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Effect of beeswax modification on the lipid matrix and solid lipid nanoparticle crystallinity

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Abstract

The influence of a heterolipid (Phospholipon 90G®, P90G), which directly modifies the surface of solid lipid nanoparticles (SLN), and goat fat on the crystallinity of beeswax matrix and the SLN prepared therefrom was studied. Lipid matrices composed of 30% (w/w) of P90G in beeswax and in 1:1 mixture of beeswax and goat fat were formulated and characterized. SLN containing polysorbate 80 with or without P90G were formulated by hot high-pressure homogenisation and characterized by particle size and zeta potential measurements. Differential scanning calorimetry (DSC) and wide-angle X-ray diffraction (WAXD) patterns of the SLN were compared with the lipid matrices. Results showed WAXD of the lipid matrices containing P90G contain amorphous portions. Most SLN formulated possessed low *z*-average diameters and polydispersity indices, which remained constant after 3 months, with high-negative potentials after 4 weeks and crystallized into stable modification within 48 h of preparation. The overall result indicated that P90G and goat fat influenced the crystallinity of beeswax matrix and its SLN, but the crystallinity of the mixed lipid matrix (1:1 beeswax/goat fat) and its SLN, beeswax containing P90G and its SLN was lower than that of beeswax alone and its SLN. There was no increase in crystallinity of SLN on storage. Modification of beeswax with P90G or goat fat offers a way of improving the SLN formulated with beeswax in terms of reduction of its crystallinity responsible for its low-drug incorporation efficiency.

Keywords: Beeswax; Goat fat; Mixed lipid; Phospholipon 90G®; Modification; Solid lipid nanoparticles; Crystallinity; Drug delivery

1. Introduction

Solid lipid nanoparticles (SLN) are an alternative carrier system to polymer nanoparticles or liposomes. They consist of physiological and biocompatible lipids, which are suitable for the incorporation of lipophilic, hydrophilic and poorly water-soluble active ingredients. Improved bioavailability, protection of sensitive drug molecules from the outer environment (water and light) and controlled release characteristics were claimed by incorporation of drugs in the solid lipid matrix [1]. Due to the rigidity of the nanoparticles, the mobility of an incorporated drug is reduced, preventing drug leakage. A major disadvantage of SLN is their inherent low-drug incorporation due to the crystalline structure of the solid lipid.

The high crystalline order of nanoparticles prevents incorporation of high amounts of drug molecules, but the use of triglyceride mixtures results in particles with a less ordered matrix [2]. Moreover, addition of a second matrix component may specifically alter the crystallization behaviour of the lipid

Abbreviations: SLN-1, SLN prepared with 30% (w/w) P90G in beeswax as lipid matrix and 0.3% (w/w) polysorbate 80; SLN-2, SLN prepared with 30% (w/w) P90G in beeswax as lipid matrix and 0.6% (w/w) polysorbate 80; SLN-3, SLN prepared with 30% (w/w) P90G in beeswax as lipid matrix and 1.0% (w/w) polysorbate 80; SLN-4, SLN prepared with beeswax as lipid matrix and 1.0% (w/w) polysorbate 80; SLN-A, SLN prepared with 1:1 mixture of beeswax and goat fat as lipid matrix and 1.0% (w/w) polysorbate 80; SLN-B, SLN prepared with 30% (w/w) P90G in 1:1 mixture of beeswax and goat fat as lipid matrix without polysorbate 80; SLN-C, SLN prepared with 30% (w/w) P90G in 1:1 mixture of beeswax and goat fat as lipid matrix and 0.3% (w/w) polysorbate 80; SLN-D, SLN prepared with 30% (w/w) P90G in 1:1 mixture of beeswax and goat fat as lipid matrix and 0.6% (w/w) polysorbate 80; SLN-E, SLN prepared with 30% (w/w) P90G in 1:1 mixture of beeswax and goat fat as lipid matrix 1.0% (w/w) polysorbate 80

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matrix. In objective of this study, beeswax was modified with goat fat and the nanoparticles formulated therefrom studied. The effect of the modification on crystallinity of the P90G or polysorbate 80 stabilized SLN formulated was studied. Properties of the SLN prepared with beeswax containing P90G were compared with SLN prepared with 1:1 mixture of beeswax and goat fat. Beeswax SLN containing polysorbate 80 as the only surfactant was compared with other triglycerides and it was found that the beeswax nanoparticles possessed excellent particle size stability but poor drug encapsulation efficiency despite the fact that polymorphism of waxes is very low compared with triglycerides [3,4].

Phospholipon 90G® has been used in parenteral emulsions and in formulation of liposomes, and was recently shown to be a good surface modifier for SLN [5,6] with resultant improvement in targeting and pharmacokinetics [7–9]. The phospholipid bilayer structure formed around the lipid core may increase the drug loading capacity, as biologically important molecules can be anchored on the colloidal particle surface, and surface-modification also enables stabilization of colloidal particles especially when generation of the nanoparticles is carried out in an aqueous medium [10].

Beeswax is a natural product used in pharmaceutical, cosmetics, food and other industries [11]. It is highly crystalline [12], and this adversely affects its drug holding capacity. Addition of goat fat and phospholipid (heterolipid) is expected to greatly disturb its crystal order in addition to surface modification. Goat fat on the other hand, is derived from a domestic animal, *Capra hircus* and has been characterized and used in experimental drug delivery systems [13,14].

2. Materials and experiments

2.1. Materials

Beeswax (Cera alba) Ph. Eur., sorbitol (Caesar & Loretz, Hilden Germany), thimerosal (Synochem, Germany) and polysorbate 80 (Tween 80®, Across Organics, Germany), Phospholipon 90G® (Phospholipid GmbH Köln, Germany) were used as procured. Goat fat was obtained from a batch processed according to earlier procedure [13]. Double-distilled water was used for nanoparticle preparation.

2.2. Preparation of lipid matrix containing Phospholipon 90G®

The lipid matrix composition was chosen based on previous experience with SLN prepared and evaluated in our laboratory [5,6], and contained 30% (w/w) of Phospholipon 90G® in beeswax or 1:1 mixture of beeswax and goat fat, and was prepared initially by fusion prior to nanoparticle preparation.

2.3. Preparation of nanoparticles

Solid lipid nanoparticles were prepared to contain 5.0% (w/w) lipid matrix (30% (w/w) of P90G in beeswax and 30% (w/w) of P90G in 1:1 mixture of beeswax and goat fat), 0.3, 0.6 or 1.0% (w/w) polysorbate 80, 0.005% (w/w) thimerosal, 4.0% (w/w) sorbitol and enough double-distilled water to make 100% (w/w). SLN without P90G or polysorbate 80 were also prepared. The nomenclatures used for the different batches of SLN formulated are presented in the key below. Melt-homogenisation technique was adopted. In each case, the lipid matrix was melted at 75 °C, which is more than 10 °C above the melting temperature and avoids the lipid memory effect and makes new crystallization possible [15]. The double-distilled water containing the polysorbate 80, thimerosal and sorbitol at the same temperature, was added to the molten lipid matrix, mixed very well and dispersed at 75 °C with Ultra-Turrax (T25 basic, Ika® Staufen Germany) at 24,000 rpm for 5 min and immediately passed through a heated high pressure homogeniser (EmulsiFlex-C5, Avestin Canada) at a pressure of 1000 bars for 20 cycles.

2.4. Photon correlation spectroscopy (PCS)

Particle sizes were determined by PCS at 25 °C using a Multi Angle Particle Size Analyser (Zetasizer 3 Model AZ6004, Malvern England) modified with a 35 mW He–Ne laser (Model 127-35, Spectra Physics USA). The detection was performed at a scattering angle of 90° in a cell AZ10 equilibrated at 293 K and at an accumulation time of 180 s. Samples were diluted with filtrated double-distilled water (0.2 μm Sterifix® filter) and data were analysed by the cumulants method assuming spherical particles. Particle size studies of SLN were done 24 h, 1, 4 and 12 weeks after preparation.

2.5. Measurement of zeta potential

The zeta potentials of the formulated SLN were determined after 1 month of preparation in a Zetasizer Nano Series (Nano-ZS, Malvern Instruments England). Each sample was diluted with bidistilled water and the electrophoretic mobility determined at 25 °C and dispersant dielectric constant of 78.5 and pH of 7. 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 [16]:

$$U_E = \frac{2\varepsilon Z f(K_a)}{3\eta} \quad (1)$$

where Z is the zeta potential, U_E the electrophoretic mobility, ε the dielectric constant, η the viscosity of the medium and $f(K_a)$ is the Henry's function.

2.6. Wide angle X-ray diffraction (WAXD) studies

To study the crystalline characteristics, WAXD studies were done on the lipid matrices and all the SLN as earlier described [13,17]. Wide angle X-ray studies were done using an X-ray generator (PW3040/60 X'Pert PRO, Fabr. DY2171, PANalytical Netherlands) connected to a copper anode (PW3373/00 DK 147726 Cu LFF). WAXD diffractograms were obtained 3 months after lipid matrices preparation and 48 h, 1 week, 1 month and 3 months after SLN preparation.