A Novel Nano-Capsule of α-Lipoic Acid as a Template of Core-Shell Structure Constructed by Self-Assembly

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Abstract

We developed a novel nano-capsule using the micellization ability of α-lipoic acid for the purpose of stabilizing α-lipoic acid. α-Lipoic acid is an optically active substance consisting of a carboxyl group and a five-membered ring. The disulfide bond in the five-membered ring is unstable and easily degraded by light or heat stress, resulting in the sulfurous smell that has often interfered with formulations, especially for cosmetics. It was found that the present encapsulation technology could drastically improve α-lipoic acid stability, that is, stabilize the unstable disulfide bond in α-lipoic acid by orienting the hydrophobic moiety of α-lipoic acid inside of the nano-capsule with an inorganic shell. The surface of the nano-capsule was covered by polyethylene chains and inorganic ions, forming a core-shell structure. Besides the nano-capsule showed mono-dispersity of the size distribution around 10 nm in diameter. This article reports on the formulation and the physicochemical properties of the nano-capsule of α-lipoic acid.

Keywords: A-Lipoic acid; Micellization; Nonionic surfactant; Nanocapsulation; Zeta potential; Drug delivery system

Introduction

α-Lipoic acid is present in all kinds of prokaryotic and eukaryotic cells and is well known to engage in energy metabolism as an essential compound. In living systems, α-lipoic acid binds to lysine groups of cells and is well known to engage in energy metabolism as an essential component of the skin.

However, there are problems with the use of α-lipoic acid, such as poor solubility in water, photo-instability, and thermal instability [9-11]. The decomposition of α-lipoic acid is undesirable not only because of the loss of content but also because of the unpleasant odor accompanying the release of sulfur compounds. In addition, α-lipoic acid may irritate skin or mucosa when administrated directly because of the loss of content but also because of the unpleasant odor [9-11]. The decomposition of α-lipoic acid is undesirable not only because of the loss of content but also because of the unpleasant odor [9-11]. The decomposition of α-lipoic acid is undesirable not only because of the loss of content but also because of the unpleasant odor [9-11]. The decomposition of α-lipoic acid is undesirable not only because of the loss of content but also because of the unpleasant odor [9-11]. The decomposition of α-lipoic acid is undesirable not only because of the loss of content but also because of the unpleasant odor [9-11].

In the pharmaceutical and cosmetic industries, nanotechnology is being aimed at facilitating the use of various water-insoluble or poorly water-soluble compounds, improving cutaneous permeability, extending produce life cycles in vivo, and enhancing stability. Many nano-medicines have been developed to provide more effective and convenient administration routes, to lower therapeutic toxicity, and to achieve the targeted delivery and controlled release of the drugs [14]. There has been a steady increase in the number of commercially available nanoparticle-based therapeutic products over the last few decades. Nanomaterials have unique physicochemical properties, such as ultra-small size, large surface area to mass ratio, and high reactivity, which makes them different from bulk materials even with the same composition. Thus, it is expected that a nano-scale composite containing α-lipoic acid can mitigate its disadvantageous properties, including solubility, stability and even bioavailability.

We developed a novel nanotechnology aimed at increasing α-lipoic acid’s potency and promoting its utilization for pharmaceuticals, supplements and cosmetics. In this study, we discovered the micelle formation of α-lipoic acid molecules, and we successfully constructed a novel nano-capsule containing α-lipoic acid via a self-assembly process. Furthermore, the physicochemical properties of the nano-capsule and the effects of nano-encapsulation on the stability of α-lipoic acid are also emphasized in this article.

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Experimental Section

Materials and reagents

α-Lipoic acid in the industrial grade was purchased from Tateyamakasei, Japan. Sucrose laurate (Ryoto Sugar Ester L-1695, Mitsubishi-Kagaku Foods, Japan), polyoxyethylene (20) stearyl ether (BS-20, Nikko Chemicals, Japan), polyoxyethylene (60) hydrogenated castor oil (HCO-60, Nikko Chemicals, Japan), polyglyceryl monolaurate (Decaglyn 1-L, Nikko Chemicals, Japan), polyglyceryl monomonyristate (Decaglyn 1-M, Nikko Chemicals, Japan), polyglyceryl monooleate (Decaglyn 1-OV, Nikko Chemicals, Japan) and polyglyceryl monostearyl (Decaglyn 1-508V, Nikko Chemicals, Japan) were used as nonionic surfactants. Calcium chloride, magnesium chloride, sodium carbonate, and disodium hydrogen phosphate were purchased from Wako Pure Chemical Industries, Japan. All chemicals were used without further purification.

Preparation of α-lipoic acid aqueous solution

Distilled water (13 mL) and 1 N NaOH (4.8 mL) were mixed, and 1.0 g of α-lipoic acid was added to the solution. After 10 minutes of stirring to completely dissolve the α-lipoic acid, the pH-value of the solution was adjusted to be in the range of 7–7.5 with 1 N NaOH. Then, the solution was heated at 70°C–80°C for 30 minutes with stirring. After cooling to room temperature, distilled water was added to obtain a predetermined concentration of α-lipoic acid (eg. 5% α-lipoic acid in the solution requires 20 mL of additional water). All the processes were carried out under shading.

Preparation of α-lipoic acid nano-capsule

Distilled water (13 mL) and 1 N NaOH (4.8 mL) were mixed, and 1.0 g of α-lipoic acid was added to the solution. After 10 minutes of stirring to completely dissolve α-lipoic acid, the pH-value of the solution was adjusted to be in the range of 7–7.5 with 1 N NaOH. A given amount of a nonionic surfactant and 20 mL of distilled water were further added to the solution, and the solution was stirred at 70°C–80°C for 30 minutes. After cooling to room temperature, distilled water was added to obtain a predetermined concentration of α-lipoic acid (eg. 5% α-lipoic acid in the solution requires 20 mL of additional water). All the processes were carried out under shading.

Surface tension measurements

Surface tensions of α-lipoic acid aqueous solutions were measured with a surface tensiometer (CBVP-A3, Kyowa Interface Science, Japan) using the Wilhelmy plate (platinum, ca. 5-cm perimeter). All solutions were prepared in previously deionized, distilled water. The solutions were filtered through 0.8 μm filter units (Sartorius, Minisart®) before measurements. The instruments were calibrated against distilled water

Zeta potential measurements

Zeta potential measurements were performed by ELSZ (Otsuka Electronics, Japan) with a semiconductor laser (λ=660 nm). Sample solutions were diluted 5 times with distilled water and filtered through 0.8 μm filter units (Sartorius, Minisart®). The measurements were run in a glass cell at 25°C. All processes were carried out under shading.

Dynamic Light Scattering measurements

The particle size of the α-lipoic acid nano-capsule was evaluated by the Dynamic Light Scattering method. The measurements were carried out by a fiber-optics particle analyzer FPAR1000 (Otsuka Electronics, Japan) with a semiconductor laser (λ=660 nm). The sample solution was filtered through a 0.8 μm filter unit (Sartorius, Minisart®) and measured at 25°C.

Transmission Electron Microscopy analysis

The form and size of the nano-capsule was evaluated using the negative staining method of Transmission Electron Microscopy (TEM) analysis. The analysis was performed using JEM-2000FX (JEOL, Japan) at accelerating voltage 200 kV, where a small drop of an aqueous solution of α-lipoic acid nano-capsule was deposited onto a carbon-coated grid. The sample solution was diluted 100 times with distilled water, and negative stain preparation was performed with uranyl acetate. Preparations and observations were carried out at room temperature.

Stability test of α-lipoic acid contained in nano-capsule

To evaluate the effect of the mixed micelle formation on the stability of α-lipoic acid, the aqueous solutions containing α-lipoic acid and different amounts of polyoxyethylene (20) stearyl ether (1, 2, 5 and 8%) were prepared by the same procedure as described above. To evaluate the effect of the nano-capsulation on the stability of α-lipoic acid in aqueous solution, the α-lipoic acid nano-capsules in the aqueous solution containing polyoxyethylene (20) stearyl ether (5 or 8%), MgCl₂ (X₂=0.5), and Na₂CO₃ (X₂=0.2) were prepared by the same procedure as described above. After the solutions were stored at 50°C for 1 month, the appearances of the samples were visually inspected and the concentrations of α-lipoic acid in the solutions were determined using High-Performance Liquid Chromatography (HPLC).

In order to assess the effects of the nano-capsulation on the stability of α-lipoic acid without solvent, powdery α-lipoic acid nano-capsules was prepared by freeze-drying the nano-capsule solution. The untreated α-lipoic acid powder was used as a control. After the samples were heated at 60°C for 3 hours, the changes in the sample appearances and the concentration of α-lipoic acid with time were evaluated by visual inspection and HPLC, respectively. The residual concentration of α-lipoic acid after storing was expressed as a relative percentage to the initial content in all the measurements.

The HPLC system consisted of LC-20AD pump, auto injector SIL-10AFA, column oven CTO-10AVF, and degasser DGU-20A3 (Shimadzu, Japan). Separation was carried out isocratically on a LiChrospher RP-18 column (4.0×250 mm; Merck, Germany) using the mobile phase of methanol-5 mM potassium dihydrogen phosphate buffer (pH 3.1) acetonitrile (1160:920:180, v/v/v) at a flow rate of 0.8 mL/min and temperature of 35°C with UV detection at 215 nm. For the solid samples, 100% or 50% methanol were used as a solvent.

Results and Discussion

Micellization of α-lipoic acid

No studies on micelle formation with α-lipoic acid have been reported. To confirm micelle formation of the α-lipoic acid molecules,
and to determine its critical micelle concentration (CMC), the surface tension measurements were carried out at various concentrations (4.9 mM–242 mM) of α-lipoic acid in the aqueous solution. The change in the surface tension is shown as a function of the α-lipoic acid concentration in figure 1. The surface tension decreases proportionally with increasing α-lipoic acid concentration and reaches a plateau region at a specific value, ca. 55 mN/m, at around 100 mM. The figure demonstrates that the α-lipoic acid molecules behave like a surface active agent, reducing the surface energy. The CMC of α-lipoic acid was given by the breakpoint of the surface tension curve, namely corresponding to 80 mM. Over this concentration, micellar colloids should be present in dynamic equilibrium with α-lipoic acid monomers in the solution. Therefore, it was revealed that the α-lipoic acid molecules spontaneously aggregated in water, regardless of the fact that it’s a small molecule with weak hydrophobic interactions.

The interface of the α-lipoic acid micelle should be negatively charged due to dissociation of the carboxyl group in water under neutral conditions and consequently oppositely charged divalent ions (such as Ca$^{2+}$ or Mg$^{2+}$) adsorb on the interface. Hence, the interfacial property of the α-lipoic acid micelle would be useful for the template of inorganic shell formation. Yamaguchi et al. [15] had investigated a mixed micelle system consisting of an amphiphilic carboxylic acid and a nonionic surfactant and demonstrated successful formation of a homogeneous mixed micelle in an aqueous medium [15]. Since α-lipoic acid as an amphiphilic molecule has the micellization ability, it was thought that α-lipoic acid could form a mixed micelle with a nonionic surfactant in water when the concentration of α-lipoic acid exceeds its CMC. For anionic amphiphiles, higher ionic environment suppresses dissociation of the surface charge of micelle, and finally it would result the loss of the hydrophilicity (water solubility). It is well known that precipitation or sedimentation of amphiphilic molecules occurs in this system. A dispersion force is necessary to prevent phase separation and sedimentation, and it was generated by hydrophilic coordination of the micelle surface using an additional substance such as nonionic surfactants with polyoxyethylene chains. The hydration function on

![Figure 1: Plot of the surface tension of the α-lipoic acid aqueous solutions at 25 °C as a function of the α-lipoic acid concentration.](image)

![Scheme 1: Schematic preparation process of the α-lipoic acid nano-capsule with core-shell (MgCO$_3$) structure.](image)

Table 1: The hydrophilic-lipophilic balance (HLB) values referred to the product catalogs.

| surfactant | surfactant (%) | α-Lipoic acid (%) | metal (M) | M$^\alpha$-Lipoic acid | CO$_3^{2-}$/M$^\alpha$ | appearance |
|------------|----------------|-------------------|-----------|------------------------|-----------------------|------------|
| blank      | 0              | 1                 | Mg        | 0.5                    | -                     | s          |
|            | 0              | 1                 | Ca        | 0.5                    | -                     | s          |
|            | 0              | 1                 | Ca        | 0.5                    | -                     | s          |
| Polyoxyethylene (20) stearyl ether | 1               | 1                 | Mg        | 0.5                    | 0.2                   | t          |
|            | 2               | 1                 | Mg        | 0.5                    | 0.2                   | t          |
|            | 5               | 1                 | Mg        | 0.5                    | 0.2                   | t          |
|            | 5               | 1                 | Ca        | 0.5                    | 0.2                   | s          |
|            | 8               | 1                 | Ca        | 0.5                    | 0.2                   | s          |
|            | 8               | 1                 | Mg        | 0.5                    | 0.2                   | t          |
|            | 10              | 1                 | Ca        | 0.5                    | 0.2                   | s          |
|            | 10              | 1                 | Ca        | 0.25                   | 0.2                   | t          |
|            | 10              | 0.5               | Ca        | 0.5                    | 0.2                   | t          |
|            | 10              | 0.5               | Ca        | 0.25                   | 0.2                   | t          |
| Polyoxyethylene (60) hydrogenated castor oil | 10              | 1                 | Ca        | 0.5                    | 0.2                   | s          |
|            | 10              | 1                 | Ca        | 0.25                   | 0.2                   | t          |
|            | 10              | 0.5               | Ca        | 0.5                    | 0.2                   | s          |
|            | 10              | 0.5               | Ca        | 0.25                   | 0.2                   | t          |
| sucrose laurate | 5               | 1                 | Mg        | 0.5                    | 0.2                   | t          |
|            | 10              | 0.5               | Ca        | 0.5                    | 0.2                   | s          |
|            | 10              | 0.5               | Ca        | 0.25                   | 0.2                   | t          |

\(t=transparent; \ s=separate\)

Table 2: Influence of nonionic surfactants, divalent cations (Ca$^{2+}$, Mg$^{2+}$) and a divalent anion (CO$_3^{2-}$) on appearance of the solution containing α-lipoic acid.

![Scheme 1: Schematic preparation process of the α-lipoic acid nano-capsule with core-shell (MgCO$_3$) structure.](image)

the micelle surface can prevent the sedimentation, and enables the micelles to finely disperse in the aqueous solution even if the divalent cations were bound on the surface of the α-lipoic acid micelle. In turn, together with nonionic surfactant and inorganic ions, α-lipoic acid is incorporated as core domain into inorganic shell layer in dynamic non-equilibrium in the solution. Thus, we aimed to encapsulate the mixed micelle containing α-lipoic acid and the nonionic surfactant with a coating of inorganic ions in order to improve the poor chemical stability of α-lipoic acid.

Construction of α-lipoic acid nano-capsule

Effect of nonionic surfactant on encapsulation: The process of constructing the α-lipoic acid nano-capsule is illustrated in scheme 1. Initially, various kinds of commercially available nonionic surfactants were considered. Table 1 shows the nonionic surfactants under consideration and their respective compatibilities. The solution, which was composed of 8% nonionic surfactant, 1% α-lipoic acid, and water, was assessed by visual inspection. Among the surfactants, the compatibilities with α-lipoic acid were rather different; the chemical structures of both the hydrophilic and hydrophobic groups seemed less
important than the HLB number of the surfactant when evaluating the potential to form mixed micelles. Specifically, it was determined that the surfactants with relatively higher HLB should preferably be utilized for encapsulation.

For the following step, we examined the influences of the added inorganic ions and their quantities on the mixed micelle formation. The surfactants used were sucrose laurate, polyoxyethylene (20) stearyl ether, and polyoxyethylene (60) hydrogenated castor oil, which were all compatible with α-lipoic acid. Table 2 shows the results of the evaluations of the mixed micellar solutions containing the inorganic ions. A water solution containing 1% α-lipoic acid and the inorganic ions was used as a control. Since this control solution resulted in precipitation when the inorganic ions were added to the 1% α-lipoic acid aqueous solution, it was evident that α-lipoic acid formed a water-insoluble complex with these inorganic ions. In the presence of a nonionic surfactant, phase separation and sedimentation were not observed even when inorganic ions were present in the same solution, which indicates that α-lipoic acid can form mixed micelles with nonionic surfactants. The inorganic ions depress the solubility of α-lipoic acid in water by forming electrical interactions with its carboxyl group; however, when the nonionic surfactant is mixed into the α-lipoic acid micelle, the nonionic hydrophilic group allows the micelle to maintain stable dispersion in the ionic solution. Regarding the amount of polyoxyethylene (20) stearyl ether, one of the surfactants utilized in this experiment, the phase state was independent of the surfactant concentration (from 1% to 8%) in the cases where Mg\(^{2+}\) was employed as the divalent cation. A simple way to take this non ideality into account is to employ the theory of regular two-component surfactant system [16]. If the CMC of a binary mixture (C) is determined against the net mole fraction a then the micellar composition α, and a may be obtained from the equation (1) by using the α value,

\[
\alpha = \ln \left( \frac{\alpha C}{\alpha C_i} \right) / (1 - \alpha)^2
\]

Where \(C_i\) is the value obtained from the CMC of the component 1 surfactant solution.

Generally, a single parameter value of α is determined by averaging the α values against each α value over the entire composition range. As is understood from equation (1) that change with composition of a binary surfactant solution the CMC value increases, and reaches plateau composition region at higher component 1 fraction, as far as equation (1) shows. Thus, this would be the reason why the increase concentration (1~8%) of nonionic surfactant is independent of the phase state. Also, it was realized at this point that the hydrophilic groups of the nonionic surfactants were remarkably effective in preventing phase separation with sedimentation and/or precipitation in the ionic aqueous system.

**Effect of divalent ions on surface potential of nano-capsule:** The adsorption of the divalent cation, Mg\(^{2+}\), onto the surface of the mixed micelle of α-lipoic acid and the nonionic surfactant was monitored by the zeta potential measurement. Figure 2 shows the plot of the zeta potential of the mixed micelle as a function of the molar ratio of Mg\(^{2+}\) to α-lipoic acid (X\(_{Mg}\)) at constant α-lipoic acid (1%) and polyoxyethylene (20) stearyl ether (2%) concentrations. The negative potential significantly decreased with X\(_{Mg}\) in the range of 0-0.5 and kept constant at approximately -5 mV over X\(_{Mg}\)=0.5. When Mg\(^{2+}\) ion localized in the vicinity of the carboxyl group of α-lipoic acid in the mixed micelle, the dissociated carboxyl group should be saturated stoichiometrically by just half-mole of Mg\(^{2+}\) ion to α-lipoic acid, theoretically to completely shield the negative charge on the surface. And the results shown in figure 2 were consistent with this theoretical speculation, supporting the idea that the Mg\(^{2+}\) ions localized near the carboxyl groups of α-lipoic acid and shielded the negative charge. However, the negative charge on the micelle surface did not reach to zero even in the presence of an equimolar amount of Mg\(^{2+}\). Essentially, it should reach zero at half molar Mg\(^{2+}\) ion because a large part of surface charge must be balanced by the adsorption of counter-ions into the region between the surface and the outer Helmholtz plane. The simple model that has been proposed initially by Stern [17] and refined by Grahame [18] cannot account for the possibility of negative charge maintained in the inner region. Therefore, the reason why the negative electric charge of the zeta potential was observed even at higher concentrations of Mg\(^{2+}\) ion might be because a PEG chain was hydrated by a water molecule. The water molecules possess electric dipole capable of hydrogen bonding with the oxygen atoms of the polyoxyethylene chains of the nonionic surfactant.

The influence of the CO\(_3^{2-}\) ion on the zeta potential was also examined by addition of Na\(_2\)CO\(_3\) to the Mg\(^{2+}\) coated micelle. The amount of Mg\(^{2+}\) was fixed at X\(_{Mg}\)=0.5. Figure 3 shows the plot of the zeta
potential of the mixed micelle as a function of the molar ratio of $\text{CO}_2^-$ to $\text{Mg}^{2+}$ ($X_{\text{CO}}$). The negative potential slightly increased with $X_{\text{Mg}}$, initially, and beyond $X_{\text{Mg}}=0.1$ the potential remained constant at approximately -8 mV. The increase in the negative potential suggested adsorption of $\text{CO}_2^-$ on $\text{Mg}^{2+}$, i.e., formation of a $\text{MgCO}_3$ shell surrounding the mixed micelle. Also, the remaining negative charge might be due to the hydration of polyethylene chains of the nonionic surfactant. The dipole of a water molecule normally interacts with an oxygen atom of a polyethylene chain in aqueous media. Thus it is expected that the nonionic surfactant system possession of polyethylene chains implies some negative zeta potential. The absolute value of the zeta potential would depend on the chain length of the polyethylene function and the measured temperature.

Morphological analysis of nano-capsule: The hydrodynamic diameter of the α-lipoic acid nano-capsule and its distribution in the aqueous solution were determined by DLS. The nano-capsule evaluated here consists of 1% α-lipoic acid, 5% polyoxyethylene (20) stearyl ether, $X_{\text{Mg}}=0.5$, and $X_{\text{CO}}=0.2$. As seen in figure 4, the mean particle size of the nano-capsule was estimated to be approximately 10 nm with a narrow distribution. The scattering data was also expressed by a correlation function with single retention time constant, which implies that the system contains a homogeneous particle-size with little polydispersity.

Figure 5 shows a TEM micrograph of the α-lipoic acid nano-capsule in an aqueous solution (the same composition of the nano-capsule as in the DLS sample). It was verified that the capsule has a spherical shape with a diameter of approximately 10 nm, which is consistent with the results obtained by the DLS measurement. Moreover, aggregations between the nano-capsules were not observed within the measured concentration range of capsule.

Both the DLS measurement and the TEM analysis demonstrated that the procedure we developed successfully produced finely-dispersed spherical particles approximately 10 nm in diameter. A complex of both α-lipoic acid and surfactants was previously reported by Souto et al. [19], however, the particle size of the obtained product was in the range of hundreds of nanometers and the solution was not transparent. Furthermore, it was necessary to use special equipment such as a high-pressure homogenizer, i.e., high mechanical energy was necessary to generate the system solubilizing α-lipoic acid. In addition, there were some reports of complex formation of α-lipoic acid with chitosan [20] and with vitamin A or vitamin E [21]. To our knowledge, however, it had not been established so far how to prepare a transparent solution containing α-lipoic acid. The novel nano-encapsulation developed in this study employed only sequential addition of the components and the common mixing process that requires no special equipment, and the transparent solution produced thereby consisted of the mono-dispersed nano-capsule with the well-controlled size in several nanometers.

Effect of nano-encapsulation on stability of α-lipoic acid in water media: Aqueous solutions of the α-lipoic acid nano-capsule with different concentrations of polyoxyethylene (20) stearyl ether (1%-8%) were stored at 50°C for one month to examine the effects of mixed-micelle formation and nano-encapsulation on the stability of α-lipoic acid. As a control, the aqueous solution of 1% α-lipoic acid was stored under the same conditions. The content of α-lipoic acid in the solution was determined by HPLC after one month of storage. Table 3 shows that the developed encapsulation improves the stability of α-lipoic acid in the aqueous solution in comparison with the naked α-lipoic acid solution, and the remaining amount of α-lipoic acid becomes higher in proportion to the concentration of polyoxyethylene (20) stearyl ether. At 1% α-lipoic acid, more than 5% polyoxyethylene (20) stearyl ether was required to obtain sufficient stability of α-lipoic acid, and, interestingly, the threshold weight ratio of α-lipoic acid and polyoxyethylene (20) stearyl ether was equal to 1:5, corresponding to their equimolar ratio. This would give us a quantitative understanding about the stability of α-lipoic acid, but we had not got any adequate analysis of these phenomena yet. It might be due to the balance of molecular occupation cross section of each α-lipoic acid and nonionic surfactant, in turn the interface energy of capsule may get limitlessly closer to zero at the equimolar ratio of α-lipoic acid and polyoxyethylene (20) stearyl ether.

| Sample | Residual ratio (%) |
|--------|-------------------|
| reference (surfactant free) | 92.6 |
| Polyoxyethylene (20) stearyl ether 1% | 91.2 |
| Polyoxyethylene (20) stearyl ether 2% | 95.3 |
| Polyoxyethylene (20) stearyl ether 5% | 94.3 |
| Polyoxyethylene (20) stearyl ether 8% | 96.4 |

Table 3: Effect of nano-encapsulation and the concentration of polyoxyethylene (20) stearyl ether on the stability of α-lipoic acid in the aqueous solution.
For the next step, the effect of the inorganic shell on the stability of α-lipoic acid was evaluated in the presence of a mixed micelle solution containing of 5% or 8% polyoxethylene (20) stearyl ether. Both the nano-capsule solutions of α-lipoic acid and the mixed micelle solutions were stored at 50°C for 1 month, and afterwards the remaining amount of α-lipoic acid was determined by HPLC measurement. As shown in table 4, the presence of the inorganic ion somewhat stabilized α-lipoic acid and it was concluded that the stability of α-lipoic acid is dependent not only on the nonionic surfactant but also on the inorganic coating.

Moreover, no appearance changes such as sedimentation or the solution becoming cloudy were observed in these nano-capsule solutions one month after storing at room temperature or even 50°C. It was also proven that the nano-capsule remained dispersed stably in water for the long-term.

**Stability of α-lipoic acid nano-capsule at solid state:** α-Lipoic acid is a yellowish crystal or powder at room temperature. It has been reported that the α-lipoic acid polymerizes by itself over-drying at above 40°C and an elastic compound is produced [22]. To evaluate the effects of nano-encapsulation on the stability of α-lipoic acid in a solid state, nano-capsules prepared by freeze-drying were analyzed under high temperature conditions at 60°C. Figure 6 shows the change in the remaining amount of α-lipoic acid after being stored at 60°C. It was observed that upon heating, the naked α-lipoic acid powder immediately turned into the elastic compound, and the content of α-lipoic acid significantly decreased; however, the active content of the α-lipoic acid nano-capsules after freeze-drying was nearly unchanged for at least two hours. It was thought that the encapsulation technology must be useful for stabilization of the disulfide bond in the molecule, and as a result it prevents self-polymerization between α-lipoic acid molecules. From these results, we expect that placing the disulfide bond in α-lipoic acid in the hydrophobic domain inside of the micelle could effectively prevent oxidation or cleavage of the disulfide moiety.

**Conclusion**

In this study, we discovered the micelle formation of α-lipoic acid, and we successfully constructed a novel nano-capsule using α-lipoic acid as a template. After forming mixed micelles with α-lipoic acid and a non-ionic surfactant, we confirmed localization of the divalent inorganic ions on the mixed micelle surface by zeta potential measurements. The Dynamic Light Scattering measurements and transmission electron microscopic observations verified that the nano-capsule had a spherical form and its average diameter was approximately 10 nm with a relatively small size distribution; furthermore, satisfactory dispersion of the nano-capsule in water was sustained long term without aggregation. We also revealed that the problematic instability of α-lipoic acid was significantly improved by the developed nano-encapsulation, not only in the liquid state but also in the solid state.

Furthermore, the novel nano-encapsulation involved only common mixing processes and sequential addition of the components. The procedure is very convenient, and it is not necessary to use any special equipment such as a high-pressure homogenizers.

We also emphasize that the improvement in the stability of α-lipoic acid by our encapsulation technology can also improve its absorptive efficiency and bioavailability. Hereafter, our research will provide perspective on applications of α-lipoic acid to several fields such as supplements, cosmetics, and medicines.

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