Available online at www.sciencedirect.com
ScienceDirect
European Journal of Pharmaceutics and Biopharmaceutics 67 (2007) 48–57
European Journal of Pharmaceutics and Biopharmaceutics
www.elsevier.com/locate/ejpb

Research paper

Solid lipid nanodispersions containing mixed lipid core and a polar heterolipid: Characterization

A.A. Attama [a,b,*], B.C. Schicke [a], T. Paepenmüller [a], C.C. Müller-Goymann [a]

[a] *Institut für Pharmazeutische Technologie, Technische Universität Carolo-Wilhelmina zu Braunschweig, Braunschweig, Germany*
[b] *Department of Pharmaceutics, University of Nigeria, Nsukka, Nigeria*

Received 7 September 2006; accepted in revised form 5 December 2006
Available online 16 December 2006

Abstract

This paper describes the characterization of solid lipid nanodispersions (SLN) prepared with a 1:1 mixture of theobroma oil and goat fat as the main lipid matrix and Phospholipon 90G® (P90G) as a stabilizer heterolipid, using polysorbate 80 as the mobile surfactant, with a view to applying the SLN in drug delivery. The 1:1 lipid mixture and P90G constituting the lipid matrix was first homogeneously prepared by fusion. Thereafter, the SLN were formulated with a gradient of polysorbate 80 and constant lipid matrix concentration by melt-high pressure homogenisation. The SLN were characterized by time-resolved particle size analysis, zeta potential and osmotic pressure measurements, differential scanning calorimetry (DSC) and wide angle X-ray diffraction (WAXD). Transmission electron microscopy (TEM) and isothermal heat conduction microcalorimetry (IMC) which monitors the in situ crystallization were also carried out on the SLN containing P90G and 1.0 % w/w of polysorbate 80. The results obtained in these studies were compared with SLN prepared with theobroma oil with and without phospholipid. Particle size analysis of SLN indicated reduction in size with increase in concentration of mobile surfactant and was in the lower nanometer range after 3 months except SLN prepared without P90G or polysorbate 80. The lipid nanoparticles had negative potentials after 3 months. WAXD and DSC studies revealed low crystalline SLN after 3 months of storage except in WAXD of SLN formulated with 1.0 % w/w polysorbate 80. TEM micrograph of the SLN containing 1.0 % w/w polysorbate 80 revealed discrete particles whose sizes were in consonance with the static light scattering measurement. In situ crystallization studies in IMC revealed delayed crystallization of the SLN with 1.0 % w/w polysorbate 80. Results indicate lipid mixtures produced SLN with lower crystallinity and higher particle sizes compared with SLN prepared with theobroma oil alone with or without P90G, and would lead to higher drug incorporation efficiency when used in formulation of actives. Mixtures of theobroma oil and goat fat would be suitable for the preparation of nanostructured lipid carriers. SLN of theobroma oil containing phospholipid could prove to be a good ocular or parenteral drug delivery system considering the low particle size, particle size stability and in vivo tolerability of the component lipids. SLN prepared with lipid admixture, which had higher increase in $d_{90\%}$ on storage are suitable for preparation of topical and transdermal products.

Keywords: Mixed lipid; Theobroma oil; Phospholipon 90G®; Solid lipid nanoparticles; Nanostructured lipid carriers; Characterization; Drug delivery

1. Introduction

Nanosized controlled drug delivery devices consisting mainly of nanoemulsions, liposomes and lipid nanoparticles have been proposed recently for oral, topical and parenteral administration of drugs. Solid lipid nanoparticles (SLN) consist of solid lipids in nanosized range dispersed in aqueous medium. SLN combine the advantages and avoid the disadvantages of other colloidal carrier systems, and are regarded as an alternative carrier system to other colloidal drug delivery systems [1–3]. When optimised, SLN exhibit high physical stability, protection of incorporated labile actives against degradation and excellent in vivo tolerability [4–6]. However, these systems generally exhibit a low drug pay-load capacity and drug

* Corresponding author. Department of Pharmaceutics, University of Nigeria, Nsukka 410001, Nigeria. Tel.: +234 42 771911; fax: +234 42 771709.
E-mail address: aaattama@yahoo.com (A.A. Attama).

0939-6411/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejpb.2006.12.004

expulsion during storage due to transition to highly ordered lipid particles [7].

Crystallinity of lipid matrices affects the functional properties of SLN derived therefrom. Lipid mixtures can result in increased or decreased crystallinity. Directly after preparation, lipids crystallize partially in higher energy modifications (α, β') with more imperfections in the crystal lattice [8–10]. If however, a polymorphic transition to β modification takes place during storage, any incorporated drug could be expelled from the lipid matrix and it can then neither be protected from degradation nor released in a controlled manner. To overcome such phenomenon, use of mixtures of lipids which do not form highly ordered crystalline arrangement is needed. Such lipid matrix could be achieved by using solid lipid and liquid lipid [11] or solid lipid mixtures of complex nature such as mono-, di- or triglycerides of different chain lengths [7]. Mixture of lipids also modifies the polymorphic properties of the individual lipids, and has been shown to generate lipid matrices of low crystallinity [12]. Lipid nanoparticles produced from such lipid mixtures involving solid lipid and liquid lipid have been termed nanostructured lipid carriers [10,11].

It was the objective of this study to formulate and characterize SLN from physically structured lipid matrix composed of 1:1 mixture of theobroma oil and goat fat. This lipid matrix has been characterized in an earlier study [13]. The SLN were compared with those formulated with theobroma oil alone. As adjuncts, Phospholipon 90G® and polysorbate 80 were used to further stabilize the formulated SLN for improved performance as drug delivery systems. Use of Phospholipon 90G® as lipid nanoparticle surface modifier has been reported [14,15]. However, in this study a lipid matrix composed of the 1:1 mixture of theobroma oil and goat fat, and Phospholipon 90G® was first formulated and used in the formulation of SLN. For the SLN, characterization procedures such as static light scattering, differential scanning calorimetry (DSC), wide angle X-ray diffraction (WAXD), transmission electron microscopy (TEM) and isothermal heat conduction microcalorimetry (IMC) were employed to investigate the colloidal lipid dispersion.

Theobroma oil obtained from the seeds of *Theobroma cacao* is a lipid with many applications in pharmaceutical and food industries. The polymorphic transformation in theobroma oil is well documented [16,17]. Goat fat on the other hand is extracted from *Capra hircus*, an animal domesticated for meat purposes. This fat is easily sourced in Nigeria and represents a potential for drug delivery applications because of its crystal properties [13]. Goat fat consists mainly of triglycerides of C16, C18 and C18:1 fatty acids which are somewhat similar to the fatty acid profile of theobroma oil [18]. However, the location of these fatty acids in their triglycerides differs, with theobroma oil being more homogeneous. A 1:1 binary mixture of these two lipids resulted in mixture of crystals with high degree of disorder that may be favourable for drug loading [13]. Despite possessing good crystal properties, natural lipids are better tolerated in vivo than semi-synthetic lipids. Goat fat has been used in the formulation of self-emulsifying drug delivery systems and has been shown to be stable to rancidity after degumming and deodorization [19,20].

2. Materials and methods

2.1. Materials

Phospholipon 90G® (P90G) (Phospholipid GmbH, Köln, Germany) is a purified, deoiled and granulated soy lecithin with a phosphatidylcholine content of at least 90%. Thimerosal (Synochem, Germany), sorbitol, theobroma oil (Caesar & Loretz, Germany) and polysorbate 80 (Tween 80®) (Across Organics, Germany) were used as procured from their manufacturers without further purification. Goat fat was obtained from a batch processed according to earlier procedure [19]. Bidistilled water was used for nanoparticle preparation.

2.2. Formulation of the lipid matrix

The lipid matrix used in SLN formulation corresponded to 30 % w/w of P90G in 1:1 mixture of theobroma oil and goat fat was prepared by fusion. This concentration of P90G has been shown to produce nanoparticles with good qualities [15]. The lipids were weighed with an electronic balance (Type L2200P-xD2, Sartorius AG, Göttingen, Germany), melted together at 60 °C on a hot plate (RCT basic, IKA®, Staufen, Germany) and stirred until solidification.

2.3. Formulation of solid lipid nanoparticles (SLN)

SLN were formulated to contain 5 % w/w of lipid matrix (30 % w/w of P90G in 1:1 mixture of theobroma oil and goat fat), 0.0, 0.1, 0.5 or 1.0 % w/w of polysorbate 80 (corresponding, respectively, to SLN-0M, SLN-1M, SLN-2M and SLN-3M), 4 % w/w of sorbitol, 0.005 % w/w of thimerosal and enough bidistilled water to make 100 % w/w. SLN containing 1.0 % w/w polysorbate 80 but no P90G (SLN-4M) was also prepared ('M' stands for mixed lipid). The hot homogenisation technique was adopted. In each case, the lipid matrix was melted at 60 °C and the water containing polysorbate 80, thimerosal and sorbitol at the same temperature was added to the molten lipid matrix with gentle stirring with a magnetic stirrer. The mixture was further dispersed with a mixer (Ultra-Turrax T25 basic, Ika Staufen, Germany) at 24,000 rpm for 5 min to produce the hot primary emulsion. The hot primary emulsion at 60 °C was immediately passed through a heated high pressure homogeniser (EmulsiFlex-C5, Avestin, Canada) at a pressure of 1000 bars for 20 cycles to produce the nanoparticles, which were collected in a hot container and allowed to recrystallize at room temperature. For comparison, SLN containing

theobroma oil and similar concentrations of polysorbate 80 and P90G as in those of 1:1 lipid mixture were similarly prepared and labelled SLN-0S, SLN-1S, SLN-2S, SLN-3S and SLN-4S ('S' stands for single lipid).

2.4. Particle size analysis

The mean hydrodynamic (peak) and size distribution (width) of the SLN were assessed at 25 °C by polarization intensity differential scattering (PIDS) with a Multi-Wavelength Coulter Counter (LS 13 320, Beckman Coulter, USA). Prior to measurement, the samples were diluted with demineralised water to PIDS obscurity of not less than 40%. Data were collected during 60 s and further analysed using the diameter 50% ($d_{50\%}$), 75% ($d_{75\%}$) and 90% ($d_{90\%}$). Size distribution by volume was calculated by applying an optical module created by the instrument software. Measurements were performed in triplicate. Particle size measurements were done 24 h, 1 week, 1 and 3 months after SLN preparation.

2.5. Zeta potential measurement

The zeta potential of a colloidal sample determines whether the particles within a liquid will tend to flocculate (stick together) or not. It is the potential within the hydrodynamic shear or slipping plane of the electric double layer of a charged particle. The zeta potentials of the formulated SLN were determined after 3 months of preparation in a Zetasizer Nano Series (Nano-ZS, Malvern Instruments, England). Each sample was diluted with bidistilled water (pH 7.0) and the electrophoretic mobility determined at 25 °C and dispersant dielectric constant of 78.5. The obtained electrophoretic mobility values were used to calculate the zeta potentials using the software DTS Version 4.1 (Malvern, England) and applying Henry equation [21]

$$U_{\mathrm{E}} = \frac{2\varepsilon Z f(K_a)}{3\eta} \tag{1}$$

where Z is the zeta potential, U_{E} is the electrophoretic mobility, ε is the dielectric constant, η is the viscosity of the medium and $f(K_a)$ is the Henry's function. The electrophoretic mobility was determined in an aqueous medium, thus the Henry's function in this case is 1.5 and is referred to as Smoluchowski approximation [21].

2.6. Osmotic pressure measurements

The osmotic pressures of the nanodispersions were determined by the freezing point depression method using a Semi-Micro Osmometer (LI 89 OSM, Knauer, Berlin, Germany), calibrated with bidistilled water (0 mOsmol/kg) and sodium chloride solution (400 mOsmol/kg). Average of three determinations for each batch of SLN was taken as the osmotic pressure.

2.7. Wide angle X-ray diffraction (WAXD)

WAXD was used to investigate the crystalline character of the formulated SLN. Wide angle X-ray studies were done on the formulated SLN using an X-ray generator (PW3040/60 X'Pert PRO, PANalytical, Netherlands) connected to a copper anode (PW3373/00 DK, 147726 Cu LFF) which delivered X-ray of wavelength, $\lambda = 0.1542$ nm, at a voltage of 40 kV and an anode current of 25 mA. The SLN samples were filled into cube-shaped sample holders and WAXD measurements were taken with a Goniometer (PW3050/60 MPD-System, PANalytical, Netherlands) from 3.0° to 33.0° in 0.015° steps (1 s per step). The interlayer spacing d was calculated from the scattering angle θ, using Bragg's equation

$$n\lambda = 2d\sin\theta \tag{2}$$

where λ is the wavelength of the incident X-ray beam and n is the order of the interference. WAXD diffractograms were obtained 24 h, 1 and 3 months after SLN preparation.

2.8. Differential scanning calorimetry (DSC)

The degree of crystallinity of the lipid nanoparticles was determined on a calorimeter (DSC 220C) connected to a disc station (5200H, Seiko, Tokyo, Japan). Approximately 5 mg of each SLN was weighed into an aluminium pan and sealed hermetically, and the thermal behaviour determined in the range of 10 to 125 °C at a heating rate of 5 °C min^{-1}. Baselines were determined using an empty pan, and all the thermograms were baseline-corrected. Transition temperatures were determined from the endothermic peak minima while transition enthalpies were obtained by integration of the endothermic transitions using linear baselines. The analyses were done 24 h, 1 and 3 months after SLN preparation.

2.9. Transmission electron microscopy (TEM)

The TEM of SLN containing 1.0 % w/w polysorbate 80 was done to study their morphologies. The samples were shock-frozen in melting nitrogen at 63 K between two flat gold holders, fractured at 173 K in a BAF 400 instrument (Balzers, D-Wiesbaden, Germany) and shadowed with platinum/carbon (2 nm) at 45° and with pure carbon at 90° for replica stabilization. After cleaning with chloroform-methanol mixture (1:1), the replicas on uncoated grids were fixed onto a sample holder and placed in the vacuum chamber of a transmission electron microscope (Leo 922, Leo D-Oberkochen, Germany), and viewed under low vacuum at 200 kV.

2.10. Isothermal heat conduction microcalorimetry (IMC)

IMC was used to study the rate of recrystallization of the formulated SLN. Crystallization exotherms of SLN containing 1.0 % w/w polysorbate 80 were recorded in an isothermal heat conduction microcalorimeter (Thermal

Activity Monitor®, TAM 2277, Thermometric AB, Jarfalla, Sweden). Two calorimeter units were installed and run concurrently. A 2 g quantity of the SLN was weighed into 3 ml glass ampoule, equilibrated for 30 min at 20 °C, and the heat flow arising from changes in crystal properties measured at the range of −3000 to 3000 μW. Heat flow signals were monitored by a Digitam Software (Thermometric AB, Jarfalla, Sweden). Data obtained from the IMC measurements were thereafter analysed to determine the isothermal crystallization kinetics of the lipid nanoparticles using the kinetics described by the Avrami equation [22,23]

$$1 - X_t = \exp(-kt^n) \quad (3)$$

where X_t represents the crystallized fraction at time t, k (time^{-1}) is the crystal growth rate constant of isothermal crystallization and n is the Avrami exponent related to the type of nucleation and growth mechanisms of crystals particularly with regard to the dimensionality of growth.

3. Results and discussion

3.1. Particle size and zeta potential

The result of the particle size analysis carried out on the SLN expressed as volume distribution is presented in Table 1. Particle size decreased with increase in mobile surfactant concentration in context of decreasing interfacial tension and increase in the rate of particle disintegration during homogenisation. Melt high pressure homogenisation adopted in the formulation of the nanoparticles has been shown to produce small particles [24], and the use of two substances with emulsifying capabilities has been shown to perform better than single emulsifier [25]. In this study, there was higher growth in particle sizes of SLN formulated with mixed lipid compared with SLN formulated with theobroma oil alone, as seen by the increase in the $d_{75\%}$ and $d_{90\%}$ values within 1 and 3 months. This increase in the $d_{75\%}$ and $d_{90\%}$ values may preclude the use of SLN prepared with mixed lipid as parenteral or ocular drug delivery systems, where high degree of particle size stability is pertinent in addition to a small initial size. This SLN may perform better as drug delivery systems for oral, topical or transdermal application, where strict limit in particle size and particle size stability may be overlooked. It is interesting to note that SLN formed without polysorbate 80 (SLN-0M and SLN-0S) but had significantly ($p < 0.05$) higher $d_{75\%}$ and $d_{90\%}$ values after 3 months indicating less stable products (Table 1) compared with SLN containing P90G. $d_{90\%}$ values are sensitive to larger particles as high values indicate presence of micrometer particles. Polysorbate 80 was needed to further stabilize the system as its presence reduced the $d_{75\%}$ and $d_{90\%}$ values of the SLN formulations throughout the period of study (SLN-1M, SLN-2M and SLN-3M, and SLN-1S, SLN-2S and SLN-3S). SLN-3M prepared with mixed lipid, which contained 1.0 % w/w polysorbate 80, had the best particle size distribution within the mixed lipid class as its $d_{90\%}$ value was lowest after 3 months, but its values were, however, sig-

Table 1
Particle size distribution of the solid lipid nanodispersions

Batch	Mean volume distribution (μm ± SD)*											
	24 h			1 week			1 month			3 months		
	$d_{50\%}$	$d_{75\%}$	$d_{90\%}$	$d_{50\%}$	$d_{75\%}$	$d_{90\%}$	$d_{50\%}$	$d_{75\%}$	$d_{90\%}$	$d_{50\%}$	$d_{75\%}$	$d_{90\%}$
SLN-0M	0.089 ± 0.007	0.111 ± 0.010	0.140 ± 0.011	0.094 ± 0.002	0.121 ± 0.012	0.173 ± 0.011	0.182 ± 0.004	0.552 ± 0.019	0.668 ± 0.016	0.575 ± 0.005	5.052 ± 1.045	34.735 ± 4.076
SLN-1M	0.086 ± 0.003	0.105 ± 0.006	0.125 ± 0.009	0.090 ± 0.001	0.114 ± 0.002	0.151 ± 0.007	0.098 ± 0.012	0.126 ± 0.011	0.557 ± 0.021	0.241 ± 0.047	0.482 ± 0.033	0.937 ± 0.099
SLN-2M	0.088 ± 0.003	0.108 ± 0.005	0.129 ± 0.006	0.090 ± 0.000	0.111 ± 0.000	0.137 ± 0.006	0.112 ± 0.007	0.124 ± 0.011	0.355 ± 0.022	0.173 ± 0.033	0.299 ± 0.012	0.634 ± 0.017
SLN-3M	0.082 ± 0.005	0.099 ± 0.007	0.116 ± 0.009	0.064 ± 0.002	0.073 ± 0.002	0.123 ± 0.005	0.115 ± 0.011	0.183 ± 0.022	0.340 ± 0.011	0.142 ± 0.007	0.185 ± 0.075	0.479 ± 0.042
SLN-4M	0.678 ± 0.022	0.871 ± 0.023	1.042 ± 0.129	0.693 ± 0.002	0.880 ± 0.017	1.364 ± 0.384	0.883 ± 0.052	1.962 ± 0.917	17.565 ± 1.605	0.874 ± 0.070	6.176 ± 4.999	40.047 ± 2.946
SLN-0S	0.090 ± 0.002	0.113 ± 0.003	0.138 ± 0.002	0.094 ± 0.001	0.173 ± 0.001	0.214 ± 0.003	0.121 ± 0.001	0.169 ± 0.001	0.244 ± 0.011	0.532 ± 0.018	2.877 ± 0.054	12.373 ± 2.137
SLN-1S	0.090 ± 0.001	0.111 ± 0.002	0.133 ± 0.001	0.091 ± 0.002	0.113 ± 0.003	0.135 ± 0.002	0.104 ± 0.001	0.138 ± 0.001	0.177 ± 0.001	0.128 ± 0.006	0.151 ± 0.003	0.298 ± 0.022
SLN-2S	0.096 ± 0.002	0.121 ± 0.000	0.149 ± 0.001	0.097 ± 0.002	0.121 ± 0.000	0.147 ± 0.003	0.102 ± 0.002	0.132 ± 0.002	0.174 ± 0.001	0.122 ± 0.007	0.139 ± 0.011	0.263 ± 0.013
SLN-3S	0.086 ± 0.002	0.107 ± 0.001	0.132 ± 0.003	0.089 ± 0.000	0.111 ± 0.001	0.132 ± 0.000	0.087 ± 0.001	0.126 ± 0.002	0.141 ± 0.002	0.112 ± 0.002	0.120 ± 0.012	0.246 ± 0.021
SLN-4S	0.680 ± 0.067	0.792 ± 0.059	0.885 ± 0.032	0.590 ± 0.001	0.692 ± 0.001	0.785 ± 0.002	0.573 ± 0.004	0.668 ± 0.007	0.761 ± 0.011	0.600 ± 0.002	0.709 ± 0.001	0.817 ± 0.003

SLN-0M to 3M (mixed lipid matrix) and SLN-0S to 3S (single lipid matrix) represent SLN formulated with 0.0, 0.1, 0.5 and 1.0 % w/w of polysorbate 80, respectively. SLN-4M (mixed lipid matrix) and SLN-4S (single lipid matrix) contain 1.0 % w/w polysorbate 80 and no P90G. *SD is the standard deviation ($n = 3$).

nificantly ($p < 0.05$) higher than the values for SLN-3S prepared with equivalent concentration of polysorbate 80 but containing only theobroma oil (single lipid). SLN prepared without P90G (SLN-4M) possessed significantly ($p < 0.05$) higher particle size compared with SLN prepared with P90G (SLN-1M, SLN-2M and SLN-3M) indicating P90G is needed for stabilization.

The particle sizes of SLN prepared with theobroma oil alone were within the lower nanometer range after 3 months of storage as the $d_{90\%}$ values were low throughout the period of study, signifying very low or absence of micrometer particles compared with SLN prepared with mixed lipid. But SLN-4S containing no P90G had higher particle size also confirming the need for inclusion of P90G to achieve stable lipid nanoparticles. A reported study using theobroma oil (cacao butter) and curdlan produced nanoparticles with low particle sizes and polydispersity indices [26]. There was almost no difference ($p < 0.05$) in the sizes of SLN-1S and SLN-2S after 1 and 3 months. This shows that low concentration of polysorbate 80 can stabilize SLN prepared with theobroma oil and phospholipid.

SLN prepared with theobroma oil alone are promising drug delivery system for parenteral or ocular drug candidates, considering the particle size and the in vivo tolerability of the components – both the lipids and the surfactant. Particle size, particle size distribution and stability are a major issue considered by formulation scientists when formulating dispersed systems especially those intended for parenteral or ocular administration. For parenteral disperse system dosage forms, particle size has to be maintained within a safe range to not only prevent thrombosis or blockade of blood vessels, but also to make administration possible. For ocular administration, irritation and tear wash out may occur on administration of large sized particles, since smaller particles are better tolerated [27]. Very small particles as nanoparticles possess adhesive properties [28], which could help prolong the residence time of the dosage form in the cul-de-sac, prevent tear wash out (due to tear dynamics), and increase ocular bioavailability.

The magnitude of zeta potential gives an indication of the potential stability of a colloidal system. Large negative or large positive zeta potential is required for colloidal dispersion stability. The general dividing line between stable and unstable suspension is generally taken as either +30 mV or −30 mV [21]. SLN prepared with mixed lipid possessed negative zeta potentials, with magnitude which was lowest in SLN-0M prepared without polysorbate 80 (Fig. 1). This

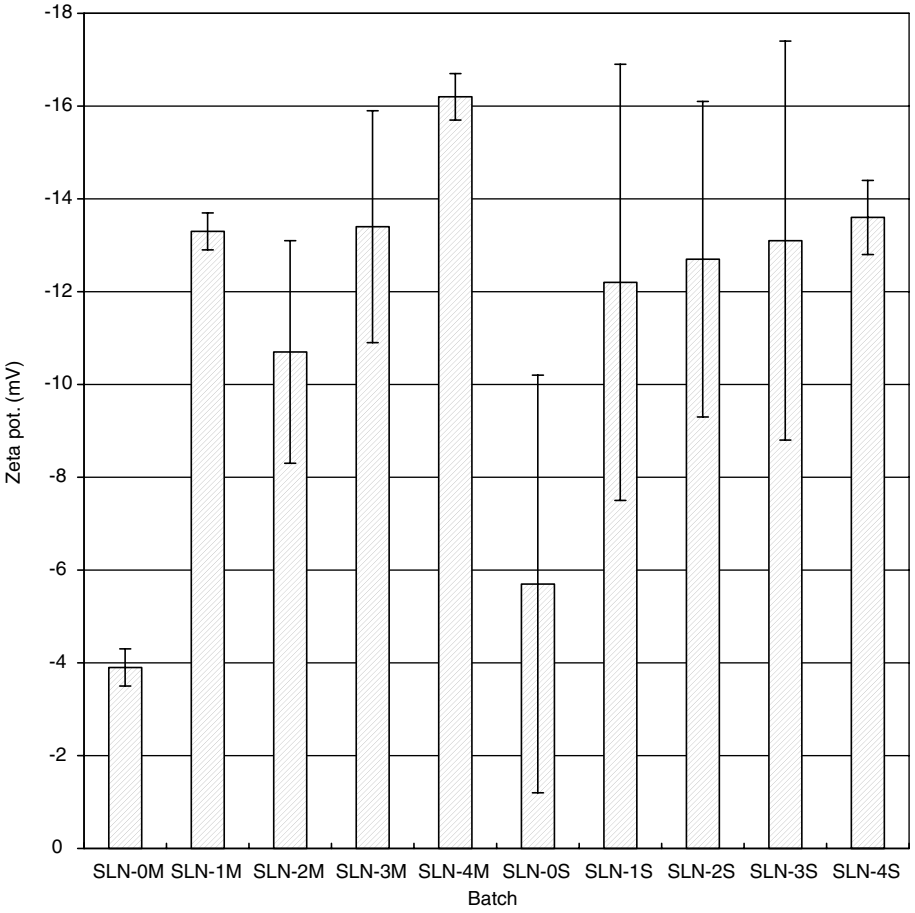

Fig. 1. Zeta potentials of the SLN after 12 weeks. SLN-0M to 3M (mixed lipid matrix) and SLN-0S to 3S (single lipid matrix) represent SLN formulated with 0.0, 0.1, 0.5 and 1.0 % w/w of polysorbate 80, respectively. SLN-4M (mixed lipid matrix) and SLN-4S (single lipid matrix) contain 1.0 % w/w polysorbate 80 and no P90G ($n = 4$).

shows that other SLN with higher zeta potentials were more stable compared with SLN-0M. This result is similar to the particle size result and further proves additional surfactant is needed in P90G stabilized nanodispersions. For the SLN containing polysorbate 80, the values of zeta potentials were -13.3 ± 0.4 mV, -10.7 ± 2.4 mV, -13.4 ± 2.5 mV and -16.2 ± 0.5 mV, respectively, for SLN-1M, SLN-2M, SLN-3M and SLN-4M while a value of -3.9 ± 0.4 was obtained for SLN-0M. This result indicates that SLN-1M, SLN-2M, SLN-3M and SLN-4M have similar interfacial properties. Similar results were obtained for SLN prepared with theobroma oil alone. SLN-0S containing no polysorbate 80 possessed the absolutely lowest zeta potential value. The zeta potential values obtained for these SLN were -12.2 ± 5.2, -12.7 ± 6.3, -13.1 ± 4.3 and -13.6 ± 0.8 mV, respectively, for SLN-1S, SLN-2S, SLN-3S and SLN-4S, while a potential of -5.7 ± 4.5 mV was recorded for SLN-0S. In comparison with the zeta potentials obtained for the mixed lipid SLN, there was no significant difference ($p < 0.05$) between the values of the SLN containing polysorbate 80, and showed they possess similar interfacial properties. The added P90G synergised with polysorbate 80 at the interface to further improve the surface properties, since P90G have been shown to modify surfaces of SLN [14]. Surface-modified SLN are potential delivery systems as biological macromolecules such as proteins and peptides, and diagnostics could be tethered to the structures formed at the surface and their cellular trafficking improved. SLN can penetrate cells and can be used for intracellular delivery of some drugs and sensitive macromolecules. The zeta potentials obtained for the lipid nanodispersions would contribute to stability of the dispersion, and presence of phospholipid structures on the surface would provide spaces for attachment of drugs and other biomolecules.

3.2. Differential scanning calorimetry (DSC)

In order to determine their degree of crystallinity, the SLN were investigated by DSC. Within 24 h and 1 month, the systems displayed several diffuse transitions upon heating which were, however, difficult to evaluate due to their low heat of transition (Figures not shown). After 3 months, SLN-1M, SLN-2M and SLN-3M presented melting transitions with peak minima, respectively, at 31.3 ± 0.3, 32.2 ± 0.2 and 31.1 ± 0.3 °C (Fig. 2a), which were close to those detected for the lower melting transition of the lipid matrix containing phospholipid. DSC thermograms of theobroma oil showed a single peak at 34.7 ± 0.6 °C with a total enthalpy of 152.7 ± 0.42 mJ/mg while goat fat alone showed two endothermic peak transitions and total enthalpy similar to earlier report [13]. The 1:1 mixture of theobroma oil and goat fat had three melting peaks at 24.7 ± 0.3, 34.3 ± 0.4 and 44.8 ± 0.2 °C with total enthalpy of 96.3 ± 2.4 mJ/mg, while on addition of 30 % w/w P90G as used in this study, the resulting lipid matrix presented a peak at 35.4 ± 0.3 °C and a shoulder at 46.5 ± 0.2 °C, with a total enthalpy of 77.8 ± 1.7 mJ/mg. In comparison,

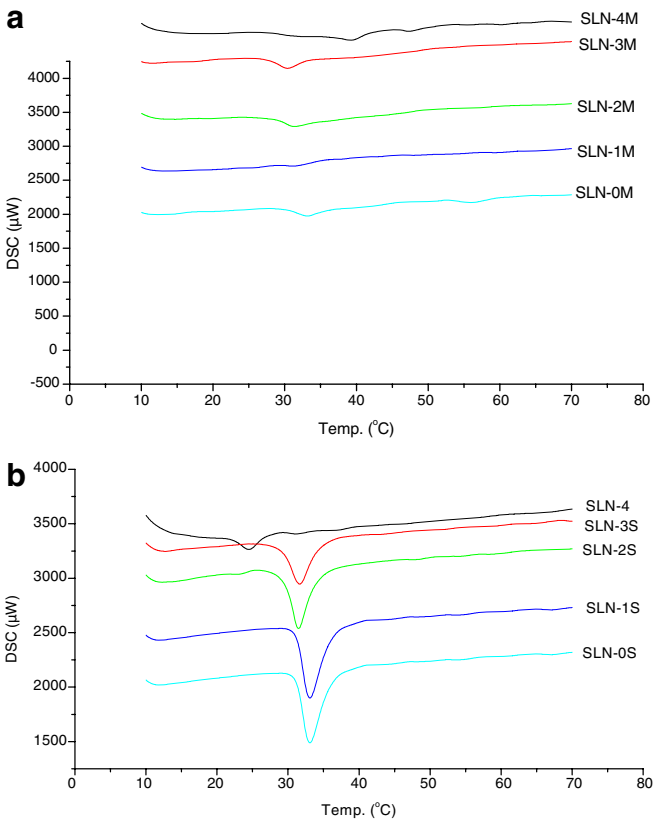

Fig. 2. DSC thermograms of the SLN after 3 months. SLN-0M to 3M (mixed lipid matrix) and SLN-0S to 3S (single lipid matrix) represent SLN formulated with 0.0, 0.1, 0.5 and 1.0 % w/w of polysorbate 80, respectively. SLN-4M (mixed lipid matrix) and SLN-4S (single lipid matrix) contain 1.0 % w/w polysorbate 80 and no P90G.

there was a significant reduction ($p < 0.5$) of the melting enthalpy of the phospholipid-containing lipid matrix showing a reduction in crystallinity.

SLN-4M without P90G had two melting peaks, which occurred at higher temperatures (38.7 ± 0.3 and 47.8 ± 0.4 °C) compared with the SLN containing P90G showing SLN-4M crystallized in a more stable state. SLN-0M on the other hand presented two melting transitions with peak minima at 33.7 ± 0.2 and 56.3 ± 0.4 °C, with the higher melting transition occurring at a temperature higher than those detected for the other SLN (Fig. 2a). This may be due to recrystallization of the SLN in higher melting β modification. On evaluation, the transition enthalpies obtained for the SLN were 3.14 ± 0.67, 0.52 ± 0.21, 3.76 ± 0.77, 3.09 ± 0.61 and 4.72 ± 0.37 mJ/mg, respectively, for SLN-0M, SLN-1M, SLN-2M, SLN-3M and SLN-4M after 3 months. These values were low attesting further to their low degree of crystallinity.

SLN-0S, SLN-1S, SLN-2S and SLN-3S showed peak minima of the endothermic transitions at 32.8 ± 0.3, 32.8 ± 0.2, 31.4 ± 0.2 and 31.6 ± 0.4 °C, respectively (Fig. 2b), while SLN-4S presented melting transitions at 24.4 ± 0.5 °C, with melting signals at about 32 and 37 °C. SLN-4S crystallized in stable and unstable modifications, and change to the more stable form on storage would lead

to increase in crystallinity and expulsion of any incorporated drug. The lower melting peak would belong to unstable modification while the higher peaks belong to stable modification. The P90G added to some SLN therefore modified the crystallization of the lipid particles. On evaluation, the enthalpies of the transitions were found to be 4.53 ± 0.98, 4.73 ± 1.09, 4.72 ± 1.12, 4.37 ± 0.71 and 4.49 ± 0.61 mJ/mg, respectively, for SLN-0S to 4S. In comparison, the melting transitions and the enthalpies of SLN-0S to 3S prepared with theobroma oil alone were significantly different ($p < 0.05$) from those of SLN-0M to 3M prepared with mixed lipids. The lower enthalpy recorded for the SLN prepared with mixed lipid indicates they are less crystalline than the SLN prepared with theobroma oil alone, and shows that mixed lipids, which do not form highly ordered matrix, could be used in the preparation of nanostructured lipid carriers or SLN.

3.3. Wide angle X-ray diffraction (WAXD)

The crystalline state of SLN was also investigated by WAXD studies. Figs. 3a and b represent the diffractograms obtained for the SLN after 3 months. All the SLN prepared with mixed lipids remained amorphous 24 h and 1 month after preparation (Figure not shown). After 3 months, weak intensity reflections were detected at $2\theta = 19.3°$ $d = 4.60$ Å for SLN-1M, SLN-2M and SLN-3M, while three very weak reflections were detected for SLN-0M at $2\theta = 6.0°$ $d = 14.73$ Å, $2\theta = 19.3°$ $d = 4.60$ Å and $2\theta = 23.1°$ $d = 3.87$ Å (Fig. 3a). SLN-4M presented a reflection at $2\theta = 19.7°$ $d = 4.67$ Å slightly different from the other SLN prepared with mixed lipid. However, the intensity remains low compared with SLN prepared with theobroma oil. These weak and very weak reflections detected for the SLN were in the stable β modification and may signify that further modification leading to highly ordered particles would not occur. Their intensities denote low degree of crystallinity. SLN-0M presented roughly three reflections, which further highlight the DSC result where it presented two separate endothermic processes, with the one of higher temperature belonging to the stable β modification of the higher melting lipid fraction.

SLN formulated with theobroma oil only remained amorphous 24 h after preparation (Figure not shown). After 1 month, SLN-2S and SLN-3S remained amorphous but SLN-0S, SLN-1S and SLN-4S presented some weak reflections at $2\theta = 13.0°$ $d = 6.81$ Å and $2\theta = 19.3°$ $d = 4.60$ Å, respectively (Figure not shown). After 3 months of preparation, only SLN-3S remained amorphous while others presented characteristic β-modified reflections at $2\theta = 19.3°$ $d = 4.60$ Å (Fig. 3b). There was thus a delay in recrystallization of the SLN consistent with reported work [24], despite an overall increase in crystallinity. These SLN presented Bragg reflections characteristic of stable β-modification after 3 months. Their intensities were higher than those of mixed lipid nanoparticles, which signify higher degree of order, or higher amount of crystalline material.

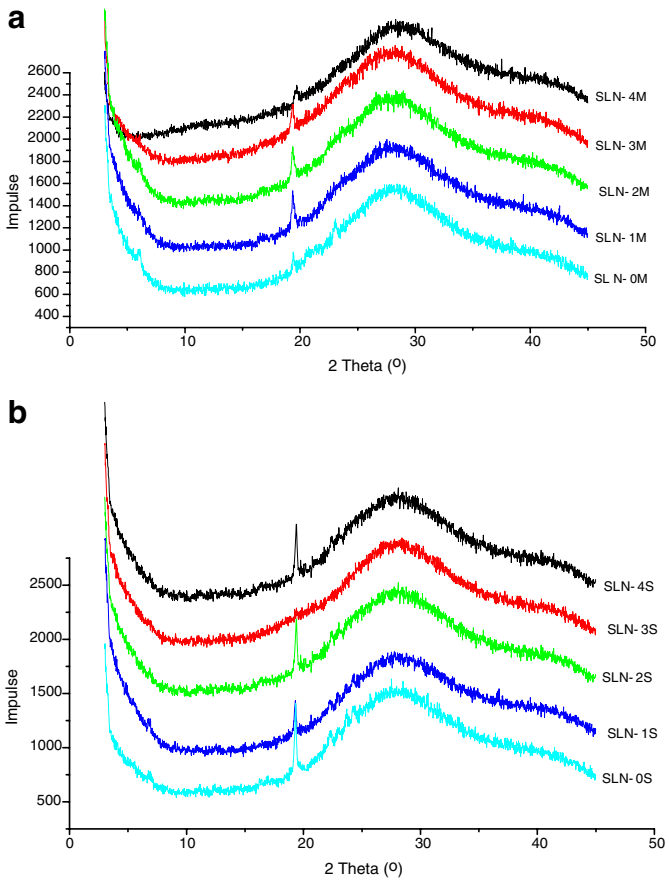

Fig. 3. WAXD diffractograms of the SLN after 3 months. SLN-0M to 3M (mixed lipid matrix) and SLN-0S to 3S (single lipid matrix) represent SLN formulated with 0.0, 0.1, 0.5 and 1.0 % w/w of polysorbate 80, respectively. SLN-4M (mixed lipid matrix) and SLN-4S (single lipid matrix) contain 1.0 % w/w polysorbate 80 and no P90G.

This suggests mixed lipids would also be suitable for preparation of nanostructured lipid carrier. The diffuse halo of SLN-3S, which persisted after 3 months, belongs either to amorphous or liquid crystalline state of the particles. SLN prepared with theobroma oil alone were more crystalline than SLN prepared with 1:1 mixture of theobroma oil and goat fat at equivalent concentrations of polysorbate 80. SLN-1M to 3M would achieve higher drug encapsulation when used in drug formulation, compared with SLN-1S to 3S, since the latter would lead to partial drug expulsion because of higher crystallinity.

3.4. Osmotic pressure measurements

The osmotic pressures obtained for the different nanodispersions using the freezing point depression method are presented in Fig. 4. Osmotic pressure values recorded for all the SLN ranged from 238.67 ± 3.51 to 293.00 ± 1.00 mOsmol/kg, with SLN-2S and SLN-0M having the highest and lowest values, respectively (Fig. 4). The freezing point depression method is the most commonly used method as it provides a rapid means of determining sample osmolality. Pharmaceutical manufacturers and researchers

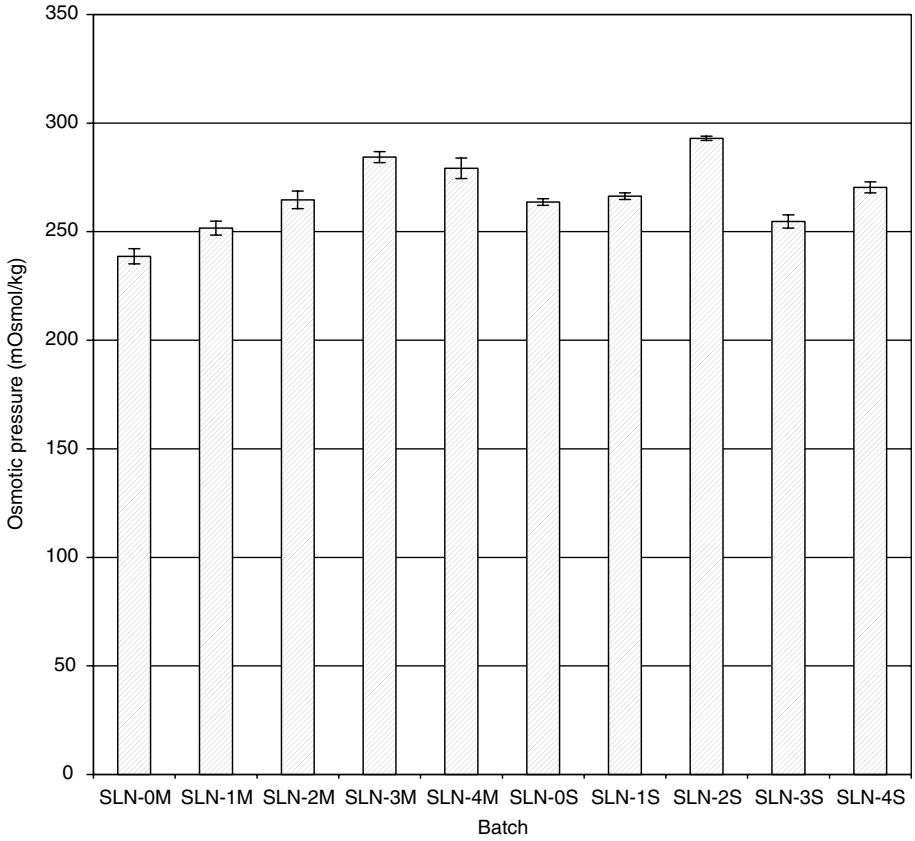

Fig. 4. Osmotic pressures of the SLN after 4 weeks. SLN-0M to 3M (mixed lipid matrix) and SLN-0S to 3S (single lipid matrix) represent SLN formulated with 0.0, 0.1, 0.5 and 1.0 % w/w of polysorbate 80, respectively. SLN-4M (mixed lipid matrix) and SLN-4S (single lipid matrix) contain 1.0 % w/w polysorbate 80 and no P90G ($n = 3$).

can benefit from routine osmolality testing in the area of drug development. Pharmaceutical and therapeutic consideration of osmotic effects concerns the side effects of ophthalmic and parenteral formulations due to abnormal osmotic pressure. With the osmometer used here, serum has an osmolality of approximately 290 mOsmol/kg. The values recorded for these SLN did not grossly deviate form this approximate value. It is thus expected that the hypoosmolar SLN (SLN-0S, SLN-1S and SLN-3S) would not cause a major osmotic upset when injected or instilled into the eye considering the rapid dilution that may occur on administration. Higher quantity of sorbitol or other isotonizing agents may be required to raise the osmotic pressure. The hypoosmolar SLN containing mixed lipid core will not pose any problem since the preparations are suitable for topical, transdermal or oral application.

3.5. Transmission electron microscopy (TEM)

The TEM of SLN-3M and SLN-3S are presented in Fig. 5. TEM revealed well defined anisometric structures of ellipsoidal to disc-like shapes. If the particles were viewed edge-on, they appear as rods or needles. Given particle dimensions are only estimates due to the fact that a particle observation exactly top-on and edge-on, respectively, is rare and cannot be discriminated from slight particle twists, in addition [29]. The size and shape of a nanoparticle are influenced by the velocity of lipid crystallization, the lipid hydrophilicity and the shape of the lipid crystals [24]. The anisometric shape may have resulted from the long delay in recrystallization of the SLN as detected by IMC measurement, as particles may not grow uniformly in three dimensions. The TEM micrographs also show a range of particle sizes, but due to the dimensional limitation of TEM micrograph interpretation, a conclusive statement on the range of particle size cannot be made therefrom. Although the systems contained high amount of phospholipids, no spherical multilamellar vesicles indicating liposome formation were detected rather, layered structures such as terraces and steps being characteristics of crystalline solid lipids are observed within the particles especially in SLN-3M nanoparticles. From the TEM micrographs, SLN-3M appears to be more crystalline than SLN-3S, suggesting SLN-3S to be present in the liquid crystalline state at least partially as detected by WAXD.

3.6. Isothermal heat conduction microcalorimetry (IMC)

Isothermal microcalorimetry can provide precise and rapid knowledge about possible solid state transition processes. Microcalorimetry is an analytical technique that has found numerous applications within the pharmaceutical

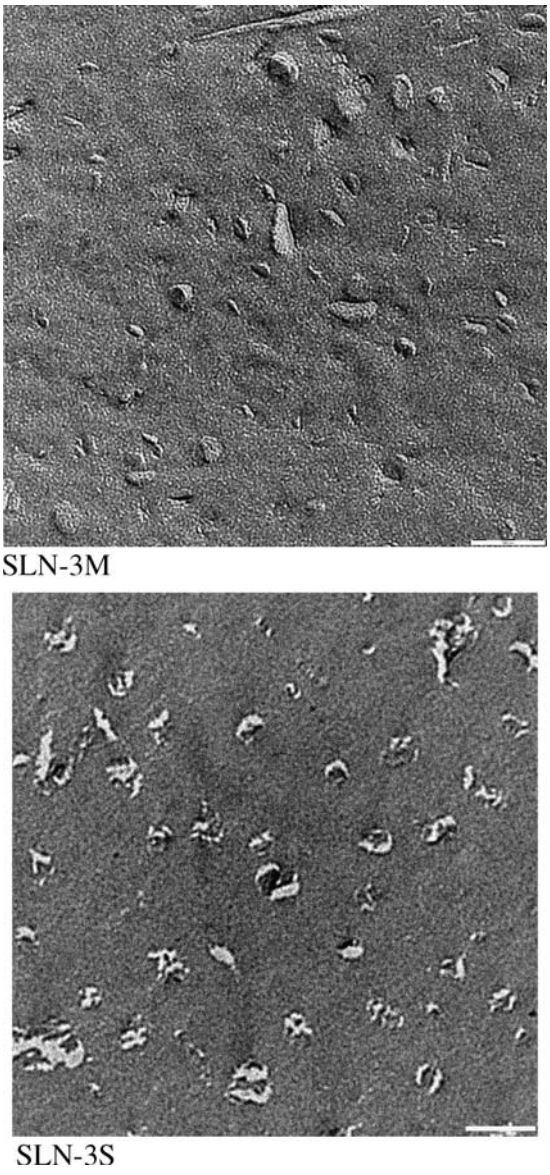

Fig. 5. TEM micrographs of the SLN containing 1.0 % w/w polysorbate 80. SLN-3M (mixed lipid matrix) and SLN-3S (single lipid matrix) (Bar = 100 nm).

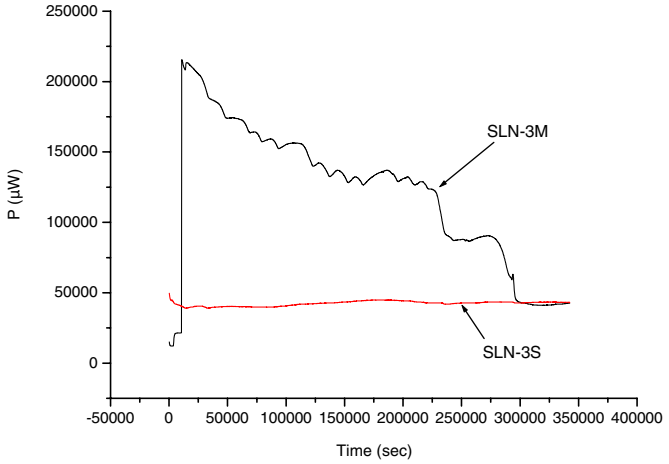

Fig. 6. Crystallization exotherms of the SLN containing P90G and 1.0 % w/w polysorbate 80.

environment especially in solid state pharmaceutics such as physical form characterization [30]. Preformulation issues such as amorphicity and polymorphism can be assessed with ease, precision and confidence. The excellent sensitivity and long term baseline stability make it an ideal method for preformulation studies. SLN-3M prepared with mixed lipid containing P90G and 1.0 % w/w polysorbate 80 showed delayed crystallization (Fig. 6). There was initial increased heat flow shown by the positive slope but crystallization proceeded non-spontaneously making it impossible for a definite baseline to be attained even after long period of study. There was thus a long delay in recrystallization of the lipid nanoparticles. Long delay in recrystallization of nanoparticles has been noted [24]. This result guides the formulator in drug content determination as analysis done before complete recrystallization of the system may lead to false high value of drug encapsulation efficiency. It was not possible to evaluate the kinetics involved in the isothermal crystallization of the SLN. SLN-3S prepared with theobroma oil containing P90G and 1.0 % w/w polysorbate 80 also showed even greater delay in recrystallization. This result is almost consistent with WAXD finding where SLN-3S remained largely amorphous after 3 months of storage. IMC gives a short term report of the progress of crystallization. Long delay in crystallization as shown in this case may not be detected by short term IMC measurement. Since both WAXD and IMC studies confirmed almost absence of crystalline interior of SLN-3S particles, it indicates that this SLN exists as highly disordered or liquid crystalline particles, proven by the presence of a small endothermic transition with low enthalpy detected by DSC measurement after 3 months and a characteristic TEM micrograph. It was not possible to evaluate the Avrami equation (Eq. (3)) in order to determine the crystallization kinetic parameters in the SLN.

4. Conclusions

The results of this study show that it is possible to formulate solid lipid nanoparticles with good properties with mixed lipid consisting of 1:1 mixture of theobroma oil and goat fat and Phospholipon 90G®. At least 0.5 % w/w polysorbate 80 would be needed to produce stable SLN with 1:1 mixture of theobroma oil and goat fat and Phospholipon 90G®. The mixed lipid used in this study yielded SLN of lower crystallinity compared with SLN formulated with theobroma oil alone, which is necessary for increased drug loading capacity. It is thus envisaged that use of mixed lipids, which do not form highly crystalline matrix, would overcome the problem of partial or total drug expulsion encountered with use of high purity lipids in the formulation of solid lipid nanoparticles and nanostructured lipid carriers. In terms of nanostructured lipid carriers,

use of mixed lipids that result in a matrix of low crystallinity may be better than a solid lipid and liquid lipid mixed together from the viewpoint of handling, possible sustenance of the incorporated drug and stability.

Acknowledgements

This work is a product of research fellowship awarded by Alexander von Humboldt Stiftung, Bonn-Bad Godesberg, Germany. Dr. A.A. Attama wishes to acknowledge the support (Ref. No. IV-NRI/1112681 STP). We also thank Phospholipid GmbH, Köln, for providing sample of Phospholipon 90G®, and Kirsten Nebelung of Institute of Pharmaceutical Technology, and Physics Department of Technical University Braunschweig for TEM studies.

References

[1] R.H. Müller, M. Karsten, G. Sven, Solid lipid nanoparticles (SLN) for controlled drug delivery – a review of the state of the art, Eur. J. Pharm. Biopharm. 50 (2000) 161–177.
[2] R.H. Müller, S.A. Ruge, Solid lipid nanoparticles (SLN) for controlled drug delivery, in: Submicron Emulsions in Drug Targeting and Delivery, Harwood Academic Publishers, Amsterdam, 1998, pp. 219–234.
[3] M. Gulati, M. Grover, S. Singh, M. Singh, Lipophilic drug derivatives in liposomes, Int. J. Pharm. 165 (1998) 129–168.
[4] S.A. Wissing, SLN als innovatives Formulierungskonzept für pflegende und protective dermale Zubereitungen. PhD Thesis, Frei Universität Berlin, 2002.
[5] V. Jenning, M. Schafer-Korting, S. Gohla, Vitamin A-loaded solid lipid nanoparticles for topical use: active release properties, J. Control. Rel. 66 (2000) 115–126.
[6] R.H. Müller, M. Radtke, S.A. Wissing, Solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) in cosmetic and dermatological preparations, Adv. Drug Deliv. Rev. 54 (2002) S131–S155.
[7] S.A. Wissing, O. Kayser, R.H. Müller, Solid lipid nanoparticles for parenteral drug delivery, Adv. Drug Deliv. Rev. 56 (2004) 1257–1272.
[8] C. Freitas, R.H. Müller, Correlation between long-term stability of solid lipid nanoparticles (SLN) and crystallinity of the lipid phase, Eur. J. Pharm. Biopharm. 47 (1999) 125–132.
[9] J.W. Hagemann, Thermal behaviour and polymorphism of acylglycerides, in: N. Garti, K. Sato (Eds.), Crystallization and Polymorphism of Fats and Fatty Acids, Marcel Dekker, New York, 1988, pp. 9–96.
[10] M. Radtke, R.H. Müller, NLC – nanostructured lipid carriers: the new generation of lipid drug carriers, New Drugs 2 (2001) 48–52.
[11] V. Jenning, A.F. Thünemann, S.H. Gohla, Characterization of a novel solid liquid nanoparticle carrier system based on binary mixtures of liquid and solid lipids, Int. J. Pharm. 199 (2000) 167–177.
[12] A.A. Attama, B.C. Schicke, C.C. Müller-Goymann, Further characterization of theobroma oil-beeswax admixtures as lipid matrices for improved drug delivery systems, Eur. J. Pharm. Biopharm. 64 (2006) 294–306.
[13] A.A. Attama, C.C. Müller-Goymann, A critical study of physically structured lipid matrices composed of a homolipid from *Capra hircus* and theobroma oil, Int. J. Pharm. 322 (2006) 67–78.
[14] M.A. Schubert, C.C. Müller-Goymann, Characterization of surface modified solid lipid nanoparticles (SLN): influence of lecithin and non-ionic emulsifier, Eur. J. Pharm. Biopharm. 61 (2005) 77–86.
[15] M.A. Schubert, B.C. Schicke, C.C. Müller-Goymann, Thermal analysis of the crystallization and behaviour of lipid matrices and lipid nanoparticles containing high amounts of lecithin, Int. J. Pharm. 298 (2005) 242–254.
[16] J.A. Solís-Fuentes, M.-R. Hernández-Medel, M.-C. Durán-de-Bazúa, Determination of the predominant polymorphic form of mango (*Mangifera indica*) almond fat by differential scanning calorimetry and X-ray diffraction, Eur. J. Lipid Sci. Technol. 107 (2005) 395–401.
[17] A.G. Marangoni, S.E. McGauley, Relationship between crystallization behaviour and structure of cocoa butter, Cryst. Growth Des. 3 (2003) 95–108.
[18] F. Ulberth, M. Buchgraber, Analytical platforms to assess the authenticity of cocoa butter, Eur. J. Lipid Sci. Technol. 105 (2003) 32–42.
[19] A.A. Attama, I.T. Nzekwe, P.O. Nnamani, M.U. Adikwu, O.C. Onugu, The use of solid self-emulsifying systems in the delivery of diclofenac, Int. J. Pharm. 262 (2003) 23–28.
[20] A.A. Attama, M.O. Nkemnele, In vitro evaluation of drug release from self micro-emulsifying drug delivery systems using a biodegradable homolipid from *Capra hircus*, Int. J. Pharm. 304 (2005) 4–10.
[21] Zetasizer Nano Series User Manual 2005. Issue 2.2, Malvern Instruments England.
[22] M. Avrami, Kinetics of phase change. I. General theory, J. Chem. Phys. 7 (1939) 1103–1112.
[23] M. Avrami, Kinetics of phase change. II. Transformation-time relations for random distribution of nuclei, J. Chem. Phys. 8 (1940) 212–224.
[24] W. Mehnert, K. Mäder, Solid lipid nanoparticles production, characterization and applications, Adv. Drug Deliv. Rev. 47 (2001) 165–196.
[25] H. Bunjes, M.H.J. Koch, K. Westesen, Influence of emulsifiers on the crystallization of solid lipid nanoparticles, J. Pharm. Sci. 92 (2003) 1509–1520.
[26] B.-D. Kim, K. Na, H.-K. Choi, Preparation and characterization of solid lipid nanoparticles (SLN) made of cocoa butter and curdlan, Eur. J. Pharm. Sci. 24 (2005) 199–205.
[27] I.P. Kaur, A. Garg, A.K. Singla, D. Aggarwal, Vesicular systems in ocular drug delivery: an overview, Int. J. Pharm. 269 (2004) 1–14.
[28] C.C. Müller-Goymann, Physicochemical characterization of colloidal drug delivery systems such as reverse micelles, vesicles, liquid crystals and nanoparticles for topical administration, Eur. J. Pharm. Biopharm. 58 (2004) 343–356.
[29] M.A. Schubert, H. Meike, C.C. Müller-Goymann, Structural investigations on lipid nanoparticles containing high amounts of lecithin, Eur. J. Pharm. Sci. 27 (2006) 226–236.
[30] M.A. Phipps, L.A. Mackin, Application of isothermal microcalorimetry in solid state drug development, Pharm. Sci. Technol. Today 3 (2000) 9–17.