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Preparation of a solid-in-oil nanosuspension containing L-ascorbic acid as a novel long-term stable topical formulation

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ABSTRACT

L-Ascorbic acid (AA, vitamin C) easily decomposes into inactive compounds in aqueous solutions and this has limited its topical use. This work reports the preparation of a solid-in-oil nanosuspension (SONS) containing AA and validation of its basic storage stability. Although AA itself is water-soluble, it can readily be nanosuspended in squalane via complex formation involving a combination of sucrose erucate (i.e. lipophilic surfactant) and sucrose monolaureate (i.e. hydrophilic surfactant) to yield SONS with a very low moisture content (<500 ppm). To extract encapsulated AA, a lipase-based enzymatic degradation technique was used to degrade a formulation phase making it easier for AA to distribute into an extraction solution. Our results demonstrate that almost all the encapsulated AA (95.3%) was readily extracted from the SONS upon addition of medium-chain triglyceride, which offers the possibility of degrading the formulation phase using lipase. Finally, its storage stability study was investigated at 25 °C over 90 days under protection from light. An aqueous solution containing AA was used as a control. Compared with the control, the SONS markedly increased the stability of AA due to its low moisture content and, thus, the potential usefulness SONSs as a novel long-term stable topical formulation of AA has been proved.

1. Introduction

L-Ascorbic acid (AA, Vitamin C) is an important antioxidant that protects the skin by scavenging and destroying free radicals and reactive oxygen-derived species (Darr et al., 1992). As an UV photoprotection agent, it also has a synergistic effect when used in conjunction with vitamin E, a lipophilic vitamin (Bissert et al., 1990; Lin et al., 2003). AA is also used topically because of its skin-depigmenting activity (Zhai and Maibach, 2001) and its ability to reduce wrinkles by promoting collagen synthesis (Murad et al., 1981). Because of these favorable effects, AA has long been used in pharmaceutical and cosmetic preparations (Pinnell et al., 2001; Kameyama et al., 1996). However, its rapid degradation in aqueous solutions is still a major drawback to the design of a variety of topical formulations of AA and this has limited its use.

The most likely degradation pathway for AA in aqueous solutions (see Scheme 1) is as follows: the first degradation step of AA is oxidation to L-dehydroascorbic acid (DHA) followed by irreversible hydrolyzation to 2,3-L-diketogulonate (2,3-KDG), a primary inactive compound (Bode et al., 1990; Simpson and Ortwerth, 2000). According to this degradation pathway, many authors have reported that AA or ascorbyl palmitate (AP, one of its derivatives) could be protected against degradation by incorporation into formulations containing emulsified systems (Gallarate et al., 1999; Raschke et al., 2004; Farahmand et al., 2006), and colloidal carrier systems (Kristl et al., 2003; Stevanović et al., 2007) in which the interfaces act as a barrier for oxygen to prevent encapsulated AA (or AP) from undergoing oxidation-induced degradation, the first degradation step of AA. In addition, Kristl et al. have reported that the hydrophilic part (i.e. the ascorbyl residue) of AP is highly stable since this part is exposed to a less polar environment (Kristl et al., 2003), suggesting that the ascorbyl residue would be protected against moisture-induced degradation, the second degradation step of AA, in systems where hydrolysis of DHA (i.e. oxidized AA) is limited. In fact, since its water-soluble characteristics make it difficult for AA to exist in less polar environments (e.g. an oil-based formulation without an aqueous phase), little is known about the potential of oil-based formulations for use as a long-term stable topical formulation for AA.

Solid-in-oil nanosuspensions (SONSs) are unique formulations for improving the dispersibility (or solubilization) of hydrophilic target drugs in an oil phase. Due to its ability to enhance the penetration of target hydrophilic drugs (which have poor transdermal permeability) through the stratum corneum (i.e. the intrinsic

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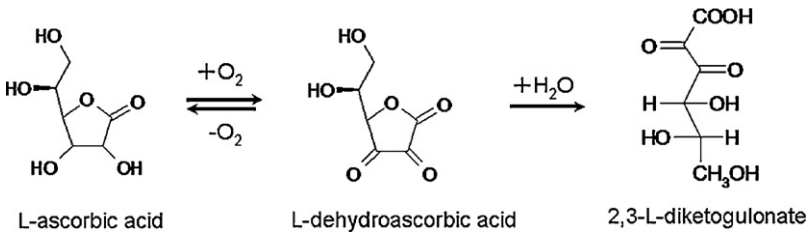

Scheme 1. The degradation pathway of AA in aqueous solutions.

barrier function of the skin), we successfully used potent candidate carriers to enhance the dermal/transdermal permeability of hydrophilic drugs [e.g. diclofenac sodium (Piao et al., 2008), and proteins (Tahara et al., 2008)]. Due to this unique formulation in which the nanosized solid phase of the target drug with nanocoated lipophilic surfactants is highly dispersed in the oil phase (Tahara et al., 2008), AA would be protected against oxidation resulting from nanocoated surfactants acting as a barrier to prevent oxygen (Gallarate et al., 1999), as well as limiting hydrolysis due to its lower moisture content (Shephard et al., 1999). Due to the synergistic effect on the protection of AA against degradation, the preparation of a long-term stable topical formulation of AA is an attractive prospect. In addition, the SONS containing AA has the following potential advantages: (a) avoidance of thermal degradation because the preparation does not involve heating, (b) enhancement of dermal/transdermal permeability, (c) easy mixing with vitamin E to prepare a homogeneous phase to obtain a synergistic effect for UV-protection (Lin et al., 2003).

In addition, target drugs could be encapsulated in stable condition in an oil phase (e.g. triglycerides) via complex formation but this would make it difficult to distribute in aqueous solutions (e.g. simulated gastrointestinal solutions) unless the oil phase is degraded completely by lipase, an enzyme which is widely present in the human gastrointestinal tract (Toorisaka et al., 2003; Yoshiura et al., 2008; Piao et al., 2006). The characteristics of S/O suspensions would limit the recovery of encapsulated target drugs from oil-based nanosuspensions for target drug quantification. In an approach to overcome this limitation, we devised a special strategy for recovering encapsulated target drugs (e.g. AA) via decomposition of the oil phase (formulation phase) by lipase-based enzymatic degradation. Because of the degradation of triglycerides under mild reaction conditions (e.g. lower temperature as well as weaker agitation), enzymatic degradation appears to be a promising method of extracting AA from SONSs.

In the present study, we propose a novel method for the preparation of a long-term stable topical formulation for AA using solid-in-oil nanosuspensions. Squalane, which is widely used in cosmetic formulations, was selected as the formulation phase. After complex formation with a combination of lipophilic surfactant and hydrophilic surfactant, AA undergoes ready stable nanosuspension in squalane to yield a solid-in-oil nanosuspension. Although squalane is not degraded by lipase, the results obtained indicate that almost all the encapsulated AA is readily extracted from the oil-based nanosuspension upon addition of a medium-chain triglyceride. Finally, the basic storage stability of AA was investigated.

2. Materials and methods

2.1. Materials

Sucrose erucate (commercial name, ER290; HLB = 2) and sucrose monolaureate (commercial name, L1695; HLB = 16) were kindly supplied by Mitsubishi-Kagaku Foods (Tokyo, Japan). L-Ascorbic acid (AA), squalane and sodium taurocholate from ox bile salt were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Medium-chain triglyceride (MCT) was purchased from NOF (Tokyo, Japan). Lipase from *Candida rugosa* (819 IU/mg at pH 7.2) was purchased from Sigma (St. Louis, USA).

2.2. Preparation of a SONS containing AA

Ten millilitres milli-Q water, obtained using a Millipore ultrapure water system (Millipore. Co., USA), containing 100 mg AA and/or appropriate amount of L1695, and 20 ml cyclohexane solution containing appropriate amount of ER290 were poured into a 50 ml round-bottom flask, and mixed in a homogenizer at 26,000 rpm for 2 min to form a W/O emulsion. The resulting emulsion was rapidly frozen in liquid nitrogen, and lyophilized using a freeze-drying machine (FD5N; Eyela, Toko, Japan) for 24 h to yield an AA-surfactants complex. And then squalane was added into the AA-surfactants complex and thoroughly dispersed with gentle stirring (50 rpm) for 12 h with protection from light at room temperature to yield an S/O nanosuspension containing AA. The concentration of AA in the SONSs formulation was set at 10 mg/g.

2.3. Determination of interfacial tension

A 10 mg/ml AA aqueous solution with/without 12.5 mg/ml L1695 was used as the aqueous phase. The oil phase (cyclohexane) contained 50 mg/ml ER290. An automated drop volume tensiometer (TVT1, Lauda, Königshofen, Germany) was used in dynamic mode for the measurement of the interfacial tension at the water-oil interface at 25 °C. The measurements were performed in triplicate for each drop formation time.

2.4. Measurement of particle size and moisture content of $SONS_{AA}$

The size distribution of SONSs was determined by dynamic light scattering (DLS) using a computerized inspection system (Nano-ZS, Malvern, UK). The viscosity and refractive index of each formulation was measured using an automatic micro-viscometer (AWMn, Anton Parr GmbH, Graz, Austria) and a refractive-index detector (RA-500, Kyoto electronics MFG. Co. Ltd., Japan). The moisture content of the SONSs containing AA was measured using a Karl Fischer moisturemeter (CA-200; Mitsubishi Chemical. Co. Ltd., Tokyo, Japan).

2.5. Extraction study

Extraction studies were carried out at 37 ± 0.5 °C with gentle stirring (1000 rpm). As an extraction solution, phosphate buffer (20 mM, pH 7.2) containing 1000 IU/ml lipase and 20 mM sodium taurocholate was prepared. Then, 100 mg of a SONS sample and 400 mg of MCT were poured into a 13 ml vial and mixed by vortexing. Then, 10 ml extraction solution was added for lipase-based enzymatic degradation and 1 ml of sample was passed through a 0.45 μm polyvinyl difluoride filter (Millipore Co. Bedford, USA) by

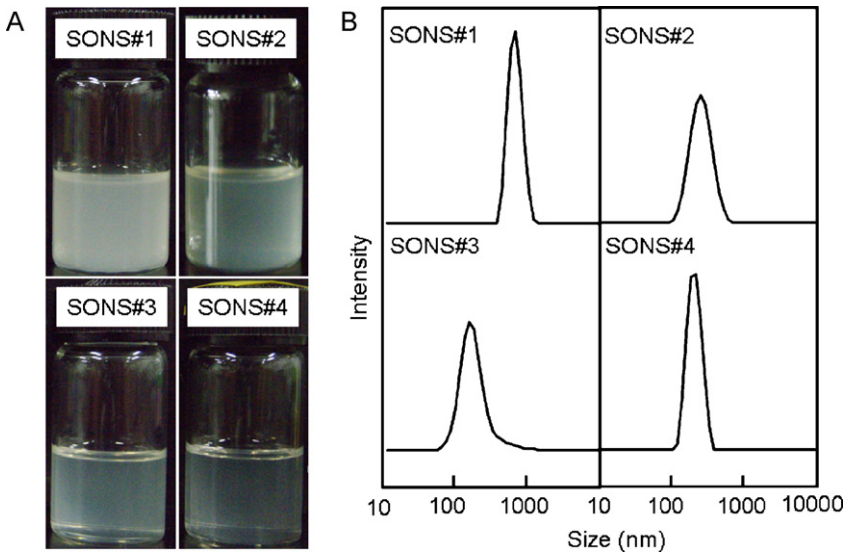

Fig. 1. Photograph of the solid-in-oil nanosuspensions containing L-ascorbic acid prepared in this study (A) and the size distribution of the solid-in-oil nanosuspension containing L-ascorbic acid determined by DLS analysis (B).

centrifuging for 5 min at 2900 rpm. AA was quantified by HPLC. The experimental conditions for the HPLC analysis were as follows: column, Inertsil NH_2 (250 mm × 4.6 mm I.D.); mobile phase, CH_3COOH:H_2O:CH_3CN = 2:87:11 (v/v/v); flow rate, 1.5 ml/ml; column temperature, 40 °C; Detection of AA was carried out at 243 nm; injection volume, 5 μl. The AA content of the formulations (%) was calculated as follows.

$$\text{AA in the formulations}\,(\%) = \frac{\text{Peak area}_{\text{Sample}}}{\text{Peak area}_{\text{Standrad solution}}} \times 100 \qquad (1)$$

where 20 mM phosphate buffer solution (pH 7.2) containing 100 μg/ml AA was used as a standard solution.

2.6. Storage stability of SONS containing AA

The SONS containing AA was stored in a well-sealed 13 ml vial. During storage, samples were kept at 25 °C in a thermostatic chamber (M.BR-2412FL; TAITEC, Tokyo, Japan) in the dark. The AA remaining in the formulations as well as the size distribution were measured over time. An aqueous solution containing 10 mg/ml AA was used as a control.

3. Results and discussion

3.1. Preparation and characteristics of SONS containing AA

To obtain a SONS containing AA, AA complexed with surfactants was first prepared by the formation of a water-in-oil (W/O) emulsion (Okazaki et al., 1997; Toorisaka et al., 2003). The viscous semi-solid obtained was readily and highly dispersed in nanosize squalane. It is well known that nanosized suspension with a uniform size distribution exhibit excellent physical stability. To obtain a SONS of a smaller size as well as a uniform size distribution, a mixture of surfactants systems was used which can provide a better performance (e.g. reduction in interfacial tension as well as an improvement in the stability of the water/oil interface) than single surfactant (Scamehorn, 1986). In view of the benefits of a low content of surfactant upon the transdermal delivery, the weight ratio of the surfactants to AA in this study was selected in the range of 10–14 (10–14 wt% in the final formulation, generally more surfactants are used for the preparation of microemulsions containing AA), and four types of AA-loaded SONS were prepared with ER290 and/or L1695. The composition and basic properties of the SONSs are summarized in Table 1. The appearance of the SONS is shown in Fig. 1(A), while the size distributions measured by DLS are shown in Fig. 1(B). For the formulations prepared without L1695, the mean size of AA in SONS#1 was about 587 nm, while that in SONS#2 was reduced to 220 nm, showing that nanosized AA in the oil-based formulation was obtained with the range of the weight ratio from 10 to 14. As opposed to these formulations without addition of L1695, the mean size of AA in both SONS#3 and SONS#4 (prepared with a combination of ER290 and L1695) was reduced to 148 nm and 178 nm, respectively. However, SONS#3 had the widest size distribution (PDI = 0.29) of all formulations, in particular, a larger size of AA (>500 nm) was found, implying that the surfactants added were not enough to encapsulate AA completely. On increasing the weight ratio to form SONS#4, the sharpest size distribution (PDI = 0.19) of all formulations and no increase in the size of AA was obtained, indicating that AA was encapsulated completely at that weight ratio. Also, we confirmed that the optimal weight ratio of ER290 to L1695 pointed at the presence of 10 due to an excess amount of added L1695 led to an increase in the size of AA in the SONSs (data not shown).

The results obtained raise the question as to why the SONSs with a smaller size of AA were prepared by combination of ER290 and L1695. We confirmed that the droplet size in the W/O emulsion with the mixture surfactants was smaller than that of ER290 alone (data not shown). To gain further insight into the change in size of AA caused by addition of L1695, the interfacial tension of the W/O emulsion (by combination of 5% ER290 in cyclohexane with 1% AA and/or L1695 in water) was investigated. The results showed that the interfacial tension upon combination of ER290 and L1695 was reduced from 18.9 mN/m to 3.56 mN/m. According to the above results, the reduction in the W/O droplet size might be associated with a reduced size of AA in the oil-based formulations after lyophilization. Another possibility is due to protection of AA against leakage, because L1695 has a high surface-activity, preferentially oriented around the W/O interface rather than AA (which has no surface-activity). Thus, the transfer of AA could be avoided because diffusion of the non-ionized and part of the ionized AA into the continuous phase was limited (Gallarate et al., 1999; Davis, 1981).

In addition, the residual amount of water in all SONS formulations was estimated to be lower than 500 ppm, suggesting that most

Table 1
The compositions and basic formulation properties of the SONSs containing AA. The data represent the mean ± SD from three experiments.

No.	AA (g)	L1695 (g)	ER290 (g)	Squalane (ad. g)	Size (nm)	PDI	Moisture content (ppm)
SONS#1	0.1	–	1	10	586 ± 56	0.21 ± 0.05	478 ± 40
SONS#2	0.1	–	1.4	10	220 ± 10	0.24 ± 0.07	456 ± 148
SONS#3	0.1	1	1	10	148 ± 4	0.29 ± 0.07	487 ± 89
SONS#4	0.1	0.125	1.25	10	178 ± 10	0.19 ± 0.08	461 ± 169

of the water phase (>99.5%) was removed during the lyophilization process.

3.2. Recovery of AA

Due to the instability of AA in terms of pH, ionic strength and temperature, it was necessary to investigate the effect of the stability of AA under the enzymatic conditions on the measurement of AA. The results obtained are shown in Fig. 2. According to these results, AA remaining in the standard solution (i.e. pH 7.2 aqueous PBS solution) was gradually reduced to 97.5% at 1 h and to 93.5% over 2 h compared with its initial concentration, implying a weaker effect on the measurement of AA within 1 h.

Next, using the enzymatic degradation for extracting encapsulated AA, MCT, which was easily degraded by lipase (Piao et al., 2007), was selected as the oil phase for addition to the SONS. Based on a previous *in vitro* release evaluation of the oral S/O suspensions (Piao et al., 2006), sodium taurocholate, a hydrophilic surfactant, was added to promote dispersibility of the SONS into the extraction solution to decompose the formulation phase by lipase effectively. From the results obtained, it was found that the maximum recovery of AA upon addition of MCT was as high as 95.3% at 1 h, while it was only 20% even over 2 h from the SONS if no MCT was added. These results demonstrate clearly that MCT-induced enzymatic degradation makes it easier for encapsulated AA to distribute into the extraction solution.

3.3. Stability studies

The storage-physical stability of the SONSs was evaluated via crystal precipitate observation, and size determination over 90 days at 25 °C in the dark. Crystal precipitates were observed in all formulations apart from SONS#4 over 7 days, while a small crystal precipitate but no significant discoloration was observed in SONS#4 over 90 days. The change in the size of AA in SONS#4 was from 178 nm to 122 nm over 28 days followed by no change in size from 28 days to 90 days, suggesting the change in size over time is the result of the crystal precipitates of AA which are not encapsulated in the complex.

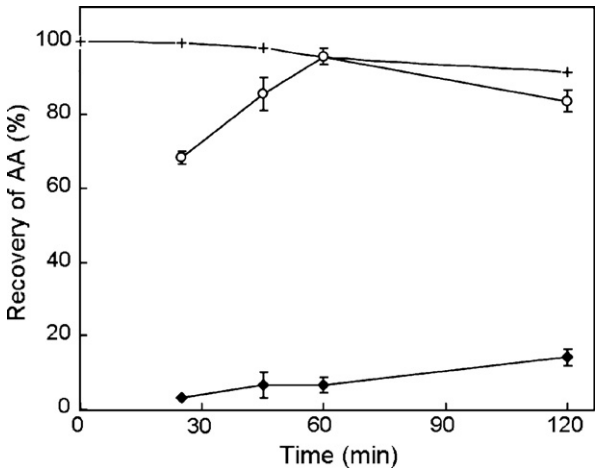

Fig. 2. The effect of the addition of MCT on the recovery of AA from the SONS#4 at 37 °C via lipase-based enzymatic degradation. (+), standard solution; (○), SONS#4: MCT = 1:4 (w/w); (♦), no addition of MCT.

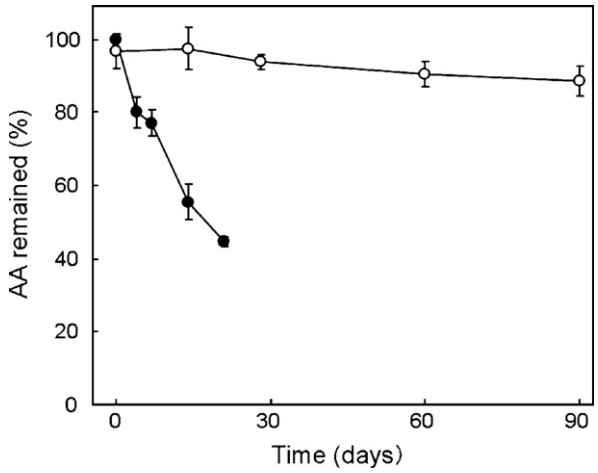

Fig. 3. The temporal percent content of AA remaining in SONS#4 (○) compared with the control, the aqueous solution containing 10 mg/ml of AA (●) at 25 °C over 90 days ($n = 3$). All the SONS samples were assayed after MCT-induced enzymatic degradation by lipase at 37 °C for 1 h.

For the storage-chemical stability study, SONS#4 was used due to its good storage stability and the aqueous solution containing 10 mg/ml of AA was used as the control. Encapsulated AA in SONS#4 was determined according to Eq. (1) after extraction of AA following MCT-induced enzymatic degradation for 1 h (see Section 3.2). Fig. 3 shows that temporal AA remained in SONS#4 compared with the control. The results showed that only 44.7% of AA remained in the control over 21 days, while 88.5% of AA remained in SONS#4 over 90 days. Together with the results of the moisture content in the SONSs shown in Table 1, these results indicated that the increased stability of AA in the solid-in-oil nanosuspensions is due to the very low moisture content. With respect to the mass loss of AA during the storage study, it can be concluded that one of reason involved the crystal precipitate of AA. Another reason was the moisture-induced chemical degradation. However, Shephard et al. studied the effect of the moisture content on the degradation of AA in the solid phase (Shephard et al., 1999). It was noted that less moisture-induced degradation (<2.5%) was found over 42 days at 50 °C when the moisture content in the air (where AA might be oxidized by oxygen to form DHA) was lower than 1%. Considering the above-mentioned study, the mass loss of AA by chemical degradation was low. As mentioned above, although very little crystal precipitate was observed, it was confirmed that most of the AA (88.5%) was encapsulated in stable condition in SONS#4.

4. Conclusion

As a long-term stable topical formulation for L-ascorbic acid, we successfully prepared a solid-in-oil nanosuspension containing L-ascorbic acid. Due to its unique formulation with a very low

moisture content, AA was readily stabilized in the SONS. Hopefully, the present SONS could be used to successfully formulate compounds that are not chemically stable in an aqueous environment.

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References

Bissert, D.L., Chatterjee, R., Hannon, D.P., 1990. Photo protective effect of superoxide-scavenging antioxidants against ultraviolet radiation-induced chronic skin damage in the hairless mouse. Photodermal. Photoimmunol. Photomed. 7, 56–62.

Bode, A.M., Cunningham, L., Rose, R.C., 1990. Spontaneous decay of oxidized ascorbic acid (dehydro-L-ascorbic acid) evaluated by high-pressure liquid chromatography. Clin. Chem. 36, 1807–1809.

Davis, S.S., 1981. Liquid membranes and multiple emulsions. Chem. Ind. 19, 670–683.

Darr, D., Combs, S., Manning, T., Pinnell, S., 1992. Topical vitamin C protects porcine skin from ultraviolet radiation-induced damage. Br. J. Dermatol. 127, 247–253.

Farahmand, S., Tajerzadeh, H., Farboud, E.S., 2006. Formulation and evaluation of a vitamin C multiple emulsion. Pharm. Dev. Technol. 11, 255–261.

Gallarate, M., Carlotti, M.E., Trotta, M., Bovo, S., 1999. On the stability of ascorbic acid in emulsified systems for and cosmetic use. Int. J. Pharm. 188, 233–241.

Kameyama, K., Sakai, C., Kondoh, S., Yonemoto, K., Nishiyama, S., Tagawa, M., Murata, T., Ohnum, T., Quigley, J., Dorsky, A., Bucks, D., Blanock, K., 1996. Inhibitory effect of magnesium L-ascorbyl-2-phosphate (VC-PMG) on melanogenesis in vitro and in vivo. J. Am. Acad. Dermatol. 34, 29–33.

Kristl, J., Volk, B., Gašperlin, M., Šentjurc, M., Jurkovič, P., 2003. Effect of collioidal carriers on ascorbyl palmitate stability. Eur. J. Pharm. Sci. 19, 181–189.

Lin, J.Y., Selim, M.A., Shea, C.R., Grichnik, J.M., Omar, M.M., Monteiro-Riviere, N.A., Pinnell, S.R., 2003. UV photoprotection by combination topical antioxidants vitamin C and vitamin E. J. Am. Acad. Dermatol. 48, 866–874.

Murad, S., Grove, D., Lindberg, K.A., Reynolds, G., Sivarajah, A., Pinnell, S.R., 1981. Regulation of collagen synthesis by ascorbic acid. Proc. Natl. Acad. Sci. U.S.A. 78, 2879–2882.

Okazaki, S.Y., Kamiya, N., Abe, K., Goto, M., Nakashio, F., 1997. Novel preparation method for surfactant-lipase complexes utilizing water in oil emulsions. Biotechnol. Bioeng. 55, 455–460.

Piao, H., Kamiya, N., Hirata, A., Yokoyama, H., Fujii, T., Shimizu, I., Ito, S., Goto, M., 2007. Reduction of gastric ulcerogenicity during multiple administration of diclofenac sodium by a novel solid-in-oil suspension. Pharm. Dev. Technol. 12, 1–5.

Piao, H., Kamiya, N., Watanabe, J., Yokoyama, H., Hirata, A., Fujii, T., Shimizu, I., Ito, S., Goto, M., 2006. Oral delivery of diclofenac sodium using a novel solid-in-oil suspension. Int. J. Pharm. 313, 159–162.

Piao, H., Kamiya, N., Hirata, A., Fujii, T., Goto, M., 2008. A novel solid-in-oil nanosuspension (SONS) on the transdermal delivery of diclofenac sodium. Pharm. Res. 25, 896–901.

Pinnell, S.D., Yang, H., Omar, M., Riviere, N.M., Debuys, H.V., Walker, L.C., Wang, Y., Levine, M., 2001. Topical L-ascorbic acid: percutaneous absorption studies. Dermatol. Surg. 27, 137–142.

Raschke, T., Koop, U., Dusing, H.J., Fibry, A., Sauermann, K., 2004. Topical activity of ascorbic acid: from in vitro optimization to in vivo efficacy. Skin Pharmacol. Physiol. 17, 200–206.

Scamehorn, J.F., 1986. Phenomena in Mixed Surfactant Systems. American Chemical Society, Washington, AD.

Shephard, A.B., Nichols, S.C., Braithwaite, A., 1999. Moisture induced solid phase degradation of L-ascorbic acid Part 1: a kinetic study using tristimulus colorimetry and a quantitative HPLC assay. Talanta 48, 585–593.

Simpson, G.L.W., Ortwerth, B.J., 2000. The non-oxidative degradation of ascorbic acid at physiological conditions. Biochim. Biophys. Acta 1501, 12–24.

Stevanović, M., Savić, J., Jordović, B., Uskoković, D., 2007. Fabrication, in vitro degradation and the release behaviors of poly (DL-lactide-co-glycolide) nanospheres containing ascorbic acid. Colloids Surf. B: Biointerfaces 59, 215–223.

Tahara, Y., Honda, S., Kamiya, N., Piao, H., Hirata, A., Hayakawa, E., Fujii, T., Goto, M., 2008. A solid-in-oil nanodispersion for transcutaneous protein delivery. J. Control. Release 131, 14–18.

Toorisaka, E., Ono, H., Arimori, K., Kamiya, N., Goto, M., 2003. Hypoglycemic effect of surfactant-coated insulin solubilized in a novel solid-in-oil-in-water (S/O/W) emulsion. Int. J. Pharm. 252, 271–274.

Yoshiura, H., Tahara, Y., Hashida, M., Kamiya, N., Hirata, A., Fujii, T., Goto, M., 2008. Design and in vivo evaluation of solid-in-oil suspension for oral delivery of human growth hormone. Biochem. Eng. J. 41, 106–110.

Zhai, H.Z., Maibach, H.L., 2001. Skin whitening agents. Cosmet. Toilet. 116, 20–25.