, scanning electron microscopy and Differential Scanning Calorimetry. Formulation F2 resulted in least SLN particle size. The F3-SLN dispersion showed higher entrapment efficiency of 78.7%. Scanning Electron Microscopy of prepared SLN particles showed that prepared particles were spherical in shape and surface was rough. The Differential Scanning Calorimetry of the SLN particles showed conversion of drug from crystalline to amorphous state. The prepared SLN particles were dispersed in HPMC Carbopol gel. The resulting SLN loaded gels were evaluated for appearance, spreadability and in vitro drug release. The SLN loaded gels showed good appearance and spreadability. The invitro drug release of SLN gel was evaluated using Franz diffusion cell using dialysis membrane 70 with phosphate buffer pH 7.4 as a dissolution media. All the formulations showed drug release till 24hrs. It was concluded that Miconazole Nitrate loaded SLN gel formulation containing Carbopol 940 in combination with Hydroxy Propyl Methyl Cellulose was suitable for topical application since it showed prolonged drug release.

Keywords: Carbopol 940, Hydroxy Propyl Methyl Cellulose, Miconazole nitrate, solid lipid nanoparticle.

Introduction

Delivery of drug to the skin is an effective & targeted therapy for local dermatological disorders.[1] Topical application have many advantages over the conventional dosage forms, especially to avoid some serious

systemic adverse effect [2], when the drug is delivered topically it can penetrate deeper into skin and since MN is a BCS class II drug have better penetration & hence it shows better absorption.[3,4] Topical route of drug delivery has gained popularity because it avoids the first pass effect, gastrointestinal irritation & metabolic degradation associated with the oral administration. Solid lipid nanoparticles are the advanced drug delivery system in which the active ingredient is incorporated into lipid carrier (e.g. Triglycerides, fatty acid, steroids, partial glycerides & waxes) & it is stabilized by using the biocompatible surfactant such as poloxamer, polysorbate, lecithin.[5] SLN particle size ranges from 50 to 1000 nm.[6] Poorly soluble drugs can be formulated in the form of SLN alone, the SLN has been considered as promising carriers for drug delivery. The SLN have higher entrapment efficiency for hydrophobic drugs in the core compared with conventional liposomes.[7]

Gels are homogeneous, semisolid preparations. It consists of two-phase system in which organic particles are not dissolved but merely dispersed throughout the continuous phase & large organic particles are dissolved in continues phase. They are normally prepared with the suitable gelling agents like HPMC, Carbopol & sodium CMC etc.[8] Gels often provide a faster release of drug substance[9] fungal infections are very common in human beings especially in tropical regions.[10,11] Fungal infection traditionally have been divided into systemic & superficial [12] the antifungal agent are developed against some fungus to least the serious fungal infections.

The antifungal agents are miconazole nitrate, ketoconazole, fluconazole etc. all those drugs work by slowing the growth of fungi that cause infection (https://www.medscape.com/view). Miconazole nitrate is a broad spectrum antifungal drug.[13,14]

MN has limited dissolution properties because of its poor aqueous solubility which also limits its antifungal activity. MN has a higher efficacy in the treatment of anaerobic & protozoal bacterial infection of dermal, buccal, vagina & oropharyngeal candidiasis.[15,16]

Experimental

Material and Methods:

Miconazole nitrate was received as a gift sample from Micro Labs limited, Hosur, HPMC and Carbopol 940, stearic acid, tween 80, glycerol was kindly provided by East Point College of Pharmacy.

Compatibility test by Infrared spectroscopy (FTIR)

The infrared spectroscopy was used to ensure the compatibility between drug and other excipients. The physicochemical characterization was performed using FTIR spectroscopy. About 1-2mg of Samples were mixed with the KBr pellets compressed into a disc, and then scanned from 4000 cm^{-1} to 400cm^{-1}.

Preparation of MN loaded SLN dispersion:

SLNs loaded with the Miconazole Nitrate were prepared by Micro emulsion and homogenization method. Miconazole Nitrate were dissolved in methanol and mixed with acetone solution containing stearic acid. The above mixture is then sonicated for 20 minutes. The lipid mixture is added drop wise to the aqueous solution (tween 80 solutions) & stirred at 3000 rpm for 50 minutes at 70° C temperature. The mixed solution is then transferred to the icy water bath and stirred for 4 hours at 3000rpm.[17] Different formulations of drug loaded SLN was prepared by varying the concentration of stearic acid as shown in the table 1.

Table 1: Quantity of substances taken for preparation of Solid lipid Nano Particles based gel.

Formulation code	Drug (mg)	Stearic acid (mg)	Tween 80 (%)	Carbopol 940 (mg)	HPMC (mg)
F1	100	1000	1.5	500	500
F2	100	1000	2	500	500
F3	100	1000	2.5	500	500
F4	100	1500	1.5	500	500
F5	100	1500	2	500	500
F6	100	1500	2.5	500	500

HPMC - Hydroxy Propyl Methyl Cellulose

Formulation of miconazole nitrate loaded SLN Based gel:

SLN gel was prepared by taking the required quantity of carbopol940 & HPMC are weighed & dispersed in small quantity of distilled water to prepare aqueous dispersion. The dispersion was than hydrated for 2 to 3 hours. The glycerol was added subsequently to the aqueous dispersion then 1% of Miconazole Nitrate was incorporated in it. Triethanolamine was added to the above dispersion using overhead stirrer.[18-21]

Characterization of MN Loaded SLN Dispersion

The SLNs characterization parameters are particle size, particle distribution, zeta potential, drug entrapment efficiency the details are described below:

Particle size, particle size Distribution Zeta potential:

The mean particle size and polydispersity index of SLN for size distribution was measured using Malvern Master sizer.

Drug Entrapment efficiency: The encapsulation efficiency of nanoparticles was determined by dissolving the nanoparticles in distilled water and then separating the nanoparticles from the aqueous medium by ultracentrifugation at 5300rpm for 70 min. The supernatant solution was separated and diluted with methanol. The amount of free Miconazole nitrate in the supernatant was measured by UV spectrophotometer at 272 nm. The Miconazole nitrate entrapped in the nanoparticles was calculated by using below formula:

$EE=(Wa-Ws/Wa)\times100$

Where EE is entrapment efficiency, Wa stands for mass miconazole nitrate added to the formulation and Ws stands for analyzed weight of drug in supernatant.[22-25]

Scanning electron microscopy

The morphological characteristic of SLN was determined by Scanning electron microscopy. One drop of sample was placed on a slide & excess water is left to dry at room temperature, then the slide was attached to the specimen holder using a double coated adhesive tape & gold coated under vacuum using sputter coater for 10mins and investigated.[26]

Differential scanning calorimeter Analysis (DSC)

The SLN were analyzed by using differential scanning calorimeter as maintaining a constant scanning speed. The DSC Thermograms were obtained at temperature range of 30-250^{0}C & scanned rate of 10^{0}C/ min. [27-29]

Evaluation of Miconazole Nitrate Loaded SLN Gel

Physical appearance:

The prepared SLN gel was observed visually for the color, homogeneity.

Spreadability study of SLN gel:

1g of SLN gel was placed between horizontal plates. A 100g of weight was placed on the upper glass plate for 5 minutes to compress the formulation. The time in seconds to sperate the slides was taken as the measure of spreadability. The spreadability was calculated by using the formula

$S=M\times L/T$

Where S is spreadability, M is weight tied on upper slide, L is the length of glass slide & T is time taken

Invitro drug release studies of SLN based gel

The invitro drug release studies were performed by using Franz diffusion cell to evaluate the amount of MN released from each formulation. It consists of donor Dialysis membrane 70 having pore size 2.4nm, the surface area of release membrane was 3.14cm^2. The receptor medium was approximately 45ml & composed of phosphate buffer pH 7.4 stirred by magnetic bead at 100rpm to avoid differences in concentration within the acceptor medium & to minimize stagnant layers. SLN based gels (equivalent to 1mg of drug (MN) were placed in the donor compartment. During the experiments, the solution in receptor side was maintained at 37^{o}C$\pm$ 0.5^{o}C. After certain time intervals 3ml of the sample medium were withdrawn from receiver compartment and same volume of freshly prepared receptor medium were added. The samples were analyzed by uv-visible spectrophotometer at 272 nm. For each formulation, the release studies were performed in triplicate.

Results and Discussion

FTIR Analysis

Data acquired from FTIR Spectrophotometric studies of drug excipients mixtures stored at 40°C$\pm$2/75%$\pm$5 RH, indicates no significant changes in spectra. FTIR Spectra of Miconazole Nitrate and mixture of Miconazole nitrate with excipients indicated no characteristic changes in spectra. The results of the FTIR studies proved there is no incompatibility between miconazole nitrate and other selected excipients.

Particle size, particle size distribution & zeta potential

The particle size was determined by using Malvern Mastersizer. The particle size of different formulation F1 to F6 are 300, 163.5, 330, 1002, 257 and 205 nm respectively. F4 formulation showed highest particle size since it contained high concentration of stearic acid and least concentration of tween 80. Formulation F2 was

smallest SLN particles since it contained lesser amount of stearic acid and intermediate concentration of tween 80.

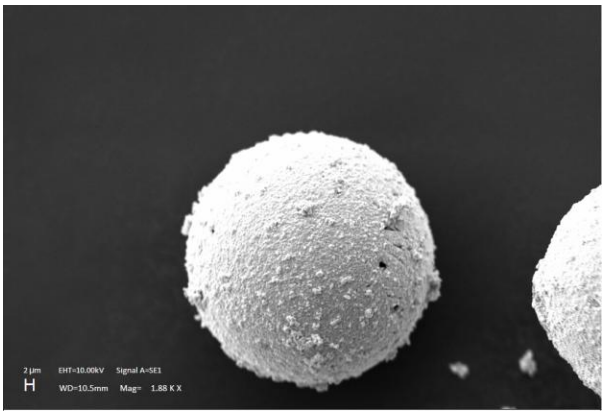
Drug entrapment efficiency

From the results obtained, it was observed that the F3 have a higher entrapment as compared to other formulation, F3 have 78.7% where as other formulation like F1 have 70.04%, F2 have 70.7%, F4 have 75.04%, F5 have 63% & F6 have 52.14% entrapment efficiency. Formulations containing 1000mg stearic acid showed EE in 70% range with highest for F3. However, formulation containing 1500mg stearic acid showed comparatively lower EE in the range 50-75%.

Scanning Electron Microscopy

The morphology of MN solid lipid nanoparticles is characterized by scanning electron microscopy. It was evident from SEM (figure 1) of Formulation F3 that the shape of SLN particles was spherical and surface was rough.

Figure 1: SEM of F3 Formulation.



Differential scanning calorimeter Analysis (DSC)

Differential Scanning Calorimetry (DSC) is one of the most widely used calorimetric techniques to characterize the solubility and physical state of drug in lipid vesicles. The DSC analysis of the pure drug MN (figure 2) and the prepared SLN of MN (figure 3) were studied. The DSC analysis for pure MN showed a large endothermic peak at 186.13 °C, which represents the melting point of MN. This peak disappeared in the DSC thermogram of MN SLN particles prepared with stearic acid and Tween 80. The disappearance of the melting endotherm of MN suggested the presence of drug in a more soluble amorphous state. The change in melting behavior of MN could be due to the inhibition of its crystallization and solubilization in

solid lipid nanoparticles since the drug was dissolved in the methanol. Therefore, it could be concluded that the MN in the prepared SLN particles was in an amorphous form. The physical state transformation of a drug to an amorphous or partially amorphous state leads to a high-energy state and high disorder, resulting in enhanced solubility in stearic acid and hence better permeation across skin.

Figure 2: DSC for Miconazole Nitrate.

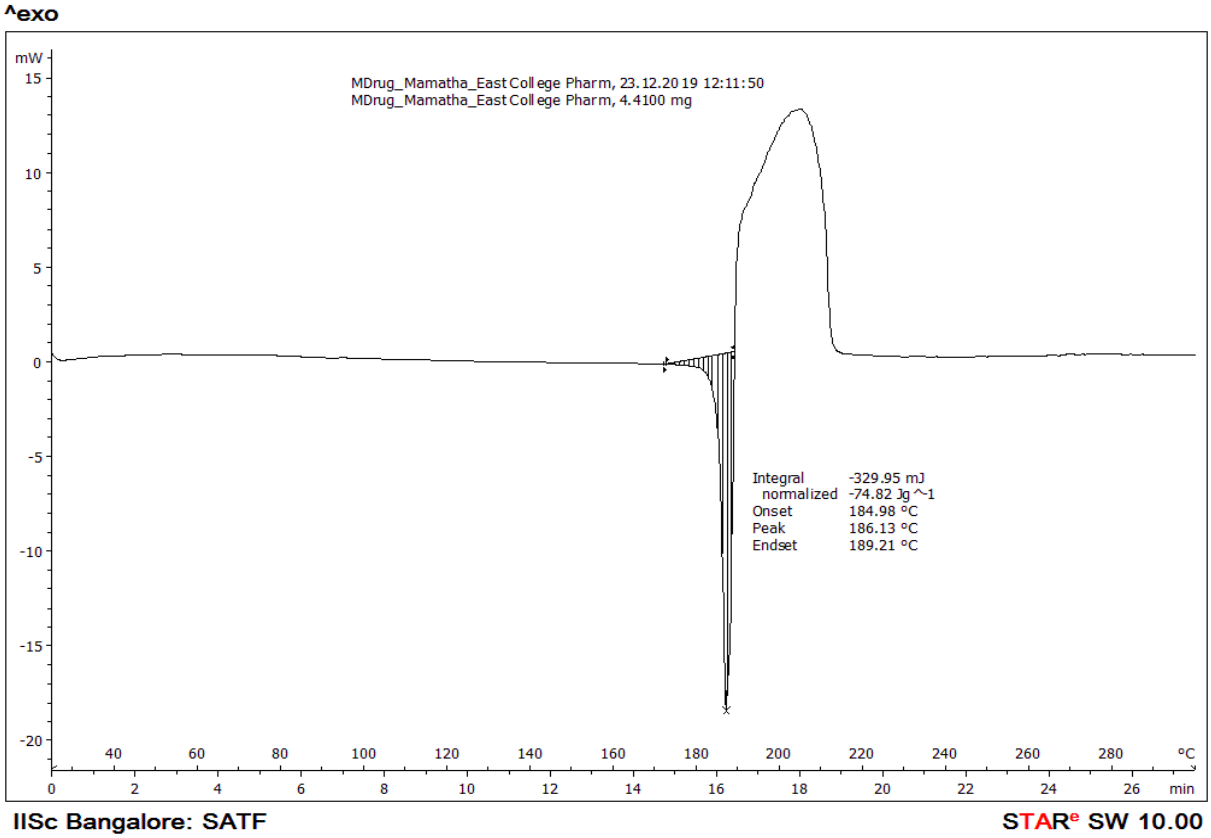
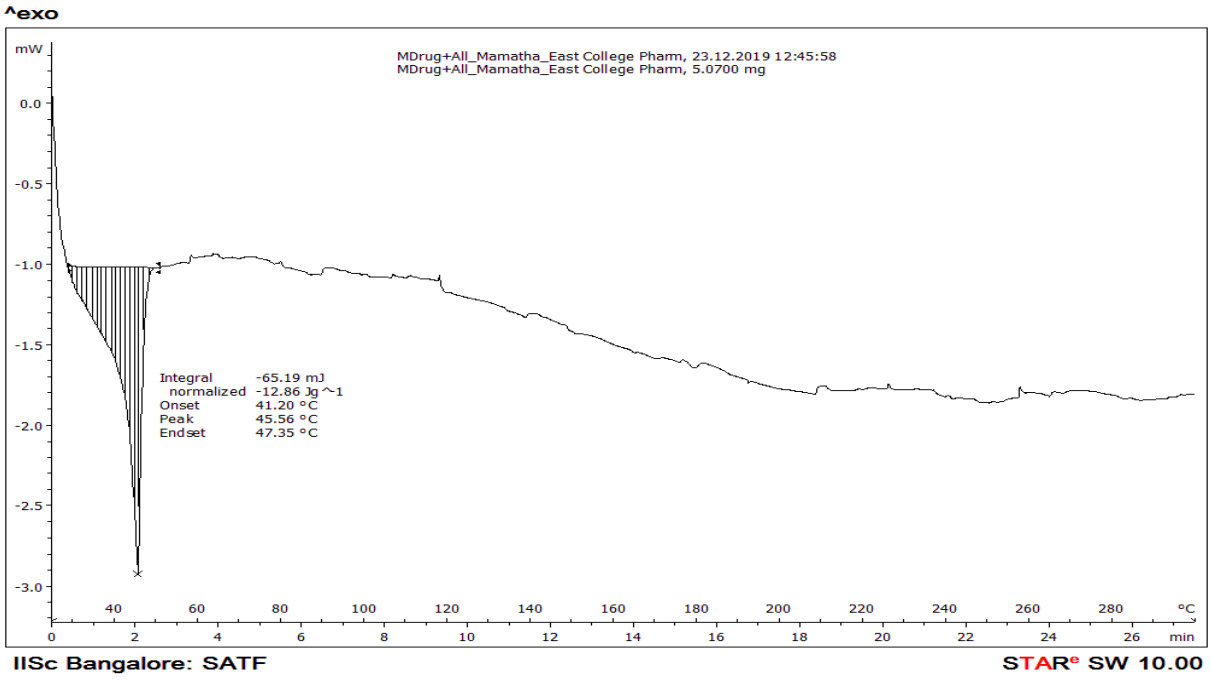


Figure 3: DSC Graph for Miconazole nitrate loaded SLN



Evaluation of Miconazole Nitrate Loaded SLN Based Gel

Physical appearance:

All the formulation was found to be homogenous. The physical appearance of the MN loaded SLN gel was found to be off white in color, had smooth texture and were translucent.

Spreadability study of SLN gel:

The Carbopol 940 and HPMC in combination shows good spreadability (table 2). Higher the value of spreadability better the formulation of the SLN based gel. The highest spreadability was observed for the formulation F6 with the spreadability value of 236.91 which may be due to the smaller SLN particle size. The spreadability for formulation F4 was lesser which may be attributed to the larger particle size of the SLN particles.

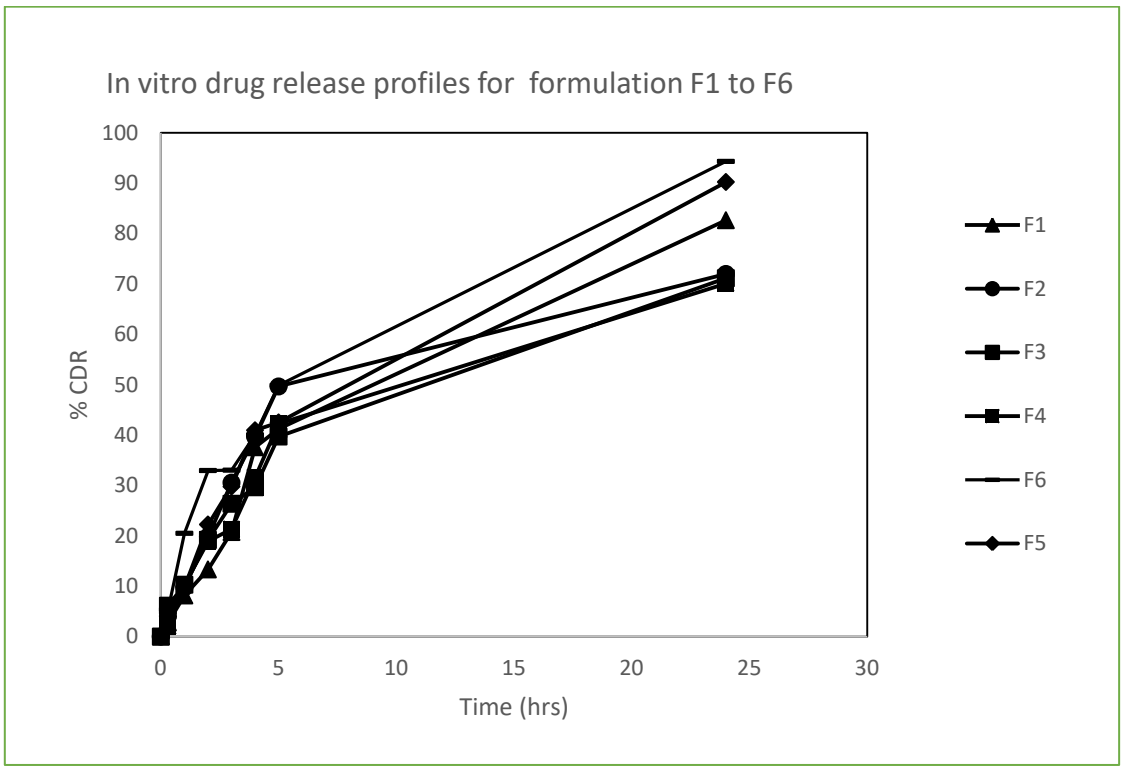
Table 2: Spreadability of SLN loaded gel.

Sl. No.	Formulation code	Spreadability (g.cm/sec)
1	F1	219.64
2	F2	200.04
3	F3	226.2
4	F4	209.7
5	F5	222.3
6	F6	236.91

In vitro release study of SLN gel

The invitro studies were performed to compare the release rate of the drug from the SLN gel formulation. The details are shown in figure 4 Percent of drug release at the end of 24hrs was found be maximum for formulation F6 (94.3%) and minimum for formulation F4 ((94.3%). There was no direct effect of stearic acid on the drug release of the formulations. however minimum drug release for F4 may be attributed to the larger size of SLN particles. Similarly, maximum drug release for formulation F6 may be due to comparatively smaller size of SLN particles.

Figure 4: In vitro drug release profiles for formulation F1 to F6.



Conclusion

The Miconazole nitrate loaded solid lipid nanoparticle-based gel was successfully prepared for topical delivery. The SLN dispersion was prepared by Micro emulsion and homogenization method, the physicochemical characterization includes particle size, particle size distribution, scanning electron microscopy, drug entrapment efficiency, spreadability, physical appearance, and drug release profile was carried out. It was shown that stearic acid had no much influence on the entrapment but however size of the SLN had an effect on drug release and spreadability of gels. The invitro drug release of SLN gel showed prolonged drug release. It was concluded that Miconazole Nitrate loaded SLN gel formulation containing Carbopol 940 in combination with HPMC was suitable for topical application & shows better results for antifungal action

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References

1. Kikwail L, Babu RJ, Prado RA, Kolot A, Armstronhg CA, Ansel JC et al. Invitro and invivo evaluation of topical formulations of spantide II. AAPS PharmasciTech 2005; 6(4): 562-72.

2. Whitehouse MW. Anti-inflammatory glucocorticoid drugs: reflection after 60years. Inflammo pharmacology. 2011; 19(1): 1-19.
3. Glavas-Dodov M, Fredro-Kumbaradzi K, Goracinova S, Calis S, Simonoska M, Hincal AA. Fluorouracil, in topical liposome gels for anticancer treatment- formulation and evaluation. Acta Pharmaceutica. 2003; 53(4): 241-250.
4. Rupal J, Kaushal Shetty JC, Mallikarjuna SC and Dipti P. Preparation and evaluation of topical gel of valdecoxib. International journal of Pharmaceutical science and drug Research. 2010; 2(1): 51-54
5. Uner M and Yenes G. Impotance of solid lipid Nanoparticles [SLN] in various administration routes and future perspectives. International journal of Nanomedicine. 2007; 2(3): 289-300.
6. Zur Muhlen A, Schwarz C, & Mehnert W. Solid lipid nanoparticles (SLN) for controlled drug delivery, drug release & R mechanism. European Journal of pharmaceutics. 1998; 45(2): 149-155
7. Rajashekar Kammari, Sudip K. Das, in; Nanoparticulate System for Therapeutic & Diagnostic Application, 2017
8. Vijay Kumar Singh et al.m vol 3, issue 7, 2013 www.ajprcom
9. Abdel- Hamid S.M., Abdel- Hady S.E., EI-Shamy A.A, EI- Dessouky H.F. Formulation of an antispasmodic drug as atopical local anesthetic. Int. J. Pharm. 2006; 326: 107.
10. Dash K, Basak S, Ray S. A study on Superficial fungal infection from west Bengal. A Brief Report. J Life Sci. 2009; 1: 51-55
11. Van Minnebruggen G, Francosis IEJA, Cammue BPA, The vissen K. Over view on past, present & future antimycotics. The open mycolgy Journal. 2010; 4: 22-32
12. Bennett JE., In: Hardman J.G & Limbird L.E. Eds; The pharmacological basis of therapeutics; Antimicrobial agent: Antifungal agent; 10^{th} ed., 2001.p.1295
13. Bensadoun RJ, Daoud J, Gueddari B, et al. Comparison of the efficacy & Safety of miconazole 50-mg mucoadhesive buccal tablets with miconazole 500-mg gel in the treatment of oropharyngeal candidiasis. Cancer. 2008; 112(1): 204-211.
14. Menes AI, Silva AC, Catita JA, et al. Miconazole-loaded nanostructured lipid carriers (NLC) for local delivery to the oral mucosa:improving antifungal activity. Colloids Surf Biointerfaces. 2013;11:755-763.

15. Ellepola AN, Samranayake LP. Antimycotic agents in oral candidosis: An overview:2. Treatment of oral candidosis. Dent Update. 2000; 27(4): 165-174.
16. Cerderia AM, Mazzotti M, Gander B. Miconazole Nanosuspensions: influence of formulation variables on particle size reduction & physical stbility. Int J Pharm. 2010; 396: 210-218.
17. Dongefei L, Sunmin J, Hong S, Shan Q, Juanjuan.L, Qing, Z. Diclofenac sodium-loaded solid lipid nanoparticles prepared by emulsion/solvent evaporation method. J.Nanopart Res.2010;10(1007):1105-10.
18. Lala R.R., Awari N.G. Nanoemulsion-Based Gel Formulations of COX-2 Inhibitors for Enhanced Efficacy in Inflammatory Conditions. Appl Nanosci. 2014; 4: 143–51.
19. Bhalekar M R, Pokharkar V, Madgulkar A, Patil N, Patil N K. Preparation and Evaluation of Miconazole Nitrate-Loaded Solid Lipid Nanoparticles for Topical Delivery. AAPS PharmSciTech. 2009; 10(1): 289–96.
20. Sanad A R, Abdelmalak N S. Formulation of a Novel Oxybenzone-Loaded Nanostructured Lipid Carriers (NLCs). AAPS. PharmScitech. 2010; 11(4): 1684-94
21. Joshi M, Patravale V. Nanostructured Lipid Carrier (NLC) Based Gel of Celecoxib. Int. J.Pharm. 2008; 346: 124-32
22. Doktorovova S, Araujo J. Formulating fluticasone propionate in novel PEG-containing nanostructured lipid carriers (PEG-NLC). Colloids and Surfaces B: Biointerfaces. 2010; 75: 538–42.
23. Biswal B, Karna N, Nayak J, Joshi V. Formulation and Evaluation of Microemulsion based topical Hydrogel containing Lornoxicam. Journal of Applied Pharmaceutical Science. 2014; 4(12): 77-84.
24. Mazumder B, Dey S, Bhattacharya S, Sarkar S, Mohanta B. Studies on Formulation and Characterization of Cellulose-Based Microspheres of Chlorpheniramine Maleate. Arch Pharm Sci& Res. 2009; 1: 66-74.
25. Sachan NK, Bhattacharya A. Modeling and Characterization of Drug Release from Glutinous Rice Starch Based Hydrogel Beads for Control Drug Delivery. Int. J. of health research. 2009; 2(1): 93-99
26. Nasr M, Mansour S, Mortada ND. Lipospheres as Carriers for Topical Delivery of Aceclofenac: Preparation, Characterization and In Vivo Evaluation. AAPS PharmSciTech. 2008; 9(1): 154-62.

27. Ahmed A, Ghourab M, Gad S, Qushawy M. The application of Plackett-Burman design and response surface methodology for optimization of formulation variables to produce Piroxicam niosomes. Int. J. Drug Dev. Res. 2013; 5: 121–30.
28. Ahmed T. Preparation of transfersomes encapsulating sildenafil aimed for transdermal drug delivery: Plackett–Burman design and characterization. J. Liposome Res. 2015; 25: 1–10.
29. Abdallah M. Transfersomes as a transdermal drug delivery system for enhancement the antifungal activity of nystatin. Int. J. Pharm. Pharm. Sci. 2013; 5: 560–67.

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