Nigerian Journal of Pharmaceutical Research
Vol. 8 No 1 pp. 218 - 228 (September 2010)

IN VITRO EVALUATION OF POLYSORBATE -20- BASED NIOSOMAL FORMULATIONS OF SALICYLIC ACID

C. O. Esimone[1], N. C. Obitte*[2], A. N. Onuora[3] and V.I. Onyishi[2].
[1]*Dept. of Pharmaceutics and Pharmaceutical Technology, Nnamdi Azikiwe University, Awka, Anambra State.*
[2]*. Dept. of Pharmaceutical Technology and Industrial Pharmacy, University of Nigeria, Nsukka.*
[3]*Department of Pharmaceutics, University of Nigeria, Nsukka*

*Correspondence: Nicholas Obitte (obittenick@yahoo.com)

Abstract
Niosomes are multiparticulate non-ionic surfactant vesicular systems that consist of lipid (often cholesterol) and a non-ionic surfactant. The objective of the study was to attempt a preliminary formulation of salicylic acid into niosomes for the possible potential improvement of its dermal permeation into warty and corny skin surfaces for expected easy and fast desquamation (for warts and corns) and enhanced penetration in fungal infections. The niosomes were formulated with polysorbate-20 and cholesterol using the Lipid Film Hydration Technique. Veegum and cetylpyridinum were incorporated in subsequent batches to investigate the effect of ionic components on the stability of the niosomes. Drug release profiles in Krebs-Ringer solution and distilled water respectively were studied to also evaluate niosome stability and release characteristics. Results showed that batch A_2 with a concentration ratio of 15 mM: 15 mM of polysorbate-20 and cholesterol, gave the highest encapsulation efficiency (EE), while batch A_4 with a ratio of 5 mM: 5 mM recorded the least EE. The presence of the charged components had a varied effect on the stabilities, release profiles and EEs of the niosomes depending on the molar concentration and the osmotic concentration of the drug release medium. We therefore conclude that the niosomes-stabilized salicylic acid is a potential alternative formulation approach for anticipated improvement on the permeation of salicylic acid into corns, warts and some other inflammatory conditions of the skin.

Keywords: nonionic surfactant, stability, encapsulation efficiency, Veegum, cetylpyridinum, vesicle, salicylic acid, warts.

INTRODUCTION

Niosomes are one of the vesicular drug delivery systems which involve the entrapment of active pharmaceutical ingredient/drug using a lipid (often cholesterol) and a non-ionic surfactant. They are non-ionic surfactant-based vesicles formed from the self assembly of non-ionic amphiphiles in aqueous media resulting, in a closed bilayer structure (Florence, 1993) on the application of some energy (physical agitation or heat). This leads to an assembly where the hydrophobic portions of the molecules are shielded from the aqueous solvent and the hydrophilic portions maintain maximum contact with the aqueous medium.

Nonionic surfactant vesicles (Niosomes) are formulation carriers for transdermal/dermal, oral, rectal and parenteral (IV) drug delivery. The primary function of human skin is to act as a protective barrier and, as such, it does impose physicochemical limitations to the type of permeates that can traverse its highly stratified

*Corresponding author
ISSN 0189-8434

structure (Jones and Brown, 2008). Ointments, creams, gels, lotions and patches which are conventional transdermal delivery dosage forms have several short comings, especially as related to their limited ability in enhancing the driving force of drug diffusion (thermodynamic activity) and/or increase skin permeability (Williams and Barry, 2004). These limitations have generally been overcome via the use of penetration enhancers (Williams and Barry, 2004), supersaturated systems (Pellet *et al.*, 2003), hyaluronic acid (Brown and Jones, 2005), prodrugs (Tsai *et al.*, 1996 liposomes, niosomes and other vesicles (Schier and Bouwstra, 2003).

Meiying *et al* (2005) have reported that the multilamellar liposomes/niosomes prepared by the lipid hydration method increased the total penetration of clotrimazole through the vaginal mucosa. Niosomes have also been shown to give desirable interactions with human skin when applied in topical preparations by improving especially the horny layer characteristics, both by reducing trans-epidermal water loss and by increasing smoothness via replenishing lost skin lipids (Choi and Maibach, 2005; Bhavana *et al.*, 1998; Jungiger *et al.*, 1991). Niosome drug-loaded dermal application is, therefore, aimed at preferentially showing interactions with the inflamed epidermal tissues without exerting an immediate or strong systemic action (Jungiger *et al.*, 1991) [11].

Salicylic acid, the model drug for our present study, is a weak acid whose keratolytic action is employed for the local treatment of warts, corns, fungal infections and certain types of eczematous dermatitis. It functionally causes the tissue cells to swell, soften and desquamate (Hardman and Limbird, 2001). Our objective in this work is to encapsulate salicylic acid in niosomes with a view of improving its dermal therapeutic properties as mentioned above.

EXPERIMENTAL

Materials

The following materials were used in the study as procured from their respective manufacturers: cholesterol, sodium hydroxide, isopropanol (BDH, England), salicylic acid, chloroform, ferric chloride (Merck, W. Germany), Tween 20 (Merck, Darmstadt), Cetylpyridinum, Veegum (Sigma USA) and distilled water obtained from an all-glass still in our laboratory.

METHODS

Preparation of salicylic acid niosomes

Three batches of salicylic acid niosomes were prepared using the lipid film hydration technique (Ferreira, 1993). Different concentrations of Tween 20, cholesterol, cetylpyridinum and Veegum were incorporated into the formulations; with the last two appropriately added as charged particles. Table 1 shows the different quantities of excipients used for the three batches.

For batch A, the appropriate quantities (Table 1) of Tween 20 and cholesterol were weighed and transferred to a test tube. The mixture was then dissolved with 1.0 ml of chloroform containing 15 mg of salicylic acid. The organic solvent was evaporated completely in a water bath maintained at 60 ^{0}C.The resultant film was hydrated with 10 ml of distilled water maintained at 60 ^{0}C, vortexed thoroughly with a vortex mixer for 10 min, and finally centrifuged at 11,000 rev/min for 30 min. Batches B and C were prepared following the same procedure except that there was an inclusion of cetyl pyridinum or Veegum to the mixture of Tween 20 and cholesterol.

Drug entrapment studies

The sediment (niosome) and the supernatant obtained after centrifugation were assayed spectrophotometrically (Tognella, 1993) for salicylic acid content using a spectrophotometer (SP6-450 UV/Vis, Pye Unicam, England). Exactly 0.5 ml of sediment was introduced into a test tube and 1. 0 ml of isopropanol added to break up the niosomes. The solution was made up to 10 ml with O.1 N HCl. Thereafter, 0.5 ml of ferric chloride ($FeCl_3$) was added and the absorbance read off at 540 nm. Similarly, the total drug content of the supernatant (control) was determined following the same procedure.

% Drug Entrapment (Leena *et al.*, 2004) or Encapsulation Efficiency=

$$\frac{\text{Mass of Drug in Niosomes}}{\text{Mass of Drug Used in the formulation}} \times 100$$

***In vitro* drug release**

The niosomal preparation with the best encapsulation efficiency was chosen from batch A, and two each from batches B and C. A 100 ml beaker containing 20 ml volume of the dissolution medium (Krebs-Ringer solution or distilled water) maintained at room temperature was placed on a hot plate with a magnetic stirrer (Heidoph, Durban) contained in the medium, operating at a speed of 50rpm. Exactly 2.0 ml of the relevant niosome formulations (samples 2, 6, 7, 14 and 15) were separately introduced into the 20ml dissolution medium. About 1.0ml of the medium was withdrawn at predetermined time intervals using 1ml pipette. The 1.0ml suspension was filtered and appropriately diluted with distilled water. A fresh 1.0ml of the dissolution medium was replaced to maintain a constant volume. Ferric chloride solution (0.5 ml) was added to the withdrawn suspension, filtered and the absorbance reading taken spectrophotometrically at 540 nm.

In vitro release study was also carried out for the control (free salicylic acid) in Krebs-Ringer solution. Exactly 15mg of pure salicylic acid powder was dissolved in 20 mls of Krebs-Ringer solution and stirred vigorously at 50rpm, to dissolve at room temperature. Exactly 1.0 ml of the solution was withdrawn at predetermined time interval and replaced with a fresh one. In each case the withdrawn solution was filtered and diluted appropriately with distilled water and the corresponding absorbance read off at 540 nm spectrophotometrically.

Preparation of calibration curve for salicylic acid

A 1 mg% w/v stock solution of salicylic acid was prepared in distilled water. From the stock, five solutions of concentrations equivalent to 0.1 mg%, 0.2 mg%, 0.3 mg%, 0.4 mg% and 0.5 mg% w/v were prepared by diluting appropriately with distilled water and filtereing. Their corresponding absorbance readings were then determined spectrophotometrically after the addition of 0.5 ml of ferric chloride solution. Thereafter absorbance was plotted against concentration to obtain the Beer Lambert's constant, K.

RESULTS AND DISCUSSION

Table 2 shows the encapsulation (entrapment) efficiencies of the formulations. The ranks of encapsulation efficiencies for batch A are of the order: Batch $A_2 > A_3 > A_1 > A_4$. Batch A_2 with 15:15 mM ratios of Tween 20 and cholesterol respectively encapsulated the greatest amount of salicylic acid, (44.2 mg%), while A_4 with a ratio of 5:5 recorded the least encapsulation efficiency (EE). Release studies were carried out to evaluate the stability and release profile of salicylic acid from the niosomes. Figures 1 and 2 show the release profile of salicylic acid niosomes in Krebs-Ringer

solution and distilled water respectively, while Fig. 3 shows the release profile of pure free salicylic acid (unencapsulated/non-niosome) in Krebs-Ringer solution. Drug release from the niosomes was faster in Krebs-Ringer solution than in distilled water while drug release from the unencapsulated drug was generally faster from the two media. Under stressed environment (i.e. in Krebs-Ringer solution) therefore, the niosomes exhibited reduced stability compared to their stability in distilled water.

The observed rank of encapsulation efficiency result agrees with Santucci *et al*'s report (Santucci *et al.*, 1993) that if the ratio of surfactant/cholesterol was above or below 1:1, it would be very difficult to prepare niosomal vesicles and that the optimal concentration of lipid/surfactant for maximum yield is 15 mM.

The inclusion of charged components had a remarkable effect on the encapsulation (entrapment) efficiencies of the niosomes. Out of the batch B formulations containing charged particles, when 1 mM of Veegum, 1mM or 3mM of Cetylpyridinum were added, the encapsulation efficiencies (EE) were almost the same, (38 %, 36.68 % and 37.8 % respectively). The presence of these ionic surfactants did not have a noticeable effect on their EEs. When the concentration of Tween 20 was held constant at 15 mM, while cholesterol decreased from 14-10 mM and veegum increased from 1-5 mM, the EE sharply dropped from 38 to 2.53% and 2.11% at 3 and 5 mM concentrations respectively. For cetylpyridinum-containing niosomes, it increased (very slightly) from 36.68 to 37.80% at 3 mM and decreased to 29.47% at 5 mM. The above results therefore show that at 1, 3 and 5 mM of veegum, niosomes became much leaky, especially B_8 and B_{10} with reduced entrapment capacity (2.53 and 2.11 %) than niosomes containing equimolar concentrations of cetylpyridinum. This implies that the higher the concentration of Veegum in the niosome the lower the Encapsulation Efficiency.

On the other hand when cholesterol was maintained at 15 mM while polysorbate-20 decreased from 14-10 mM, and veegum increased from 1-5 mM, there was an increase in EE from 3.6 to 10.74 % at 3 mM and sharp decrease to 1.90 % at 5 mM; whereas for cetylpyridinum, EE increased consistently from 2.26 to 6.68 %. It is evident that the two charged components caused the niosomes to be generally leaky with decreased capacity for drug entrapment than when polysorbate-20 was maintained at) 15 mM. These observations agree with a previous report (Santucci *et al.*, 1993) that inclusion of charged components like cationic and anionic components into niosomal preparations affects the properties and the nature of the niosome. In addition we assert that in the presence of charged components 15 mM of polysorbate 20 is the optimum concentration for optimum drug (salicylic) entrapment, otherwise there will be low encapsulation efficiency as was the case above.

The distilled water environment was more benign than that of Krebs-ringer solution. However, when compared with the unencapsulated salicylic acid, the vesicle could be seen to be relatively resistant to the osmotic stress or shock impinged upon it by the Krebs-Ringer solution. This is an indication of improved stability of the niosomal formulation over the unencapsulated or non-niosome salicylic acid.

Carafa *et al.*, (1981), in their studies, have established that the physical stability of niosome is determined by its resistance to an osmotic stress and to non-stressed conditions. This resistance to osmotic stress tends to be

Table 1: Quantities of ingredients in millimole used for the preparation of salicylic acid niosomes

Batch	S/NO	Tween 20	Cholesterol	Cetylpyridinum	Veegum®
A	1	20	20	-	-
	2	15	15	-	-
	3	10	10	-	-
B	4	5	5	-	-
	5	15	14	1	-
	6	15	14	-	1
	7	15	12	3	-
	8	15	12	-	3
	9	15	10	5	-
	10	15	10	-	5
	11	14	15	1	-
C	12	14	15	-	1
	13	12	15	3	-
	14	12	15	-	3
	15	10	15	5	-
	16	10	15	-	5

Table 2: Encapsulation Efficiencies of the Salicylic Acid Niosomes

Batch no		Tween 20 (mM)	Cholesterol (mM)	Cetylpyridinum (mM)	Veegum® (mM)	Encapsulation Efficiency (mg %)
A	1	20	20	-	-	15.0
	2	15	15	-	-	44.20
	3	10	10	-	-	35.53
	4	5	5	-	-	4.47
B	5	15	14	1	-	36.68
	6	15	14	-	1	38.0
	7	15	12	3	-	37.80
	8	15	12	-	3	2.53
	9	15	10	5	-	29.47
	10	15	10	-	5	2.11
C	11	14	15	1	-	2.26
	12	14	15	-	1	3.63
	13	12	15	3	-	2.53
	14	12	15	-	3	10.74
	15	10	15	5	-	6.68
	16	10	15	-	5	1.90

*Corresponding author
ISSN 0189-8434

Table 3: T_{25} values of the Niosomes in Krebs-Ringer solution

Batch No	T_{25} (Min)
Batch A_2	2
Batch B_6	3
Batch B_7	4
Batch C_{14}	4
Batch C_{15}	6

Table 4: T_{25} values of the Niosomes in distilled water

BATCH	T_{25} (Min)
Batch A_2	60
Batch B_6	6
Batch B_7	23
Batch C_{14}	23
Batch C_{15}	24

Table 5: T_{25} values of Free Salicylic acid in Krebs-Ringer solution

SAMPLE	**T_{25} (Min)**
Free Salicylic Acid	**0.2**

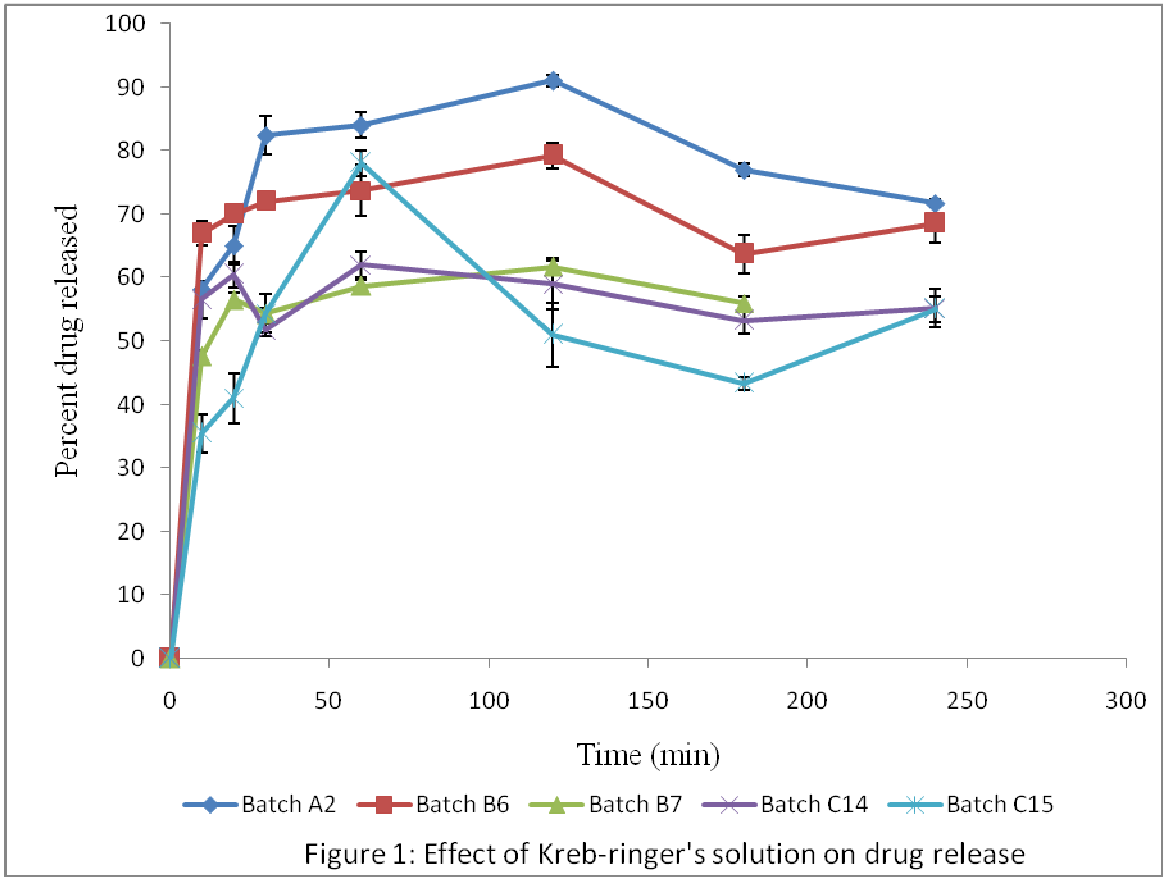

Figure 1: Effect of Kreb-ringer's solution on drug release

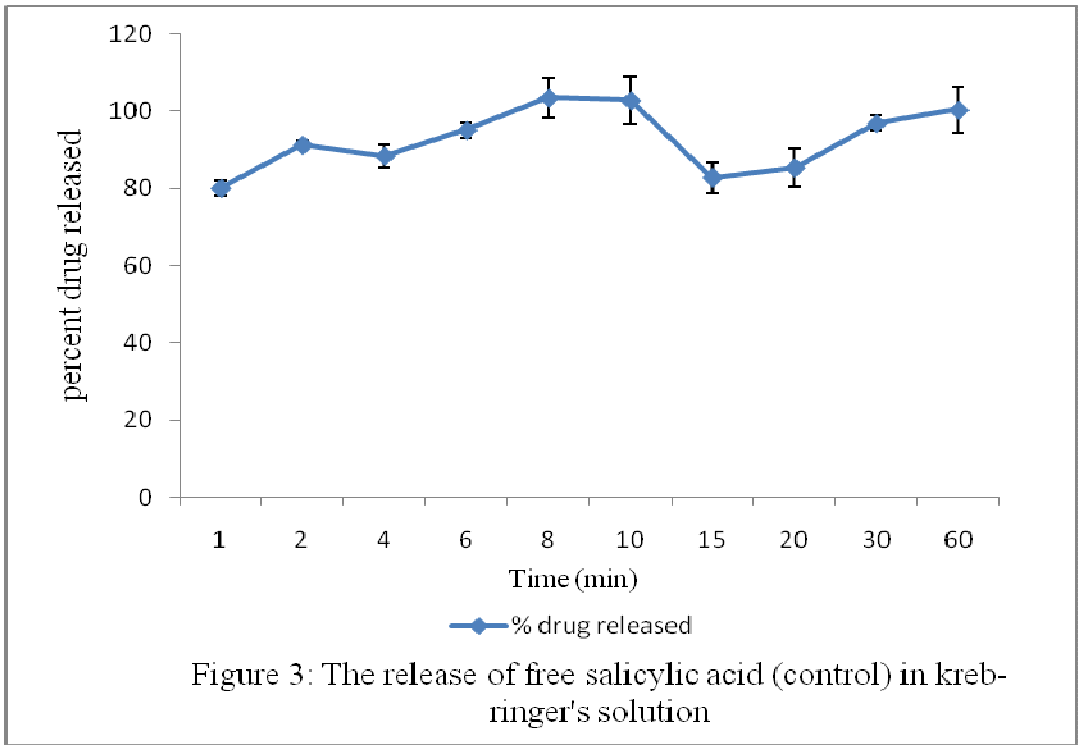

Figure 3: The release of free salicylic acid (control) in kreb-ringer's solution

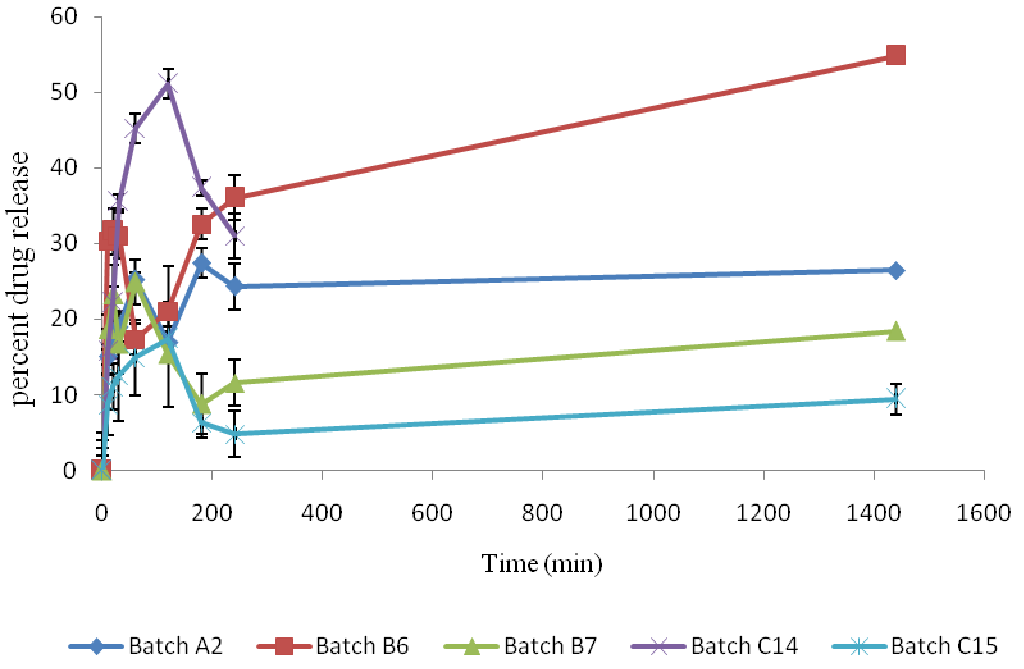

Figure 2: Effect of distilled water on the release of salicylic acid

low if the hydration, permeability and fluidity of the lipid membrane are significant. In addition stability studies of niosomes are important because they predict its protectiveness over the drug in case of threat to leakage due to contact with electrolytes *ex/in vivo*.

Batch A_2 with the highest EE, also recorded the highest T_{25} (time for 25 % of drug to be released) of 60 min in distilled water but least T_{25} (2min) in Krebs-Ringer solution, as shown in table 3. It seemed to have been most affected by environmental stress considering its entrapment efficiency. On the other hand batch B_6 containing 1mM veegum which had a higher EE (38%) witnessed a drastic lowest T_{25} (6 min) in distilled water and even a lower T_{25} (3 min) in krebs-ringer solution. The niosome membrane permeability to the aqueous solution of this concentration of the charged particles may have been high. The low concentration of the charged components may be responsible. High EE, therefore, may not necessarily guarantee or predict a slow release and/or high stability.

Stability in Krebs-Ringer solution was found to increase with higher molar concentration of the charged components. Batch C_{15} having 5 mM of cetylpyridinum was most stable in the solution, as its T_{25} (6 min) value was relatively higher than others. On the other hand batch B_6 having 1mM of Veegum which recorded relatively high EE of 38% was the least stable as its low T_{25} is an indication of fast release. The bilayer membrane probably became highly permeable. This slightly higher stability in Krebs-Ringer solution for batches B_7, C_{14} and C_{15} than A_2 and B_6 could be due to an optimized ion-dipole interaction which existed between the cholesterol and the ionic charges.

In addition, sometimes the incorporated drug does participate in the micro-structure of the system, and may even influence it due to molecular

interactions, especially if the drug possesses amphiphilic and/or mesogenic properties (Costas *et al.*, 2006). Therefore, when developing niosomes and its related formulations, the goal is to obtain systems with optimized drug loading and release properties, long shelf-life and low toxicity.

Niosomes to an extent (like solid lipid nanoparticles), possess occlusive properties which can increase the water content of the skin and favour drug penetration into the skins (Muller and Dingler, 1998; Jenning *et al.*, 2000). In addition the aqueolipoidal nature of the bilayer architecture liberally advantages its stability in aqueous environment while still exerting its lipid characteristics. Although Solid lipid nanoparticles may be more aqueous-stable with respect to drug encapsulation and retention (because of its solid nature), however the stability studies we carried out above indicated that some of the noisome batches maintained stability in stressed conditions. Warty skin texture is so tough and dry that sometimes optimal local therapeutic drug accumulation may not be achieved easily with conventional creams and ointments. Hence the need to exploit the unique property of niosomes which supports enhanced penetration into the local skin surface without or with insignificant systemic absorption.

CONCLUSION

Salicylic acid has been formulated into niosomes for possible topical or dermal use with entrapment efficiencies of 35-44 % in some of the formulations. The optimal molar concentration of the lipid/surfactant level of the preparation was 15 mM: 15 mM. The results obtained did show that salicylic acid niosomes may possibly offer enough barrier protection while ensuring adequate drug release for maximum topical therapeutic effect against corns, warts or eczematous dermatitis without any significant systemic absorption. Future studies will attempt an evaluation of skin permeation studies of this preparation.

REFERENCES

Bhavana V, Ajay JK, Jain NK. Proniosome Based Transdermal Delivery of Levonorgestrel for Effective Contraception. J Cont Rel. 1998; 54:149-65.

Brown MB, Jones SA. Hyaluronic Acid-A Unique Topical Vehicle for Localized Delivery of Drugs to the Skin. JEDV. 2005; 19:308-18.

Carafa M, Santucci E, Alhaique F, Covello T, Murtas E, Riccieri FM, Luccania G, Torisi MR. Preparation and Properties of New Unilamellar Non-Ionic Surfactant Vesicles. Int J Pharm. 1981; 60:51-9.

Choi MJ, Maibach HI. Liposomes and Niosomes as Topical Drug Delivery Systems. Skin Pharmacol Physiol. 2005; 18, 5:209-19.

Costas K, Sofia A, Katerina K, Sotira C. Recent Advances in Novel Drug Delivery Systems. Azojon. 2006; 2:1-11. Http://www.Azonano.Com/Oars.Asp. Accessed from the internet on 27/4/2006.

Ferreira SH. The Role of Interleukins and Nitric Oxide in the Mediation of Inflammatory Pain and Its Control by Peripheral Analgesics. Drugs. 1993; 46 (1):1-9.

Florence AT. New Drug Delivery Systems. Chemistry and Industry. 1993; 20:1000-1004.

Hardman JG, Limbird LE. Goodman and Gilman's The Pharmcological Basis of Therapeutics. New York; McGraw-Hill Medical Publishing Division, 2001.

Jenning V, Gysler A, Schafer-Korting, Gohla

Jones SA, Brown MB. Evolution of Transdermal Drug Delivery. www.pharmaquality.com. Accessed from the internet on 10/09/2008

Jungiger HE, Hofland HEJ, Bouwstra JA . Liposomes and Niosomes Interactions with Human Skin. Cosmet Toil. 1991; 106:45-50.

Leena P, Jonanna A, Samuli H, Karjalainen, Jouni H. Improved entrapment efficiency of hydrophilic drug substance during nanoprecipitation Of Poly l lactide nanoparticles. AAPS Pharm Sci Tech. 2004; 5(1), article 16.

Meiying N, Zhongwei G, Huaizhong P, Heming Y, Kai X. Preparation and *In vitro* evaluation of liposomal/niosomal delivery systems for antifungal drug, Clotrimazole. Ind J Exp Biol. 2005; 43:150-57.

Muller RH, Dingler A. The Next Generation after the Liposomes-Solid Lipid Nanoparticles (SLN^{TM}, Lipopearls^{TM}) as dermal carrier in cosmetics. Eurocosmetics.1998; 7/8:19-26.

Pellet M, Raghavan SI, Hadgraft J, Davis AF. The Application of Supersaturated Systems to Percutaneous Drug Delivery. In-Guy RH, and Hadraft J eds. Transdermal Drug Delivery. Marcel Dekker. 2003.

Santucci E, Carafa M, Coriellor T, Martas E, Ricciert FM, Alhaique F, Modesti A, Modica A. Vesicles from polysorbate 20 and cholesterol- a simple preparation and a characterization. STP Pharm Sci. 1993; 6:29-32.

Schier H, Bouwstra J, Liposomes. Niosomes as Topical Drug Carriers-Dermal and Transdermal Drug Delivery. J Control Rel. 1994;30:1-15

SH. Vitamin A-loaded Solid Lipid Nanoparticles for Topical use-Occlusive Properties and Drug Targeting to the Upper Skin. Eur J Pharm Biopharm. 2000; 49:211-218.

Tognella S. Nimesulide-New Clinical Opportunities. Drugs. 1993; 46(1):275-276.

Tsai JC, Guy RH, Thornfeldt CR, Gao WN, Feingold KR, Elias PM. Metabolic Approaches to Enhance Transdermal Drug Deliv. I. Effect of Lipid Synthesis Inhibitors. J Pharm Sci. 1996; 85:643-48.

Williams AC, Barry BW. Penetration Enhancers. Adv Drug Del Rev. 2004; 56:603-18.