IJPSR (2015), Vol. 6, Issue 2 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 18 June, 2014; received in revised form, 28 August, 2014; accepted, 17 October, 2014; published 01 February, 2015

IN-VITRO EVALUATION OF SHEA BUTTER-BASED NYSTATIN MICROPARTICLES

H. C. Nwuke [1], I. T. Nzekwe [*2], C. O. Agubata [3], A. A. Attama [1], K. C. Ofokansi [1], O. Okorie [4], V. C. Okore [1] and C. O. Esimone [5]

Department of Pharmaceutics [1], University of Nigeria, Nsukka, Enugu State, Nigeria
Department of Pharmaceutics and Pharmaceutical Technology [2], Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.
Department of Pharmaceutical Technology and Industrial Pharmacy [3], University of Nigeria, Nsukka, Enugu State, Nigeria.
Department of Pharmaceutics and Pharmaceutical Technology [4], University of Port Harcourt, Rivers State, Nigeria.
Department of Pharmaceutical Microbiology and Biotechnology [5], Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

Keywords:

Microparticles; Antifungal, Nystatin, Shea Butter, Lipid

Correspondence to Author:

I. T. Nzekwe

Department of Pharmaceutics and Pharmaceutical Technology, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria

E-mail: pharmzekwes@gmail.com

ABSTRACT: Nystatin, an antifungal drug for mucosal infections has a large dosing frequency and is rapidly inactivated by acids. In order to increase drug stability and reduce dosing frequency and reduce dosing frequency and gastric irritation, formulation into inert lipid matrices as solid lipid microparticles may be utilized. The formulations were done from Shea butter extract using either Tween 20 or Tween 65. The mixture was homogenized in each case to enable the incorporation of drug into solid lipid microparticles. The properties of the microparticles were evaluated by determination of size, percentage recovery, encapsulation efficiency and drug release in simulated gastric fluid (without enzymes). The antimicrobial activities of the microparticles were then investigated by the agar-diffusion inhibition zone diameter method against *Candida albicans*, using pure nystatin dispersed in DMSO as a control. Micron-sized particles with a narrow size distribution were obtained. Though drug encapsulation efficiency and loading were very low, good activity was exhibited against the test organism, *in vitro*. The antimicrobial activity and release profile obtained with Batch A containing Tween 65 indicated that it could be used to formulate sustained release nystatin microparticles with good anti-candida activity.

INTRODUCTION: Many antimicrobial agents are limited by poor pharmacokinetics, leading to variable and incomplete absorption. Colloidal particles formed from solid lipid systems are an attractive option for drugs due to their ease of preparation, stability, potential for slow release or depot effects and capacity to improve the absorption of lipophilic drugs.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.6(2).624-29

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.6(2).624-29

Several such systems exist such as solid lipid nanostructured [1, 2, 3] lipid carriers [4-5], solid lipid microparticles [6] as well as liposphereres [7, 8] etc. Though these several types of lipid-based carrier systems exist each with its own merits and demerits, selection of a carrier system and its excipients should be dictated by the physical properties of the drug, the route of administration sought, safety/toxicity considerations, drug loading and scalability, and also ease of preparation. Solid lipid microparticles, because of their large exposed surface areas can potentially improve contact area between drug and skin or mucosa, enhance drug permeability and, by their matrix nature, protect acid-labile drugs from inactivation. In addition,

they can serve as slow release "depot" from which drug is released by diffusion or surface erosion, maintaining adequate drug levels over a long period of time. This can reduce dosing frequency, reduce side-effects, improve compliance and help prevent microbial resistance which can stem from low drug pressures. Though they can also be prepared by several different techniques [9], hot-homogenization technique is a very convenient scalable and modifiable method which has gained wide acceptance. Solid lipid microparticles have been used as oral [10, 6] topical [11, 12] and parenteral deliveries [13].

Nystatin, a polyene antifungal produced from *Streptomyces noursei* is structurally similar to amphotericin B and they share the same mechanism of action. It is not absorbed and the oral preparation has been used rather in treating mucocutaneous mycoses 14 such as oral and intestinal candidal infections. Due to instability due to long exposure to light, heat and air [15] or acid inactivation [16], the goal of application of solid lipid microparticle technology to formulation of nystatin is to preserve molecular stability, and possibly confer sustained release effect, since its dose frequency in most conditions is fairly high (four times a day). The hydrophobic core of solid lipid systems provides a cloistered microenvironment where the drug is shielded from the activities of reactive species in solution such as hydrogen ions. In addition, attainment of higher drug stability can lead to reduced dose size and lead to miniaturization, thereby saving cost. Therapeutic activity at reduced doses can also help contain the incidence of dose-related side effects, such as gastrointestinal disturbances.

Shea butter is a cream coloured fatty substance extracted from the nuts of *Vitellaria paradoxa*, which grows abundantly in the savannah regions of West and Central Africa. It is widely consumed as food, being the most important source of dietary fatty acid and glycerol, [17, 18] and also finds use in cosmetic preparations for its moisturizing quality. On the other hand, polysorbates have long been used to facilitate the solubilisation of poorly soluble drugs [19] and to stabilize emulsions [20, 21].

Following some success in the development of intralipid-based parenteral nystatin with reduced toxicity [14], it is necessary to study the applicability of Shea butter as lipidic material in the re-formulation of oral nystatin as solid lipid microparticles using common surfactants. It is hoped that this can increase acid stability and confer sustained release effects. Moreover, the use of a homolipid instead of admixtures will waive the complex regulatory considerations involved in mixed lipid systems or admixtures.

MATERIALS AND METHODS:

Materials

Tween 65 and Tween 20 (Merck), n-hexane (May and Baker). Nystatin (Mekophar, Vietnam) was procured locally from a pharmacy shop. Distilled water was obtained from an all-glass still. Raw shea butter seeds were purchased from the local market. All other reagents were of analytical grade and were used as such without further treatment.

Extraction of shea butter

A 10 kg quantity of the yellow waxy shea butter from the local market was extracted using 1.5 liters of n-hexane. The system was left undisturbed for 24 hrs after which the supernatant was decanted into an evaporating dish where the solvent was allowed to evaporate, leaving only the yellow semi-solid.

Extraction of pure nystatin from tablets

Sixty tablets of nystatin equivalent to 30, 000 units of nystatin were first ground and then extracted with agitation over 4 hours using a mixture of glacial acetic acid (25 ml) and methanol (250 ml). The system was filtered and the solvent evaporated at room temperature under a current of air. The percentage recovery was 95 %.

Preparation of solid lipid microparticles

A preliminary study was first conducted which investigated the influence of varying drug and surfactant ratios on formulation isotropicity. This established that stable samples could be prepared from Tween 65 and 20 according using the quantities shown in **Table 1**. The three batches were prepared using the same procedure. Briefly, 1 g quantity of nystatin was dispersed in 1ml of dimethyl sulfoxide (DMSO) and a 10 g of melted shea butter was incorporated into the dispersion. Surfactant dissolved in 45ml of warm water was introduced into the drug-Shea butter dispersion

followed by high speed homogenization in a vortexing mixer (Sonik, Japan) for 5 minutes. Three batches of solid lipid microparticles were produced according to the quantities shown in **Table 1**. The systems were allowed to stand for 24 hours after homogenization before analyses.

TABLE 1: QUANTITIES OF INGREDIENTS USED IN PREPARING THREE BATCHES OF SOLID LIPID MICROPARTICLES

Batch	Shea butter (g)	Nystatin (g)	Tween type	Tween conc.
A	10	1	65	2.5 g
B	10	1	20	1.5 ml
C	10	0	20	1.5 ml

Micrography

A small quantity of each preparation were dropped on a microscope slide, covered with a cover slip and viewed under oil immersion in a microscope enabled with a camera. The sizes of the individual microspheres in each batch were measured using a meter rule, while correcting for the magnification of the microscope.

Percentage recovery and encapsulation efficiency

The solid lipid microparticles were recovered by filtration and weighed. Percentage recovery is the ratio of the final weight of the microparticles to the sum of the weights of drug and excipients, expressed as a percentage.

$$Percentage\ Recovery = \frac{W1}{W2+W3} \quad (1)$$

Where W1 is the mass of micropartciles; W2 is the mass of nystatin and W3 is the combined masses of Shea butter and Tween.

Encapsulation efficiency (EE) is the ratio of actual to theoretical drug content, also expressed as a percentage. These determinations were done for each formulation.

$$(EE,\%) = \left(\frac{Actual\ drug\ entrapped}{theoretical\ amount\ of\ drug\ added}\right) \times 100\,\% \quad (2)$$

Antimicrobial activity

A Saboraud dextrose agar plate was seeded with *Candida albicans*. A hole was bored in each of four sections of the plate and each hole was filled with one of the batches (the fourth was pure nystatin dispersed in DMSO. The plates were appropriately labeled underneath and incubated at 25 ^{o}C for 48 hours. This was done in triplicate. The inhibition zone diameters produced were measured at the end of the period [22].

Drug release studies

Equal amount (100 mg) of batches A and B SLMs (containing nystatin) was encapsulated in a size 300 gelatin shell. The capsule was placed in a basket which was immersed in simulated gastric fluid (SGF) (without enzymes) at 37 ^{o}C. Stirring was achieved by means of a magnetic stirrer assembly operating at 50 rpm. At suitable time intervals, a 2 ml sample was withdrawn from the medium and analyzed for content of nystatin by taking absorbance readings in a Pye Unicam SP6-450 UV/Vis spectrophotometer (Philips, England) at 305 nm (previously determined). The corresponding concentrations of drug were calculated using a calibration plot previously obtained in the medium.

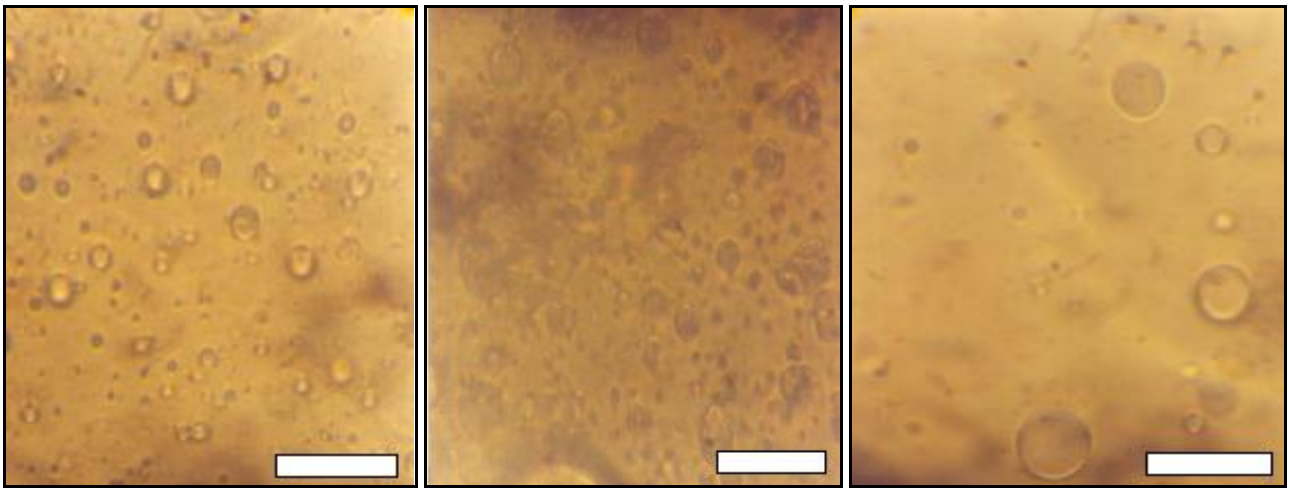
RESULTS AND DISCUSSION:

Size, morphology and drug loading

The micrographs are presented in **Figure 1**. The mean particle sizes of batches A, B and C were 16.25 µm (± 0.01), 29.69 µm (± 0 0.01) and 31.3 µm (± 0.02) respectively. The particles were roughly microspherical in shape, which is not always the case [6, 9]. Surface characteristics differed, being smooth in Batch C (no drug) and roughest in Batch B.

The explanation offered is that the surface is affected by the presence of drug crystals [8] and also nature of excipients used [23]. Such surface-residing drug loads could be quantified using X-ray photoelectron spectroscopy [9], particularly if the drug molecule contains functional growths absorbing differently from matrix material.

Low standard deviations indicated that size distribution was narrow, as previously reported [8]. Though light microscopy could be easily used in this way to determine size and morphology, it is not suitable for use in counting the rather high numbers of particles (300 – 500) needed to fully unravel particle size distribution and detect the presence of submicronic aprticles [9].



A B C

FIG. 1: MICROGRAPHS OF BATCHES OF NYSTATIN SOLID LIPID MICROPARTICLES (BAR REPRESENTS 50 µm)
Batch A contained nystatin, shea butter and Tween 65
Batch B contained nystatin, shea butter and Tween 20
Batch C contained shea butter and Tween 20 without nystatin

In this case, only about 32 particles or less were counted for each batch. Though drug loading should lead to increased size [24], unloaded batch (C) exhibited higher particle sizes and may have a vesicular nature.

Optimum particle size is dependent on intended application and route of administration. For particles intended to be absorbed topically, sizes need be smaller than 3 µm (such as solid lipid nanoparticles) [7, 25] in order to eliminate kinetic barriers. For drugs which need not undergo absorption like nystatin, the thermodynamic stability of the microparticle system would be an important parameter, in order to prevent size growth with time. This growth tendency is more likely in nanosubdivision, due to the high surface energies. As with all such lipid-based carrier systems, small particle sizes are can be used by using including high pressure homogenization step, using high concentrations of surfactants [1] or reducing the concentration of lipid which would affect homogenization efficiency.

Very low encapsulation efficiency was obtained for the two batches. This could be due to a number of factors such as low drug solubility in the lipid. In general, higher encapsulation efficiencies are obtained with lipophilic drugs which dissolve in the lipid matrix such as gonadotropin-releasing hormone [26]. Entrapment can be promoted by dissolving the drug first in a solvent in which it is soluble, such as methanol or propylene glycol.

Trapping may also be promoted by heating the drug to melting, in order to promote formation of mixed drug-lipid crystals. This temperature has been determined for nystatin by Samein[27]as 158-160 °C, who was able to get higher drug trapping by aiding drug solubilization using a co-solvent system of propylene glycol and glyceryl monostearate. However, this higher temperature could affect the physical stability of the lipid and cause polymorphic changes.

TABLE 2: ENCAPSULATION EFFICIENCY, PERCENTAGE RECOVERIES, AND INHIBITION ZONE DIAMETERS OF THREE BATCHES OF NYSTATIN SOLID LIPID MICROSPHERES.

Batch	Encapsulation Efficiency (%)	Percentage recovery (%)	Mean IZD* (mm) ± SEM
A	9	77	31.0 ± 0.47
B	3.5	90.4	35.7 ± 2.13
C	-	92	12. 0 ± 0.47
Pure nystatin	-	-	41.3 ± 0.55

*Except for C, the rest contained equal nystatin concentrations (20 mg/ml).
A contained Shea butter, Tween 65 and nystatin
B contained Shea butter, Tween 20 and nystain
C contained Shea butter, Tween 20 and no drug
Pure nystatin was dispersed in DMSO.
No of replicates equals 3. The inhibition zone diameter was measured after incubation at 25 ^{o}C for 48 hours against Candida albicans.

In order to promote trapping and modify drug release, mixed lipid systems such as Shea butter and goat fat admixtures [8] have been tried successfully, but concerns about the regulatory

burdens on development of such complex systems abound. Moreover, techniques which promote drug trapping may not necessarily promote drug release, and this would affect antimicrobial activity. Drug crystal extrusion may also occur in highly crystalline lipid

Antimicrobial activity and drug release

The inhibition zone diameters (IZD) produced by the two formulations of equal nystatin content (20 mg/ml), as well as batches C (no drug) and D (pure drug), are presented in **Table 2**. Inhibition zone is seen in Batch C, which contained no nystatin. This could be due to the small antimicrobial activity of Tween 20 [28], which also explains the slightly higher activity seen in Batch B.

The release curve for pure nystatin suggests delayed drug release but this may be due to poor drug wettability in the absence of surfactant, in contrast to the others. The poor percentage drug release obtained for all samples in 120 minutes may be associated with degradation of drug in acid environment, and correlates with pharmacopoeial prescription that analysis should be done within 30 minutes of dissolution in hydrochloric acid. After the onset of drug release, Batch A approximated zero order kinetics (r^2 of 0.9). Non-swelling matrix systems may be expected to show good correlation with Higuchi square root law, but this is not the case. Complex mechanisms or combinations of mechanisms may therefore be involved.

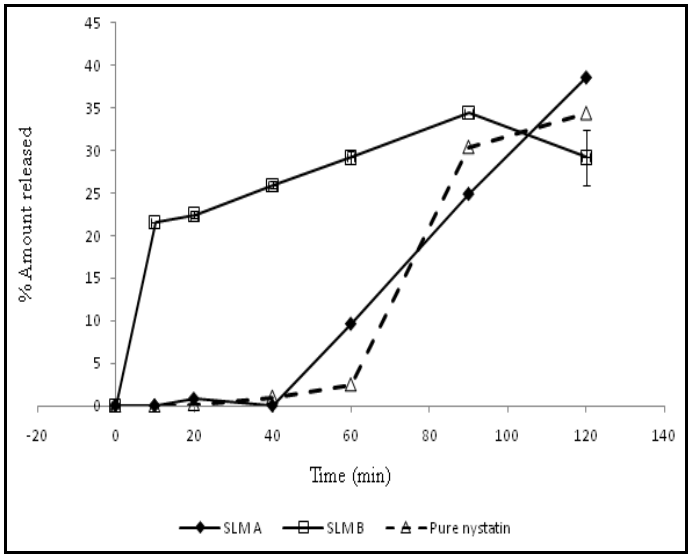


FIG. 2: RELEASE PROFILE OF BATCHES OF NYSTATIN SLM IN SIMULATED GASTRIC FLUID.

Equal quantity (100 mg) of SLM preparation or pure nystatin was used in the test in total medium volume of 100 ml.

TABLE 3: CORRELATION COEFFICIENTS FOR DIFFERENT KINETIC TREATMENTS OF NYSTATIN RELEASE DATA FROM TWO SLM BATCHES

Kinetic model	Zero order	First Order	Higuchi
R^2 (Batch A)	0.90	0.89	0.70
R^2 (Batch B)	0.53	0.56	0.78

Zero order release controlled entirely by dissolution would be an advantage in development of sustained release formulations. This is particularly relevant in developing low-frequency nystatin regimens which would also avoid the toxicities seen in fluctuating-concentration systems. This will minimize dose dumping and reduce toxicity without sacrificing efficacy [29].

CONCLUSIONS: The foregoing discussion reveals that nystatin can be loaded into SLMs for oral delivery in the management of mucocutaneous mycoses. The lipid ingredient used is readily available, and the formulation does not involve many processing steps. Though drug entrapment was very poor, formulation of nystatin in Shea butter-Tween 65 SLM (Batch A) preserves the antimicrobial activity of nystatin against *Candida albicans*, *in vitro* and also exhibits sustained release profile. This has the advantages of economy and efficacy, and safety as it guarantees non-fluctuating drug levels in intestinal fluid. It can also improve compliance due to reduced dosing frequency. Further studies will seek to improve entrapment efficiency.

REFERENCES:

1. Mulla JS and Khazi IM: Influence of process variables on particle size of solid lipid nanoparticles. Indian Journal of Novel Drug Delivery 2009; 1(1): 47-49.
2. Gasco MR, Priano L and Zara GP: Solid lipid nanoparticles and microemulsions for drug delivery: The CNS. Progress in Brain Research 2009; 180: 181-192.
3. Cavalli R, Marengo E, Rodriguez L and Gasco MR: Effects of some experimental factors on the production process of solid lipid nanoparticles. European Journal of Pharmaceutics and Biopharmaceutics 1996; 43 (2): 110-115
4. Müller RH, Radtke M, Wissing SA: Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations. Advanced Drug Delivery Reviews 2002; 54 (Suppl 1): S131–55.
5. Varshosaz J, Eskandari S and Tabakhian M: Production and optimization of valproic acid nanostructured lipid carriers by the Taguchi design. Pharmaceutical Development and Technology 2010; 15(1): 89–96.

6. Mezzena M, Scalia S, Young PM, Traini D: Solid Lipid Budesonide Microparticles for Controlled Release Inhalation Therapy. AAPS Journal 2009; 11 (4): 771-778.
7. Nasr M, Mansour S, Mortada ND and El Shamy AA: Lipospheres as Carriers for Topical Delivery of Aceclofenac: Preparation, characterization and *in vivo* Evaluation. AAPS PharmSciTech 2008; 9 (1): 154-162.
8. Esimone CO, Attama AA, Osonwa UE, Nwakile CD and Onochie ITO: Formulation and evaluation of goat fat and shea butter based lipospheres of benzyl penicillin**.** International Journal of Pharmaceutical Science and Research 2012; 3(4): 1022-1027.
9. Jaspart S, Piel G, Delattre L and Evrard B: Solid lipid microparticles: formulation, preparation,characterization, drug release and applications. Expert Opinion Drug Delivery 2005; 2(1): 75 87.
10. Nnamani PO, Attama AA, Ibezim EC and Adikwu MU: SRMS142-based solid lipid microparticles: application in oral delivery of glibenclamide to diabetic rats. European Journal of Pharmaceutics and Biopharmaceutics 2010; 76(1): 68-74.
11. Passerini N, Gavini E, Albertini B, Rassu G, Di Sabatino M, Sanna V, Giunchedi P, Rodriguez L: Evaluation of solid lipid microparticles produced by spray congealing for topical application of econazole nitrate. Journal of Pharmacy and Pharmacology 2009; 61(5): 559-67.
12. Martins RM, Siqueira S, Fonseca MJ and Freitas LA: Skin penetration and photoprotection of topical formulations containing benzophenone-3 solid lipid microparticles prepared by the solvent-free spray-congealing technique. Journal of Microencapsulation, Early Online 2014; 1-10.
13. Li LC, Zhu L, Song JF, Deng JS, Bandopadhyay R and Wurster DE: Effect of solid state transition on the physical stability of suspensions containing bupivacaine lipid microparticles. Pharmaceutical Development and Technology 2005; 10(2): 309-18.
14. Semis R, Nili SS, Munitz A, Zaslavsky Z, Polacheck I and Segal E: Pharmacokinetics, tissue distribution and immunomodulatory effect of intralipid formulation of nystatin in mice. Journal of Antimicrobial Chemotherapy 2012; 67: 1716–1721.
15. Nichols WK: Anti-Infectives. Remington: The Science and Practice of Pharmacy. Lippincott Williams and Wilkins, Philadelphia, Edition 20, 2000: 1507-1561.
16. British Pharmacopoeia. Her Majesty's Stationery Office, London, Vol. I, 2001: 1190.
17. Letchamo W, Khoo BK and Hartman TG: Evaluation of the quality of West African shea butter (*Vitellaria paradoxa*). International Society for Horticultural Science 2007; 756: 273–288.
18. Honfo FG, Hell K, Akissoé N, Coulibaly O, Fandohan P and Hounhouigan J: Effect of storage conditions on microbiological and physicochemical quality of shea butter. Journal of Food Science and Technology 2011, 48(3):274–279.
19. Saha P and Kou JH: Effects of solubility of excipients on permeation of poorly water-soluble Compounds across Caco-2 cell monolayer. European Journal of Pharmaceutics and Biopharmaceutics 2000; 50: 403-411.
20. Trotta M, Gallarate M, Pattarino F and Morel S: Emulsion containing partially water- miscible solvents for preparation of drug nanosuspensions. Journal of Controlled Release 2001; 76: 119-128.
21. Kim CK, Cho YJ and Gao ZG: Preparation and evaluation of biphenyl dimethyldicarboxylate microemulsion for oral delivery. Journal of Controlled Release. 2001; 70: 149-155.
22. Okore VC: Pharmaceutical Microbiology: Principles of the pharmaceutical application of antimicrobial agents. El' Demak Publishers, 2005.
23. Savolainen M, Khoo C, Glad H, Dahlqvist **C** and Juppo AM: Evaluation of controlled-release polar lipid microparticles. International Journal of Pharmaceutics 2002; 244: 151-161.
24. Passerini N, Perissutti B, Moneghini M, Voinovich D, Albertini B, Cavallari C and Rodriguez L: Characterization of carbamazepine-Gelucire 50/13 microparticles prepared by a spray-congealing process using ultrasounds. Journal of Pharmaceutical Sciences-US 2002; 91 (3): 699-707.
25. Escribano E, Calpena, AC, Queralt J, Obach R and Domenech J: Assessment of diclofenac permeation with different formulations: anti-inflammatory study of a selected formula. European Journal of Pharmaceutical Sciences 2003; 19: 203–210.
26. Del Curto MD, Chicco D and D'Antonio M: Lipid microparticles as sustained release system for GnRH antagonist (Antide). Journal of Controlled Release 2003; 89: 297-310.
27. Samein LH: Preparation and evaluation of nystatin loaded-solid-lipid nanoparticles for topical delivery. International Journal of Pharmacy and Pharmaceutical Sciences 2014; 6 (Suppl 2): 592-597.
28. Lawrence MJ and Arthur HK: Handbook of Pharmaceutical excipients. American Pharmaceutical Association, Washington, Third Edition, 2000: 416-418.
29. Sheppard D, Lampiris HW and Katzung GW: Basic and Clinical Pharmacology. The McGraw-Hill Company, Singapore, Ninth Edition, 2001: 793.

How to cite this article:
Nwuke HC, Nzekwe IT, Agubata CO, Attama AA, Ofokansi KC, Okorie O, Okore VC and Esimone CO: *In-Vitro* Evaluation of Shea Butter-Based Nystatin Microparticles. Int J Pharm Sci Res 2015; 6(2): 624-29.doi: 10.13040/IJPSR.0975-8232.6 (2).624-29.