Original Article

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Effect of surfactant on lycopene-loaded nanostructured lipid carriers

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ABSTRACT: Nanostructured lipid carriers (NLC) have gained high interest as enhancing drug delivery systems *via* topical application during the last few years. NLC can enhance stability of many active substances against environmental stress. The extremely small size of NLC plays an important role in skin penetration. The unchanged size of NLC upon storage indicates its physical stability. The aim of this work was to investigate the effect of surfactant type on physical properties and stability of lycopene-loaded NLC. The preparation of the NLC was achieved by means of high pressure homogenization. The results indicate that different types of surfactant yield NLC with different properties. We also explored the effect of contact angle on the size of the NLC. It was found that the small contact angle gave NLC with small size. Among two small contact angle surfactants, Plantacare 1200 gave lycopene-loaded NLC with smaller size, higher zeta potential and narrower size distribution. The particle size, size distribution, and zeta potential of lycopene-loaded NLC prepared with Plantacare 1200 was unchanged during 30 days of storage. It was concluded that Plantacare 1200 is the most suitable surfactant for lycopene-loaded NLC. The chemical stability of lycopene entrapped in the NLC was significantly enhanced.

Keywords: NLC, surfactant, orange wax, lycopene, stability

1. Introduction

Nanostructured lipid carrier (NLC), the second generation innovative lipid nanoparticle that acts as a bioactive carrier system, has been developed to overcome some potential limitations of the solid lipid nanoparticle (SLN). NLC has attracted increasing scientific and commercial attention during the last few years (*1,2*) due to the lower risk of systemic side effects and is suitable for transdermal administration (*3,4*). It has benefits over SLN in improving release properties (*5-7*). NLC can reduce irritation, increase absorption of the active compound to skin, and protect photolabile agents from light. In addition, the expulsion of drug entrapped in NLC during storage is minimized or avoided. NLC is an alternative carrier to other drug carrier systems such as liposomes and polymeric nanoparticles because it has combined the advantages of other colloidal carriers and avoided their disadvantages. These includes high amounts of drug payload, increasing drug stability, the possibility to control drug release and targeting, and avoidance of organic solvents (*8*).

Many water-insoluble active compounds were reported to be successfully incorporated in NLC and showed high advantages in skin permeation (*9,10*). Lycopene, a potential natural antioxidant found in tomatoes and tomato-based food products, watermelon, and pink grapefruit (*11*) has so far become one of the most interesting molecules. It is an acyclic carotene with 11 conjugated double bonds and possesses the highest antioxidant activity among common carotenoid compounds (*12*). Lycopene functions as an antioxidant, anti-inflammatory, anti-cancer, and anti-mutagenic agent and exhibits a high physical quenching rate constant for singlet oxygen *in vitro* (*13-15*). However, lycopene is water insoluble and hardly diffuses *via* the transdermal pathway when applied tropically. Lycopene is an unstable molecule. Because of many conjugated double bonds in its molecule, it is very susceptible to oxidation when exposed to air and light (*16*). NLC were reported to give excellent protection to incorporated labile drugs from degradation (*17*). The utilization of NLC to increase stability of lycopene is a challenge and has not yet been reported anywhere. Moreover, it is known that for a sufficient residence time and optimal penetration *via* the transdermal route in order to obtain the maximum therapeutic efficiency, the size of NLC plays the most important role (*18*). The unchanged size of NLC upon storage indicates its physical stability as well as its effectiveness. As NLC is derived from the emulsion system, the NLC hence is mainly stabilized by a surface active molecule or a so-called surfactant. In the process of NLC production, the spreading of surfactant on the surface of an oil droplet of lipid is one of the main reactions required for desirable NLC. One impact for this interaction is contact angle, an angle which is created at the point where the three phases composed of gas, liquid and solid

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meet (*19*). It is known that contact angle influences the spreading of liquid onto the solid substrate (*20,21*). At the present time, there are very few systematic investigations of the quantitative relationship between surfactant solution and the spreading behavior of the solid lipids.

In the present study, several surfactants which showed a different contact angle to the major lipid of NLC were first investigated. We hypothesized that contact angle might be a useful tool for selection of a suitable surfactant for a small size NLC within a short time. The main aim of this study was to evaluate the effect of surfactant type on stability of lycopene loaded NLC.

2. Materials and Methods

2.1. *Materials*

Orange wax was provided by Koster Keunen, LLC (Connecticut, USA). Plantacare 1200 (lauryl glucoside) were from Cognis (Dusseldorf, Germany). C-1216 (sucrose laurate), C-1816 (sucrose stearate), C-1616 (sucrose palmitate), C-1815 (sucrose stearate) surfactants were obtained from Surfhope® SE Cosme marketed by Mitsubishi-Kagaku Foods Corporation (Japan). Lycopene was obtained in the form of an oily solution containing 40-47 mg/L of tomato lycopene in rosemary oil under the trade name of "Lycosol" (LiBiol, Germany). Tetrahydrofuran (THF) was from Rankem, New Delhi, India. Ultra-purified water was obtained from a MilliQ Plus system, Millipore (Schwalbach, Germany).

2.2. *Preparation of lycopene-loaded NLC*

NLC formulations using orange wax as a major lipid core were produced using a high pressure homogenizer (HPH) (Micron LAB40, Homogenizer Systems, Germany). The melted lipid phase containing orange wax (90%, w/w) and lycopene oil solution (10%, w/w) was dispersed in a hot surfactant solution (75°C), obtaining a pre-emulsion by high speed stirring using an Ultra-Turrax T25 (Janke and Kunkel GmbH, Staufen, Germany) at 12,000 rpm for 30 sec. This hot pre-emulsion was further processed by HPH applying five cycles at 500 bar and 75°C. The lipid dispersion was cooled at ambient conditions to room temperature and solidified to obtain the aqueous NLC dispersions.

2.3. *Contact angle measurement*

Contact angle between lipid surface and surfactant was determined by means of goniometry. The solid surface was prepared on a microscopic glass slide using an appropriate amount of lipid mixture composed of 90% (w/w) orange wax and 10% (w/w) lycopene oil solution. The series of 0.1% (w/v) aqueous solutions of five different surfactants; C-1216, C-1816, C-1616, C-1815, and Plantacare 1200 were freshly prepared. The contact angle between the lipid surface and a single drop of the surfactant solution was determined 15 sec after the droplet was put onto the lipid surface using a Contact Angle Meter G1 (Krüss, Hamburg, Germany).

2.4. *Particle size and zeta potential measurement*

Analysis of the particle size, size distribution, and zeta potential was carried out by means of photon correlation spectroscopy (PCS) with a Malvern Zetasizer IV (Malvern Instruments, UK). The PCS yielded mean particle size (z-ave) and polydispersity index (PDI) which indicated the width of the size distribution. The z-ave and PDI values were obtained by averaging at least ten measurements at a fixed angle of 90° in 10-mm diameter cells at 25°C. All samples were diluted with purified water to have a suitable scattering intensity before measurement. For measuring zeta potential the sample was dispersed in purified water adjusted with sodium chloride solution (0.9%, w/v) to a conductivity of 50 μS/cm. The experiments were done in triplicate.

2.5. *Stability test*

To evaluate the physical stability of lycopene-loaded NLC, the samples of NLC dispersions were stored at 25°C over a period of 30 days. The changes of particle size, size distribution, and zeta potential against storage time were determined using PCS. The chemical stability of lycopene-loaded NLC was investigated as follows. Lycopene-loaded NLC dispersion containing 40 μg/mL lycopene and lycopene solution in THF of equivalent lycopene concentration were exposed to light at 50°C for 24 h. The amount of lycopene remaining in each sample during storage time was determined periodically using UV/visible spectroscopy at 475 nm with a UV-2450 double-beam spectrophotometer (Shimadzu, America) and THF as a solvent for dissolving lycopene in the NLC.

2.6. *Statistical analysis*

Statistical analysis of differences between different treatments was performed using analysis of variance (ANOVA). In all cases, $p < 0.05$ indicates the level of significance.

3. Results and Discussion

It is well known that surfactant is a group of hydrophilic-lipophilic molecules which are active at the interface of two immiscible liquid phases. The surfactant can produce huge advantages in pharmaceutical fields. By chemical structure, surfactant can be divided into two main groups of ionic and non-ionic surfactants with different solubility properties defined by the value of hydrophilic-lipophilic balance (HLB). The lower HLB surfactants give a w/o emulsion while the higher HLB molecules provide an o/w emulsion. Because NLC is derived from an o/w

emulsion system, the surfactants used in this study were of a higher HLB group which are preferably dissolved in an aqueous external phase of the emulsion. Moreover, as we emphasized developing the lycopene-loaded NLC for transdermal application, the surfactants of non-ionic groups particularly with a basic chemical structure of a sugar ester group were used in this study because they were claimed to be skin friendly. Besides their good compatibility with the skin, these surfactants also showed a drug enhancement effect in percutaneous absorption (*22,23*).

3.1. *Effect of surfactant on contact angle*

The contact angle is defined geometrically as the angle formed by a liquid at the three phase boundary where a liquid, gas, and solid intersect. It can be determined by goniometry (*24*). This technique is based on analysis of the shape of a drop of a test liquid on a test solid. A low contact angle value indicates well spreading or wetting while a high value indicates poor wetting or incompatibility. A zero contact angle represents complete wetting. In this study, five different surfactant solutions dropped on the solid surface composed of orange wax and lycopene oil solution demonstrated different contact angles as shown in Figure 1. It was obviously seen that the smallest contact angle of 38° was obtained from C-1216 followed closely by Plantacare 1200 which showed a contact angle of 39°. The other three surfactants; C-1816, C-1616, and C-1815, exhibited contact angle values higher than 45°. Considering the properties of surfactant, it was found that they are similar in basic properties. All five surfactants are non-ionic sugar esters with similar HLB values. The different contact angles obtained from these surfactants therefore were considered to be due to the number of carbon atoms existing in the hydrophobic chain of each molecule. The chemical structure of C-1216 (sucrose laurate) and Plantacare 1200 (lauryl glycoside) as shown in Figure 2 indicate that these two surfactants have 12 carbon atoms per molecule whereas the other surfactants such as sucrose stearate (C-1815 and C-1816) and sucrose palmitate (C-1616) possess 18 and 16 carbon atoms, respectively. It was considered that the surfactants with the shorter hydrocarbon chain length of C12 were more appropriate for the surface of lycopene-orange wax than that of C15-C18.

3.2. *Effect of surfactant on the particle size of the NLC*

In this study, lycopene loaded NLC formulations of five different surfactants were produced. The orange wax was used as a major lipid component. Orange wax is light reddish-brown to orange in color with properties of sunscreen-enhancing (*25*), antioxidant (*26*), and antimicrobial activities (*27*). Lycopene also has an anti-stress effect related to inhibition of harmful effects from UV exposure, because it is an effective agent against solar stress (*28,29*). The development of lycopene loaded NLC in this study was expected to promote an additional biological action of both orange wax and lycopene as well as to enhance their bioavailability *via* the NLC system. Moreover, lycopene was used as the oil solution in order to reduce the degree of organization of lipid matrix of orange wax after the process of NLC production by hot high pressure homogenization. The results of this study revealed that the five different surfactants produced lycopene-loaded NLC with different sizes as shown in Figure 3. It was found that only two surfactants, C-1216

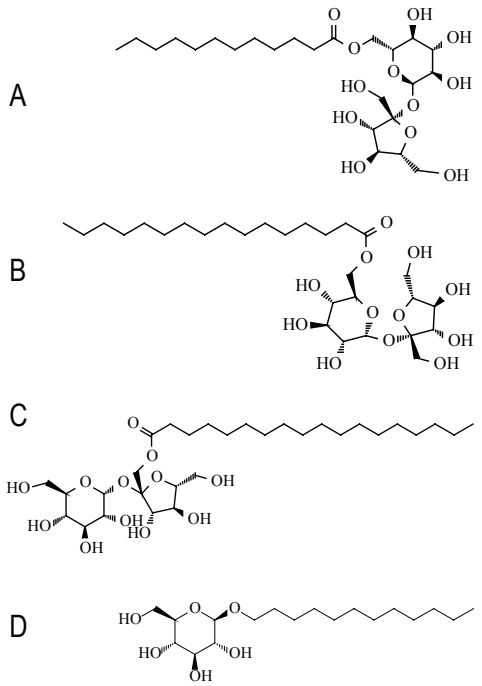

Figure 2. Chemical structure of sucrose laurate (A), sucrose palmitate (B), sucrose stearate (C), and lauryl glucoside (D).

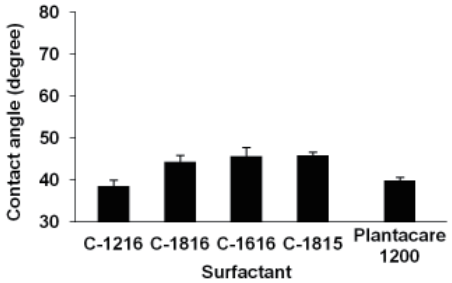

Figure 1. Contact angle between surfactant solution and lipid surface of orange wax and lycopene mixture.

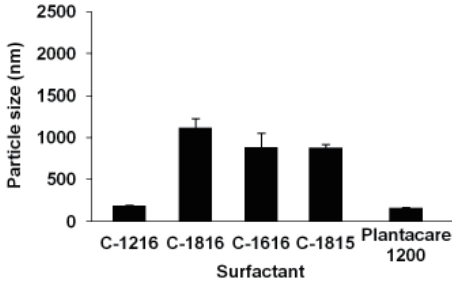

Figure 3. Particle size of lycopene-loaded NLC stabilized by different surfactants.

and Plantacare 1200, could yield the extremely smallest size NLC with z-ave of approximately 170-190 nm. The NLC particles obtained from C-1616, C-1816, and C-1815 were obviously greater with a z-ave higher than 900 nm. Considering this effect with the contact angle values obtained from the five surfactants mentioned above, it was found that the contact angle value and the particle size of the NLC were closely related. The results demonstrated that the small particle size of lycopene-loaded NLC was obtained from the surfactants with small contact angles of less than 40°. According to the famous Young equation (*30*), surfactants with lower surface tension give smaller contact angles. Moreover, a surfactant solution with lower surface tension can reduce more surface or interfacial free energy. Considering the data obtained from this experiment, it could be presumed that the small particle size of the lycopene-loaded NLC obtained from the small contact angle surfactants was due to the high capacity of decreasing interfacial free energy between the two immiscible lipid and aqueous phases during the emulsion forming process.

3.3. *Effect of surfactant on physical stability of the NLC*

Two surfactants, C-1216 and Plantacare 1200, were selected to use in this study because they showed the smallest contact angle value and high ability to produce the smallest size lycopene-loaded NLC. The NLC formulations obtained from both surfactants were evaluated for their particle size (z-ave), size distribution (PDI), and zeta potential. The results demonstrated that the mean size of the freshly prepared NLC of both formulations was not significant different with a z-ave of approximately 170-190 nm. However, the PDI and zeta potential of these particles were different. The PDI of lycopene-loaded NLC obtained from C-1216 was 0.3 whereas that of Plantacare 1200 was 0.1. The zeta potential of the NLC obtained from C-1216 was −52 mV whereas that obtained from Plantacare 1200 was −62 mV. Keeping these formulations at 25°C for 30 days caused the zeta potential of the NLC obtained from C-1216 to decrease to −47 mV whereas no significant change of zeta potential in the NLC was obtained from Plantacare 1200 as seen in Figure 4. The particle size and size distribution of the lycopene-loaded NLC were observed at day 7, day 14, and day 30 in comparison with those freshly prepared (day 0). The results are shown in Figures 5 and 6, respectively. It was found that the particle size of the NLC obtained from C-1216 was increased obviously during 30 days of

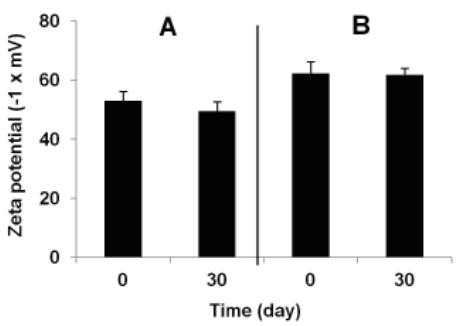

Figure 4. Effect of storage time on zeta potential of lycopene-loaded NLC stabilized by C-1216 (A) and Plantacare 1200 (B).

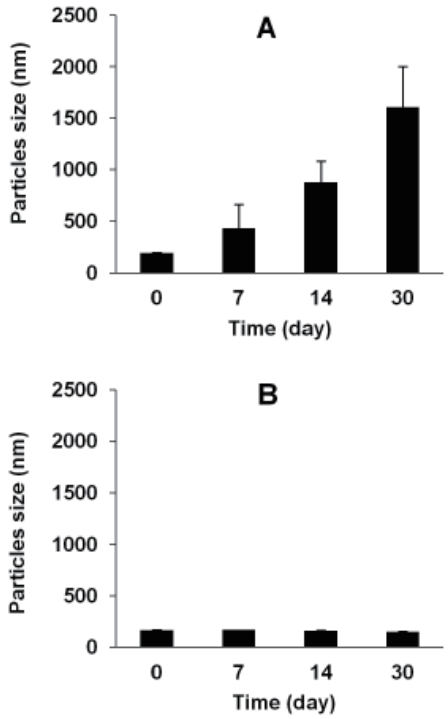

Figure 5. Effect of storage time on particle size of lycopene-loaded NLC stabilized by C-1216 (A) and Plantacare 1200 (B).

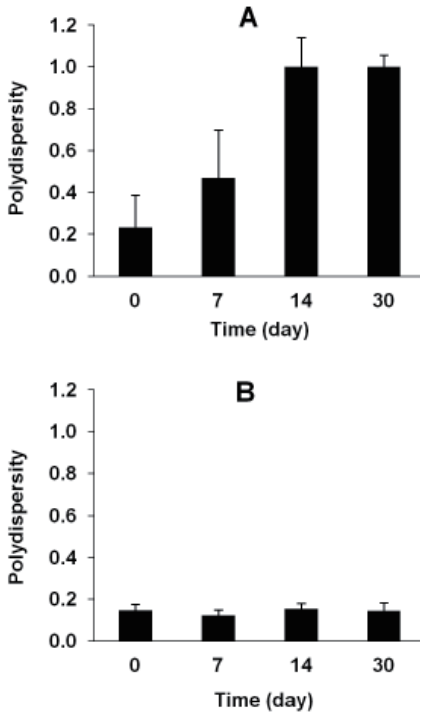

Figure 6. Effect of storage time on size distribution of lycopene-loaded NLC stabilized by C-1216 (A) and Plantacare 1200 (B).

storage whereas the size and PDI of that obtained from Plantacare 1200 showed no significant change. Therefore the lycopene-loaded NLC obtained from Plantacare 1200 were revealed to be more stable than that from C-1216. The good narrow size distribution with constant small particle size of the NLC obtained from Plantacare 1200 throughout the period of 30 days might be due to the strong effect of Plantacare 1200 to produce an extremely high zeta potential at the surface of the NLC system. The high zeta potential of the Plantacare 1200 based NLC was considered to be due to the hydroxyl ions caused by Plantacare 1200 molecules surrounding the surface of the NLC nanoparticles. The high zeta potential resulted from the electrical charge on this particle surface which repulsed the charge from other particles, and therefore made a stable NLC system. This is a reason why the lycopene-loaded NLC stabilized by Plantacare 1200 showed higher stability overtime than those made from C-1216. The results of this investigation demonstrated the influence and mechanism of the surfactant on enhancing NLC stability. Moreover, it can be assumed that Plantacare 1200 is the most suitable surfactant for producing lycopene-loaded NLC having orange wax as a major lipid at this moment.

3.4. *Chemical stability of the lycopene*

This experiment was done in order to study the chemical stability of lycopene in solution in comparison with that loaded in the NLC. The results revealed that lycopene in solution decreased rapidly during the first 4 h of storage and further gradually decomposed as demonstrated in Figure 7A. The plot of log lycopene versus the storage time showed a linear regression with y = 1.674 – 0.072x (r^2 = 0.995). It was found that the degradation profile of lycopene was extremely slowed when incorporated in the NLC stabilized by C1216 and Plantacare 1200 as shown in Figures 7B and 7C, respectively. The plot of log lycopene during the storage time showed a linear regression with y = 1.596 – 0.006x (r^2 = 0.992) and y = 1.599 – 0.004x (r^2 = 0.997) for C1216 and Plantacare 1200 systems, respectively. These results suggest that lycopene in the samples followed a first-order chemical degradation. The first-order kinetic calculation demonstrated that the half life of lycopene in solution was 9.6 h whereas that entrapped in the NLC was 117.5 and 192.5 h stabilized by C-1216 and Plantacare 1200, respectively. This result revealed that lycopene was most chemically stable when entrapped in the Plantacare-1200 NLC.

4. Conclusion

It was found that surfactant type influenced the quality of lycopene loaded NLC. The results of this study demonstrate that the contact angle is a key tool to overcome the small size NLC. Surfactant type also showed an important role on the zeta potential of the NLC. Plantacare 1200 was proven to be the best surfactant for lycopene-loaded NLC using orange wax as a major lipid. Lycopene was chemically stabilized by entrapping in the NLC.

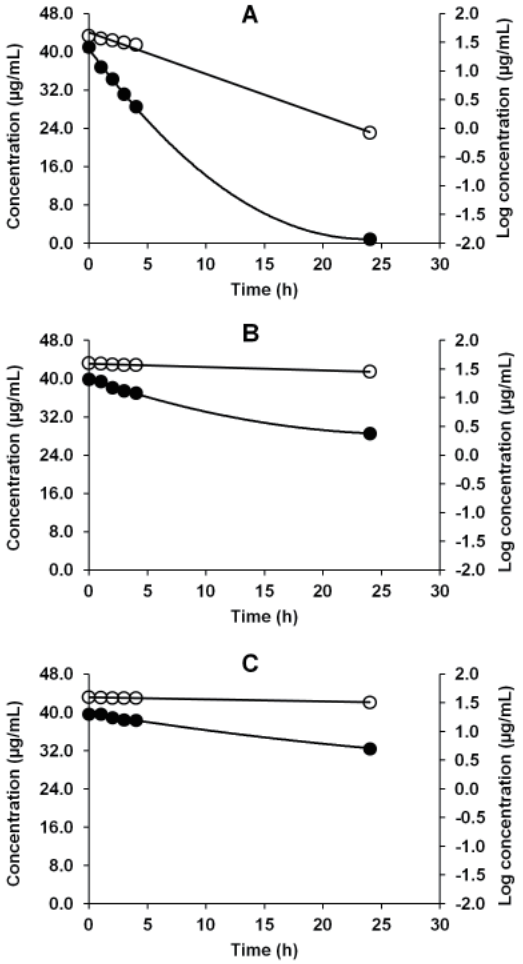

Figure 7. Stability profiles of lycopene concentration (●) and log concentration (○) in solution (A), C-1216 (B) and Plantacare-1200 (C) NLC dispersions.

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