Surface modified solid lipid microparticles based on homolipids and Softisan® 142: preliminary characterization

Nnamani PO1, 2*, Ibezim EC1, Attama AA1, Adikwu MU1, 3

1Drug Delivery Research Unit, Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka 410001, Enugu State, Nigeria
2ICS–UNIDO International Center for High Technology and New Materials, AREA Science Park, Padriciano 99, Trieste, Italy
3Science and Technology Education Post–Basic (STEP–B) Abuja, Nigeria

ABSTRACT

Objective: To preliminarily investigate three different lipid matrices consisting of two natural homolipids from Capra hircus (goat fat) and Bovine Spp. (tallow fat) and one semi-synthetic lipid (Softisan® 142) separately structured with Phospholipon® 90G (P90G) as potential delivery systems for poorly water soluble drugs. Methods: The structured lipid matrices were characterized by differential scanning calorimetry (DSC) and employed to prepare solid lipid microparticles (SLMs) by the melt homogenization method using gradient concentrations of polysorbate 80 and at different emulsification times of 2, 5 and 10 min using a Silverson mixer. The SLMs were analyzed for morphology and particle size, thermal properties, stability studies and determination of injectability. Results: The results showed that SLM production was optimum at 5 % of lipid matrices, 1.5 % of polysorbate 80 and emulsification time of 5 min. Increase in polysorbate 80 concentrations decreased the particle size of the SLMs. The SLMs were well formed, spherical, smooth and non–porous with particle sizes in the ranges of (13.90 ± 2.10) μm – (0.09 ± 0.01) μm for SLMs produced from the structured – tallow fat; (13.40 ± 1.30) μm – (0.10 ± 0.01) μm for the structured goat fat and (13.40 ± 2.00) μm – (2.10 ± 1.00) μm for the structured Softisan® 142 lipid matrices. DSC traces showed that Softisan® 142 was the most crystalline of all three bulk matrices due to its high enthalpy (–7.962 mW/mg) while tallow fat was the least (–5.067 mW/mg) but addition of P90G to the matrices lowered their enthalpies mostly in the structured goat fat matrices. The SLMs when stored at 4–6 °C were most stable and syringeable with 27 G needle. Conclusions: This suggests that structured goat fat matrices with the enthalpy of –2.813 mW/mg will mostly favour drug loading of some poorly soluble drugs more than tallow fat (–4.892 mW/mg) and Softisan® 142 (–5.501 mW/mg).

1. Introduction

Microsized controlled drug delivery devices e.g. microemulsions and solid lipid microparticles (SLMs) have recently been proposed for different routes of drug administration. The composition of SLMs is equivalent to solid lipid nanoparticles (SLNs), but is in the micrometer size range. SLMs avoid the disadvantages of other particle carrier systems and offer the possibility of controlled drug release and drug targeting[1–4]. When optimized, SLMs exhibit high physicochemical stability, a high potential for large scale production and excellent biocompatibility[5] especially when natural lipids are concerned[6].

Recently, lipid drug delivery systems are making waves in research due to their intrinsic advantages[5,6]. Yet as bulk materials, lipids crystallize immediately after preparation in higher energy modifications with more imperfections in the crystal lattice and at such may affect the functional properties of SLMs prepared. As a result, these systems suffer from low drug entrapment due to the crystalline nature of the solid lipids whether of natural or synthetic origin[7]. In addition, the presence of emulsifiers, the preparation method and the high–shear dispersion may account for changes in the crystallinity of matrix constituents compared with bulk materials. Lipid mixtures can result in increased or decreased crystallinity[8].
Moreover, the addition of a second matrix component may somewhat alter the crystallization behaviour of the matrix. This may lead to amorphous or only partially crystallized metastable systems[7]. Polymorphic transformations may cause changes in active and auxiliary substance solubilities and melting points. In particular, the conversion of one polymorph into another may change the physical properties of the substance[2, 9, 10]. To overcome such a phenomenon, use of mixtures of lipids which do not form highly ordered arrangement is needed. Mixtures of lipids also modify polymorphic properties of the individual lipids and have been shown to generate lipid matrices of low crystallinity[11].

Here we report that the homolipids (goat fat and tallow fat) and semi–synthetic lipid (Softisan® 142) were separately modified with a heterolipid (P90G) at a ratio of 4 : 1 and characterized. These physically structured lipid matrices were formulated into SLMs with a gradient of polysorbate 80 (0.00, 0.75, 1.50 and 2.00 % w/w, as the mobile surfactant) at various emulsification times (2, 5, 10 min) and characterized.

Homolipids and heterolipids have been lipidic excipients of interest in lipid drug delivery systems. Goat fat (Capra hircus) is ordinarily a waste product in abattoirs in Nigeria and represents a potential for drug delivery due to its crystal properties. It consists mainly of triglycerides and has been used in the formulation of self–emulsifying drug delivery systems[12, 13] and solid lipid nanodispersion[14]. Goat fat is a natural lipid and has good tolerability in vivo as well as having been shown to be stable to rancidity after degumming and deodorization[15, 15].

Tallow fat (Bovine Spp.) is a solid fat extracted from the tissues and fatty deposits of animals, especially suet (the fat of cattle, sheep or oxen). It is white, odourless, tasteless and consists chiefly of triglycerides of oleic, stearic and palmitic acids. It has a wide range of industrial applications[16]. Tallow can be stored for extended periods without the need for refrigeration provided it is kept in an airtight container to prevent oxidation[16].

Softisan® 142 is a semi–synthetic lipid consisting of hydrogenated coco–glycerides with wide application in drug and cosmetic delivery[17] whereas Phospholipon® 90G has been used in parenteral emulsions and in liposome formulation and has been reported as a good surface modifier[18–21]. This perhaps suggests that the bilayer structure of the phospholipids around the inner lipid core can enhance drug entrapment and further stabilize the particles especially when generated in an aqueous medium[20].

Goat fat and tallow fat are natural homolipids used in food, pharmaceutical and other industries[12, 17] while Softisan® 142 is semi–synthetic. They are highly crystalline and this adversely affects their drug loading capacities. Addition of a heterolipid, P90G is, however, expected to largely disturb the crystal arrangement and further modify the surface of the generated particles with the aim of improving their drug–holding capabilities and to ascertain the order of their possible utilization in the delivery of some poorly–water soluble drugs.

2. Materials and methods

2.1. Materials

Phospholipon® 90G (P90G) (Phospholipid GmbH Köhn, Germany) is a purified, deoiled and granulated soy lecithin with phosphatidylecholine content of at least 90 %. Softisan® 142 (Pastillen, Germany), sorbic acid (Fischer Co., New Jersey), sorbitol (BDH, England) and polysorbate 80 (Tween® 80) (Uniqema, Belgium) were used as they were procured from their manufacturers without further purification. Homolipids (goat fat and tallow fat) were obtained from a hatch processed in our Pharmaceuticals laboratory according to an earlier procedure[12, 14]. Distilled water (Lion water, Nigeria) was used for SLM preparation.

2.2. Extraction of goat fat

Goat fat was extracted from the adipose tissue of Capra hircus according to an earlier method[11, 12]. To summarize, the adipose tissue of Capra hircus was collected from freshly slaughtered goat, manually freed of extraneous materials, crushed and boiled in distilled water for 45 min, filtered through a muslin cloth and allowed to solidify at room temperature. The solid fat was manually removed and bleached/deodourized by passing it through a mixture of activated charcoal and bentonite (2 : 1) at 100 °C at a ratio of 10 g of the fat and 1 g of the column material. The above procedure was also applied to extract tallow fat from Bovine spp.

2.3. Differential scanning calorimetry (DSC)

The thermal behaviour was determined using a DSC (NETZSCH DSC 204 F1, Germany) with an empty standard aluminum pan used as reference. DSC scans were recorded at heating rates of 10 °C/min between 35 °C and 190 °C under a 20 ml/min nitrogen flux with sample size of 3–5 mg. The DSC thermograms of pure goat fat, tallow fat, and Softisan® 142, as bulk materials, and mixtures with P90G and SLMs were assessed. All determinations were baseline–corrected.

2.4. Formulation of the lipid matrix

The lipid matrix used in the formulation corresponded to a 4 : 1 mixture of goat fat and P90G; tallow fat and P90G and Softisan® 142 and P90G respectively and were prepared by fusion. The lipids were weighed with an electronic balance (Mettler H8, Switzerland), melted together at 60 °C on a thermo–regulated water bath shaker (Heto, Denmark) and stirred until solidification.

2.5. Formulation of solid lipid micro Particles (SLMs)

SLMs were formulated to contain 5 % w/w of the previously molten lipid matrices (i.e. 4 : 1 mixture of goat fat and P90G; tallow fat and P90G and Softisan® 142 and P90G), gradients of polysorbate 80 (0.00, 0.75, 1.50, and 2.00 %
w/w), 4.00 % w/w of sorbitol, 0.10 % w/w of sorbic acid and enough distilled water to make 100.00 % w/w. The hot homogenization method was adopted. In each case, the lipid matrix was melted at 60 °C and the aqueous phase containing polysorbate 80, sorbitol and sorbic acid at the same temperature was added to the molten lipid matrix and stirred gently with a magnetic stirrer (SR 1U5 52188, Remi Equip., India). The mixture was further dispersed with a mixer (Silverson L4R, Adelphi Manufac., England) at 6 200 rpm for different emulsification times (2, 5, and 10 min) to produce the hot primary emulsion which was collected in pre-warmed containers and allowed to recrystallize at room temperature.

2.6. Morphology and particle size analysis

Particle size analysis was carried out on the SLMs within one week of production using a digital light microscope (Leica Diestar, Germany) and images captured with a Moticam 1000 camera. The morphology (shape and surface) and size of the particles were also noted. The SLMs were subjected to time-resolved particle size analyses for 12 months at 6 month intervals to check the effect of storage on particle size.

2.7. Thermal properties of the SLMs (DSC)

The thermal behaviour of the SLMs was assessed using a DSC (NETZSCH DSC 204 F1, Germany) with an empty standard aluminum pan used as reference. DSC scans were recorded after baseline correction at heating rates of 10 °C /min between 35 and 190 °C under a 20 mL/min nitrogen flux with sample size of 3 – 5 mg.

2.8. Stability studies of the formulations

The physical stability of the microparticles was evaluated for 12 months under different temperature conditions. Some 6 mL volumes of each SLM batch were stored in closed glass bottles and placed at 4–6 °C and 25 °C out from direct light. Aliquot samples were withdrawn every 6 months to determine particle size and morphology as described above.

2.9. Determination of injectability

Injectability, defined as the smallest needle gauge that a microparticle sample can pass through was determined according to the previously reported method [13]. The injectability was determined by pushing 4 mL of sample from a 5 mL plastic disposable syringe through hypodermic needles ranging from 18 to 27 guage within 20 sec. The formulation was first tested using the smallest needle (27 G). If the entire content of the sample passed through a 27 G needle, its injectability was recorded as 27 otherwise, the study was repeated using 25 G needle, followed by the next smaller gauge needle.

2.10. 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-values < 0.05 – 0.001.

3. Results

3.1. Differential scanning calorimetry

The DSC results are shown in Figures 1–9. The melting endotherm for goat fat showed a transition endothermic peak at 53.7 °C with an enthalpy of −6.420 mW/mg (Figure 1) while its corresponding P90G-structured matrix exhibited a melting peak at 50.8 °C with an enthalpy of −2.813 mW/mg (Figure 2). The DSC trace of tallow fat was 54.5 °C with an enthalpy of −5.067 mW/mg (Figure 3) and that of the structured matrix was 52.2 °C with an enthalpy of −5.501 mW/mg (Figure 4). The DSC thermogram of Softisan® 142 showed an endothermic peak at 46.8 °C with an enthalpy of −7.962 mW/mg (Figure 5) and that of its corresponding structured matrix was 43.3 °C with an enthalpy of −4.892 mW/mg (Figure 6). Here we observed a melting point value for goat fat that was slightly different from that in the literature reported by Attama and Muller–Goymann, 2006 and the possible reason may be a question of sensitivity of the DSC machine used.

SLMs prepared from these matrices generally showed increased endothermic temperatures which may be due to the presence of other formulation excipients such as polysorbate 80, sorbic acid and/or sorbitol. The DSC traces for the SLMs formulated with the structured goat fat showed a wide peak at 118.1 °C with an enthalpy value of −12.1 mW/mg (Figure 7) while the DSC traces of the SLMs prepared from P90G-structured tallow fat matrix indicated two endothermic transitions which occurred at 104.4 °C with an enthalpy of −13.31 mW/mg and at 108.8 °C with an enthalpy of −14.67 mW/mg for the higher peak (Figure 8). Normally, the highest peak is attributed to a stable modification whereas the fraction responsible for the lower peak is attributed to an unstable modification [11]. The SLMs formulated using the structured-Softisan® 142 showed a peak endothermic temperature at 104.3 °C with an enthalpy of −16.58 mW/mg (Figure 9).

3.2. Physicochemical properties

The result of the particle size analysis for the optimized SLMs is presented in Table 1. Optimum concentration of 1.5 % w/w concentration of polysorbate 80 was employed in the SLM production at the emulsification time of 5 min. The SLMs obtained were uniformly dispersed with uniformly sized particles. For all the SLM formulations, significant differences in particle size were observed after 10 minutes. The microparticulate dispersions were stable, odourless and did not show sedimentation even after centrifugation (3 000 rpm for 90 min).

3.3. Morphology and particle size analysis

The particle sizes within one week of formulation were in the range of (13.90 ± 2.10) μm – (0.09 ± 0.01) μm for the structured – tallow fat; (13.40 ± 1.30) μm – (0.10 ± 0.01) μm for the structured – goat fat and (14.30 ± 2.00) μm – (2.10
± 1.00) μm for the structured Softisan® 142 lipid matrices (Table 1). Time resolved particle size analysis showed that the SLMs increased in size probably due to crystallization of the previously molten lipid matrices[11, 14]. This tendency was most pronounced in the SLMs formulated from the structured–goat fat matrices (25.3±5.3) μm followed by tallow fat (19.4 ± 2.8) μm and Softisan® 142 (17.5 ± 2.0) μm.

The microparticles clearly show a homogeneous monolayer coating of the surfactant at the periphery of the lipid core (Figure 10). Upon storage, the shape of the SLMs remained intact (spherical) in the dispersions obtained from P90G–structured goat and tallow fat matrices according to the observation by Kim et al[6]. However, the SLMs obtained from P90G–structured Softisan® 142 slightly changed becoming somewhat crystal–like which may point towards higher crystallinity.

3.4. Stability studies of the formulations

Storing the formulations at 4–6 °C and 25 °C did not make the SLMs sediment. The formulations remained uniformly dispersed. However, the samples stored at 4–6 °C appeared better than those at 25 °C. This suggests that there was slight crystal growth at ambient temperature especially in the formulation made from P90G–structured Softisan® 142 matrices.

3.5. Determination of injectability

To further confirm the observed crystal growth at room temperature, the samples stored at this temperature were not syringeable with 27 G needle after 6 months whereas those at 4–6 °C remained syringeable (27 G) even up to 12 months. By implication, the SLMs were best stored at 4–6 °C. However, their being syringeable may suggest a potential for parenteral drug delivery.

Figure 1. DSC thermogram of goat fat.

Figure 2. DSC thermogram of P90G–structured goat fat matrix.

Figure 3. DSC thermogram of tallow fat.

Figure 4. DSC thermogram of P90G–structured tallow fat matrix.

Figure 5. DSC thermogram of pure Softisan® 142.

Figure 6. DSC thermogram of P90G–structured Softisan® 142 matrix.

Figure 7. DSC thermogram of SLM formulation based on P90G–structured goat fat matrix.

Figure 8. DSC thermogram of SLM formulation based on P90G–structured tallow fat matrix.

Figure 9. DSC thermogram of SLM formulation based on P90G–structured Softisan® 142.
Table 1
Effects of different polysorbate concentrations and emulsification times on SLM mean diameter.

| Formulation | Polysorbate 80 percentage (w/w) | Emulsification time (min) | Particle mean diameter ± SD (μm) |
|-------------|----------------------------------|---------------------------|----------------------------------|
|              |                                  |                           | Goat fat and P90G | Tallow fat and P90G | Softisan®142 and P90G |
| SLM 1a       | 0.75                             | 2                         | 13.40±1.30          | 13.90±2.10          | 13.4±2.0            |
| SLM 1b       | 0.75                             | 5                         | 12.90±1.00          | 12.80±1.00          | 12.8±1.0            |
| SLM 1c       | 0.75                             | 10                        | 10.10±0.75          | 10.00±1.00          | 10.5±1.0            |
| SLM 2a       | 1.50                             | 2                         | 8.60±2.00           | 8.00±2.40           | 8.9±2.2             |
| SLM 2b       | 1.50                             | 5                         | 5.30±2.50           | 5.50±2.50           | 5.0±2.5             |
| SLM 2c       | 1.50                             | 10                        | 3.50±2.20           | 2.00±1.20           | 2.1±1.0             |
| SLM 3a       | 2.00                             | 2                         | 0.10±0.01           | 0.09±0.01           | ND                  |
| SLM 3b       | 2.00                             | 5                         | ND                  | ND                  | ND                  |
| SLM 3c       | 2.00                             | 10                        | ND                  | ND                  | ND                  |

ND implies not determined. Results are the mean of 3 measures ±SD.

4. Discussion

We observed that an increase in polysorbate 80 concentration reduced the particle size of the SLMs. At 2.00 % w/w of polysorbate 80, the particle size was difficult to determine possibly due to the fact that they were no longer within the micrometer range. Higher emulsification times of 10 min generally produced smaller but gelled SLMs making them unpleasant for intending-orally actives. They may however be more relevant in topical and transdermal drug delivery systems as their minute particles would not present any coarse feel. The formulations containing 0.75 % w/w of polysorbate 80 generally had some un-emulsified entities (otherwise called dead-ends) at 2 min emulsification times. This made it impossible for this concentration to be selected for subsequent production. Even when the emulsification time was increased to 5 or 10 min, there was still separation probably due to the small concentration of the surfactant (0.75 %) not being able to completely lower the interfacial tension to prevent partitioning of the particles. However, the optimized SLMs were spherical in shape with smooth surfaces and a ring of the surfactant coat on the inner core.

Mixtures of lipids modify the properties of the individual lipids. We observed that the structuring of these bulk crystalline matrices with P90G generally produced matrices with lower melting endotherms as well as enthalpies. Reduction in enthalpy generally suggests less crystallinity of the lipid matrices[11]. This is perhaps due to the presence of the unsaturated phospholipid molecules of P90G in the ordered structures of the matrices that caused a broadening and a shift of the solid lipid – to – liquid crystal transition peak towards lower temperatures. In other words, the varied
fatty acid contents of these lipids may have interacted in such a manner as to partly disorder the crystal arrangement of the individual lipids since their fatty acids vary widely in chain length and degree of saturation\[11, 14\]. Our observation therefore, agrees with the current literature that has proved P90G as a good surface modifier for solid lipid particles\[17, 18\] with possible improvement in targeting and pharmacokinetics\[13-20\] whereby the phospholipoids bilayer structure formed around the lipid core may increase the drug loading capacity, as biologically important molecules can be anchored on the colloidal particle surfaces, and surface-modification also enables stabilization of colloidal particles especially when generation of the microparticles is carried out in an aqueous medium\[19\].

The decrease in enthalpy between the SLMs produced using the P90G--structured goat fat suggests that they were less crystalline than those of P90G--structured tallow fat. Further comparison with the SLMs obtained from the structured--Softisan® 142 suggests that the latter was the most crystalline of all. This is because the SLMs from the structured Softisan® 142 matrices showed the highest enthalpy value of −16.58 mW/mg. The increase in enthalpy confirms high amounts of crystals upon storage due to delayed crystallization from fractions of a supercooled amorphous melt. This was further confirmed by the shape of the SLMs after 6 months storage which was somewhat crystal–like and/or prismatic.

We, therefore, conclude that SLMs were successfully prepared by the hot homogenisation method which is simple, reproducible, scaleable and cheap. The optimized processing parameters have shown that lipid structuring modifies the properties of the individual lipids and may increase or decrease crystallinity. Our observation shows that structuring of goat and tallow fats (natural homolipids) achieved larger distortions in the crystal arrangement of their matrices than with structured–Softisan® 142 (semi–synthetic homolipid) matrix thereby creating numerous defects/spaces for possible drug localization. Yet, structured–goat fat matrices (at the analyzed ratio combinations) demonstrated the best potentials for use as an SLM drug delivery system among the three investigated matrices followed by the structured–tallow fat matrices. By implication, they can be employed as SLM carriers to orally deliver some poorly water–soluble drugs belonging to biopharmaceutical classification scheme (BCS) classes II and III.

**Conflict of interest statement**

We declare that we have no conflict of interest.

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