African Journal of Biotechnology Vol. 9(37), pp. 6158-6161, 13 September, 2010
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 ©2010 Academic Journals

Full Length Research Paper

Effect of nanoliposomes on the stabilization of incorporated retinol

Sungho Ko[1] and Seung-Cheol Lee[2]*

[1]Korea Food Research Institute, 516 Baekhyun-dong, Bundang-gu, Seongnam, 463-746, Republic of Korea.
[2]Department of Food Science and Biotechnology, Kyungnam University, Masan, 631-701, Republic of Korea.

Accepted 27 August, 2010

Nanoliposomes containing retinol were prepared, and the stability of the incorporated retinol was evaluated. Average particle size of nanoliposomes was 98 nm. Retinol was readily incorporated into nanoliposomes with incorporation efficiencies higher than 99%. The bare retinol in the buffer was almost degraded within 2 days of storage, while at least, over 20% of the initial retinol in nanoliposomes was kept until 8 days in all experimental conditions tested. The incorporated retinol in nanoliposomes was more rapidly degraded as temperature increases under both dark and UV light condition during the whole period of storage, and UV light slightly decreased the stability of retinol in nanoliposomes.

Key words: Retinol, nanoliposome, stability.

INTRODUCTION

Vitamin A is one of the fat-soluble vitamins essential for vision, cellular differentiation, organ development during embryonic and fetal development, and membrane structure and function (Harold, 1975). Retinol, the most reduced form of the vitamin, satisfies requirements for all known functions of vitamin A. Also, retinol, one of the essential micronutrients, is obtained mainly in form of preformed retinol from meat and provitamin A carotenoids, such as β-carotene from plant tissue. However, both preformed retinol and provitamin A are sensitive to air, oxidizing agents, ultraviolet (UV) light and low pH values (Eitenmiller et al., 1999; Christen, 2000).

Liposome technology has significant potential to improve formulation such as solubility, stability and tumor delivery-related issues that hinder the clinical advancement of these novel camptothecins. Liposomes are hydrophilic vesicles consisting of one or more concentric bilayers enclosing aqueous compartments (Berrocal and Abeger, 1999; Keller, 2001). More recent liposome investigations mostly use small vesicles (50-150 nm in diameter) because this size range is a compromise among loading efficiency, stability and distribution in the organisms (Liand, 2004; Sulkoski et al., 2005). However, their physical and chemical instability during manufacturing and storage is a major obstacle that prevents many promising nanoliposomal candidates from moving from the experimental scale to the market (Zhang and Pawelchak, 2000).

In previous study, we investigated the effect of cholesterol and β-sitosterol in multilamellar vesicles (MLVs) on the kinetics of retinol degradation in aqueous buffers (Lee et al., 2005a; Lee et al., 2005b; Lee et al., 2008). It appears that the effect of cholesterol in MLVs on the stability of incorporated retinol was greater at lower temperatures, at neutral pH levels and in the dark. Therefore, the aim of this study is to determine the effect of nanoliposome on the stability of incorporated retinol under various conditions such as temperature, darkness and UV light.

MATERIALS AND METHODS

Materials

All *trans* retinol, L-α-phosphatidylcholine (PC) isolated from soybeans were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and reagents were of analytical grade or purer.

*Corresponding author. E-mail: sclee@kyungnam.ac.kr. Tel: +82-55-249-2684. Fax: + 82-55-249-2995.

Abbreviations: MLVs, Multilamellar vesicles; **UV,** ultraviolet; **PC,** L-α-phosphatidylcholine; **IE,** incorporation efficiency.

Instruments

Nanoliposome was collected by centrifugation so that the effects on the incorporation efficiency could be examined with a Hitachi preparative ultracentrifuge (SCP 55H, Hitachi Koki Co. Ltd., Japan).

Preparation of liposomes containing retinol

Liposomes were produced by the dehydration-rehydration procedure of Kirby and Gregoradis (1984) with slight modification. Retinol was added to phospholipid at a ratio of 0.01:1 (wt : wt) in a 50 ml round bottomed flask. Then the mixtures were dissolved in 13 ml of chloroform/methanol solvent mixture (2:1, v/v). The solvent was evaporated on a rotary evaporator (EYELA N-1000, Tokyo Rikakikai Co., Tokyo, Japan) at 30℃ to deposit a dry lipid film on the wall of the flask. The flask was removed from the evaporator and residual solvent was removed under nitrogen stream at 4℃. 30 ml of 10 mM phosphate buffer (pH 7.0) (Lee et al., 2002) and 0.5 g of glass beads were added to assist hydration of the lipids. The solution was then mixed on the rotary evaporator (without vacuum) to form MLVs by hydrating the lipids. Next, the suspension was pressed with French Press (SLM-AMINCO, Spectronic Instruments, Inc. (New York, NY, USA)/ 20,000 psi cell) to reduce the size of MLVs (Lee et al., 2008) and then centrifuged for 1 h at 110,000 ×g. The supernatant was removed and the pellet was washed with 13 ml of the appropriate buffer (Masahiro et al., 2007). The size of the prepared liposomes was measured with a particle size analyzer (LS230 Small Volume Module, Coulter Co., USA).

Analytical method

Retinol in nanoliposomes or buffer was analyzed using a colorimetric assay (Subramanyam and Parrish, 1976). The solution (0.15 ml) of liposome containing retinol was mixed with 0.45 ml of a chloroform/methanol solvent mixture (2:1, v/v) and centrifuged for 3 min at 4,200 ×*g*. A 0.1 ml aliquot of the organic solvent layer was transferred to the test tube and 1 ml of 20% $SbCl_3$ solution was added successively. Then the absorbance of the solution was measured at 620 nm immediately by using a UV–VIS spectrophotometer (UV 1601, Shimadzu, Japan).

Stability of incorporated retinol in liposomes/buffers during storage

The aliquot (0.2 ml) of the nanoliposome suspension of incurporated retinol was placed in glass vials and saturated with oxygen by equilibrating against the atmosphere for 2 h in the dark. Vials were wrapped in aluminum foil or treated with UV light and stored at various temperature (4, 25, 37 and 50℃) conditions under dark for 10 days. Incorporation efficiency (IE) was calculated as follows:

IE (%) = The amount of retinol incorporated in nanoliposomes × 100/ The amount of total retinol

RESULTS

Incorporation of retinol into nanoliposomes

MLVs liposomes into which retinol was incorporated were prepared by dehydration-rehydration method, and then nanoliposomes were successfully obtained by pressing MLVs with French press under neutral condition, pH 7.0.

Table 1. The particle size of nanoliposomes containing retinol and incorporation efficiency of retinol into nanoliposomes.

Mean size of liposome (nm)	Incorporation efficiency (%)[1]
98 ± 48	99.97 ± 0.01

Retinol : phosphatidylcholine = 0.01:1(w/w).
[1]Mean ± standard deviation of triplicate measurements.

Mean size of nanoliposomes was 98 ± 48 nm (Table 1). In general, nanoliposomes with diameters of 100 nm are frequently used as a carrier in drug delivery systems due to their better distribution in the organisms (Sulkoski et al., 2005). Retinol was incorporated into the liposome bilayers. The incorporation efficiency of nanoliposomes was 99.97 ± 0.01% as shown in Table 1. This result indicates that retinol could be successfully incorporated into nano size of liposomes

Stability of incorporated retinol of liposomes/buffer

The effects of temperature and UV light on the stability of retinol incorporated into nanoliposomes were investigated. The time dependent degradation of the incorporated retinol into nanoliposomes in the aqueous solution was observed at various temperature ranges of 4, 25, 37 and 50℃ for 10 days. The results were plotted as percentage of the remaining retinol versus time. As shown in Figure 1A, 89% of the initial retinol incorporated in nanoliposomes in the solution at 4℃ remained on day 2 of storage, and then the remaining retinol gradually decreased by about 68 and 60% until day 8 under dark and UV exposure, respectively. Moreover, from day 8 to 10, retinol was degraded with 18% more in the dark condition, whereas it was done with 38% more under UV light. These results show that UV light slightly accelerated the degradation of retinol. However, the bare retinol in the buffer was rapidly degraded as much as about 96% within only 2 days in all experimental conditions tested, indicating that nanoliposomes could act as a protecting barrier for retinol against the external environments. When stored at 25℃, the percentage of retinol degraded over time was higher than that at 4℃ (Figure 1B). Eighty and sixty percent of retinol in nanoliposome remained on day 2 in the dark and UV exposure, respectively, which shows that retinol was less stable at 25 than 4℃ and under UV light than dark condition. Furthermore, the stability of retinol in nanoliposomes progressively decreased for the whole storage period, but there was no significant difference in the retinol stability in nanoliposomes between under dark and UV light. In Figure 1C, about 50% of the initial retinol in nanoliposomes was degraded on day 2 of storage at 37℃ under both dark and UV light, showing that the retinol was less stable at 37℃ than 4 and 25℃. Then the gradual reduction was observed in

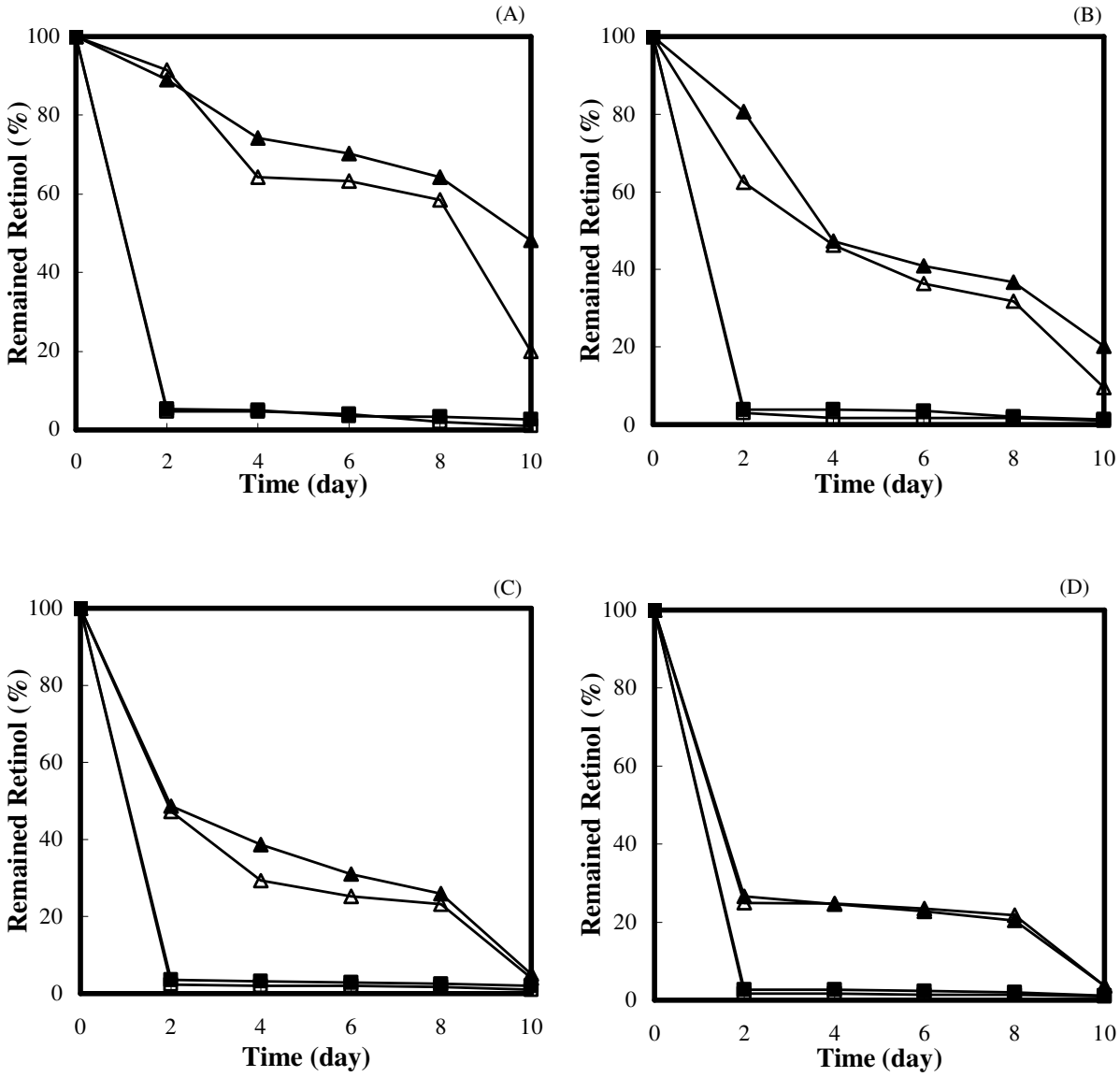

Figure 1. The effects of nanoliposomes on retinol stability. Retinol in nanoliposomes (▲, dark; △, UV light) and bare retinol in buffer (■, dark; □, UV light) were stored at various temperature ranges. A, 4℃; B, 25℃; C, 37℃; D, 50℃ in the dark and UV light, respectively.

the remaining retinol until day 10, which resulted in 96% decrease of the initial retinol in nanoliposomes in the dark and UV light. However, UV light did not significantly affect the stability of retinol in nanoliposomes. A similar trend also occurred at 50℃ (Figure 1D). A large decrease (about 75%) of retinol in nanoliposomes was observed on day 2 at 50℃ under both dark and UV light. The retinol was degraded to a greater extent at 50℃ than 4, 25, and 37℃. On day 10, most of the remaining retinol in nanoliposomes was degraded. The reduction trend of the amount of the incorporated retinol in nanoliposomes throughout the period of storage was similar between under dark and UV light, implying that nanoliposomes could protect retinol against UV light to some extent. These results indicate that nanoliposomes could act as an efficient protecting barrier for retinol against environmental influences such as temperature and UV light.

DISCUSSION

We have investigated the effect of nanoliposomes on the stabilization of incorporated retinol under a variety of conditions such as temperature, UV light, and time. Retinol was efficiently incorporated into nanoliposomes at neutral buffer. The incorporation efficiency of retinol was over 99% in nanoliposomes. This incorporation efficiency of active materials is affected by the preparation method

of liposome, ratio of phospholipid to active materials and organization of liposome (Wu et al., 1994). It has been reported that the dehydration/rehydration method used in this study shows highest incorporation efficiency of active materials (Kirby and Gregoriadis, 1984). In our previous study, the nanoliposomes pressed by French press at same conditions without any incorporated materials was 100 nm (Lee et al., 2008) and the mean particle size of nanoliposome containing retinol in this experiment was 98 nm, indicating that retinol did not influence the size of nanoliposomes.

The stability of incorporated retinol in nanoliposomes was significantly enhanced under both dark and UV light during the whole period of storage, compared to that of bare retinol. The retinol in nanoliposomes was more rapidly degraded as temperature increased and was not considerably affected by UV light, implying that nanoliposomes could act as a barrier for retinol against UV light to some extent. The greatest protective effect of nanoliposome was shown under dark condition at 4℃. The effects of temperature and light are consistent with a report that stable gel phase lipids provide the greatest protection against retinol degradation, perhaps by severely reducing the permeability of oxygen and light. These results have shown that nanoliposomes could act as an efficient protecting barrier for retinol against environmental influences.

When compared to the previous results on the stability of retinol incorporated in MLV (Lee et al., 2005a, b), the retinol stability in nanoliposomes was less than in MLV. Nevertheless, nanoliposomes can be used as effective carriers capable of encapsulating active materials for various delivery applications by enhanced bioavailability, such as cell permeability, absorption rate and target delivery of substances (Allen et al., 2006; Yokoyama and Okano, 1998).

These results also imply that the nanoliposome containing active materials has potential applications in improvement of the shelf life of foods, stability of cosmetic materials and drug delivery systems.

ACKNOWLEDGEMENT

This study was supported by Kyungnam University Research Fund, 2009.

REFERENCES

Allen TM, Cheng WW, Hare JI, Laginha KM (2006). Pharmacokinetics and pharmacodynamics of lipidic nano-particles in cancer. Anticancer Agents Med. Chem. 11: 513-523.

Berrocal MC, Abeger A (1999). Shelf life of a saturated vitamin E carrier system for use in the food industry. Food Sci. Technol. Int. 5: 509-513.

Christen Y (2000). Oxidative stress and Alzheimer disease. Am. J. Clin. Nutr. 71: 621-629.

Harold WG (1975). Decomposition of linoleic acid hyperoxides. Enzymic reactions compared with nonenzymic. J. Agric. Food Chem. 23: 129-136.

Keller BC (2001). Liposome in nutrition. Trends Food Sci. Technol. 12: 25-31.

Kirby CJ, Gregoriadis G (1984). Dehydration-rehydration vesicles, a simple method for high yield drug entrapment in liposomes. Biotechnology 2: 979-984.

Lee JM, Cho YJ, Park DJ, Ko SH, Lee SC (2008). Preparation of nano-liposome by sonication and pressure. Korean J. Food Sci. Technol. 40: 115-117.

Lee SC, Kim JJ, Lee KE (2005a). Effect of β-sitosterol in liposome bilayer on the stabilization of incorporated retinol. Food Sci. Biotechnol. 14: 604-607.

Lee SC, Lee KE, Kim JJ, Lim SH (2005b). The effect of cholesterol in the liposome bilayer on the stabilization of incorporated retinol. J. Liposome Res. 15: 157-166.

Lee SC, Yuk HG, Lee DH, Lee KE, Hwang YL, Ludescher RD (2002). Stabilization of retinol through incorporation into liposomes. J. Biochem. Mol. Biol. 35: 358-363.

Liand X, Mao G, Ng KYS (2004). Mechanical Properties and stability measurement of cholesterol-containing liposome on mica by atomic force microcopy. J. Colloid Interf. Sci. 278: 53-62.

Masahiro Y, Kyoko T, Masayuki ABE, Yoichi U, Masashi N, Noboru A (2007). Release of drugs from liposomes varies with particle size. Biol. Pharm. Bull. 30: 963-966.

Subramanyam GB, Parrish DB (1976). Colorimetric reagents for determining vitamin A in feeds. J. Assoc. Off. Anal. Chem. 59: 1125-1130.

Sulkoski WW, Pentak D, Nowak K, Sulkowska A (2005). The influence of temperature, cholesterol content and pH on liposome stability. J. Mol. Struct. 744-747: 737-747.

Wu TW, Zeng LH, Wu J, Fung KP (1994). Morin: A wood pigment that protects three types of human cells in the cardiovascular system against oxyradical damage. Biochem. Pharmacol. 47: 1099-1103.

Yokoyama M, Okano T (1998). Targeting of anti-cancer drug with nano-sized carrier system. Nippon Rinsho. 56: 3227-3234.

Zhang JA, Pawelchak J (2000). Effect of pH, ionic strength and oxygen burden on the chemical stability of EPL/cholesterol liposomes under accelerated condition: Part 1: Lipid hydrolysis. Eur. J. Pharm. Biopharm. 50: 357-364.