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Increased bioavailability of a transdermal application of a nano-sized emulsion preparation

T. Kotyla [a], F. Kuo [b], V. Moolchandani [c], T. Wilson [a], R. Nicolosi [a,*]

[a] *Department of Clinical Laboratory and Nutritional Sciences, Center for Health and Disease Research, University of Massachusetts Lowell, Lowell, MA 01854, USA*
[b] *Center for Health and Disease Research and the Biomedical Engineering and Biotechnology PhD Program, University of Massachusetts Lowell, Lowell, MA 01854, USA*
[c] *School of Pharmacy, University of Maryland, Baltimore, MD 21201, USA*

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Abstract

The aim of this study was to compare the transdermal application of a nano-sized emulsion versus a micron-sized emulsion preparation of delta tocopherol as it relates to particle size and bioavailability. Two separate experiments were performed using seven F1B Syrian Golden hamsters, 1 week apart. Each emulsion preparation consisted of canola oil, polysorbate 80, deionized water and delta tocopherol; the only difference between the two preparations was processing the nano-sized emulsion with the Microfluidizer® Processor. Both were formulated into a cream and applied to the shaven dorsal area. The particle size of the micron-sized emulsion preparation was 2788 nm compared to 65 nm for the nano-sized emulsion formulation. Two hours post-application, hamsters that were applied the nano-sized emulsion had a 36-fold significant increase of plasma delta tocopherol, where as hamsters that were applied the micron-sized emulsion only had a 9-fold significant increase, compared to baseline, respectively. At 3 h post-application, plasma delta tocopherol had significantly increased 68-fold for hamsters applied the nano-sized emulsion, whereas only an 11-fold significant increase was observed in hamsters applied the micron-sized emulsion, compared to baseline, respectively. Significant differences were also observed between the nano-sized and micron-sized emulsion at 2 and 3 h post-application. This study suggests that nano-sized emulsions significantly increase the bioavailability of transdermally applied delta tocopherol.

Keywords: Nano-sized emulsion; Transdermal; High shear processing; Microfluidizer

1. Introduction

Due to its antioxidant properties, vitamin E (tocopherol) is postulated to reduce the incidence and severity of disease states associated with oxidative stress, such as experimental atherosclerosis, cancer, chronic inflammation, and neurological disorders (Brigelius-Flohe et al., 2002). Moreover, a large body of evidence indicates that α-tocopherol reduces spontaneously occurring oxidative DNA damage and inhibits an early stage of carcinogenesis due to a blockage of nitrosamine formation (Ohshima et al., 1982; Azzi et al., 1995; Moore et al., 1999). Other studies suggest that vitamin E compounds have a direct effect against tumors and tumor cell growth (Kline et al., 2001). In addition, it has been reported that certain isomers of tocopherol, γ-tocopherol, in particular, have anti-inflammatory properties (Grammas et al., 2004; Singh and Jialal, 2004; Singh et al., 2005). Thus, any delivery system which could enhance the bioavailability and efficacy of substances such as tocopherol could be of great value for medical applications of nutraceuticals and pharmaceuticals.

Lipid-based nano-formulations are among the most attractive candidates for improving substance solubility and for site-specific targeting following parenteral administration (Moghimi and Agrawal, 2005). Nano-sized emulsions are a class of stable emulsions composed of surfactant and oil suspended in water with a particle diameter usually less than 100 nm (Becher, 1983; Porrasa et al., 2004; Sonneville-Aubrun et al., 2004). The reported stability of nano-sized emulsions makes them extraordinary and they are often described as "approaching thermodynamic stability" (Tadros et al., 2004). It has been sug-

* Corresponding author. Tel.: +1 978 934 4501; fax: +1 978 934 2034.
E-mail address: Robert_Nicolosi@uml.edu (R. Nicolosi).

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gested that emulsion systems offer an appealing substitute for the formulation of poorly soluble drugs such as paclitaxel and amiodarone (Constantinides et al., 2004).

Compared to typical micron-sized emulsion preparations which can be thousands of nanometers in size, nano-delivery systems with particle sizes approximately 100 nm or less have been shown to increase bioavailability and efficacy of a number of compounds such as anti-inflammatory agents (Kumar et al., 2004), insulin (Yan et al., 2002), tetanus toxoid (Vila et al., 2005) and dicumarol (Thanos et al., 2003). Support for increased efficacy of nano-sized emulsion delivery substances has also come from our laboratory, which demonstrated that nano-sized emulsions prepared on a Microfluidizer® Processor containing an anti-oxidant synergy formulation (ASF) dramatically reduced tumor size in a neuroblastoma-bearing mouse model compared to a micro-suspension of ASF (Kuo et al., 2007).

2. Materials and methods

2.1. Nano-sized emulsion and micron-sized emulsion formulations

The nano-sized emulsion was formed by making a ternary mixture of delta tocopherol (Sigma–Aldrich Inc., Missouri, USA) (4.62 mg/mL) dissolved in 10 g canola oil and 10 g polysorbate 80 (Sigma–Aldrich Inc., Missouri, USA). Delta tocopherol and not alpha tocopherol was used in this study since its background level in the blood stream of hamsters is very low, making it easier to quantitate the added amounts contributed by the micro- and nano-sized emulsions. This solution was heated (50 °C) and gently stirred with a magnetic stirrer for 10 min. Two hundred and forty milliliters of deionized/distilled water was heated (60 °C) and added to the solution. The solution was allowed to equilibrate with gentle magnetic stirring for 20 min. The delta tocopherol nano-sized emulsion was prepared using the Microfluidizer® Processor, Model M-110EH (Microfluidics Corporation, Newton, MA, USA). Microfluidizer® Processors provide high pressure and a resultant high shear rate by accelerating the product through microchannels to a high velocity for size reduction to the nanoscale range. The fluid is split into two and is pushed through microchannels with typical dimensions in the order of 75 microns at high velocities (in the range of 50–300 m/s). As the fluid exits the microchannels it forms jets which collide with jets from opposing microchannels. In the channels the fluid experiences high shear (up to 10^7 L/s) which is orders of magnitude higher than that of conventional technologies. Jet collisions result in mixing in submicron level. Therefore high shear and impact are responsible for particle size reduction and mixing of multiphase fluids in the Microfluidizer technology.

For studies described in this communication, the delta tocopherol formulation was passed through the Microfluidizer® Processor once at a pressure of 24,000 psi. The micron-sized emulsion formulation was prepared as the ternary mixture and not subjected to the Microfluidizer® Processor.

2.2. Viscosity and particle size

The viscosity of the formulations was measured using an Ubbelohde Viscometer (VWR International, Boston, MA, USA) at 25 °C. For measurement of the mean droplet size and polydispersity index (width of the particle size distribution) a dynamic laser light scattering Malvern Nano-S instrument (Malvern Instruments Inc., Southborough, MA, USA) was used.

2.3. Study design

Seven male Syrian Golden hamsters (F_1B strain, BioBreeders Inc., Fitchburg, MA, USA), 8–10 weeks of age were used in this study and were housed in individual box cages with bedding in a temperature-controlled room (20 °C) and a 12 h light cycle. They were fed a commercial non-purified diet (Ralston Pruina 4001, St. Louis, MO, USA) and water provided ad libitum. Both emulsion formulations were mixed together 1:1 with a hypoallergenic cream (PCCA, Houston, TX, USA). All hamsters were preshaven 1 day prior to application (day 0). On day 1, 1 mL of the micron-sized emulsion containing cream was applied to a 1 in^2. area of shaven skin on the hamster's dorsal side. The cream was gently rubbed onto the skin until it was no longer visible. Time point bleeds of baseline and 1–3 h post-application were collected via the retro-orbital sinus into heparinized capillary tubes under ultrapure CO_2/O_2 (50/50) gas (Northeast Airgas, Salem, NH) anesthesia and analyzed for plasma delta tocopherol concentrations. The subsequent week, this procedure was repeated using the nano-sized emulsion-formulated cream, on the same animals. The animals were maintained in accordance with the guidelines of the Committee on Animal Care of the University of Massachusetts Lowell Research Office (IACUC), and the guidelines prepared by the Committee on Care in Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (DHEW publication no. 85-23, revised 1985).

2.4. Plasma delta tocopherol analysis

Plasma delta tocopherol concentration was determined by adding 200 μL of plasma with 10 μL of retinyl acetate (internal standard; 10 μg/mL) and 200 μL of ethanol containing butylated hydroxytoluene (BHT) (10 mg/L) and 1.0 mL hexane followed by vortex mixing. The samples were centrifuged at 500 × *g* for 5 min and the organic layer transferred to a 7.0 mL brown borosilicate screw top vial. The sample residues were re-extracted with 1.0 mL of hexane and the organic layers were combined. The organic layer was evaporated under N_2 and reconstituted with 200 μL of ethanol containing BHT (10 mg/dL) and injected into an HPLC. The HPLC system is a Model 5600 CoulArray eight-channel system with two Model 580 pumps, a high-pressure gradient mixer, a PEEK pulse damper, a Model 540 autoinjector, a CoulArray Thermostatic Chamber and a serial array of eight coulometric electrodes (ESA Laboratories, Inc., Chelmsford, MA, USA). The column is a 3.0 mm × 150 mm, 3 μM, Supelcosil LC-18 (Supelco, Bellefonte, PA, USA). The mobile phase consisted of methanol/1 M Propanol/1 M ammonium acetate (78:20:2 v/v/v) at a flow rate

of 0.8 mL/min. The concentration of delta tocopherol was determined by external standardization using purified solutions of delta tocopherol standards (Sigma Chemicals, St. Louis, MO, USA).

2.5. Statistical assessment

SigmaStat software was used for all statistical evaluations (Jandel Scientific, San Rafael, CA, USA) (Snedecor and Cochouran, 1980). A repeated measures one-way analysis of variance (RM ANOVA) was used to analyze plasma delta tocopherol data between baseline, 1–3 h post-application within each treatment. When statistical significance was found by ANOVA, the Student–Newman–Keuls separation of means was used to determine differences. A paired *t*-test was used to analyze plasma delta tocopherol data between treatments at each time point. All values are expressed as mean ± S.E.M. and statistical significance was set at the minimum $p < 0.05$.

3. Results

3.1. The effect of Microfluidizer® processing of the delta tocopherol formulation on particle size and concentration

The average particle size diameter of the delta tocopherol micron-sized emulsion and the nano-sized emulsion were 2788 nm and 65 nm, respectively (Figs. 1 and 2). In Fig. 1, the third peak is cut-off, since this peak exceeds the limit of the Malvern Nano-S instrument. From this we can hypothesize that the microemulsion preparation is actually larger than the 2788 nm average displayed in the graph. The starting concentration for both preparations of delta tocopherol was 4.6 mg/mL.

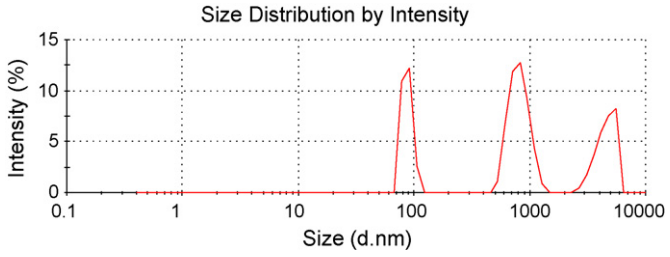

Fig. 1. Dynamic laser light scattering particle size analysis of the delta tocopherol micron-sized emulsion showing an average particle size of 2788 nm, with a range of 0.08–6 μm.

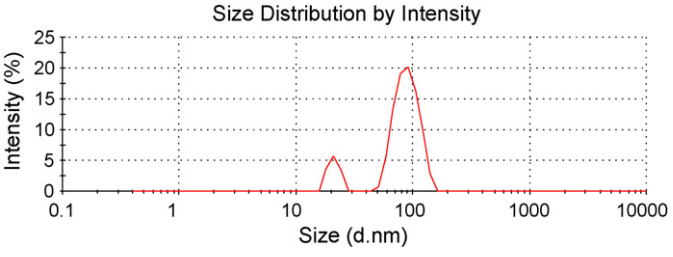

Fig. 2. Dynamic laser light scattering particle size analysis of delta tocopherol nano-sized emulsion preparation showing an average particle size of 65 nm, with a range of 10–200 nm.

The final concentration of delta tocopherol in the micron-sized emulsion formulation was 19% greater (4.13 mg/mL) compared to the nanoemulsion formulation (3.48 mg/mL). We have previously demonstrated that this loss of delta tocopherol can be attributed to a single pass through the Microfluidizer® Processor (data not shown).

3.2. Bioavailability studies of micro- versus nanoemulsions of delta tocopherol

No significant differences in plasma delta tocopherol concentrations were observed for either the nano-sized emulsion or micron-sized emulsion preparations at 1 h post-application compared to baseline (Fig. 3). However, at 2 h post-application, the hamsters that were applied the cream containing the nano-sized emulsion of delta tocopherol had significantly greater levels of plasma delta tocopherol compared to baseline (36-fold) and 1 h post-application (10-fold) (Fig. 3). At 3 h post-application, the plasma delta tocopherol concentrations of the hamsters that were applied the cream containing the nano-sized emulsion of delta tocopherol had increased significantly compared to baseline (68-fold), 1 h post-application (20-fold), and 2 h post-application (0.86-fold) (Fig. 3). By comparison, the hamsters that were applied the cream containing the micron-sized emulsion of delta tocopherol had a smaller yet significant increase of 9- and 11-fold at 2 and 3 h post-application, respectively compared to baseline but not compared to 1 h post-application (Fig. 3). More importantly between groups, the hamsters that were applied the cream containing the nano-sized emulsion of delta tocopherol had significantly higher concentrations of plasma delta tocopherol at 2 h (129%) and 3 h post-application (265%) compared to the hamsters that were applied the cream containing the micron-sized emulsion of delta tocopherol (Fig. 3).

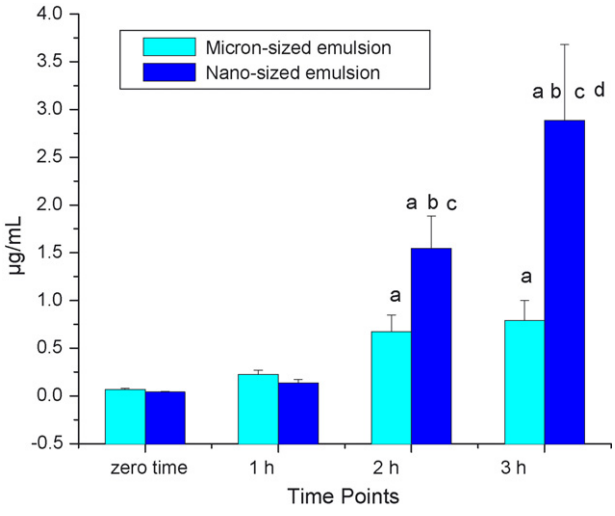

Fig. 3. Plasma concentrations of delta tocopherol from hamsters transdermally applied a nano- or micron-sized emulsion preparation of delta tocopherol. Values are means ± S.E.M. ($n = 7$) $p < 0.05$. (a) Significantly from baseline ($p < 0.05$); (b) significantly from 1 h ($p < 0.05$); (c) significantly from 2 and 3 h between treatments ($p < 0.05$); (d) significantly from 2 h within treatment ($p < 0.05$).

4. Discussion

The particle size of the delta tocopherol nano-sized emulsion had a mean diameter of 65 nm compared to the preparation of the delta tocopherol micron-sized emulsion which had an average particle size of 2788 nm. We hypothesize that since the third peak of the micron-sized emulsion preparation exceeds the limit of the Malvern Nano-S instrument, its particle size may actually be larger than the value that is shown. This vast difference in particle size was likely a major factor contributing to our findings as evidenced from the significantly higher concentrations of plasma delta tocopherol found in the hamsters applied the nano-sized emulsion preparation compared to the micron-sized emulsion applied hamsters. More support for this conclusion also comes from our laboratory (Kuo et al., 2007), which has recently reported a significant decrease in the tumor size of neuroblastoma in nude mice with a nano-sized emulsion preparation of an antioxidant synergy formula (ASF). In that study, tumor growth rate in the mice applied with the nano-sized emulsion of ASF showed a significant decrease in tumor size when compared to mice that were applied the ASF in a suspension.

In addition to our studies and those of others cited earlier demonstrating the ability of particle size to influence bioavailability of several classes of compounds (Yan et al., 2002; Thanos et al., 2003; Kumar et al., 2004; Vila et al., 2005), similar findings have been reported for penclomedine (de Smidt et al., 2004). In this study (de Smidt et al., 2004), which penclomedine was prepared as a medium chain triglyceride having particle sizes in the nanometer range (160–720 nm), the formulation with a particle size of 160 nm had the greatest bioavailability. The authors suggested that the smaller nanometer particles penetrated to a significantly greater extent and provided a larger surface area for absorption than the larger particles, thereby providing greater efficacy as a delivery system. Seki et al. (2004) reported, using dexamethasone palmitate (DMP) as the active compound with a particle size of 25 nm demonstrated, superior efficacy, a highly uniform size with stability in various conditions, a higher plasma concentration of the drug and improved distribution volume of the drug into inflammation sites, when compared to the DMP with a particle size of 300 nm compound. Wegmuller et al. (2004) reported that particle size was an important determinant of iron absorption from poorly soluble iron compounds in foods. By decreasing the particle size of the elemental iron powders, an increase in the rats' absorption of iron was demonstrated.

Although speculative, in the present study, the 42-fold smaller particle size of the transdermal delta tocopherol nano-sized emulsion preparation versus the micron-sized emulsion delta tocopherol formulation could have increased the surface to volume ratio, thereby positively affecting bioavailability. Specifically for topical application, nano-sized emulsions solubilize the poorly water-soluble nutrient or drug and deliver it to the skin cell membrane where the drug molecules are released from the nanoemulsion systems to the skin surface, thus increasing the drug skin permeation (Paolino et al., 2002).

The three main factors determining the transdermal efficacy of drugs are mobility of drug in the vehicle, release of drug from the vehicle, and permeation of drug into the skin. These factors affect either the thermodynamic activity that delivers the drug into the skin or the permeability of the drug in the skin, particularly the stratum corneum (Peltola et al., 2003). The small particle size ensures close contact to the stratum corneum thus increasing the amount of encapsulated drug/nutrient penetrating into the viable skin (Jenning et al., 2000). A common method to improve drug permeation through the skin is to use penetration enhancers. Penetration enhancers can change the structure of skin lipids and alter the skin barrier function (Paolino et al., 2002). For these reasons penetration enhancers were not used in our preparations. Other methods have been proposed to increase the permeability of drugs through the skin, i.e., iontophoresis and ultrasound, but these methods are not frequently used due to the requirement of qualified staff for their application (Paolino et al., 2002).

In conclusion, the present study suggests that nano-sized emulsion preparations provide a transdermal delivery system that increases the bioavailability of fat soluble substances such as tocopherol compared to micron-sized emulsion preparations that are produced containing the same compounds at equal concentrations.

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References

Azzi, A., Boscoboinik, D., Marilley, D., Ozer, N.K., Stauble, B., Tasinato, A., 1995. Vitamin E: a sensor and an information transducer of the cell oxidation state. Am. J. Clin. Nutr. 62, 1337S–1346S.

Becher, P., 1983. Encyclopedia of Emulsion Technology, vol. 1. Marcel Deckker, New York, pp. 129–285.

Brigelius-Flohe, R., Kelly, F.J., Salonen, J.T., Neuzil, J., Zingg, J.M., Azzi, A., 2002. The European perspective on vitamin E: current knowledge and future research. Am. J. Clin. Nutr. 76, 703–716.

Constantinides, P.P., Tustian, A., Kessler, D.R., 2004. Tocol emulsions for drug solubilization and parenteral delivery. Adv. Drug Deliv. Rev. 56, 1243–1255.

de Smidt, P.C., Campanero, M.A., Troconiz, I.F., 2004. Intestinal absorption of penclomedine from lipid vehicles in the conscious rat: contribution of emulsification versus digestibility. Int. J. Pharm. 270, 109–118.

Grammas, P., Hamdheydari, L., Benaksas, E.J., Mou, S., Pye, Q.N., Wechter, W.J., Floyd, R.A., Stewart, C., Hensley, K., 2004. Anti-inflammatory effects of tocopherol metabolites. Biochem. Biophys. Res. Commun. 319, 1047–1052.

Jenning, V., Gysler, A., Schafer-Korting, M., Gohla, S.H., 2000. Vitamin A loaded solid lipid nanoparticles for topical use: occlusive properties and drug targeting to the upper skin. Eur. J. Pharm. Biopharm. 49, 211–218.

Kline, K., Weiping, Yu., Sanders, B.G., 2001. Vitamin E: mechanisms of actionas tumor cell growth inhibitors. J. Nutr. 131, 161S–163S.

Kumar, R., Chen, M.H., Parmar, V.S., Samuelson, L.A., Kumar, J., Nicolosi, R., Yoganathan, S., Watterson, A.C., 2004. Supramolecular assemblies based on copolymers of PEG600 and functionalized aromatic diesters for drug delivery applications. J. Am. Chem. Soc. 126, 10640–10644.

Kuo, F., Kotyla, T., Wilson, T., Kifle, L., Panagiotou, T., Gruverman, I., Tagne, J.B., Shea, T., Nicolosi, R., 2007. A nanoemulsion of an anti-oxidant synergy formulation reduces tumor growth rate in neuroblastoma-bearing nude mice. J. Exp. Ther. Oncol. 6, 129–135.

Moghimi, S.M., Agrawal, A., 2005. Lipid-based nanosystems and complexes in experimental and clinical therapeutics. Curr. Drug Deliv. 2, 295.

Moore, S.R., Hill, K.A., Heinmoller, P.W., Halangoda, A., Kunishige, M., Buettner, V.L., Graham, K.S., Sommer, S.S., 1999. Spontaneous mutation frequency and pattern in Big Blue mice fed a vitamin E-supplemented diet. Environ. Mol. Mutagen. 34, 195–200.

Ohshima, H., Bereziat, J.C., Bartsch, H., 1982. Monitoring N-nitrosamino acids excreted in the urine and feces of rats as an index for endogenous nitrosation. Carcinogenesis 3, 115–120.

Paolino, D., Ventura, C.A., Nistico, S., Puglisi, G., Fresta, M., 2002. Lecithin microemulsions for the topical administration of ketoprofen: percutaneous adsorption through human skin and in vivo human skin tolerability. Int. J. Pharm. 244, 21–31.

Peltola, S., Saarinen-Savolainen, P., Kiesvaara, J., Suhonen, T.M., Urtti, A., 2003. Microemulsions for topical delivery of estradiol. Int. J. Pharm. 254, 99–107.

Porrasa, M., Solansb, C., Gonzáleza, C., Martyneza, A., Guinarta, A., Guti erreza, J.M., 2004. Studies of formation of W/O nano-sized emulsions. Colloid Surf. A 249, 115–118.

Seki, J., Sonoke, S., Saheki, A., Fukui, H., Sasaki, H., Mayumi, T., 2004. A nanometer lipid emulsion, lipid nano-sphere (LNS), as a parenteral drug carrier for passive drug targeting. Int. J. Pharm. 273, 75–83.

Singh, U., Jialal, I., 2004. Anti-inflammatory effects of alpha-tocopherol. Ann. N.Y. Acad. Sci. 1031, 195–203.

Singh, U., Devaraj, S., Jialal, I., 2005. Vitamin E, oxidative stress, and inflammation. Annu. Rev. Nutr. 25, 151–174.

Snedecor, G.W., Cochouran, W.G., 1980. Statistical Methods, 6th ed. Iowa State University Press, Ames, IA.

Sonneville-Aubrun, O., Simonnet, J.T., L'Alloret, F., 2004. Nanoemulsions: a new vehicle for skincare products. Adv. Colloid Interf. Sci. 108/109, 145–149.

Tadros, T., Izquierdo, P., Esquena, J., Solans, C., 2004. Formation and stability of Nano-sized emulsions. Adv. Colloid Interf. Sci. 108/109, 303–318.

Thanos, C.G., Liu, Z., Reineke, J., Edwards, E., Mathiowitz, E., 2003. Improving relative bioavailability of dicumarol by reducing particle size and adding the adhesive poly(fumaric-*co*-sebacic) anhydride. Pharm. Res. 20, 1093–1100.

Vila, A., Sanchez, A., Evora, C., Soriano, I., McCallion, O., Alonso, M.J., 2005. PLA-PEG particles as nasal protein carriers: the influence of the particle size. Int. J. Pharm. 292, 43–52.

Wegmuller, R., Zimmermann, M.B., Moretti, D., Arnold, M., Langhans, W., Hurrell, R.F., 2004. Particle size reduction and encapsulation affect the bioavailability of ferric pyrophosphate in rats. J. Nutr. 134, 3301–3304.

Yan, P., Zheng, J.M., Zhao, H.Y., Li, Y.J., Xu, H., Wei, G., 2002. Relationship between drug effects and particle size of insulin-loaded bioadhesive microspheres. Acta Pharmacol. Sin. 23, 1051–1056.