

Department of Chemical Engineering and Materials Science¹, Chung-Ang University; Department of Internal Medicine and Asan Institute for Life Science², University of Ulsan College of Medicine, Seoul, South Korea

Lipoic acid nanoparticles: effect of polymeric stabilizer on appetite suppression

CHUL HO PARK¹, KI-UP LEE², JOONG-YEOL PARK², EUN-HEE KOH², HYOUN-SIK KIM², JONGHWI LEE¹

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Jonghwi Lee, Department of Chemical Engineering and Materials Science, Chung-Ang University, 221, Heukseok-dong, Dongjak-gu, Seoul 156 756, South Korea
jong@cau.ac.kr

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Alpha-lipoic acid (ALA), which is common in the human body, is efficacious in appetite suppression. However, its typical formulations of salt or micronized crystals cannot satisfy the desired bioavailability requirements for appetite suppression due to low absorption and a short plasma half-life. Herein, we describe a new ALA nanoparticulate formulation produced by nano-comminution using polymeric stabilizers, such as hydroxypropyl cellulose, Pluronic® F127, and polyvinylpyrrolidone. Nanoparticles of similar sizes did not show any remarkable differences in the *in vitro* release profiles. However, the *in vivo* results from food intake studies in mice demonstrated that the hydroxypropyl cellulose case had the largest improved efficacy among the three polymeric stabilizer cases. Compared to the nanosuspension formulations, the powder formulations of nanoparticles had improved efficacy in reducing food intake for six hours, possibly because of the delayed release kinetics. Therefore, the ALA powder formulation of nanoparticles is a candidate to replace the current formulations to achieve proper appetite suppression.

1. Introduction

Alpha-lipoic acid (ALA) is a naturally synthesized substance in most prokaryotic and eukaryotic cells. In human beings, it is present in most cells in the body as the part of several multi-enzyme complexes (Fujiwara et al. 1995). ALA contains a cyclic 5-membered disulfide (S-S), which can act as an oxidant when converted into two thiol functional groups (SH) and as an antioxidant in the reverse reaction (Biewenga et al. 1997). Efficacies for the therapy of many diseases, such as diabetes (Strodtter et al. 1995), degenerative processes in neurons (Packer et al. 1997), atherogenesis (Ma et al. 2006), and acquired immune deficiency syndrome (Bilska and Wlodek 2005), have been published. Recently, it was reported that ALA causes a reduction in food take and enhances energy expenditure in rodents (Kim et al. 2004).

Salt forms of ALA using the carboxylic acid functional group have been industrially preferred due to a 4-fold higher plasma level and 3-fold faster maximum plasma concentration than solid dosage forms (microcrystal form) (Breithaupt-Grogler et al. 1999; Fuchs et al. 1997; Hermann et al. 1996). However, the form does not improve bioavailability, possibly due to the short plasma half-life (30 min) and low intrinsic bioavailability (30%) (Breithaupt-Grogler et al. 1999). Furthermore, repeated oral administration did not elevate the plasma level to greater than 25 mM (Marangon et al. 1999). As a replaceable method, Kipp et al. (2004) suggested that nanoparticle forms can increase drug dose several times compared to salt forms; further, these forms show improvement in bioavailability with respect to the dissolution rate and saturation (Choi et al. 2005).

Among the many nanoparticle preparation techniques, nano-comminution technology has been implemented in the

pharmaceutical industry since it is a relatively simple and effective process (Gao et al. 2008). However, ALA can be self-polymerized during nano-comminution due to its unstable five-membered disulfide bonds (Park et al. 2006). As one way to solve this problem, we suggested low-speed nano-comminution at a low temperature (4 °C), which successfully produced ALA nanoparticles (Park et al. 2009). This study also showed self-polymerized material content optimum in ALA nanoparticles for the improvement of bioavailability.

In general, the size of drug particles decreases with comminution time and then reaches a steady state. The steady-state sizes of nanoparticles depend predominantly on the kind of stabilizers used to prevent irreversible agglomeration (Choi et al. 2005). Also, the biocompatible polymeric stabilizers can have unique pharmaceutical properties themselves, such as mucoadhesive, stimuli-responsive, and controllable release properties, which can affect bioavailability (Broman et al. 2001; Möschwitzer and Müller 2006). This study aims to improve the bioavailability of ALA nanoparticles using various polymeric stabilizers, such as hydroxypropyl cellulose (HPC), polyvinylpyrrolidone (PVP), and Pluronic® F127 (F127). Furthermore, we will compare nano-suspensions to powder nano-formulations for appetite suppression, which will be assessed by calculating the food intake of mice over 6 h.

2. Investigations, results and discussion

2.1. Preparation of ALA nanoparticles

During the nano-comminution process, shear energy continually breaks up drug particles. The reduction in particle size results

Table: Volume-average particle sizes of samples measured by the light scattering method (comminution time: 9 h, sonication: 1 min)

Stabilizers	Mean particle size
HPC	420 ± 43 nm
F127	400 ± 38 nm
PVP	430 ± 47 nm

in an increase in the surface area such that the broken particles tend to aggregate, minimizing the thermodynamic energy of the system. Polymeric stabilizers adsorb onto the surface of the comminuted particles by a mainly thermodynamic driving force. The entropic repulsive force of the adsorbed polymer chains hinders particle aggregation. Therefore, the physical properties of polymeric stabilizers (i.e., hydrophilicity, molecular weight, chain flexibility, etc.) determine the steady-state particle sizes in nano-comminution (Choi et al. 2005). The Table shows that the three polymeric stabilizers successfully produced ALA nanoparticles of similar particle sizes, which were employed herein to exclude the influence of particle size. Additionally, X-ray diffraction studies showed that the crystal structures of ALA remained largely intact in all cases after nano-comminution (data not shown here).

In a suspension state, ALA tends to continuously self-polymerize due to water, and the kinetics depend on its storage temperature (Park et al. 2006). A solid dosage form might be better to improve the stability of ALA nanoparticles and suppress self-polymerization. To convert suspensions into solid dosage forms, a drying operation, which retains the characteristics of the nanoparticles and their related advantages, is necessary. Among the various drying methods, freeze-drying was preferentially selected because it can generally produce good re-dispersability (ability to achieve the original particle sizes of the nano-suspension upon re-constitution in water). Dispersants are often used to recover the original particle size, which hinders the aggregation of nanoparticles during lyophilization. Herein, sucrose was used as a dispersant (deChasteigner et al. 1996, 1995). The content of sucrose affects the lyoprotection effect, as shown in Fig. 1. Sucrose content below 60 wt% is not enough for lyoprotection, and the existence of micropar-

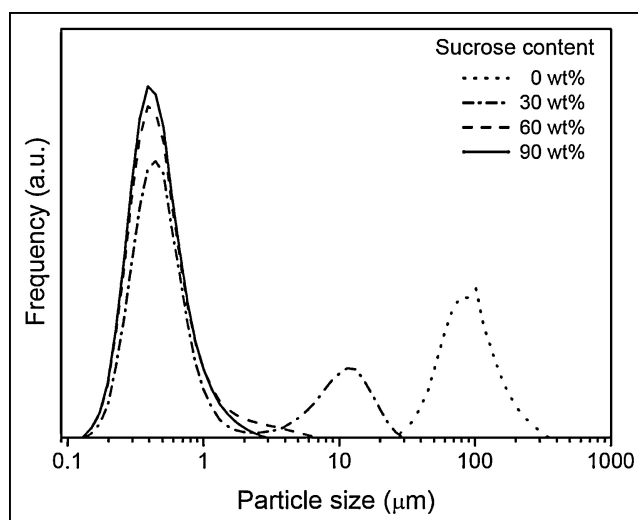


Fig. 1: Volume-average particle size distribution as a function of sucrose content (sonication: 2 min, stabilizer: HPC)

ticles is obvious upon re-constitution (poor re-dispersability). The existence of microparticles in the particle size distribution curves also depends on sonication time. That is, longer sonication can remove microparticles, and a sonication time of 2 min was arbitrarily chosen in this experiment.

Figure 2 shows additional evidence of possible aggregation of ALA nanoparticles in the 0 wt% sucrose cases. The individual ALA nanoparticles were exposed in the 0 wt% sucrose cases, while sucrose seems to completely cover individual particles in the 60 wt% sucrose cases. These results led us to choose a sucrose content of 60 wt% for *in vivo* and *in vitro* experiments.

2.2. *In vitro* release tests

In vitro release rate is an important factor in understanding the *in vivo* efficacy of drugs. The *in vitro* release rate of ALA from nanoparticulate powders relies on surface area (particle size) and the degree of self-polymerization (Park et al. 2009). As aforementioned, the type of polymer did not significantly affect particle sizes. The release profiles of ALA (Fig. 3) also show similar behavior. HPC cases show only a slightly higher

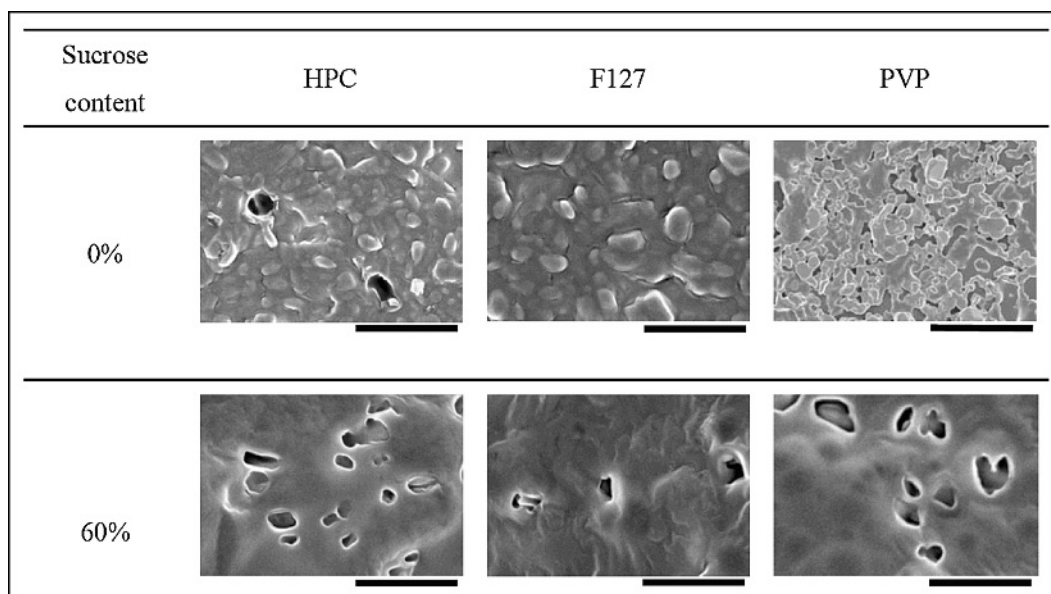


Fig. 2: SEM images of dried ALA nanoparticles having various polymer and sucrose contents. The scale bars correspond to 1 μm

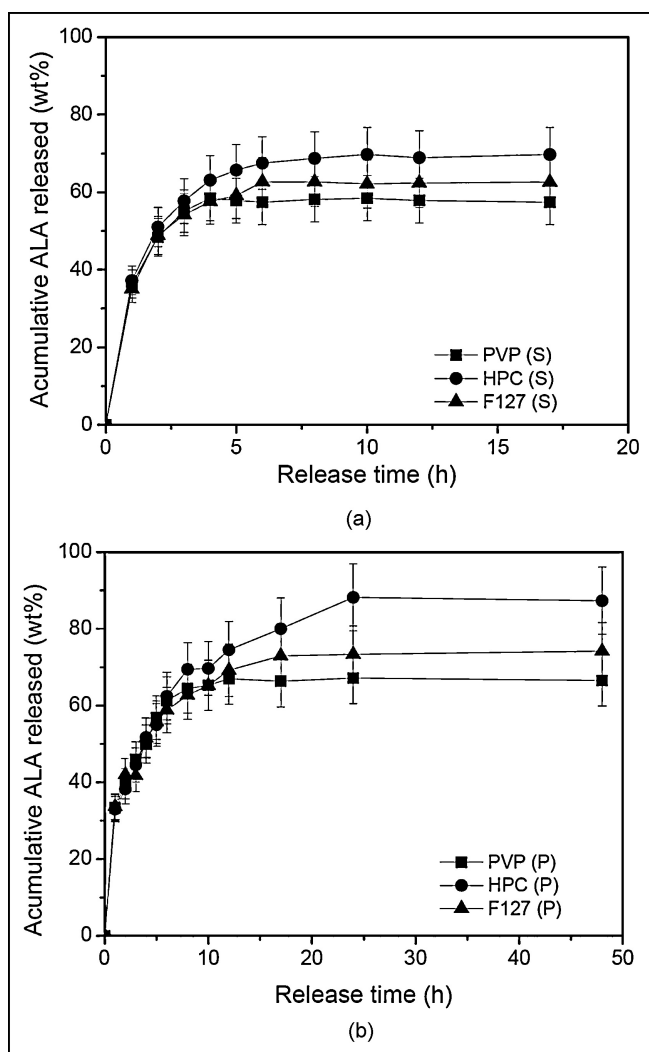


Fig. 3: Cumulative amount of ALA released for different polymers for (a) suspensions and (b) powders at pH 6.8

release profile than the others. The small differences in the release profiles do not fully reflect the differences in particle sizes of the Table though. Thus, the small (not statistically distinct) differences might occur due to different degrees of self-polymerization (Park et al. 2009).

Initial release rates of Fig. 3a and b show distinct differences between powder forms and suspensions, regardless of polymer type. The faster release rate in suspensions might be caused by the absence of the polymer swelling step, which is necessary for the release of powder forms. The conformation of adsorbed polymer chains is different in suspensions and powders. Similar to drying, re-constitution in water resulted in significant changes in chain conformation. The swelling of polymers includes complex water diffusion mechanisms, changes in polymer conformation (relaxation), and re-establishment of dynamic equilibrium between polymer adsorption and desorption. After enough water molecules are inserted into the sucrose and polymer phases, diffusion pathways to release ALA molecules through the polymer chains will be active. Therefore, the release rates of the powders are all slower than those of the suspensions, as shown in Fig. 3.

2.3. *In vivo* tests of food intake

It has been shown so far that the particle sizes and the *in vitro* release rates for all the different polymer cases are similar. Therefore, the kind of polymers may not be a determining factor

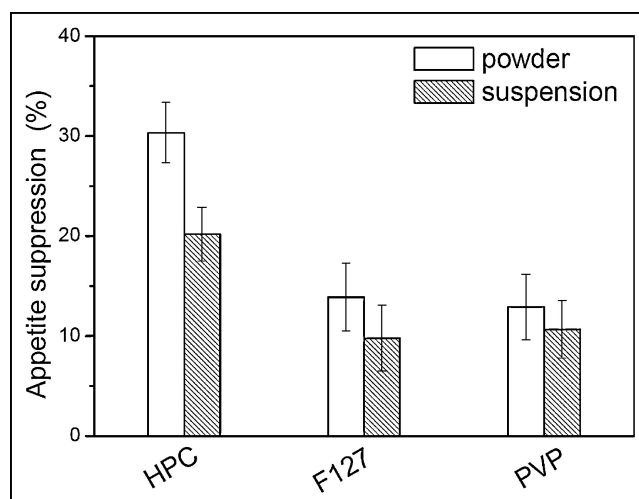


Fig. 4: Efficacies of appetite suppression during 6 h after 100 mg/kg injection of each sample

in *in vivo* efficacy tests. Figure 4, however, shows almost opposite results. Distinctly different *in vivo* efficacy results led us to consider the physical and chemical properties of polymers.

Mucoadhesion can be considered as an important aspect of polymers and can maintain the local delivery system by adhering to mucin. Since ALA nanosuspensions were used for intraperitoneal injection, the interactions between the polymers and intraperitoneal mucus could be an important factor in bioavailability. Mucoadhesion depends on molecular weight, viscosity, optimum concentration of polymeric adhesive, chain flexibility, spatial conformation, optimum hydration, charge and degree of ionization of the polymer, surrounding pH, and high initial contact time (Bernkop-Schnürch 2005). For example, the mucoadhesive effect generally increases with an increase in the molecular weight of polymers, which was explained via physical interactions (Herrero-Vanrell et al. 2005).

In Fig. 4, the appetite suppression efficacy of HPC cases was the highest among the three polymers. Because HPC has the highest molecular weight, the mucoadhesion effect of HPC could be the highest, too, and its bioavailability might be strongly improved due to the enhanced mucoadhesive property. Also, specific interactions, such as hydrogen bonding through the -OH groups of HPC, can increase the attractive interaction between mucin and mucoadhesive polymers.

Compared to the other polymers, F127 and PVP, HPC is generally known to be more mucoadhesive (Sudhakar et al. 2006). Therefore, in the HPC cases, ALA nanoparticles might be trapped on the mucin so that the long contact time, as well as the continuous release profile, improved its efficacy. Figure 5 describes this possible mechanism of the improved efficacy of HPC cases, which can be strongly localized on mucin due to the high molecular weight and -OH groups, resulting in the highest improved efficacy.

Figure 4 also shows the comparison of appetite suppression between powder and suspension forms. ALA has a short plasma half-life (30 min) (Breithaupt-Grogler et al. 1999), and released ALA can be metabolized in a relatively short time. Therefore, sustained release behavior could help to improve efficacy. According to the results shown in Fig. 3, the powder forms have longer release rates than the suspensions, and so the efficacy of ALA for appetite suppression might be sustained longer than the suspensions. This might be the reason why we have higher appetite suppression efficacy with the powder forms (Fig. 4).

In our preliminary experiments, polymers of wider spectrum and various molecular weights were tried, but many of them showed no distinct differences. Thus, we narrowed our focus to

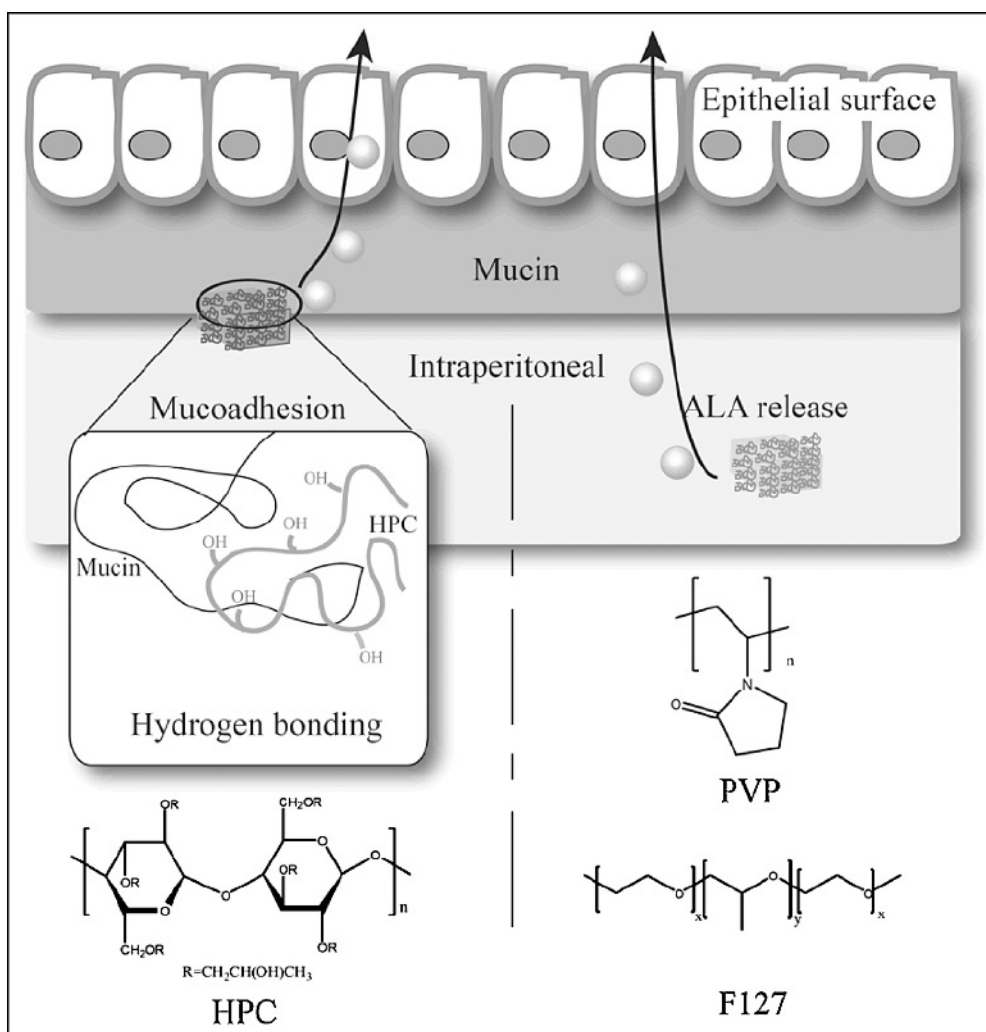


Fig. 5: Schematic diagram of mucoadhesive ALA delivery. The hydroxyl groups of HPC induce strong specific interactions with mucin compared to PVP and F127

differences in the *in vivo* results for similar particle sizes. When investigating similar particle sizes, differences in *in vivo* results of drug nanoparticles caused by different polymeric stabilizers have rarely been reported. These results show that the combination of proper polymers and other bioavailability improvement methods can provide maximum efficacy in ALA relative to the typical formulations.

3. Experimental

3.1. Materials

ALA was purchased from Antibioticos S.P.A. (Italy), while PVP (PLASDONE®, C-30 Povidone USP, 35,000 g/mol) was purchased from ISP Technologies. HPC (80,000 g/mol) and F127 (15,000 g/mol) were purchased from Sigma-Aldrich (USA). Sucrose, which was used as a dispersant, was purchased from Sigma-Aldrich. KH_2PO_4 and NaOH were received from Duck San Pure Chem (Korea). All the substances were utilized without any chemical purification.

3.2. Nano-comminution

Low-speed rotation nano-comminution was performed, as described previously (Park et al. 2009). Briefly, nano-comminution was performed in a 60-ml wide-neck amber glass with 6.8 g distilled water, 0.1 g a polymeric stabilizer, and 0.6 g ALA. The rotation speed was 100 rpm. ZrO_2 beads (1 mm, 50% v/v) were charged in the bottle. During nano-comminution, a temperature of 4 °C was maintained to suppress the chemical reactivity of ALA.

3.3. Powder preparation

After nano-comminution, ALA nanoparticles were in a suspension. To obtain a solid powder form, a freeze-drying method was used. As a dispersant, sucrose was dissolved into the suspension before freezing. The suspension was subsequently dropped into liquid nitrogen and freeze-dried in a EYELA FD-1000 freeze dryer (Tokyo, Japan) at $-55 \pm 3^\circ\text{C}$ (10^{-4} Torr).

3.4. *In vitro* release tests

For release tests, 60 wt% (in dried powders) sucrose was used. ALA was released from a dialysis tubing cellulose membrane (Sigma-Aldrich, average flat width: 25 mm) after activation in distilled water for 12 h. The release tests in simulated intestinal fluid were determined by using a Type II USP paddle apparatus under a sink conditions. The intestinal fluid (pH 6.8) at $37 \pm 0.5^\circ\text{C}$ was composed of KH_2PO_4 (8.805 g), NaOH (0.896 g), and distilled water (1 L). The volume of the dissolution medium was 500 ml, and the paddle speed was 30 rpm. Specimens were withdrawn within a tolerance of ± 10 seconds. UV/VIS spectroscopy detected the amount of cumulative ALA released, which was quantified by the λ_{max} (340 nm) absorption of 1,2-dithiolane (cyclic disulfide bond).

3.5. *In vivo* tests of appetite suppression

For the *in vivo* tests of nano-suspensions, ALA in powder form was re-dispersed in water, and the resulting suspension was used, which contained the same amount of sucrose as the powder form. Food intake was measured in male C57BL/6 mice (body weight: 20 ± 3 g, $n=6$ each). After an overnight fast, ALA nanoparticles were intraperitoneally (IP) injected (100 mg/kg), and food intake was measured for 6 h. Mice were administered the same amount of pure ALA as measured by UV/VIS spectroscopy.

3.6. Characterizations of particles

The volume-averaged particle size of ALA nanoparticles was measured in 150 ml water using a Horiba LA-910 laser light scattering analyzer with a refractive index of 1.3. The agitation and the circulation speed were set to level 3. Mean particle sizes were calculated after sonication (30 Hz) for 1 min. Morphologies of particles coated by Pt (about 3.5 nm thickness) were examined by a Hitachi S-4800 scanning electron microscope at an accelerating voltage of 4 kV. The particle structures of nanoparticles were measured by a Scintag XDS 2000 X-ray diffractometer ($\lambda = 1.54 \text{ \AA}$) at 40 kV and 40 mA.

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References

- Bernkop-Schnürch A (2005) Mucoadhesive polymers: strategies, achievements and future challenges. *Adv Drug Deliv Rev* 57: 1553–1555.
- Biewenga GP, Haenen G, Bast A (1997) The pharmacology of the antioxidant lipoic acid. *Gen Pharmacol* 29: 315–331.
- Bilska A, Wlodek L (2005) Lipoic acid - the drug of the future? *Pharmacol Rep* 57: 570–577.
- Breithaupt-Grogler K, Niebch G, Schneider E, Erb K, Hermann R, Blume HH, Schug BS, Belz GG (1999) Dose-proportionality of oral thioctic acid – coincidence of assessments via pooled plasma and individual data. *Eur J Pharm Sci* 8: 57–65.
- Broman E, Khoo C, Taylor LS (2001) A comparison of alternative polymer excipients and processing methods for making solid dispersions of a poorly water soluble drug. *Int J Pharm* 222: 139–151.
- Choi JY, Yoo JY, Kwak H-S, Uk Nam B, Lee J (2005) Role of polymeric stabilizers for drug nanocrystal dispersions. *Current Appl Phys* 5: 472–474.
- deChasteigner S, Cave G, Fessi H, Devissaguet JP, Puisieux F (1996) Freeze-drying of itraconazole-loaded nanosphere suspensions: a feasibility study. *Drug Devel Res* 38: 116–124.
- deChasteigner S, Fessi H, Cave G, Devissaguet JP, Puisieux F (1995) Gastrointestinal tolerance study of a freeze-dried oral dosage form of indomethacin-loaded nanocapsules. *STP Pharma Sci* 5: 242–246.
- Fuchs J, Packer L, Zimmer G (1997) Lipoic acid in health and disease. In: Hermann R, Niebch G (eds) *Biothiols*, Pt A, pp. 340–347.
- Fujiwara K, Okamuraikeda K, Motokawa Y (1995) Assay for protein lipoylation reaction. In: Hermann R, Niebch G (eds) *Biothiols*, Pt A, pp. 340–347.
- Gao L, Zhang DR, Chen MH (2008) Drug nanocrystals for the formulation of poorly soluble drugs and its application as a potential drug delivery system. *J Nanopart Res* 10: 845–862.
- Hermann R, Niebch G, Borbe HO, FiegerBuschges H, Ruus P, Nowak H, RiethmullerWinzen H, Peukert M, Blume H (1996) Enantioselective pharmacokinetics and bioavailability of different racemic alpha-lipoic acid formulations in healthy volunteers. *Eur J Pharm Sci* 4: 167–174.
- Herrero-Vanrell R, Vicario M, de las Heras B, Rincon A, Molina-Martinez IT (2005) *in vitro* evaluation of solutions prepared from mucoadhesive polymers. *Invest Ophthalmol Vis Sci* 46: 2034.
- Kim MS, Park JY, Namkoong C, Jang PG, Ryu JW, Song HS, Yun JY, Namgoong IS, Ha J, Park IS, et al. (2004) Anti-obesity effects of alpha-lipoic acid mediated by suppression of hypothalamic AMP-activated protein kinase. *Nat Med* 10: 727–733.
- Kipp JE (2004) The role of solid nanoparticle technology in the parenteral delivery of poorly water-soluble drugs. *Int J Pharm* 284: 109–122.
- Möschwitzer J, Müller RH (2006) Spray coated pellets as carrier system for mucoadhesive drug nanocrystals. *Eur J Pharm Biopharm* 62: 282–287.
- Ma XY, Padmalayam I, Cahoon S, Chakrabarti R, Pillarisetti S, Saxena U (2006) Lipoic acid prevents atherosclerosis lesion development in ApoE null mice. *Diabetes* 55: A497–A497.
- Marangon K, Devaraj S, Tirosh O, Packer L, Jialal I (1999) Comparison of the effect of [alpha]-lipoic acid and [alpha]-tocopherol supplementation on measures of oxidative stress. *Free Radic Biol Med* 27: 1114–1121.
- Packer L, Tritschler HJ, Wessel K (1997) Neuroprotection by the metabolic antioxidant alpha-lipoic acid. *Free Radic Biol Med* 22: 359–378.
- Park CH, Kim AR, Yun HL, Lee J (2006) Ring opening and polymerization of alpha-lipoic acid. *Polym-Korea* 30: 357–361.
- Park CH, Youn HR, Lee J, Lee KU, Park JY, Koh EH, Kim HS (2009) Improved efficacy of appetite suppression by lipoic acid particles prepared by nanocomminution. *Drug Devel Ind Pharm* 35: 1305–1311.
- Sudhakara Y, Kuotsua K, Bandyopadhyay AK (2006) Buccal bioadhesive drug delivery — A promising option for orally less efficient drugs. *J Control Release* 114: 15–40.
- Strodter D, Lehmann E, Lehmann U, Tritschler HJ, Bretzel RG, Federlin K (1995) The influence of thioctic acid on metabolism and function of the diabetic heart *Diabetes Res Clin Pract* 29: 19–26.