Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 109 (2008) 167–171
Food Chemistry
www.elsevier.com/locate/foodchem

Stabilization of α-lipoic acid by complex formation with chitosan

Kyoko Kofuji *, Michiko Nakamura, Takashi Isobe, Yoshifumi Murata, Susumu Kawashima

Faculty of Pharmaceutical Sciences, Hokuriku University, Ho-3, Kanagawa-machi, Kanazawa 920-1181, Japan

Received 20 August 2007; received in revised form 16 October 2007; accepted 5 November 2007

Abstract

α-Lipoic acid (ALA) is an essential cofactor in mitochondrial multi-enzyme complexes related to energy production. However, it is unstable under light or heat, and its decomposition is accompanied by an unpleasant odor. Therefore, its stabilization by complex formation with the cationic polymer chitosan (CS) was investigated. The ALA dissolved in demineralized water was efficiently adsorbed on the precipitated insoluble CS particles, and an ALA–CS complex was obtained. The amount of ALA adsorbed on CS was affected by the CS species and the quantity ratio of ALA to CS. The ALA from the ALA–CS complex was released immediately by changing the pH. When ALA was incubated at 65 °C, it melted and polymerized. In addition, some decomposition of ALA was also observed in the physical mixture of ALA with CS. However, the ALA–CS complex did not decompose at all under the same conditions. Thus, the stabilization of ALA was achieved by complex formation with CS. CS is useful as a material for the stabilization of ALA, leading to its clinical use.

Keywords: α-Lipoic acid; Chitosan; Stability; Complex

1. Introduction

α-Lipoic acid (ALA), that is, 1,2-dithiolane-3-pentanoic acid, which is also referred to as 6,8-thioctic acid, is an essential cofactor in mitochondrial multi-enzyme complexes related to energy production. ALA is also known to be a powerful antioxidant (Biewenga, Haenen, & Bast, 1997; Packer, Witt, & Tritschler, 1995). Unlike other antioxidants, ALA is both fat- and water-soluble and is easily absorbed and transported across cell membranes. Therefore, it is widely used as a drug in the prevention of various chronic diseases associated with oxidative stress, and is administered daily as supplements for diet, anti-aging, diabetes, cardiovascular disease (Hagen et al., 1999; Jacob et al., 1999; Maritim, Sanders, & Watkins, 2003; Ruhe & McDonald, 2001; Smith, Shenvi, Widlansky, Suh, & Hagen, 2004; Wollin & Jones, 2003).

However, ALA is unstable under light or heat, and gradually decomposes at room temperature. Temperature more than the melting point (59–62 °C) of ALA causes its immediate polymerization, and renders it unusable because polymerized ALA is insoluble in almost all solvents (Wagner et al., 1956). Furthermore, the decomposition of ALA is accompanied by an unpleasant odor due the sulfur contained in it. Therefore, the stabilization of ALA is necessary in the product of various fields such as supplements and cosmetics (Segall et al., 2004; Souto, Müller, & Gohla, 2005).

Chitosan (CS) is an abundant natural polysaccharide that is nontoxic, biocompatible, and biodegradable (Mi, Tan, Liang, & Sung, 2002; Paul & Sharma, 2000). In addition, it is a cationic polymer; it interacts with an anionic material or metal ion. ALA may be expected to form an electrostatic complex with CS due to the carboxyl group in the ALA and the amino group in the CS.

In the present study, the preparation of an ALA–CS complex and its stabilization were investigated.

* Corresponding author. Tel.: +81 76 229 1165; fax: +81 76 229 2781.
E-mail address: k-kofuji@hokuriku-u.ac.jp (K. Kofuji).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.11.078

2. Experimental

2.1.1. Materials

(±)-α-Lipoic acid (ALA), alginic acid, (Alg-H) and poly-L-lysine hydrobromide were purchased from Sigma–Aldrich Inc. (USA). Various chitosans were received as generous gifts from Koyo Chemical Co., Ltd. (Japan), Kyowa Tecnos Co., Ltd. (Japan), Nippon Kayaku Food Techno Co., Ltd. (Japan), Yaegaki Bio-industry, Inc. (Japan), and Yaizu Suisankagaku Industry Co., Ltd. (Japan). Chitin (Chitin 1000) was purchased from Seikagaku Corporation (Japan). D(+)-Glucosamine and *N*-acetyl-D(+)-glucosamine were purchased from Wako Pure Chemical Ind., Ltd. (Japan). All other reagents were of the reagent grade.

2.2.2. Preparation of ALA–CS complex

ALA powder (20 mg) was added in a measuring flask and made up to 100 ml with demineralized water. The insoluble residue of ALA was removed using a filter (Advantec DISMIC-25, 0.2 μm). The filtered ALA solution (5–15 ml) and CS powder (10–100 mg) were added into a 15-ml sample tube. The suspension was shaken for 24 h at 25 °C and it was then centrifuged at 3500 rpm for 10 min. The precipitate (i.e., CS which adsorbed ALA) was washed twice with water; it was then considered as an ALA–CS complex. It was dried at room temperature for 24 h in a dish, before holding it under vacuum in a desiccator in the presence of P_2O_5. The amount of ALA in the supernatant was determined by high-performance liquid chromatography (HPLC). Simultaneously, the HPLC of the filtered ALA solution that did not contain the CS powder was also identically performed; this solution served as a blank. The amount of ALA adsorbed on the CS was calculated as the difference between the amount of ALA in the blank (A_1) and the amount of ALA that remained in the supernatant (A_2). The percentage of ALA adsorbed on CS was calculated using the following equation:

$$\% \text{ of ALA adsorbed on CS} = \{(A_1 - A_2)/A_1\} \times 100$$

Similarly, the adsorptive properties of ALA to other polymers were examined.

2.3.3. Determination of ALA

Hundred microliter of *p*-nitrophenol (6.0 μg/ml) dissolved in demineralized water as an internal standard was added to 500 μl of the sample solution containing ALA. The mixture was filtered (Millipore Cosmonice Filter W, 0.45 μm) and subjected to HPLC. A 20-μl aliquot of the sample was loaded onto a column (Nacalai tesque COSMOSIL $5C_{18}$-MS-II Waters; 150 mm × 4.6 mm) with a precolumn (Shiseido CAPCELL PAK NH_2 UG80) and eluted with 40% acetonitrile/demineralized water containing 0.1% phosphate as the mobile phase at a flow rate of 0.3 ml/min (Shimadzu LC-10AS). The ALA in the effluent from the column was detected at 333 nm using a UV spectrophotometer (Shimadzu SPD-10AVVP).

2.4.4. Release of ALA from ALA–CS complex

ALA–CS complex powder (20 mg) was added into a 1.5-ml sample tube. Various aqueous solutions (1 ml) were added into the tube. Then, the tube was centrifuged at 3500 rpm for 10 min. The ALA that was released from the ALA–CS complex existed in the supernatant. The supernatant was subjected to the HPLC described above. When acidic solutions were added to the ALA–CS complex, CS was dissolved; subsequently, a viscous solution of CS resulted. Then, the ALA released from the ALA–CS complex in an acidic solution with *p*-nitrophenol, which was the internal standard, was separated by a centrifugal filter device (Millipore Microcon YM-3) and subjected to the HPLC described above.

2.5.5. Stability study of ALA

ALA or ALA–CS complex powder (20 mg) was added into a 1.5-ml sample tube. The tube was floated in a water bath at 65 °C using a floating tube rack. The tube was periodically taken up and 0.1 N NaOH was added in the tube to release the ALA from the complex. Then, the tube was centrifuged at 3500 rpm for 10 min, and the supernatant was filtered (Millipore Cosmonice Filter W, 0.45 μm) and subjected to the HPLC described above. The stability of the ALA in a physical mixture with CS and other polymers or monosaccharides is also examined by using a similar procedure.

3. Results and discussion

3.1.1. Preparation of ALA–CS complex

CS barely dissolved in a natural pH solution. ALA, which dissolved in the demineralized water, was adsorbed on the precipitated insoluble CS particles. Consequently, a complex of ALA and CS was obtained. As shown in Fig. 1, by altering the properties of CS, its ALA adsorptive ability was manipulated. The adsorption of ALA on CS tended to increase with an increase in the molecular weight

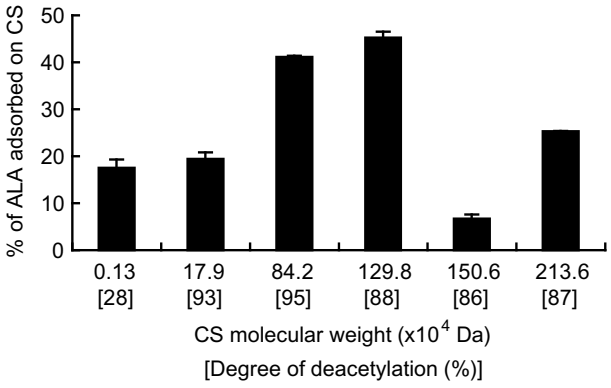

Fig. 1. Effect of CS species on the percentage of ALA adsorbed on CS. CS powder (10 mg) was added into 5 ml of ALA solution and incubated for 24 h at 25 °C. The values of the molecular weight and degree of deacetylation of CS were referred (Kofuji et al., 2005). Data represent the mean ± S.D. ($n = 5$).

of CS. However, at molecular weights >150 × 10^4 Da, CS exhibited irregular behaviors and instead tended to lower ALA adsorptive ability. Bernkop-Schnürch, Schuhbauer, Clausen, and Hanel (2004) have prepared a tablet containing ALA and CS; they have achieved sustained release of ALA from the tablet based on the ionic interactions between the cationic polymer CS and the anionic drug ALA. Thus, the adsorption of ALA on CS would be caused by an electrostatic interaction between the carboxyl group of the ALA and the amino group of the CS. However, the CS molecular weight, degree of deacetylation, and distribution of acetamide groups in a CS molecule differ for each CS polymer (Sashiwa, Saimoto, Shigemasa, Ogawa, & Tokura, 1991). Therefore, the ALA adsorptive ability of CS may be influenced by a change in the interaction between ALA and CS, which results from a change in the inter- or intra-molecular attractive and repulsive forces in CS polymers. Consequently, the selection of CS species to be used as a material for the adsorption of ALA is important; the CS with molecular weight = 129.8 × 10^4 Da and degree of deacetylation = 88% was used in the subsequent experiments. On the other hand, the adsorption of ALA was also observed on chitin, although the degree of deacetylation of chitin is low (Fig. 2). However, its ALA adsorptive ability is lower than that of CS. In contrast, Alg-H, which is an anionic polymer and barely dissolved in demineralized water, did not adsorb ALA at all. This indicates that for the adsorption of ALA on a polymer, the polymer is required to contain not acidic saccharide such as uronic acid but amino saccharide such as glucosamine and *N*-acetyl-glucosamine. In addition, about 50% of the initial amount of ALA in the preparative medium was adsorbed on CS despite changing the amount of CS (10–100 mg) addition into the preparative medium and the volume (5–15 ml) of the ALA solution. However, a greater amount of ALA was adsorbed on 1 g of ALA–CS complex with a decrease in the amount of CS added into the preparative medium. For example, when 10 mg and 100 mg of CS were added into the ALA solution (5 ml), the amounts of ALA adsorbed on 1 g of ALA–CS complex were about 40 mg and 4 mg, respectively. A greater volume of the ALA solution also increased the amount of ALA adsorbed on 1 g of ALA–CS complex. However, the residual ALA in the preparative medium, which is not adsorbed on CS, increased. For example, the amount of residual ALA doubled when the volume of the ALA solution was doubled.

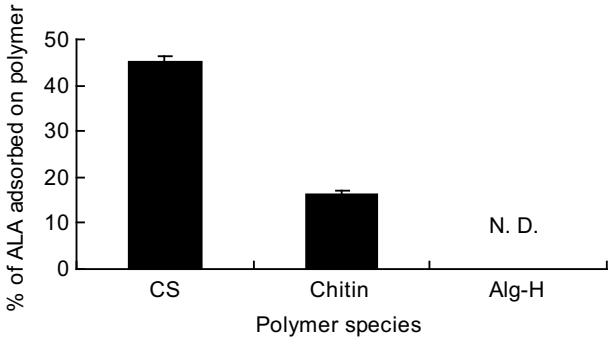

Fig. 2. Effect of polymer species on the percentage of ALA adsorbed on CS. CS (molecular weight: 129.8 × 10^4 Da, degree of deacetylation: 88%), Chitin, or Alg-H powder (10 mg) was added into 5 ml of ALA solution and incubated for 24 h at 25 °C. Data represent the mean ± S.D. ($n = 5$).

3.2.2. Release of ALA from ALA–CS complex

ALA was barely released from the ALA–CS complex in demineralized water. On the other hand, it was immediately released from the ALA–CS complex in solutions (i.e., pH 6.0 0.1 M phosphate buffer, physiological saline, or 0.1 N NaOH solution) whose pH was higher than that of the demineralized water (Fig. 3). Similarly, ALA was immediately released from the ALA–CS complex in acidic solutions also (i.e., pH 4.5 0.1 M acetate buffer or 0.1 N HCl solution), in which the amino groups of CS were protonated and CS was dissolved. CS is a positively charged polymer with a pK_a of approximately 6.2–6.5 (Domard, 1987; Park, Choi, & Park, 1983). ALA is a negatively charged drug with a pK_a of approximately 5.2–5.4 (Ruixia et al., 2004; Walton et al., 1955). Therefore, the ALA–CS complex was maintained at a pH of approximately 5.9 because the degrees of ionization of both ALA and CS may be higher at this pH value. Moreover, the degree of ionization of either CS or ALA may decrease due to alterations in the pH, and this decrease may have caused the release of ALA from the ALA–CS complex. On the other hand, it was not completely released from the ALA–CS complex in an acidic solution. This may be due to the low solubility of ALA in the acidic solution.

3.3.3. Stability of ALA

It has been known that ALA is decomposed by light or heat. The rupture of the S–S bond of the 1,2-dithiolane ring in an ALA molecule results in the disappearance of the 333-nm absorption band, which corresponds to ALA, and the formation of dihydrolipoic acid (DHLA). DHLA

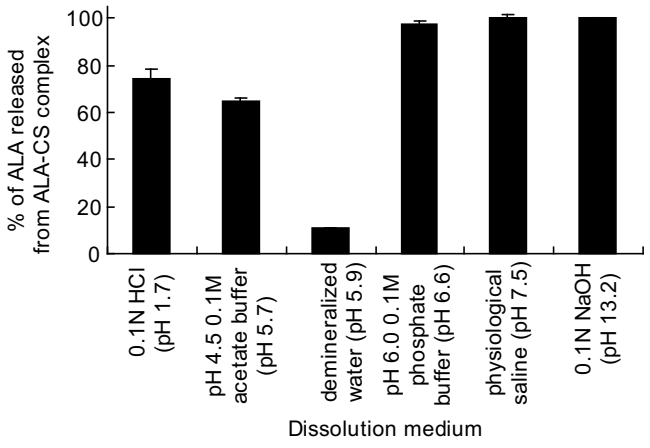

Fig. 3. Percentage of ALA released from the ALA–CS complex (4 mg ALA/g complex). Parenthesis: pH after the addition of dissolution medium to the ALA–CS complex. Data represent the mean ± S.D. ($n = 5$).

has almost the same antioxidant activity as ALA itself; however, polymeric products are also formed (Matsugo, Han, Tritschler, & Packer, 1996). In fact, the polymerization of ALA was immediately observed for incubation at 65 °C: only about 30% of the initial ALA amount remained after the incubation for 30 min, and the value of the residual ALA was almost constant although the incubation time was prolonged (Fig. 4). On the other hand, as shown in Fig. 5, the ALA–CS complex did not decompose. Complex formation with CS might increase the melting point of ALA, which may be one of the possible causes of the stabilization of ALA on heating. The effect of stabilization of ALA by the ALA–CS complex formation was not affected by the quantity ratio of ALA to CS. In addition, the stability of ALA was observed in the physical mixture of ALA with CS, although the effect of stabilization of ALA on the physical mixture with CS was less than that in the case of complex formation with CS. In the physical mixture of ALA with CS, ALA is surrounded by CS. The heat of incubation may be intercepted physically by CS. However, the effect of stabilization of ALA on the physical mixture with Alg, which is an anionic polysaccharide, was not observed. Furthermore, the effect of stabilization of ALA on the physical mixture with poly-L-lysine, which is a cationic polymer containing amino groups, was also not recognized (Fig. 6). This suggested that the effect of stabilization of ALA by the ALA–CS complex is considered to result from an interaction between ALA and CS. While the effect of stabilization of ALA was also observed in the case of its physical mixture with a monosaccharide such as glucosamine and acetyl glucosamine, which are compositions of CS. Further, the stability of ALA in its physical mixture with a monosaccharide is comparable to that in the case of its physical mixture with CS. Ruixia et al. (2004) reported that the adsorption of ALA on polystyrene-based resins containing several different polar groups (e.g., amino groups, hydroxyl groups) was caused by an electrostatic interaction and a hydrogen-bonding interaction. These indicate that the complex formation between ALA and CS might be the result of not only an electrostatic interaction but also a hydrogen-bonding interaction between ALA and CS.

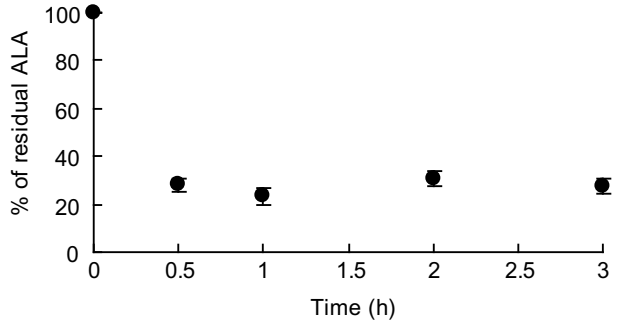

Fig. 4. Percentage of residual ALA after incubation at 65 °C. Data represent the mean ± S.D. ($n = 5$).

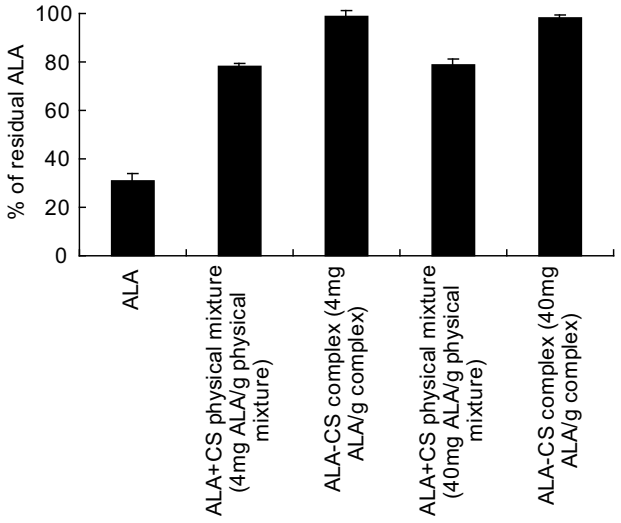

Fig. 5. Percentage of residual ALA after incubating the ALA–CS complex or the physical mixture of ALA with CS for 2 h at 65 °C. Data represent the mean ± S.D. ($n = 5$).

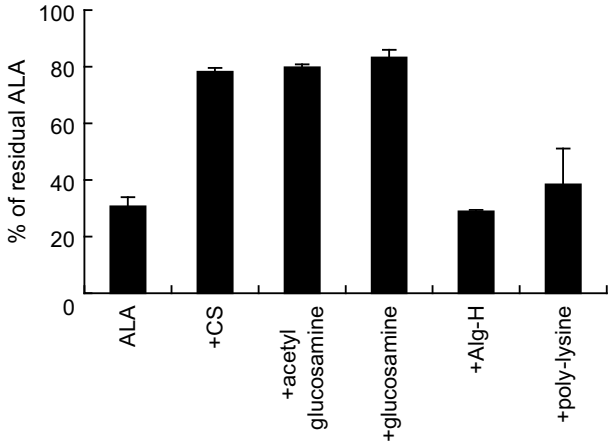

Fig. 6. Percentage of residual ALA after incubating the physical mixture of ALA with a polymer or a monosaccharide (4 mg ALA/g physical mixture) for 2 h at 65 °C. Data represent the mean ± S.D. ($n = 3$–5).

4. Conclusion

CS was able to adsorb ALA dissolved in demineralized water. ALA adsorbed on CS was immediately released by changing the pH. This implies that ALA is promptly released from the ALA–CS complex within a gastrointestinal tract after oral administration. The ALA–CS complex inhibited the decomposition of ALA under heating, namely, the stability of ALA in a product improved. Therefore, CS is a promising biocompatible and biodegradable material for the stabilization of ALA.

Acknowledgment

This work was supported by the specific research fund of Hokuriku University.

References

Bernkop-Schnürch, A., Schuhbauer, H., Clausen, A. E., & Hanel, R. (2004). Development of a sustained release dosage form for α-lipoic acid. I. Design and in vitro evaluation. *Drug Development and Industrial Pharmacy, 30*(1), 27–34.

Biewenga, G. P., Haenen, G. R. M. M., & Bast, A. (1997). The pharmacology of the antioxidant lipoic acid. *General Pharmacology, 29*(3), 315–331.

Domard, A. (1987). pH and c.d. measurements on a fully deacetylated chitosan: Application to Cu(II)–polymer interactions. *International Journal of Biological Macromolecules, 9*(2), 98–104.

Hagen, T. M., Ingersoll, R. T., Lykkesfeldt, J., Liu, J., Wehr, C. M., Vinarsky, V., et al. (1999). (R)-α-Lipoic acid-supplemented old rats have improved mitochondrial function, decreased oxidative damage, and increased metabolic rate. *The FASEB Journal, 13*(2), 411–418.

Jacob, S., Puus, P., Hermann, R., Tritschler, H. J., Maerker, E., Renn, W., et al. (1999). Oral administration of rac-α-lipoic acid modulates insulin sensitivity in patients with type-2 diabetes mellitus: A placebo-controlled pilot trial. *Free Radical Biology & Medicine, 27*(3–4), 309–314.

Kofuji, K., Qian, C., Nishimura, M., Sugiyama, I., Murata, Y., & Kawashima, S. (2005). Relationship between physicochemical characteristics and functional properties of chitosan. *European Polymer Journal, 41*(11), 2784–2791.

Maritim, A. C., Sanders, R. A., & Watkins, J. B. III. (2003). Effects of α-lipoic acid on biomarkers of oxidative stress in streptozotocin-induced diabetic rats. *Journal of Nutritional Biochemistry, 14*(5), 288–294.

Matsugo, S., Han, D., Tritschler, H. J., & Packer, L. (1996). Decomposition of α-lipoic acid derivatives by photoirradiation. Formation of dihydrolipoic acid from α-lipoic acid. *Biochemistry and Molecular Biology International, 38*(1), 51–59.

Mi, F., Tan, Y., Liang, H., & Sung, H. (2002). In vivo biocompatibility and degradability of a novel injectable-chitosan-based implant. *Biomaterials, 23*(1), 181–191.

Packer, L., Witt, E. H., & Tritschler, H. J. (1995). α-Lipoic acid as a biological antioxidant. *Free Radical Biology & Medicine, 19*(2), 227–250.

Park, J. W., Choi, K. H., & Park, K. K. (1983). Acid–base equilibria and related properties of chitosan. *Bulletin of Korean Chemical Society, 4*(2), 68–72.

Paul, W., & Sharma, C. P. (2000). Chitosan, a drug carrier for the 21st century: A review. *STP Pharma Sciences, 10*(1), 5–22.

Ruhe, R. C., & McDonald, R. B. (2001). Use of antioxidant nutrients in the prevention and treatment of type 2 diabetes. *Journal of the American College of Nutrition, 20*(5), 363S–369S.

Ruixia, W., Jinlong, C., Lianlong, C., Zheng-hao, F., Ai-min, L., & Quanxing, Z. (2004). Study of adsorption of lipoic acid on three types of resin. *Reactive & Functional Polymers, 59*(3), 243–252.

Sashiwa, H., Saimoto, H., Shigemasa, Y., Ogawa, R., & Tokura, S. (1991). Distribution of the acetamide group in partially deacetylated chitins. *Carbohydrate Polymers, 16*(3), 291–296.

Segall, A., Sosa, M., Alami, A., Enero, C., Hormaechea, F., Pizzorno, M. T., et al. (2004). Stability study of lipoic acid in the presence of vitamins A and E in o/w emulsions for cosmetic application. *Journal of Cosmetic Science, 55*(4), 449–461.

Smith, A. R., Shenvi, S. V., Widlansky, M., Suh, J. H., & Hagen, T. M. (2004). Lipoic acid as a potential therapy for chronic diseases associated with oxidative stress. *Current Medicinal Chemistry, 11*(9), 1135–1146.

Souto, E. B., Müller, R. H., & Gohla, S. (2005). A novel approach based on lipid nanoparticles (SLN®) for topical delivery of α-lipoic acid. *Journal of Microencapsulation, 22*(6), 581–592.

Wagner, A. F., Walton, E., Boxer, G. E., Pruss, M. P., Holly, F. W., & Folkers, K. (1956). Properties and derivatives of α-lipoic acid. *Journal of the American Chemical Society, 78*(19), 5079–5081.

Walton, E., Wagner, A. F., Bachelor, F. W., Peterson, L. H., Holly, F. W., & Folkers, K. (1955). Synthesis of (+)-α-lipoic acid and its optical antipode. *Journal of the American Chemical Society, 77*(19), 5144–5149.

Wollin, S. D., & Jones, P. J. H. (2003). α-Lipoic acid and cardiovascular disease. *The Journal of Nutrition, 133*(11), 3327–3330.