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Full Length Research Paper

Physicochemical properties of nanostructured lipid carriers as colloidal carrier system stabilized with polysorbate 20 and polysorbate 80

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Nanostructured lipid carriers (NLC), a colloidal carrier system offer many advantages as drug carrier. Incorporation of liquid lipid can improve the loading capacity of drugs in the NLCs. The NLC20 and NLC80 were produced by high-pressure homogenization technique, stabilized with polysorbate 20 and polysorbate 80, respectively. Transmission electron microscopy showed that these NLCs were spherical. Photon correlation spectroscopy showed that the average size of NLC80 and NLC20 were 102.8 ± 0.1 and 261.63 ± 8.56 nm, respectively, and their zeta potentials were -23.93 ± 0.75 and -30.57 ± 0.06 mV, respectively. The results suggest that NLC80 is a more stable formulation. X-ray diffractometry and differential scanning calorimetry showed that NCLs were less crystalline than the bulk lipid. The melting point depression of NLC80 was 5.71°C below bulk lipid's melting point (61.56°C), while NLC20 exhibited two melting points at 54.80 and 59.10°C. These findings suggest that polysorbate 80 was a better dispersing agent for NLC than polysorbate 20. The physicochemistry properties of the NLCs are greatly influenced by the type of surfactant used. The small size and superior particle surface to volume ratio would increase loading efficiency and bioavailability of drugs, thus making NLC a promising drug delivery system.

Key words: Nanostructured lipid carriers, colloidal delivery system, polysorbate 80, polysorbate 20, high-pressure homogenization, physicochemical properties.

INTRODUCTION

The term colloid is broadly applicable to systems consisting of at least 2 components; one dispersed in the other as fine particles in any state of matter. As pharmaceutical carriers, colloidal drug delivery systems can be subdivided into polymer systems (micelles, dendrimers, etc), self-assembled lipid systems (liposomes, emulsions, solid lipid nanoparticles, etc), drug nanoparticle systems and pro-colloidal systems (self-emulsifying oral delivery systems and liquid crystalline systems). Colloidal drug carrier systems offer many advantages as drug delivery vehicles including capability of increasing bioavailability of poorly soluble drugs, provide protection for sensitive active compounds (Dingler, 1998), and facilitate controlled release of drugs (Müller et al., 2000).

Solid lipid nanoparticles (SLN) combining the advantages of colloidal carriers, had attracted increased attention as a drug delivery system when it was introduced in 1991 (Müller et al., 2000). Since then, SLN is referred to as alternative carrier system to traditional colloidal system such as liposomes, emulsions and polymeric nanoparticles due to its exceptional stability, scaling-up potential and biocompatible components (Radomska-Soukharev, 2007). However, the disadvantages of SLN include tendency for particle growth, unpredictable gelation tendency, unexpected dynamics of polymorphic transitions and inherently low incorporation capacities

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due to the crystalline structure of solid lipids (Westesen et al., 1997; Westesen and Siekmann, 1997).

Nanostructured lipid carriers (NLC) are the second generation SLN composed of solid lipid matrix which are incorporated with liquid lipids (Zauner et al., 2001). Among the nanostructured lipid carriers that contain solid lipids together with liquid oils are, Miglyol®, α-tocopherol, etc (Souto and Müller, 2006). The presence of liquid lipids with different fatty acid C-chains produces NLC with less organized crystalline structure and therefore provides better loading capacity for drug accommodation (Müller et al., 2002). Liquid lipids are better solubilizers of drugs than solid lipids.

The application of NLC as a drug delivery system is enhanced by eliminating the use of organic solvents in the preparation stage and using the hot high-pressure homogenization technique. Polysorbate 20 and polysorbate 80 are non-ionic surfactants commonly used as excipients and emulsifiers in medications for parenteral administration. However, their efficiency in the stabilization of NLC is yet to be elucidated. In this study, the effect of polysorbate 20 and polysorbate 80 in the stabilization of NLC was investigated through the physiochemical properties of the formulated nanoparticles.

MATERIALS AND METHODS

Hydrogenated palm oil (Softisan 154®) was donated by Condea (Witten, Germany). Olive oil (Basso®) was obtained from Basso Fegele and Figli Srl (San Michele Di Serino, Italy). The reagents used were Lipoid S100, a lecithin from phospholipid (Cologne, Germany), non-ionic surfactants, polysorbate 20 and polysorbate 80 (Fisher-Scientific, USA), thimerosal and sorbitol (Sigma-Aldrich Chemie GmbH, Germany). All chemicals were pharmacopeial or reagent grade chemicals.

Lipid matrices preparation

The lipid matrices were composed of hydrogenated palm oil, Lipoid S100 and olive oil at the ratio of 7:3:3. The mixtures were heated to approximately 10°C above the melting point of the lipid matrices to avoid lipid memory effect. After stirring with a teflon-coated magnet, a yellowish-milky solution was obtained.

Nanostructured lipid carriers formation

Sorbitol, surfactant and thimerosal of 4.75, 1.0 and 0.005%, respectively, were dissolved in bidistilled water. Subsequently, this aqueous surfactant was heated to the same temperature as that of the lipid matrices. Nanostructured lipid nanoparticles containing 5% of lipid matrices were then dispersed into aqueous surfactant mixture with high-speed stirring in Ultra-Turrax® (IKA/Staufen, Germany) at 13000 rpm for 10 min to produce a hot pre-emulsion. The hot pre-emulsions were then homogenized in a high-pressure homogenizer EmulsiFlex® (Avestin, Inc./Ottawa, Canada) at 1000 bar for 20 cycles using an optimized protocol. The emulsions were allowed to recrystallize at room temperature to form NLC.

Particle size

The average diameter and polydispersity index (PDI) of NLC were calculated with the Malvern software using photon correlation spectroscopy (PCS) (Zetasizer Nano ZS, Malvern, UK). The measurements were obtained in triplicates (n = 3) and standard deviations calculated at a fixed angle of 173° and at 25°C. The aqueous NLC were diluted with bidistilled water prior to analysis to prevent back-scattering effect.

Zeta potential measurement

Laser doppler electrophoresis technique was applied to measure particle electrostatic charge. The analysis was done with Zetasizer Nano ZS (Malvern, UK) and the results were expressed as zeta potential (ZP). The measurements were performed in triplicates at pH of 7.26 ± 0.13 to mimic physiological pH.

Transmission electron microscopy

A drop of diluted NLC dispersions was placed onto the surface of a copper grid coated with carbon. Upon drying, the grids with mesh size of 300 were stained with 2% phosphotungstic acid, (PTA) (w/v) for 120 s and dried at room temperature. The NLC samples were placed onto sample holders and probed with transmission electron microscopy (TEM) (Hitachi H-7100, Japan) (AL-Haj et al., 2008).

Differential scanning calorimetry

Differential scanning calorimetry (DSC) analysis was done with Mettler DSC 822e (Mettler Toledo, Greifensee, Switzerland). Approximately 10 mg of bulk lipid and NLC were place in aluminum pans. The pan was heated and the thermograms were recorded at temperature range of 25 to 70°C at a heating rate of 5°C/min. An empty aluminum pan was used as a reference (AL-Haj et al., 2008).

Wide-angle X-ray diffraction

The crystallinity of NLCs were determined by wide-angle X-ray diffraction (WXRD) using an X-ray diffractometer (Philips, Germany), equipped with Cu Kα radiation. Samples of 10 mm in length were placed onto X-ray plates, exposed to 40 kV, 30 mA and with a scanning speed of 4°/min for 2Θ. The diffractogram was recorded between 3 to 40°.

Statistical analysis

The data obtained were statistically analyzed. The results were expressed in mean ± SD. One-way analysis of variance (ANOVA) was performed using SPSS 17.0 to evaluate the significance of data at α of 0.01.

RESULTS

Morphological imaging

The transmission electron microscopy (TEM) imaging was done within 1 week of NLC production. Figure 1 shows that NLC appeared spherical with dark-grey shading. The lighter areas in the center of the particles suggest successful incorporation of oil within the lipid matrices. The NLC80 appeared to have a much higher amount of oil within the particles. The size of particles obtained by this

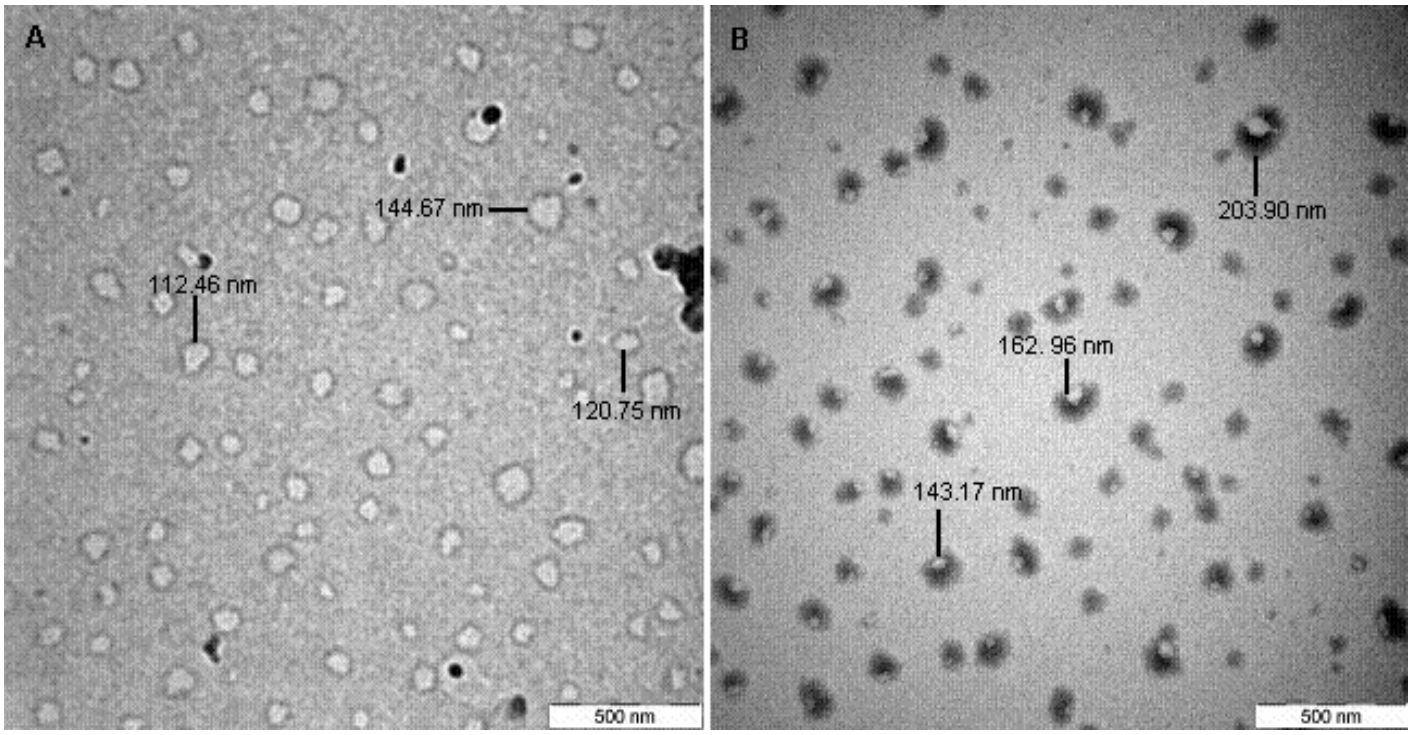

Figure 1. Transmission electron micrograph of nanostructured lipid carriers. (A) NLC80 and (B) NLC20.

Table 1. Particle size and polydispersity index of nanostructured lipid carriers.

Sample name	Z-average (nm)	PDI
NLC20	261.63 [a] ± 8.56	0.46 ± 0.03
NLC80	102.80 [b] ± 0.10	0.47 ± 0.01

Data are presented as mean ± SD. [a,b], means with different superscript are significantly different ($p < 0.01$); NLC20 and NLC 80 are nanostructured lipid carriers with polysorbate 20 and polysorbate 20 as stabilizer, respectively; Z-average represent particle diameter, while PDI is the polydispersity index.

preparation was between 90 to 130 nm.

Particle size and polydispersity index

The average diameter of NLC80 was significantly ($p < 0.01$) lower than that of NLC20 (Table 1). There was no significant ($p > 0.01$) difference between PDIs of NLC20 and NLC80.

Zeta potential

Figure 2 shows the zeta potential (ZP) of the NLC20 and NLC80. Both particles had negative ZP values with NLC80 (-30.57 ± 0.06 mV) being significantly (*p* < 0.01) more negative than NLC20 (-23.93 ± 0.75 mV).

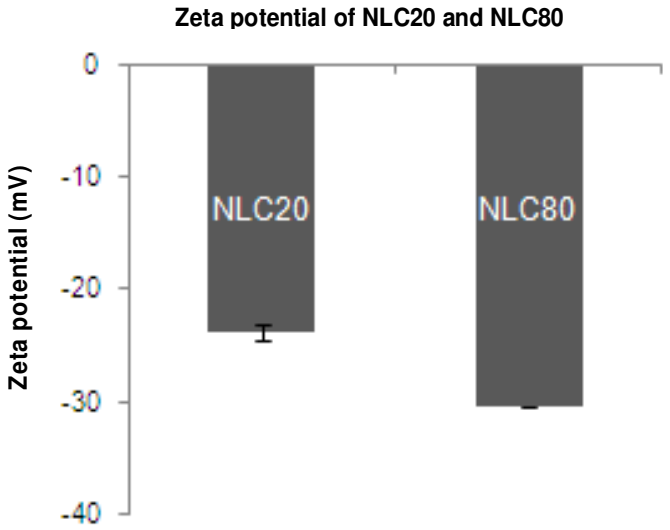

Figure 2. The zeta potential of NLC20 and NLC80. The data are presented as means ± SD.

Differential scanning calorimetry

The bulk lipid had a melting point of 61.56°C. The NLC80 appeared to have a single melting peak of 55.85°C (Figure 3). In contrast to NLC80, NLC20 showed two distinct endothermic depressions at 54.80 and 59.10°C, respectively.

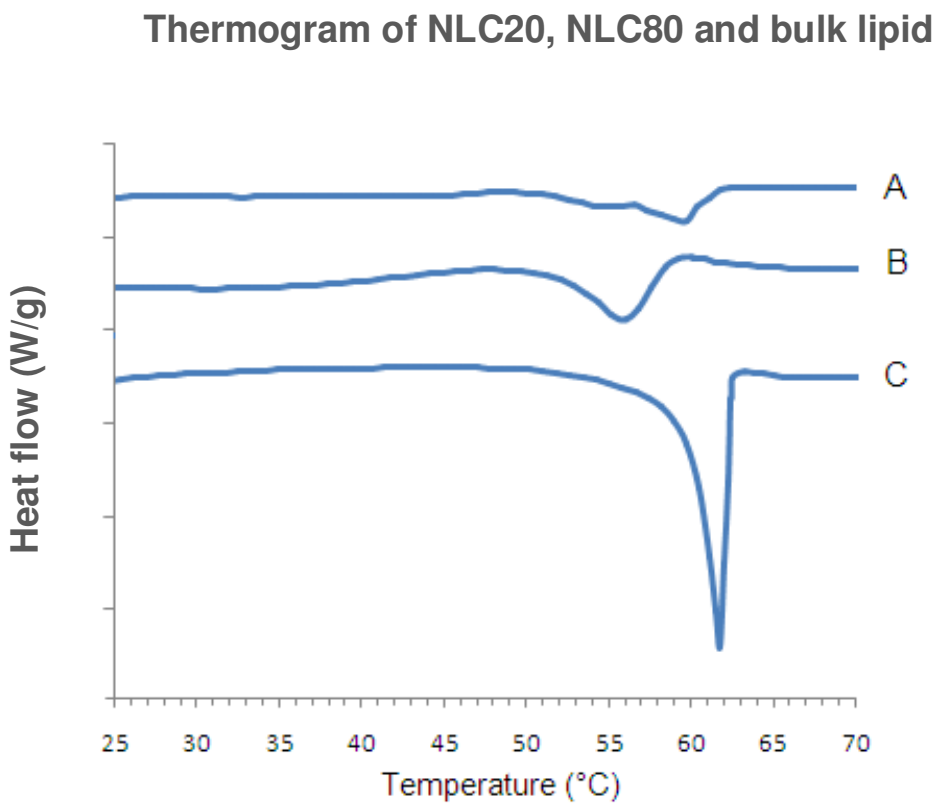

Figure 3. Thermogram of NLCs recorded as a function of temperature from 25 to 70°C. (A) NLC20; (B) NLC80 and (C) bulk lipid.

Wide-angle X-ray diffraction

To complement DSC, wide-angle X-ray diffractometry (WXRD) was used to determine the geometric scattering of X-ray from crystal planes to assess degree of crystallinity in the nanoparticles. The diffractogram paralleled the results obtained by DSC thermal analysis (Figure 4). Bulk lipid produced diffraction pattern with a relatively sharp peak, indicating that it had a crystalline structure. However, the diffraction peaks for NLC20 and NLC80 were broader and less intense than that of bulk lipid.

DISCUSSION

The NLCs obtained in this study were spherical and they are known as zero-dimensional materials (Mathur et al., 2010). In this study, NLC20 and NLC80 using polysorbate 20 and polysorbate 80 were produced, respectively as stabilizers. The microstructures of NLC20 and NLC80 seemed to be distinctively different. In the production of NLC, incorporation of solid and liquid lipid mixtures promote less perfect crystallization and thus reducing the probability of expulsion of the encapsulated drug upon storage (Müller et al., 2004). Furthermore, lipophilic active ingredients have greater solubility in liquid lipids than that of solid lipids, which would allow more flexibility for modulation of drug release and better drug-loading efficiency.

To determine the size distribution of the formulated NLCs in a more precise and quantitative manner, PCS analysis was performed. Surface energy accounts for the high dispersion efficiency of nanoparticles (Nanda et al, 2003). The integration of polysorbate 80 possibly provided more interfacial area than polysorbate 20. As a result, the average size of NLC80 was smaller than NLC20. Smaller particle size is particularly ideal for drug encapsulation because of the larger surface area which reacts with target components. Achieving particle size of less than 100 nm is more feasible for hard materials such as metal oxide than lipid nanoparticles, which are soft materials (Gupta, 2006). Nonetheless, nanoparticles of these sizes were suggested to exhibit unique physical and biological properties. NLC80 of approximately 100 nm in size was produced. Since polysorbate 80 possessed high surface activity and low toxicity and is classed as generally recognized as safe (GRAS) among surfactants, NLC80 is thus potentially an excellent drug carrier system for parenteral application. Because of the nano-scale-size, NLCs would be minimally phagocytosed by macrophages of the mononuclear phagocytic system

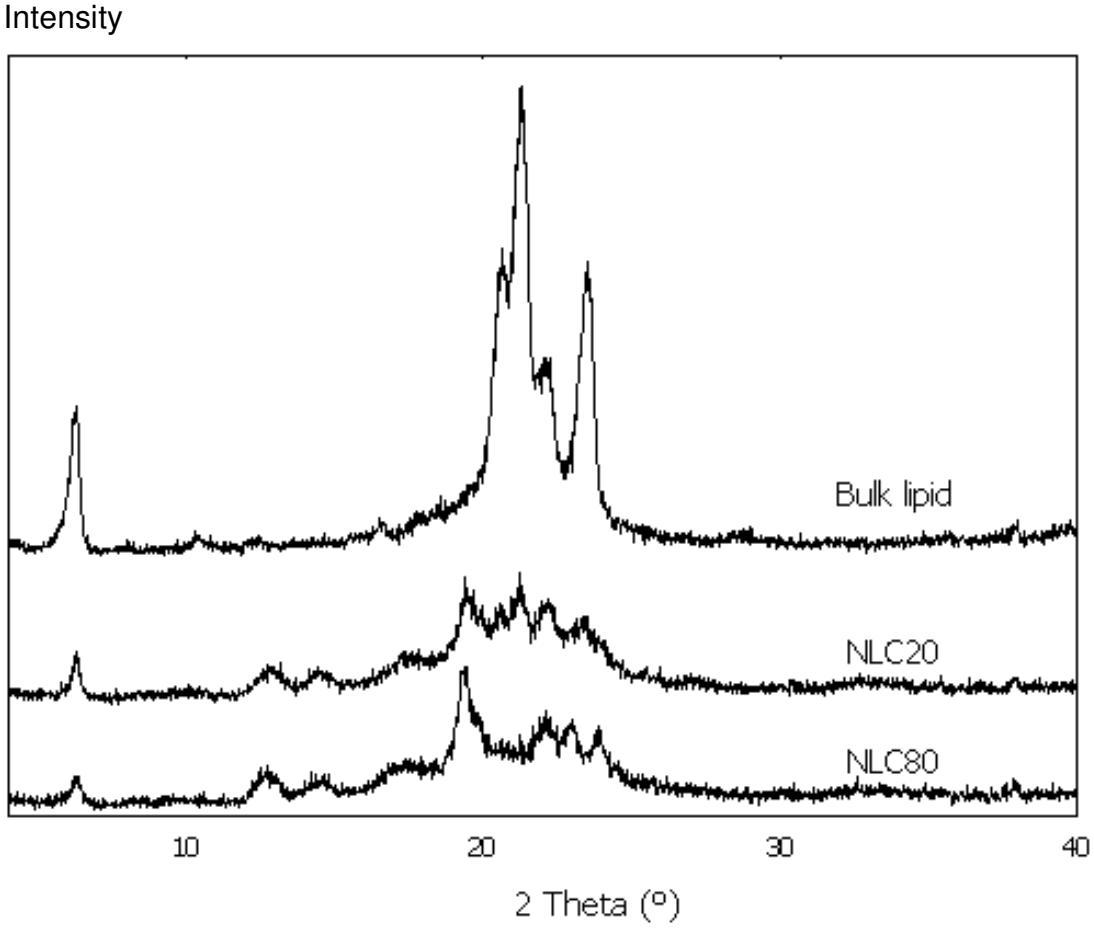

Figure 4. X-ray diffraction pattern of lipid bulk material; NLC20 and NLC80.

(Muhlen et al., 1998) and thus decreasing its destruction in the body. The sizes of the NLCs measured by TEM seemed to be larger than that measured by PCS. This discrepancy could be due to differences in principals of measurements, measuring conditions and technology applied in these techniques. In TEM, particles are exposed to high vacuum in electron beam column. Photon correlation spectrometry (PCS) technique measures the Brownian displacement, which is then related to the size of the particles (Burgess, 2006).

The PDIs of the NLC20 and NLC80 were similar and this suggest that they had similar size distribution. This study suggests that the surfactants did not affect particle size distribution and high-pressure homogenization may be recommended for the production of NLCs. Particles in the suspension exhibited electrostatic charges on their surfaces and can be expressed as ZP, which can be used to predict long-term stability of NLC suspensions. Generally, the ZP of a suspension should be either less than -30 mV or greater than +30 mV for the nanoparticles to be stable. Particle flocculation and aggregation is also less likely to occur in suspensions with higher ZP. Therefore, NLC80 with ZP of -30.57 ± 0.06 mV was more stable than NLC20.

Differential scanning calorimeter was used to determine the nanoparticles crystallization through the determination of melting temperature and the heat-flow associated with material transition as a function of time and temperature (Freitas and Müller, 1999; Muhlen et al., 1998). Using DSC, this study showed that the type of surfactant in the formulation affected crystallization behavior of NLC which gave different polymorphic forms. The lipid molecules showed three different polymorphic structures; the unstable α-, the metastable β'-, and the most stable β-modification. Most bulk lipids would exhibit β-modification or at least, predominantly β-modification (Souto and Müller, 2007). The hydrogenated palm oil showed one peak at 61.56°C, indicating the stable arrangement of bulk lipid (β-modification) (Muhlen et al., 1998) in the formulations. The decrease in NLC80 melting point to 55.85°C, which was below that of bulk lipid was attributed to the β'-modification (AL-Haj et al., 2008). This was shown earlier, where bulk lipid upon transformation into the nanoparticulate form, exhibited melting point depression (Hunter, 1986). Using hydrogenated palm oil in the formulation produced a 5.71°C depression in melting

point in the NLCs. This melting point depression was due to its less-ordered arrangements, and the need for lesser amount of energy to overcome the lattice force in the materials. The melting point depression of this colloidal system can also be attributed to the small size of nanoparticles (Jenning et al., 2000). The NLC20 recorded two melting points, where the higher melting point (59.10°C) was higher than the melting point of NLC80. It is possible that it is the imperfect dispersing of polysorbate 20 in Softisan 154® that caused the existence of the two peaks. The WXRDs show that the NLCs had diffused X-ray scattering patterns, and this was similar with the results obtained by DSC analysis, indicating that the formulations had produced NLCs low in molecular order and crystallinity (Kuntsche et al., 2004; Dong et al., 2003).

Nanostructured lipid carriers have unique characteristics that can enhance the performance of a variety of incorporated drug forms. The physicochemical properties of the NLCs are essentially influenced by the type of surfactant used. Polysorbate 80 appeared to be a better surfactant than polysorbate 20 in dispersing hydrogenated palm oil, in terms of particle size, charges and crystallization behavior. The type of stabilizer significantly affected the average size and charge but not the size distribution of the NLCs. The NLC80 was small in size and would have superior particle surface to volume ratio, better bioavailability and drug-loading efficiency than NLC20.

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