African Journal of Pharmaceutical Research & Development

Vol. 10 No.2; pp. 085-090 (2018)

PRELIMINARY CHARACTERIZATION OF MODIFIED NANOSTRUCTURED LIPID CARRIERS AS POTENTIAL DRUG DELIVERY SYSTEMS

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ABSTRACT

The objective of this study was to preliminarily investigate two solid homolipids from: natural Bos indicus (tallow fat) and semi-synthetic lipid (Precirol® ATO 5), separately structured with liquid lipid (Transcutol® HP) and/or heterolipid flakes (Phospholipon 90G) to ascertain their potentials for drug delivery. Lipid mixtures were prepared by fusion and screened by differential scanning calorimetry (DSC). Optimized lipid matrices were used to formulate nanostructured lipid carriers (NLC) by hot homogenisation method using mixed optimized surfactants concentrations (1, 2 and 3 %w/w) of Polysorbate 80, Poloxamer® 188 and Solutol® HS respectively. NLC particles were analysed for particle size, polydispersity index, surface charge, morphology, thermal properties, pH storage stability and solid state characteristics. Resultant binary lipids formed with solid lipids (Precirol ATO 5® and/or tallow fat) and liquid lipid (Trancutol P®) had better thermal properties than the individual bulk lipids or when modified with P90G. FTIR spectra showed no interactions whereas NLC production was optimum at 15 % binary lipid composition and surfactant concentrations used, at the emulsification time of 15 min. NLC particles were stable, spherically smooth and non-porous with nanometric sizes, moderate polydispersity and high negative surface charges. Since the binary lipid mixtures of tallow fat/Transcutol and/or Precirol/Transcutol had low crystallinity required for high drug encapsulation, additional to being able to form moderately polydispersed nanoparticles, it follows that the NLC formulations might serve as alternative oral delivery systems to improve solubility of some poorly soluble drugs.

KEYWORDS: **Nanostructured lipid carrier; Precirol® ATO 5; Tallow fat; Transcutol® HP; Phospholipon® 90G.**

INTRODUCTION

Lipid – based nanoparticulate systems range from simple oil solutions to complex mixtures of oils, lipid/oils, surfactants, co-surfactants and co-solvents (1, 2). They include lipid nanoparticles (solid lipid nanoparticle, nanostructured lipid carriers), lipid nanocapsules, lipid nanoemulsion, lipid nanosuspensions, liposomes, microemulsion, etc. These systems have been widely utilized as drug delivery system with tremendous success records in enhancing the bioavailability of several drugs especially poorly water soluble drugs (1, 2). The development of lipid-based nanoparticulate systems evolved following the research for a drug delivery system which could overcome the limitation of polymeric nanoparticles in terms of toxicity, use of organic solvents in formulation, high cost etc., as well as enhance the delivery of lipophillic drugs (3). Among the lipid based systems, the first generation lipid nanoparticle, known as the solid lipid nanoparticles (SLN) have shown to offer numerous advantages over others which includes a solid core for controlled drug release, stability of incorporated drug and protection of incorporated drug from degradation by chemical, moisture, light or oxidation (4). They are sub-micron colloidal carriers (with a size range of 50 to 1000 nm) composed of physiological lipids dispersed in water or in aqueous surfactant solution. SLN stands out from other

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delivery systems as a result of its biocompatibility, biodegradability, non-toxicity (generally regarded as safe (GRAS) status), low cost, feasibility of scale up, high penetration through biological barriers like the skin and increased bioavailability of lipophillic drugs through the lymphatic systems (5,6,7). In SLN, the liquid state of the oil droplets in nanoemulsions is replaced by a solid lipid, which eventually transforms into solid lipid nanoparticles in view of overcoming the drawbacks associated with liquid state of oil. However, SLN are not without limitations. The polymorphic transitions of the SLN matrix from a high-energy lipid modification immediately after preparation to low-energy lipid modification (which has a perfect crystalline lattice) on storage gives little or no room for drug encapsulation. This would invariably lead to drug expulsion from the matrix especially when a highly purified lipid is used (8, 9). Research for new drug delivery system in view of solving these aforementioned problems of SLN led to the development of a second generation lipid nanoparticle referred to as nanostructured lipid carriers (NLC) (10, 11).

NLC consists of a blend of solid lipid and oil mostly in the ratio of 70:30 up to a ratio of 99.9:0.1 (12). The presence of the liquid lipid which can be embedded in the solid lipid matrix or localized at the interface of the solid matrix and surfactant layer in lipid mixtures results generally to an imperfect crystal lattice with a depression in melting point and more space for drug loading (13). The liquid lipids have also been reported to show better drug dissolution capacity than the solid lipid thus further reducing the possibility of drug expulsion (14). In other words, lipid structuring in NLC offer better flexibility in drug payload, modulation of drug release and improved performance. With regard to the production of NLC, various lipid molecules can be mixed ranging from homolipids to heterolipids (15). Homolipids have widely been used as lipid excipients of choice in formulation of lipid-based nanoparticulate drug delivery systems (16-17). The mixtures of lipids (homolipids and heterolipids) have been reported to result in amorphous or partially crystalline metastable lipid systems which could overcome the polymorphic transformations seen in bulk lipids (18).

In the light of the above, we report some preliminary characterization of binary and ternary mixtures of a homolipid (tallow fat) and semi-synthetic lipid (Precirol® ATO 5) separately modified with a heterolipid (Phospholipon 90G, P90G) and/or a liquid lipid (Transcutol® HP) at different ratios. These lipids have GRAS-status and have been extensively used in different drug delivery systems (16-20). Phospholipon® 90G is a lecithin fraction enriched with phosphotidylcholine greater or equal to 90 % and contains oleic, linoleic, stearic and palmitic acids which are fatty acids of different chain length and degrees of saturation (18-20). On the other hand, tallow fat (*Bos indicus*) is a natural homolipid which is obtained from the tissues and fat deposits of some animals including cattle, sheep and oxen, appearing mostly white, odourless and tasteless and essentially consisting of triglycerides of palmitic, stearic and oleic acid (18). Its crystal property has been likened to *Capra hirus* (goat fat) which has been utilised extensively in various drug delivery systems (16, 18). The mixture of tallow fat and/or Precirol® ATO 5 with Transcutol® HP and/or P90G in this study was geared towards achieving lipid matrices of lowest crystallinity ideal for formulation of NLCs to improve the solubility of some poorly soluble drugs in the oily-core of the nanoparticle, hence less probability of drug expulsion but rather high drug payload.

MATERIALS AND METHODS

Materials

Phospholipon® 90G (P90G) was a gift from Phospholipid GmbH, Germany whereas Poloxamer® 188 and Solutol® HS (BASF, Germany) were received as donations. Polysorbate® 80 (Merck, Germany), Precirol® ATO 5 and Transcutol® HP were donated by Gattefossé, France. Tallow fat was obtained from a batch processed in the Department of Pharmaceutics, University of Nigeria Nsukka (UNN). Bidistilled water (Lion water, Nigeria) was used throughout the study.

Extraction of tallow fat

Tallow fat was extracted from adipose tissue of *Bos indicus* following a previously reported method (18) with some modifications. Briefly, the adipose tissue was collected from freshly slaughtered cattle and manually freed of extraneous materials. It was then boiled in double distilled water for 45 min, filtered through a muslin cloth and allowed to solidify at room temperature. The solid fat was removed and decolourized by passing it through a mixture of 1 g of column material consisting of activated charcoal and bentonite (2:1) for each 10 g of fat at 100 °C, and filtered hot in the oven using double-flutted filter papers.

Screening of starting materials

Selection of lipids

Firstly, thermal properties of the bulk lipids including tallow fat, Precirol® ATO 5, Phospholipon® 90 G (P90G) and Transcutol® HP were determined by differential scanning calorimetry (NETZSCH DSC 204 FI instrument, Germany) by heating the sample (3-5 mg) from 35 to 190 ˚C at a heating rate of 10 ˚C/min under constant flushing with nitrogen (20 ml/min). Binary lipid mixtures (1:1, 2:1 and 3:1) were obtained from each solid lipid (Precirol ATO 5® and tallow fat) and the liquid lipid (Transcutol® HP) and/or P90G respectively by fusion at a temperature of 90 ˚C for 2 h. The matrices were cooled for 48 h at room temperature for full recrystallization and afterwards re-investigated by DSC. Ternary systems were obtained from a combination of two solid lipids with the oil and/or P90G at 1:1:1, 1:2:2, 2:1:1 combinations to modify the crystal properties of each individual lipid. All samples were studied by DSC and the mixture that showed the least enthalpy values was selected for further investigation.

Surfactant combination and preparation of nanostructured lipid carriers

After optimization of lipid matrix, selection of surfactant concentration and/or combination was done through 1, 2, 3, 4 and 5 % w/w of Solutol® HS 15, Poloxamer® 188 and Polysorbate 80. Briefly, nanostructured lipid carriers (NLCs) were prepared by hot homogenization (18). To the melted lipid phase, Solutol® HS 15 (3 %w/w) and Polysorbate 80 (Tween® 80, 1 %w/w) were added whereas Poloxamer® 188 (2 %w/w) was added to the aqueous phase and heated to the same temperature before addition to the molten lipid phase. This was followed by high shear homogenization (Ultra-Turrax, T18 basic, IKA Germany) at 25,000 rpm for 15 min to produce an o/w emulsion which was allowed to cool at room temperature. Only blank NLCs (without drug) were produced and assessed in this preliminary study to understand their potentials for drug delivery.

Solid state characteristics

Differential scanning calorimetry (DSC)

The thermal properties of formulated NLCs were determined using DSC (NETZSCH DSC 204 F1, Germany) as earlier described. Briefly, samples weighing 5 mg were kept in the standard aluminium pans and sealed. Then the pans were placed under isothermal condition at 25 ˚C for 10 min. DSC analysis was performed at heating rates of 10 ˚C/min from 35 to 190 ˚C. An empty sealed pan was used as reference. The thermograms were obtained after baseline corrections. Recrystallization index (RI %) was calculated using the modified equation 1 [14, 21, 22].

$$RI\ (\%) = \frac{\Delta H\ aqueous\ lipid\ nanoparticle\ dispersion}{\Delta H\ bulk\ material\ of\ P\ or\ T\ x\ Concentration\ of\ solid\ lipid\ phase}\ X\ 100\ \ldots(1)$$

Where ΔH aqueous lipid nanoparticle dispersion and ΔH bulk material refer to enthalpies (-mW/mg) of the aqueous lipid nanoparticle (NLC) dispersion and bulk materials of Precirol® ATO 5 and/or tallow fat respectively. Concentration of solid lipid phase means the actual amount of the solid lipid in the total dispersion (e.g. 15 % w/w) dispersion of 3:1 mixture of solid:liquid lipids.

Fourier transform infrared spectroscopy (FTIR)

The compatibility between the single lipids and lipid mixtures were studied using a Shimadzu FTIR 8300 spectrophotometer (Shimadzu, Tokyo, Japan). Spectra were recorded in the wavelength region of 4000 to 400 cm^{-1} with threshold of 1.303, sensitivity of 50 and resolution of 2 cm^{-1}. In each case, the sample was dispersed in KBr and then compressed into discs by applying a pressure of 5 tons for 5 min in a hydraulic press. The pellet was placed in the light path and the spectrum obtained.

Particle characterization

Mean diameter (z-average, nm) and polydispersity index (PDI) of the NLC particles were measured by photon correlation spectroscopy (PCS) using a zetasizer nano-ZS (Malvern Instrument, Worceshire, UK), equipped with a 10 mw He-NE laser operating at the wavelength of 633 nm and a backscattering angle of 173° at 25 °C. All samples were diluted with bidistilled water to obtain a suitable scattering intensity prior to analysis. The zeta potentials (ZP) of NLC formulations (n=3) were determined via electrophoretic mobility measurements using a zetasizer nano-ZS (Malvern Instrument, Worceshtire, UK) and applying the Helmholtz-Smoluchowski equation.

Particle morphology

The morphology of NLC particles was determined using a scanning electron microscope (Hitachi S-4000 Microscope, USA). Samples were diluted with bidistilled water and deposited on film-coated copper grids to dry overnight at room temperature. The dried samples were visualized under SEM.

Stability studies

NLCs were subjected to time-dependent pH analysis to determine their short-term stability. Dispersion pH was determined using a pH meter (pHep® Hana Instrument, Italy) after 24 h of preparation and upon storage at room temperature for one, three and six months.

Statistical Analysis

Analysis of variance (ANOVA) was used to test the statistical significance of differences among groups. Statistical significance in the differences of the means was evaluated using student t-test. Differences were considered significant for p values < 0.05.

RESULTS AND DISCUSSIONS

Screening of lipids

Details of DSC traces of all matrices (bulk, binary and ternary) were respectively shown in Figure 1, 2 and 3. All binary and ternary matrices melted at lower temperatures than their corresponding bulk matrices. This agrees with earlier observations by different workers (14, 18). However, Table 1 shows the individual characteristics of each matrix in terms of enthalpy, melting peak and area. Lower enthalpies were generally observed from the lipid matrices containing a mixture of solid and liquid lipid (tallow fat and/or Precirol® ATO 5 with Transcutol® HP) rather than solid lipids alone. The observed lower melting peaks and enthalpies suggest less crystalline nature of the matrices. This is also an indication of the modifications or large distortions in crystalline agreement as a result of tiny oily droplets within the formed solid matrices which would invariably favour drug loading capacities of the lipid matrices making it impossible for drug expulsion. This is in consonance with previous reports that lipid matrices with a certain degree of disorder are considered to be ideal for formulation of nanoparticulate lipid carriers due to their high drug loading potential (19). Modification of the lipid matrices with addition of P90G also produced lipid particles with decreased melting peaks and enthalpies though much higher than those of Transcutol® HP. By implication therefore, tallow fat and/or Precirol® ATO 5 with Transcutol® HP each was selected as the optimized matrices for NLC formulations.

FTIR Analysis

FTIR spectra of the pure Precirol® ATO 5, binary lipids of Precirol® ATO 5 and/or tallow fat with Transcutol® HP are presented in Fig 4. The FTIR spectra exhibited no shifting and no loss of characteristic functional group peaks. This suggests the absence of any undesired interaction between the solid lipids (tallow fat and Pzxrecirol® ATO 4) and liquid lipid Transcutol® HP. Thus, indicating their suitability for use in formulation of NLC particles.

Particle characterization

Particle characterization of the NLC particles was necessary to understand the suitability of the formulated product quality and stability. The PCS data showed that the mean particle size of the NLC from Precirol® ATO 5/Transcutol HP and tallow fat/Transcutol HP (represented respectively as PT-NLC and TT-NLC) was 570.3 nm and 655.9 nm with a PDI of 0.58 and 0.48 respectively (Table 2). Since PDI was approx. 0.5, it could be said that the NLC particles were moderately polydispersed. In addition, both the PT-NLC and TT-NLC particles possessed a large negative surface charge with zeta potential values of approx. -29 and -32 mV respectively. This is an indication of good stability of the lipid nanoparticles which might be attributed to the application of optimized concentrations of surfactants whose anionic fractions could be the source of the high negative charge recorded since greater ionization at the interface tended to increase the electrostatic repulsion (19).

Particle morphology

SEM was used to study the shape and surface morphology of the NLC lipid particles. The SEM images were shown in Figure 5. This depicted small spherically uniform nanoparticles with no visible aggregation. However, the portion containing some incompletely-dried NLC particles appeared somewhat clustered but generally all particles were discrete.

Stability

The pH-dependent stability study was crucial in establishing the stability of the NLC particles over a

period of time (20). The mean pH values of the PT-NLC and TT-NLC particles obtained 24 h post production was 5 and 5.4 respectively as shown in Figure 6. There was slight reduction in pH values after 3 and 6 months, though statistically insignificant. This perhaps could depict the absence of any chemical reactions or physical degradation occurring in the formulations, hence indicative of stability of the formulated NLC when stored over a period of time. In other words, it could be inferred that the surface properties and product integrity of the formulations were intact during the 3 and 6 months storage, hence the formulations were stable. This further confirms the results obtained from the PCS analysis of the lipid particles. All observations were in consonance with earlier works (18, 20)

CONCLUSIONS

Nanostructured lipid carriers (NLC) were successfully prepared by hot homogenization method, which is a simple, reproducible and cost-effective technique for fproducing stable nanoparticles. The optimised process parameters have shown that lipid structuring modifies individual properties of the bulk lipids and may decrease or increase lipid crystalline nature. While decrease in crystalline nature is more desirable, structuring solid lipids (Precirol® ATO 5 and/or tallow fat) with liquid lipid (Transcutol® HP) produced more distorted (less crystalline) lipid matrices (than those with P90G). These amorphous matrices would expectedly have more spaces for drug entrapment ideal for use in formulating NLC particles of lipophillic drugs. An outlook from this study therefore would be to utilize these matrices for the delivery of ciprofloxaxin (a practically water-insoluble drug) and gentamicin sulphate (water-soluble drug) to see the improvements therein.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest concerning the work.

ACKNOWLEDGMENT

The work embodied in this research was the preliminary part of Master's degree work carried out by Pharm. A.A. Ugwu. The authors are thankful to her for the work. We also appreciate Prof. Dr. CM Lehr for making his laboratory (Helmholtz Institute for Pharmaceutical Research Saarland University (HIPS) Germany, available for the particle characterization. We also thank Dr. Chiara Rossi for SEM analysis.

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