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Novel lipid-based dermal microgels of Neobacin®

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This study investigates the potential of novel microgles based on solid lipid microparticles (SLMs) as a sustained delivery system for neobacin®, a topical antibiotic drug powder. Matrices generated from sunseed oil and goat fat (1:9, 2:8 and 3:7) was surface-modified with Phospholipon® 90G and employed to formulate SLM-based microgels. The microgels were characterized in terms of *in vivo* wound healing in rats, *in vitro* permeation, membrane drug retention studies and antimicrobial activity against various microorganisms using standard cup-plate agar diffusion method. The 3:7 microgels exhibited sustained release property, achieving 34% drug permeation over 12 h, 64% membrane drug retention and largest growth inhibition zone diameters (IZD) on all organisms, whereas commercial neobacin® gel achieved 35% drug permeation at 4 h and 72% membrane drug retention. *In vivo* wound healing followed this order 3:7>1:9>2:8 better than neobacin® powder. Neobacin® microgel formulation despite rapid degradation possessed greater wound healing and antimicrobial property than the conventional powder form of neobacin®.

Key words: Microgels, surface-modified solid lipid microparticles, sustained release, neobacin®.

INTRODUCTION

Skin is the largest, easily accessible organ of the body for local and systemic drug administration with numerous advantages compared to other routes (Souto et al., 2011; Reszko et al., 2009). However, it is an excellent barrier that naturally prevents transport of molecules into and out of the body despite several strategies proposed to overcome the barrier functions of the *stratum corneum* (Higaki et al., 2005; Trommer and Neubert, 2006).

Nano/micro carriers have shown skin penetration enhancement properties and include nano/micro-

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Abbreviations: SMSLMs, Surface-modified solid lipid microparticles; **SLMs,** solid lipid microparticles; **P90G,** Phospholipon® 90G; **IZD,** inhibition zone diameters; **IU,** International units; **MWCO,** molecular weight cut-off; **SRMS,** solidified reverse micellar solutions.

emulsions, solid lipid nano/micro-particles produced from lipid materials of various fatty acids, triglycerides, oils, phospholipids and waxes of GRAS status (Acosta et al., 2011; Lv et al., 2009). However, these liquid or colloidal dispersions have low viscosity, thus providing only a short-term adherence onto the skin; hence inconvenient for applications. Incorporation into semi-solids (for example, creams) or hydrogels can increase adhesivity and convenience for use. Recently, many products that take advantage of nanotechnology have been manufactured (Lv et al., 2009; Senyigit et al., 2010; Eskandar et al., 2010; Pardeike et al., 2009; Korting and Schafer-Korting, 2010).

In practice, a wide range of antibacterial action is provided by the overlapping spectra of bacitracin and neomycin (neobacin®) in the treatment of infections. Neobacin® which is available as a powder, 5 g for wound dressing, contains 5 mg of neomycin sulphate and 250 IU of bacitracin zinc in each gram of powder. But as a dressing powder, it is without moisture, thus impedes fast migration of epithelial cells to the wound surface thereby delaying healing as a result of scab formation. In addition, it causes allergic dermatitis especially in disrupted skin (Fox et al., 2006; Dainiak et al., 2010; Atiyeh et al., 2009). Development of an alternative to powder-type neobacin® dosage form that would provide sustained delivery is a major challenge. We report here, surface-modified solid lipid microparticles (SMSLMs) of neobacin® generated from a blend of liquid (sunseed oil) and solid (goat fat) lipids at 1:9, 2:8 and 3:7 respectively, surface modified with phospholipid and Tween 80 as a mobile surfactant. The objective of the study was to confer some hydrophobic character to the freely water soluble neomycin sulphate to enhance its permeation through the skin for better drug release. To improve adhesivity, convenience and moist-driven wound healing devoid of scabbing, we therefore, encapsulated the SMSLMs into Carbopol 940® hydrogels (microgels).

MATERIALS AND METHODS

The materials include Neobacin® powder (Drugfield Pharmaceuticals Ltd, Nigeria), Carbopol 940® (B. F. Goodrich, U.S.A.), propylene glycol, triethanolamine (Spectrum Chem. Mfg. Corp., California), ethanol (BDH, England), polysorbate 80 (Uniqema, Belgium), Phospholipon® 90G (Phospholipid GmbH Köln, Germany), sunseed oil (double refined) vitamin A fortified (Kelwaram Chanrai Group Lagos, Nigeria), and goat fat (Pharmaceutics Laboratory, University of Nigeria). Distilled water (Lion water, Nigeria) was used for all preparation.

Lipid matrix preparation and formulation of SLMs

Goat fat was obtained as earlier described (Attama et al., 2007; Nnamani et al., 2010; Attama and Müller-Goymann, 2006). Mixtures of sunseed oil and goat fat in the ratio of 1:9; 2:8 and 3:7 were prepared by fusion at 60°C on a thermo-regulated water bath shaker (Heto, Denmark) and stirred until solidification. Surface-modification was done with 20% phospholipid (SRMS). The lipid matrices were characterized in a differential scanning calorimetry (DSC) machine (NETZSCH DSC 204 F1, Germany) at 35-190°C under a 20 ml/min nitrogen flux at a heating rate of 10°C/min. All determinations were baseline-corrected.

SLMs were formulated to contain 5% w/w of each SRMS, 1.5% w/w polysorbate 80 and enough distilled water to make 100% (w/w). By adding neobacin® (10% w/w) to each of the SRMS prior to SLM production by melt emulsification, neobacin- loaded SLMs were obtained. The thermal properties of all SLM formulations were determined by DSC.

Characterization of SLMs

Particle size, injectability, time-dependent pH stability and storage stability studies of samples stored at room and refrigeration temperatures were carried out. The drug encapsulation efficiency was determined indirectly after centrifugation in a membrane concentrator (MWCO 5000, Vivascience AG, Hannover, Germany). Drug concentration of the aqueous continuous phase was determined by a UV/Vis spectrophotometer (Unico 2102, England) at 252 nm considering the initial amount of drug loaded using the equation:

$$EE\ (\%) = \frac{Real\ drug\ loading}{Theoretical\ drug\ loading} \times 100 \quad (1)$$

Preparation of microgels

Carbopol 940® (1% w/w) was dispersed in sufficient distilled water and stirred on a magnetic stirring device (SR 1UM 52188, Remi Equip., India) at 600 rpm for 30 min. Propylene glycol (10% w/w) was added and the mixture further neutralized with triethanolamine to pH 5.5. Neobacin® drug powder was incorporated into one batch of hydrogel while plain hydrogel batch (not containing drug or SLMs) was also produced. To prepare microgels, Carbopol 940® was dispersed in 85 ml of the SLMs (with/without drug) and stirred thoroughly to ensure uniform dispersion. All products were dispensed in lacquered aluminium tube, securely closed and stored at room temperature until used. All formulations were 100% dispersions according to Table 1.

Characterization of microgel formulations

Physical examination

The semi-solid formulations were physically examined for colour, homogeneity, spreadability and consistency. The pH was also evaluated.

In vitro permeation studies

Franz diffusion cells with a receiver compartment volume of 20 ml and effective diffusion area of 2.84 cm^2 were used to evaluate drug delivery characteristics from the selected compositions. A Millipore membrane (0.22 µm), (Millipore Corporation, Billerica, MA) was used. The receptor phase (phosphate buffer solution, PBS, pH 7.4) was continuously stirred and kept at a temperature of 37 ± 0.1°C during the experiments. 0.5 g of the semi-solid products equivalent to 1 g of drug powder was spread uniformly on the membrane

Table 1. Composition of various optimized SLMs and semi-solid formulations.

Ingredients (g)	SMSLM compositions (g)						Neobacin hydrogel	Plain hydrogel	Neobacin® powder
	SLM-0			SLM-neobacin®					
Sunseed oil	1	2	3	1	2	3	-	-	-
Goat fat	9	8	7	9	8	7	-	-	-
Lipid blend	4	4	4	4	4	4	-	-	-
Phospholipon® 90G	1	1	1	1	1	1	-	-	-
Neobacin®	-	-	-	10	10	10	10	-	-
Polysorbate 80	1.5	1.5	1.5	1.5	1.5	1.5	-	-	-
Distilled water	88.5	88.5	88.5	73.5	73.5	73.5	-	-	-
Microgel compositions (g)									
SMSLMs	85	85	85	85	85	85	-	-	-
Carbopol 940®	1	1	1	1	1	1	1	1	-
Propylene glycol	10	10	10	10	10	10	10	10	-
Triethanolamine	4	4	4	4	4	4	4	4	-
Distilled water	-	-	-	-	-	-	75	85	-

placed in the donor compartment. At appropriate time intervals, 1 ml of the sample was withdrawn from the receiver compartment and the same amount of fresh solution was added to maintain sink condition. Each experiment was run in three independent cells. The samples were analyzed spectrophotometrically at a wavelength of 252 nm and the concentration of neobacin® in each sample was determined from a standard curve. Each data point represents the average of three determinations. The release study was carried out for 12 h period.

Skin retention studies

The Millipore membrane (0.22 µm) from each product batch of *in vitro* permeation study was removed, gently scraped with spatula to remove all remaining residues and further cleaned with cotton wool soaked in PBS before crushing. To the crushed Millipore membranes contained in different volumetric flasks, 50 ml of ethanol was separately added and mechanically shaken in a water bath shaker (Heto Denmark) at 37°C for 1 h. After which they were filtered using a Whatman No. 1 filter paper and the filtrate analyzed using UV spectrophotometer. The concentration that was retained and further eluted from the membrane was calculated using standard Beer's plot and compared with the drug concentration that permeated through the skin *ab initio*.

In vivo wound healing assay

The wound-healing properties of the microgel formulations were investigated on wound-inflicted rats and the efficacies of the formulations were compared. Thirty six adult (age: 6-10 weeks old) Wistar albino rats of both sexes (180 -250 g) were divided into nine groups of four rats each. Four groups served as controls, one group as standard and the other four as test groups. The hair around the left flank was shaved with shaving sticks and swabbed with cotton wool. A template disc (diameter 30 mm) was placed and outlined on the skin. Full thickness wounds were produced by excising the skin with sterile surgical blades. The wounds were immediately covered with a superficial smear of the semi-solid preparations (0.25 g) equivalent to 0.5 g of drug powder. A comparative assessment was done using the commercial drug powder. The treatment was in accordance with Table 1. The animals were on normal feed, clean water, and the formulations were smeared on the afflicted wounds each alternate day. Wound diameter measurements were taken before each application in triplicates and the average taken. Housing was by battery cage system. This animal experiment complied with the University of Nigeria recommendation of the committee for safe handling of experimental animals and the protocol approval was obtained.

In vitro antimicrobial activity of the formulations

The antibacterial activity of various semisolid formulations of neobacin® against various clinical isolates of microorganisms (*Staphylococcus aureus, Klebsiella pneumonia, Salmonella typhi, Pseudomonas aeruginosa and Escherichia coli*) was evaluated by the standard cup-plate method and the inhibition zone diameters measured. The organisms were seeded in sterile nutrient agar, swirled for uniform distribution and allowed to set at room temperature. Cork borer of diameter 6 mm was used to bore holes in the set agar. A stock solution of neobacin® (0.25 µg/ml) was prepared by dissolving 50 mg of neobacin® powder in a litre of distilled water and two-fold serial dilution was done to obtain different dilutions of the drug. The various neobacin® concentrations were dropped into the wells of the seeded plates, and incubated after 30 min at a temperature of 37°C ± 0.5 for 24 h.

Statistical analysis

All experiments were performed in replicates for validity of statistical analysis. Results were expressed as mean ± SD. ANOVA and Student's t-test were performed on the data sets generated using SPSS. Differences were considered significant for $p < 0.05$.

RESULTS

The thermal property of goat fat has been described

(Attama et al., 2007; Nnamani et al., 2010; Attama and Müller-Goymann, 2006) whereas sunseed oil had an endothermic peak at 39.3°C. The physical mixtures of sunseed oil and goat fat at 1:9, 2:8, and 3:7 respectively showed melting peaks at 52.13, 51.4 and 50.9°C (Figure 1). The SRMS of 1:9 matrices melted at 51.3°C whereas that of 2:8 had peak at 51. 2°C while SRMS 3:7 melted at 48.7°C. The DSC thermogram of pure neobacin® powder showed two sharp endothermic peaks at 149°C with an enthalpy of -4.711 mW/mg and 214.5°C with an enthalpy of -4.29 mW/mg while an exothermic peak appeared in between the endothermic ones at 176.8°C with an enthalpy of -0.1252 mW/mg.

For SLM-O formulated from 1:9 matrix, two broad endothermic peaks were observed at 78.0 and 123.7°C with enthalpies of -25.94 and -8.425 mW/mg, respectively. The drug-loaded SLMs produced from this matrix showed endothermic peaks at 53.8 and 80.6°C. The melting endotherms of the SLM-O from SRMS 2:8 showed broad peaks at 52.6, 89.9 and 116.7°C while the corresponding drug-loaded SLMs showed sharper endothermic peaks at 57.4, 86.3 and 122°C. However, the SLM-O produced from SRMS of 3:7 showed broad endothermic peaks at 88.8 and 118.9°C while the corresponding SLM-drug-loaded sample showed an endothermic peak at 126.0°C but with an earlier shoulder of broad peak which occurred at 108.2°C due to an unstable modification.

Table 2 shows that the SMSLMs containing no neobacin® (SLM-O) were larger than those containing neobacin® (SLM-Drug). This trend was again observed when the samples were re-analyzed after one month of storage. Photomicrographs (Figures 2 and 3) showed that the SLMs were smooth, well-formed and monodispersed. It was observed that after 3 months, all the batches formulated with SRMS 1:9 had pH in the range of 4.90±0.05 to 5.30±0.01; those formulated with SRMS 2:8 had pH in the range of 4.80±0.05–5.25±0.10, whereas the pH of SLMs formulated with SRMS 3:7 ranged from 4.70±0.10 to 5.50±0.1. The syringeability study showed that the drug-loaded SLMs that appeared smaller in size required bigger gauge needle (23 G) to be syringeable within 20 s while the non-drug-loaded SLM samples (SLM-O) were syringeable with smaller needle of 25 G.

Table 2 shows the encapsulation efficiency of the formulations. It was evident from the table that the drug encapsulation efficiencies in the formulations were very poor and varied inversely with the proportion of sunseed oil to goat fat (1:9>2:8>3:7) respectively.

The microgels had uniform colour, is somewhat white for the microgels, cream to off-white colour for the plain hydrogel containing neobacin® powder (neobacin® gel) and transparent for gel without neobacin®. They had good consistency and were quite spreadable with pH constantly in an acceptable range of 5.5 ± 0.25.

Figure 4 comparatively depicts the release of neobacin® from all formulations. The result shows that neobacin® gel achieved a maximum release of 35% at 4 h attaining faster peak than those encapsulating SMSLMs; but immediately degraded just at the point the microgels were starting to release the embedded drug at 10 h. The microgels generally showed slow release of the entrapped drug and those formulated from the 3:7 SRMS matrices achieved maximum release of 34% at 12 h, despite the low percentage encapsulation efficiency.

Table 3 shows the result of the Millipore membrane retention studies. Neobacin gel had the highest concentration of neobacin® (72%) retained on the membrane followed by neobacin microgel formulated from SLMs of SRMS 3:7 matrix (64%) while SLMs of 1:9 SRMS matrix had the least (27%).

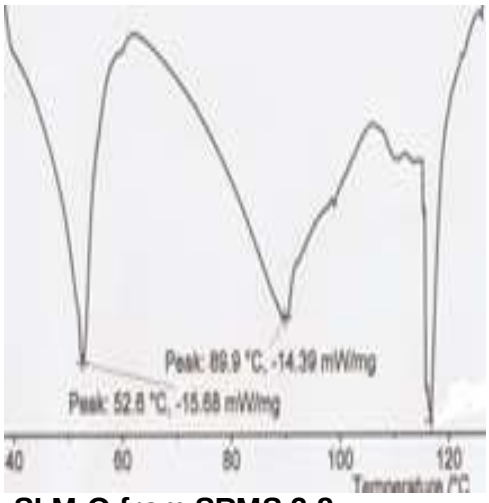
The result of the *in vivo* wound healing study is depicted in Figure 5. It is discernible from the figure that the wounds of the rats treated with neobacin microgels (3:7>1:9>2:8) healed faster than neobacin® powder. At day 12, maximum healing of 70% was recorded for the formulation produced from 3:7 SRMS whereas the drug powder could only achieve 60 % wound healing. Neobacin® gel also achieved 60 % release though at day 8 instead of day 12.

The formulations had various degrees of microbial growth inhibition (Table 4). Among the neobacin microgels, the batch formulated from SRMS 3:7 had the highest growth inhibition diameters (IZD) on all microorganisms followed by the neobacin® gel formulation while that of 2:8 microgel was the least. The drug powder showed the highest IZD of 26.11 mm on *Staphylococcus aureus* only, followed by the neobacin microgels of 3:7 which recorded 25.32 mm on the same organism (Figure 5).

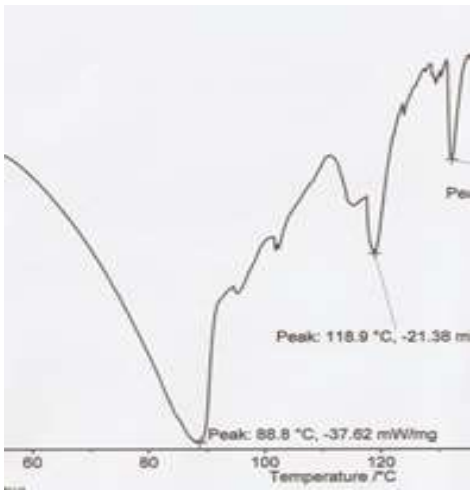
DISCUSSION

Surface-modification of the physical mixtures by phospholipid (SRMS) generated matrices that melted at lower temperatures (Attama et al., 2007; Nnamani et al., 2010; Attama and Müller-Goymann, 2006). The characteristic thermogram of neobacin® powder could be due to the fact that it is a combination drug. This shows that neobacin® loadings resulted in lower temperature of melting suggesting that the matrix generally produced less ordered crystals that required lower energy to overcome the lattice forces than the more rigid (crystalline) substance entirely contained in the SLM-O. The high enthalpy values generally observed with the drug-loaded SLMs suggest low entrapment of neobacin® in the matrices (especially the 3:7) due to inherent solubility problem of the drug. Although the SLMs were stable, monodispersed, smooth and well-formed, some appeared clustered showing tendency of an intending

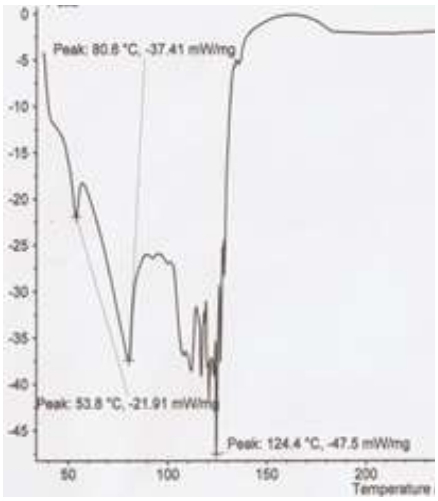
SLM-O from SRMS 1:9



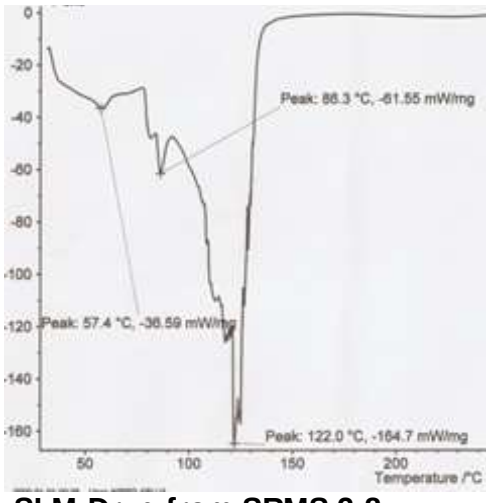
SLM-O from SRMS 2:8



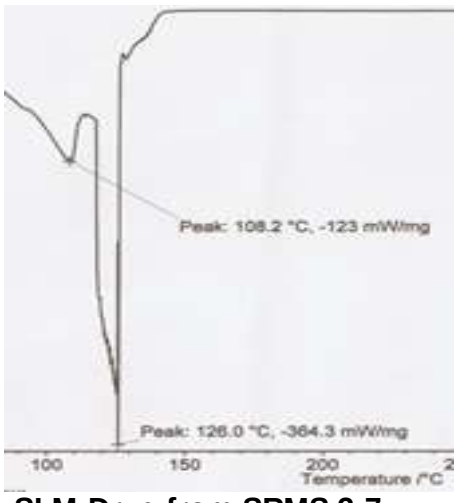
SLM-O from SRMS 3:7



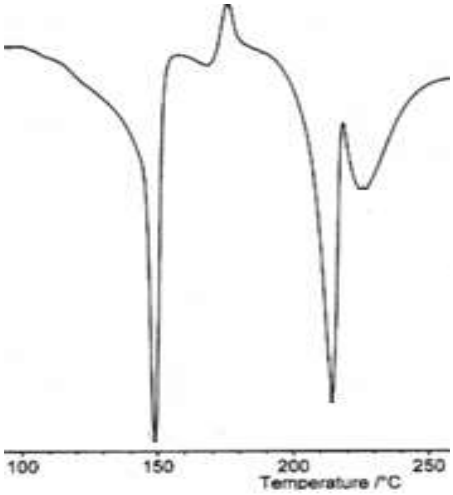
SLM-Drug from SRMS 1:9



SLM-Drug from SRMS 2:8



SLM-Drug from SRMS 3:7



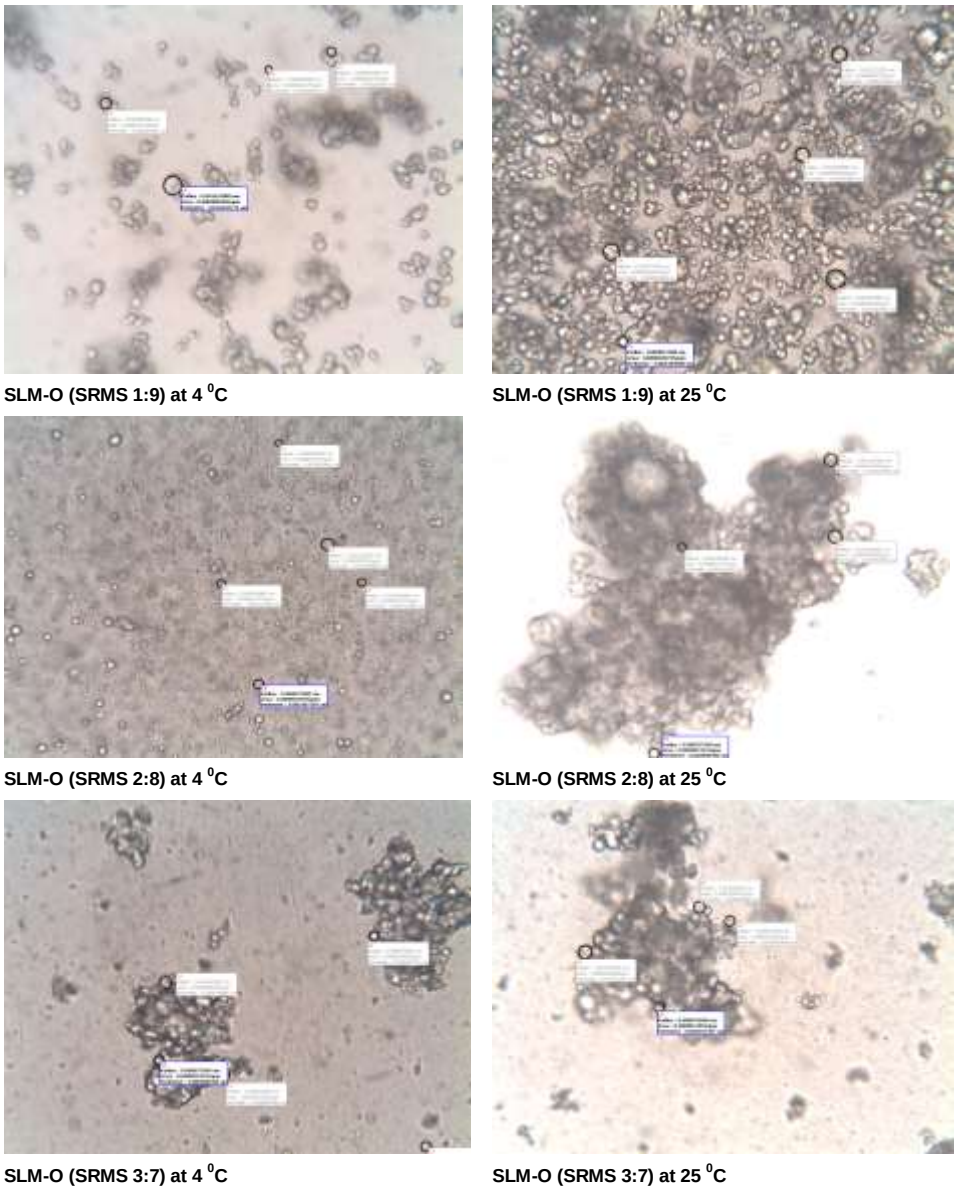
Pure neobacin® powder

Figure 1. DSC thermograms

Table 2. Properties of the formulated SLMs.

Batch	LM	Average particle size (µm)		EE (%)	S (Guage)	Thermal properties	
		4°C	25°C			M.pt (°C)	Enthalpy (-mW/mg)
SLM – O	1:9	21.67±0.01	32.35±0.02	-	25	78; 123.7; 131.8	25.94; 8.42; 5.38
SLM – O	2:8	20:68±0.01	28.16±0.02	-	25	52.6; 89.9; 116.7	15.68; 14.39; 17
SLM – O	3:7	19.69±0.01	19.85±0.01	-	25	85.8; 118.9; 132.3	37.62; 21.38; 13
SLM – D	1:9	12.50±0.02	19:17±0.03	5.9	23	58.8; 80.6; 124.4	21.91; 37.4; 47.5
SLM – D	2:8	15.00±0.01	20.02±0.03	5.0	23	57.4; 86.3; 122	36.59; 61.55; 164
SLM – D	3:7	18.08±0.01	19.83±0.01	4.3	23	126.0; 108.2	123; 364.3

LM = lipid matrix; EE = encapsulation efficiency; S = syringeability; SLM-D = neobacin®-loaded solid lipid microparticles; SLM-O = Zero-drug SLMs; M.pt = melting point, values represented with corresponding enthalpies in order of appearances.

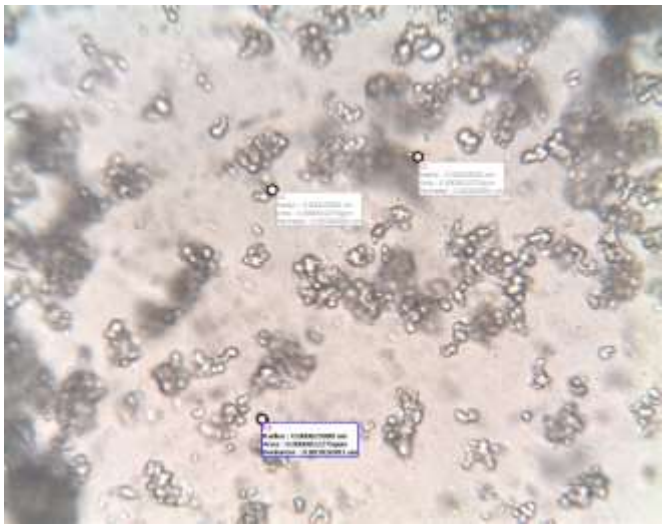


SLM-O (SRMS 1:9) at 4 ^{0}C **SLM-O (SRMS 1:9) at 25 ^{0}C**

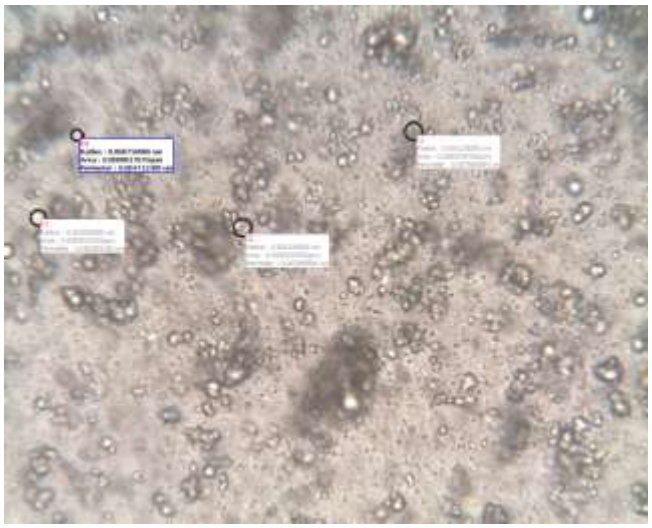
SLM-O (SRMS 2:8) at 4 ^{0}C **SLM-O (SRMS 2:8) at 25 ^{0}C**

SLM-O (SRMS 3:7) at 4 ^{0}C **SLM-O (SRMS 3:7) at 25 ^{0}C**

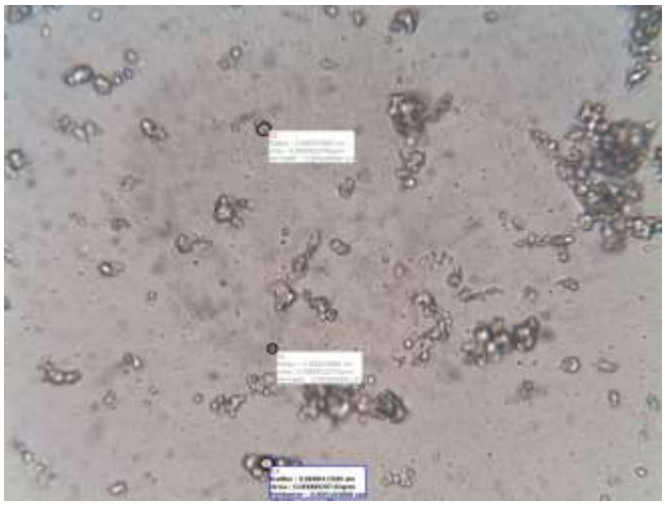
Figure 2. Photomicrographs of zero-drug SLMs at different storage temperatures (X100).



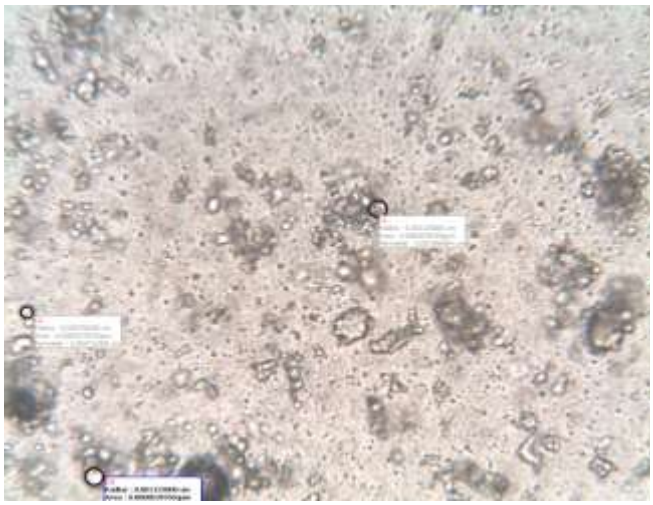
SLM-Drug (SRMS 1:9) at 4°C



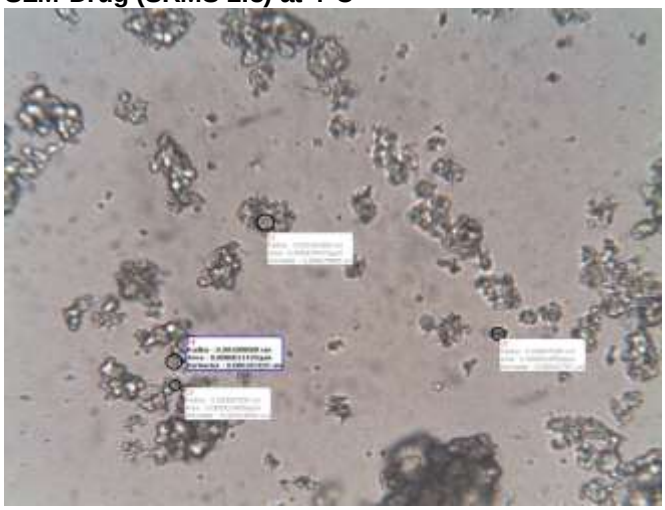
SLM-Drug (SRMS 1:9) at 25°C



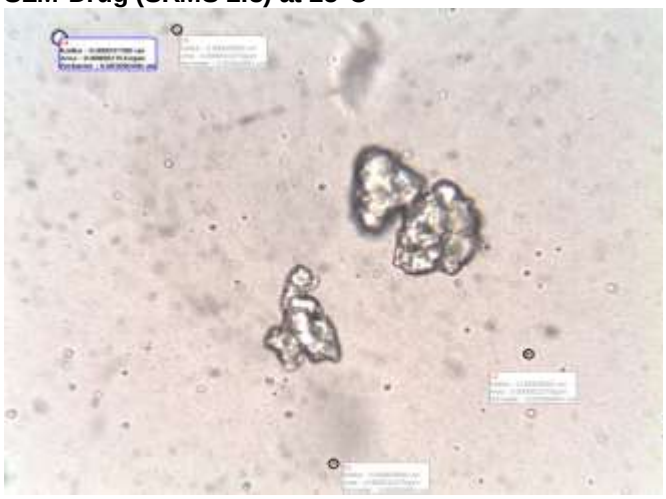
SLM-Drug (SRMS 2:8) at 4°C



SLM-Drug (SRMS 2:8) at 25°C



SLM-Drug (SRMS 3:7) at 4°C



SLM-Drug (SRMS 3:7) at 25°C

Figure 3. Photomicrographs of drug-loaded SLMs at different storage temperatures (X100).

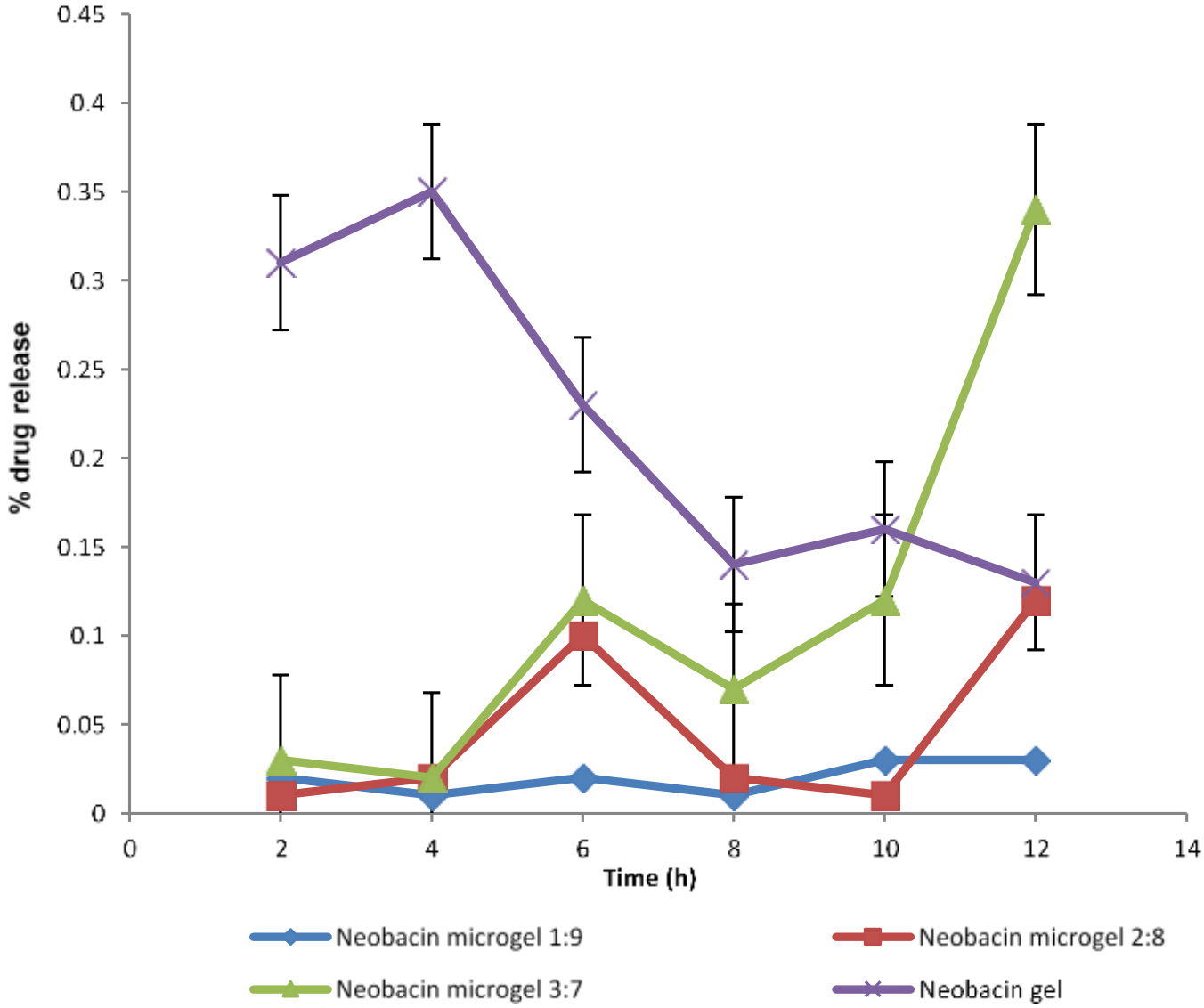


Figure 4. *In vitro* drug release study.

Table 3. Skin retention study.

Formulations	Concentration (%)
Neobacin microgel 1:9	27
Neobacin microgel 2:8	36
Neobacin microgel 3:7	64
Neobacin® gel	72

particle agglomeration which may not likely be stable upon extensive storage in the form of SLMs. Photo-micrographs indicated that SLM-O formulations without neobacin® (Figure 2) were bigger than SLM-drug loaded formulations (Figure 3). However, their shapes were best preserved at lower temperature rather than at room temperature. The result of the syringeability study suggests that even though particles of SLM-O batches

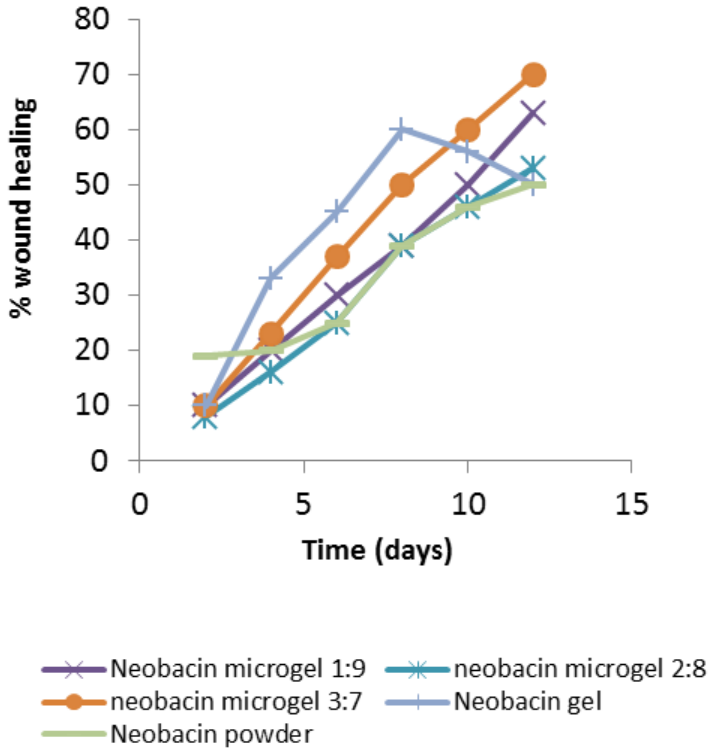


Figure 5. Percentage wound healing.

Table 4. Antimicrobial activity of neobacin® from topical drug delivery systems.

Formulations	Inhibition zone diameter, IZD (mm)*				
	S. aureus	*P. aeruginosa*	*S. typhi*	*K. pneumonia*	*E. coli*
Neobacin microgel 1:9	21.13±2.58	15.11±0.98	11.22±0.76	20.12±3.04	19.±0.7
Neobacin microgel 2:8	15.23±0.76	16.33±0.78	10.12±0.93	17.11±0.85	17±0.9
Neobacin microgel 3:7	25.32±1.64	23.33±1.97	23.23±1.56	24.33±1.88	24±1.3
Neobacin® gel	23.21±1.75	21.23±1.99	23.33±1.60	23.33±1.55	23±1.5
Gel alone	15.36±0.92	15.21±0.83	15.11±0.37	15.12±0.74	15±0.8
Neobacin® powder	26.11±2.07	21.32±1.44	22.14±1.56	23.23±1.75	22±0.6

*Values are average of three determinations.

appeared bigger, they were more collapsible upon syringing to conveniently pass through smaller needles of 25 G while the SLM-Drug samples could not, perhaps due to the little drug entrapped in the lipid domain of the microparticles. The encapsulation efficiency was very poor and varied inversely with the proportion of sunseed oil to goat fat (1:9>2:8>3:7) respectively. This could be due to low solubility of the drug in the lipid melt, which agrees with literature that hydrophilic drugs could be only 5% entrapped in a lipid particle and good loading capacity is a result of high solubility of the drug in the lipid melt (Sanna et al., 2003; Bondi and Craparo, 2010; Muchow et al., 2008; Souto et al., 2004). Moreover, the encapsulation efficiency values confirm the observations from the thermal analysis.

The microgels generally showed higher and a more prolonged release of the entrapped drug than the noebacin® gels. In addition, since microgels formulated from the 3:7 SRMS matrices achieved maximum release of 34% at 12 h, despite the low percentage EE, it means that it was available in the aqueous medium conveying the microparticles, closely attached to the carrier surface, in the vicinity of the surfactant layer, so that upon encountering the gel matrix of the polyacrylic acid, it got properly embedded in its 3-D matrix (Zhu et al., 2009; Feng et al., 2009; Kapoor and Chauhan, 2008; Bachhav

and Patravale, 2009; Padula et al., 2009). Therefore to improve the skin uptake and targeting, a semi-solid vehicle as we have demonstrated remains a standard approach which according to some report suggests that hydrogels exhibit high stability and adhesivity in addition to low toxicity (Bachhav and Patravale, 2009; Padula et al., 2009; Bhalekar et al., 2009).

The result of skin retention study showed that some neobacin® permeated through the membrane while reasonable amount was retained on the Millipore membrane (0.22 µm). This confirms the earlier observation on the size of the micro-particles and further agrees with literature that particles whose diameter were greater than 10 µm remained on the skin surface whereas those within 3 and 10 µm selectively penetrated the follicular ducts and those smaller than 3 µm randomly distributed into the hair follicles and stratum corneum (Schaefer and Lademann, 2001). Our observation is in consonance with literature reports that greater drug retention in the skin was observed while a lower concentration of drug could permeate through (Bhalekar et al., 2009; Puglia et al., 2008; Zhu et al., 2008). However, the drug release pattern is governed by the type of solid lipid matrix, not the hydrogel type (Paolicelli et al., 2009). Chen and co-workers as well as other researchers also observed that some processing parameters can affect the properties of the hydrogel such as the order of mixing the gelators (Chen et al., 2007; Sanna et al., 2010; Feng et al., 2009).

The result obtained from the *in vivo* wound healing study agrees with those of Fox et al. (2006) and Atiyeh et al. (2009) that wounds heal best when kept clean and moist rather than from dry powdery environment which forms hard scab and acts as a barrier to the development of new tissues. Semi-solid formulation provides moist environment which maintains epithelial cell viability to 97% (Atiyeh et al., 2009), and enables healing epithelial cells to freely migrate across the wound bed, promoting wound healing from the sides as well as the bottom of the wound. Wound healing is a complex and dynamic process of restoring cellular structures and tissue layers in damaged tissue as closely as possible to its normal state. Wound contraction is a process that occurs throughout the healing process, commencing in the fibroblastic stage whereby the area of the wound undergoes shrinkage. In the maturational phase, the final phase of wound healing, the wound undergoes contraction resulting in a smaller amount of apparent scar tissue. A wound without moisture will form scab (Fox et al., 2006; Atiyeh et al., 2009), as was observed from the drug powder-treated wounds. This is because the epithelial cells had to migrate from under below the scab to reach moisture that enabled them to heal the wounds but in so-doing, the epithelial cells migrated longer distances and, as a result, did not survive well, thus delayed wound healing. Better wound healing property of the microgel formulations could additionally be attributed to their occlusive nature which enhanced adherence of the drug formulation to the wound surface (Wolf et al., 2009). Moreover, the results of the antimicrobial study indicated that neobacin microgel formulated with sunseed oil:goat fat (3:7 matrix) was the overall best, having consistently inhibited the growth of all tested microorganisms, followed by the neobacin® gel and lastly conventional neobacin® powder.

Interestingly, the neobacin ® gel had maximum drug permeation of 35% at 4 h prior to degradation achieving 72% drug retention on the membrane, whereas neobacin microgel that was prepared from 3:7 SMSLM had slow initial release that intercepted the degrading neobacin® gel at 10 h prior to its achieving a maximum *in vitro* drug permeation of 34% and skin retention of 64%. In an outlook study exploiting both fast and delayed release of neobacin®, a complementary system of neobacin® gel and microgel prepared from 3:7 SMSLM would be recommended and if packaged in separate tubes could carry instruction as “After wound cleaning, apply neobacin® gel first and after 4 h, repeat application with microgel”.

Conclusions

This study has shown that microgels are better for wound healing than dry drug powder. Neobacin® gel formulation despite rapid degradation was also superior to the conventional powder form of neobacin®.

Conflict of interests

The authors have not declared any conflict of interests.

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REFERENCES

Acosta E, Chung O, Xuan XY (2011). Lecithin-linker microemulsions in transdermal delivery. J. Drug Del. Sci. Tech. 21:77-87.

Atiyeh BS, Dibo SA, Hayek SN (2009). Wound cleansing, topical antiseptics and wound healing. Int. Wound J. 6:420-430.

Attama AA, Müller-Goymann CC (2006). A critical study of novel physically structured lipid matrices composed of a homolipid from *Capra hircus* and theobroma oil. Int. J. Pharm. 322:67-78.

Attama AA, Schicke BC, Paepenmüller T, Müller-Goymann CC (2007). Solid lipid nanodispersions containing mixed lipid core and a polar heterolipid: characterization. Eur. J. Pharm. Biopharm. 67:48-57.

Bachhav YG, Patravale VB (2009). Microemulsion based vaginal gel of fluconazole: formulation, *in vitro* and *in vivo* evaluation. Int. J. Pharm. 365:175-179.

Bhalekar M, Pokharkar V, Madgulkar A, Patil N (2009). Preparation and evaluation of miconazole nitrate-loaded solid lipid nanoparticles for topical delivery. AAPS Pharm. Sci. Tech. 10:289-296.

Bondi ML, Craparo EF (2010). Solid lipid nanoparticles for applications in gene therapy: a review of the state of the art. Expert Opin. Drug Deliv. 7:7-18.

Chen H, Mou D, Du D, Chang X, Zhu D, Liu J, Xu H, Yang X (2007). Hydrogel-thickened microemulsion for topical administration of drug molecule at an extremely low concentration. Int. J. Pharm. 341:78-84.

Dainiak MB, Allan IU, Savina IN, Cornelio L, James ES, James SL, Mikhalovsky SV, Jungvid H, Galaev IY (2010). Gelatin–fibrinogen cryogel dermal matrices for wound repair: Preparation, optimisation and *in vitro* study. Biomaterials. 31:67-76.

Eskandar NG, Simovic S, Prestidge CA (2010). Mechanistic insight into the dermal delivery from nanoparticle-coated submicron o/w emulsions. J. Pharm. Sci. 99:890-904.

Feng G, Xiong Y, Wang H, Yang Y (2009). Gelation of microemulsions and release behavior of sodium salicylate from gelled microemulsions. Eur. J. Pharm. Biopharm. 71:297-302.

Fox LP, Merk HF, Brickers DR (2006). Dermatological pharmacology. In: Goodman & Gilman (Eds.), The pharmacological basis of therapeutics, McGraw-Hill Companies Inc., USA, pp. 1679-1690.

Higaki K, Nakayama K, Suyama T, Amnuaikit C, Ogawara K, Kimura T (2005). Enhancement of topical delivery of drugs via direct penetration by reducing blood flow rate in skin. Int. J. Pharm. 288:227-233.

Kapoor Y, Chauhan A (2008). Ophthalmic delivery of Cyclosporine A from Brij-97 microemulsion and surfactant-laden p-HEMA hydrogels. Int. J. Pharm. 361:222-229.

Korting H, Schafer-Korting M (2010). Carriers in the topical treatment of skin disease. In: I. Dugheta (Ed.), Handbook of Experimental Pharmacology, Wiley-VCH, Weinheim. pp. 435-468.

Lv Q, Yu A, Xi Y, Li H, Song Z, Cui J, Cao F, Zhai G (2009). Development and evaluation of penciclovir-loaded solid lipid nanoparticles for topical delivery. Int. J. Pharm. 372:191-198.

Muchow M, Maincent P, Muller RH (2008). Lipid nanoparticles with a solid matrix (SLN, NLC, LDC) for oral drug delivery. Drug Dev. Ind. Pharm. 34:1394-1405.

Nnamani PO, Ibezim EC, Attama AA, Adikwu MU (2010). Surface modified solid lipid microparticles based on homolipids and softisan® 142: preliminary characterization. Asian Pacif. J. Trop. Med. 3:205-210.

Padula C, Nicoli S, Santi P (2009). Innovative formulations for the delivery of levothyroxine to the skin. Int. J. Pharm. 372:12-16.

Paolicelli P, Cerreto F, Cesa S, Feeney M, Corrente F, Marianecci C, Casadei MA (2009). Influence of the formulation components on the properties of the system SLN-dextran hydrogel for the modified release of drugs. J. Microencapsul. 26:355-364.

Pardeike J, Hommoss A, Muller RH (2009). Lipid nanoparticles (SLN, NLC) in cosmetic and pharmaceutical dermal products. Int. J. Pharm. 366:170-184.

Puglia C, Blasi P, Rizza L, Schoubben A, Bonina F, Rossi C, Ricci M. (2008). Lipid nanoparticles for prolonged topical delivery: an *in vitro* and *in vivo* investigation. Int. J. Pharm. 357:295-304.

Reszko AE, Berson D, Lupo MP (2009). Cosmeceuticals: practical applications. Dermatol. Clin. 27:401-416.

Sanna V, Caria G, Mariani A (2010). Effect of lipid nanoparticles containing fatty alcohols having different chain length on the *ex vivo* skin permeability of Econazole nitrate. Powder Technol. 201: 32-36.

Sanna V, Kirschvink N, Gustin P, Gavini E, Roland I, Delattre L, Evrard B (2003). Preparation and *in vivo* toxicity study of solid lipid microparticles as carrier for pulmonary administration. AAPS PharmSciTech. 5: Article 27.

Schaefer H, Lademann J (2001). The role of follicular penetration: A differential view. Skin Pharmacol. Appl. Skin Physiol. 14:23-27.

Senyigit T, Sonvico F, Barbieri S, Ozer O, Santi P, Colombo P (2010). Lecithin/chitosan nanoparticles of clobetasol-17-propionate capable of accumulation in pig skin. J. Control. Rel. 142:368-373.

Souto EB, Doktorovova S, Boonme P (2011). Lipid-based colloidal systems (nanoparticles, microemulsions) for drug delivery to the skin: materials and end-product formulations. J. Drug Del. Sci. Tech. 21:43-54.

Souto EB, Wissing SA, Barbosab CM, Müller RH (2004). Evaluation of the physical stability of SLN and NLC before and after incorporation into hydrogel formulations. Eur. J. Pharm. Biopharm. 58:83-90.

Trommer H, Neubert RHH (2006). Overcoming the stratum corneum: the modulation of skin penetration. Skin Pharmacol. Appl. Skin Physiol. 19:106-121.

Wolf NB, Küchler S, Radowski MR, Blaschke T, Kramer KD, Weindl G, Kleuser B, Haag R, Schäfer-Korting M (2009). Influences of opioids and nanoparticles on *in vitro* wound healing models. Eur. J. Pharm. Biopharm. 73:34-42.

Zhu W, Guo C, Yu A, Gao Y, Cao F, Zhai G (2009). Microemulsion based hydrogel formulation of penciclovir for topical delivery. Int. J. Pharm. 378:152-158.

Zhu W, Yu A, Wang W, Dong R, Wu J, Zhai G (2008). Formulation design of microemulsion for dermal delivery of penciclovir. Int. J. Pharm. 360:184-190.