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Chitosan microspheres for encapsulation of α-lipoic acid

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Abstract

Encapsulation of α-lipoic acid (LA) was carried out using chitosan as an encapsulant matrix. Placebo and LA-loaded chitosan microspheres were prepared by a spray-drying process. Scanning electron microscopy (SEM) studies confirmed the spherical particle geometry and the smooth surface morphology of LA-loaded particles. The particle size distribution (PSD) analysis of the placebo and LA-loaded microspheres has shown that 50% of the microspheres were less than 3.53 and 7.89 μm, respectively. The structural interactions of the chitosan matrix with the encapsulated LA were studied by Fourier transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC) which revealed structural interactions of lipoic acid with the encapsulant matrix. The antioxidant activity of encapsulated lipoic acid was studied using the free-radical scavenging assay. This study demonstrated significant retention of antioxidant activity of lipoic acid (75%) after encapsulation in the chitosan matrix. Encapsulation efficiency of lipoic acid obtained in this study was 55.2% when ethanol and acetic acid (1:1 v/v) was used as incubation/extraction medium.

Keywords: Chitosan; α-Lipoic acid; Microspheres; Encapsulation efficiency; Antioxidant activity; Spray-drying

1. Introduction

Oxidative stress underlies both minor and severe cell damage with a range of physiological (e.g. muscle fatigue and tiredness) and patho-physiological consequences such as cardiovascular disease and cancer. α-Lipoic acid is an important and powerful biological antioxidant that can directly scavenge free radicals and protect cells from oxidative damage (Scott et al., 1994). Free α-lipoic acid is rapidly taken up by cells and reduced to dihydrolipoic acid (DHLA) intracellularly. As DHLA is also rapidly eliminated from cells, the extent to which antioxidant effects can be sustained remain unclear.

Supplemental doses of α-lipoic acid (LA) are rapidly metabolized and rapidly cleared from plasma and tissues (Teichert et al., 1998), suggesting that it should be taken in divided doses through out the day, rather than a single dose. A constant plasma level of LA might be highly beneficial (Bernkop-Schnurch et al., 2004) for various reasons such as given below:

(a) Immediate release spikes probably leading to toxic side effects can be avoided.
(b) The antioxidative and radical scavenging effect might be guaranteed for a prolonged period of time and not just a few hours.
(c) In addition, glucose should be stimulated continually leading to a pronounced reduction in the blood glucose level.

The design and evaluation of sustained release dosage forms based on chitosan acetate and LA in the form of tablets have been studied for oral administration. The strong retardation of drug release is based on ionic interactions between the cationic polymer and the anionic drug. The degree of ionization of the drug was found to have no significant influence on its absorption behaviour. These dosage forms might provide a constant plasma level of the drug being highly beneficial for various therapeutic applications (Bernkop-Schnurch et al., 2004). LA has been microencapsulated on a molecular level with α-cyclodextrin giving it a much improved shelf life and taste (Reuscher and Bauer, 2006). A controlled release formulation of LA comprising pharmaceutically acceptable carriers were designed to prevent degradation in the gastrointestinal tract (GIT) and to obtain desired serum levels over an extended period. Many synthetic and natural polymers comprising polyvinylpyrrolidone, glycerol phosphatidylcholine, hydroxypropylmethylcellulose phthalate, etc. have been claimed for the preparation of the controlled

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release formulation (Byrd and Janjikhel, 2001a). Medical Research Institute has developed a new, controlled release oral formulation of LA based on polymeric cellulose resins (Byrd and Janjikhel, 2001b; Byrd, 2003). Another study also reported on the preparation and characterisation of solid lipid nanoparticles containing LA. A lipid with low melting point was selected for the preparation of these nanoparticles. An entrapment efficiency of 90% was obtained and DSC analysis has confirmed the solid-like behaviour although with a very low crystallinity index (Souto et al., 2005).

In the current investigation, encapsulation of α-lipoic acid using chitosan has been undertaken. Chitosan was chosen as the encapsulant matrix due to its excellent biocompatibility and biodegradability. Lipoic acid encapsulated chitosan and placebo chitosan microspheres were prepared by spray-drying, which is a rapid, simple and cost effective process (Genta et al., 1995). The spray-dried chitosan microspheres obtained were of good morphological characteristics and a narrow size distribution. The encapsulation process may cause some structural interactions between lipoic acid and chitosan. Systematic studies to understand the effectiveness of encapsulation and the structural interactions that may result in reducing the activity or perhaps increasing the sustained release characteristics of the matrix were studied using various characterisation techniques. Microspheres were characterised by scanning electron microscopy (SEM), particle size distribution (PSD) analysis, Fourier transform infrared spectroscopy (FTIR) and differential scanning calorimetry (DSC). The antioxidant activity of encapsulated lipoic acid was studied using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay encapsulation efficiency was estimated by an extraction technique and analysed by high performance liquid chromatography (HPLC).

2. Materials and methods

2.1. Materials

LIPOEC is the product name for α-lipoic acid (LA) supplied by Cognis Corporation Cincinnati, OH, USA. Chitosan (degree of deacetylation – 90% and viscosity – 60 mPa s) was obtained from Swift chemical company, Australia. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 2-4,6-tripyridyl-s-triazine (TPTZ) were procured from Sigma–Aldrich, Australia. HPLC grade acetonitrile and AR grade ortho-phosphoric acid were obtained from Crown Scientific (Melbourne, Australia). All other reagents were of analytical grade and used as obtained.

2.2. Preparation of placebo and encapsulated lipoic acid

Placebo chitosan microspheres were prepared by spray-drying 3% (w/w) of chitosan solution prepared using 2% (w/w) aqueous acetic acid. The solution was spray-dried using a laboratory spray-dryer (Buchi). The inlet and outlet temperatures were 112 and 78 °C, respectively.

Three percent, chitosan (w/w) dissolved in 2% (w/v) aqueous acetic acid solution was added to 3% LA solution (w/w) in absolute ethanol. Chitosan and LA were taken in a weight ratio of 1:1. The mixture was stirred thoroughly to form a solution. The mixture was spray-dried using the same dryer as used for placebo preparation. The inlet and outlet temperatures were 111 and 73 °C, respectively.

2.3. Characterisation studies

SEM was carried out using a Hitachi S570 SEM at 15 kV. The samples were sprinkled on to conductive glue (Electrodag) on an aluminium SEM stub and sputter coated with gold. PSD of the microspheres was analysed in isobutanol on a Malvern Mastersizer 2000. FTIR of the conjugates was carried out on Nicolet Avatar 360 FTIR spectrometer set in transmission mode. The FTIR analysis was performed using the KBr pellet method.

2.4. Antioxidant activity

2.4.1. 2,2-Diphenyl-1-picrylhydrazyl radical scavenging activity assay

Antioxidant activity of LA-encapsulated chitosan microspheres was analysed by a modified method, as reported in literature using DPPH (Pekkarinen et al., 1999). Free LA (1 mM) solution in absolute ethanol was freshly prepared. Both the placebo and LA-loaded chitosan microsphere samples (weight of microspheres with or without 1 mM of loaded LA) were dispersed in absolute ethanol and the clear supernatant was used for the analysis. All the samples were left at room temperature for 90 min and the absorbance was measured at 516 nm. Three millilitres of 0.1 mM ethanolic DPPH was kept as control for the experiment. Free lipoic acid, placebo chitosan microspheres and LA-loaded chitosan microspheres were analysed.

$$\text{Scavenging activity (\% RSA)} = \left[\frac{1 - \text{abs (sample)}}{\text{abs (control)}}\right] \times 100$$

2.4.2. Ferric ion reducing antioxidant power (FRAP) assay

This test was used to determine the antioxidant activity of the encapsulated α-lipoic acid. The reducing capacity is expressed as a molar equivalent of Fe(II). In this method, the direct reduction of Fe(III)-2-4,6-tripyridyl-s-triazine complex (Fe(III)-TPTZ) to the Fe(II)-TPTZ form by α-lipoic acid was estimated and expressed as molar equivalent of Fe(II) (Huang et al., 2005).

20 mM $FeCl_3$ solution was prepared by dissolving 0.3244 g of $FeCl_3$ (anhydrous) in 100 mL of ultra pure water. TPTZ solution was prepared by dissolving 0.3132 g of TPTZ in 100 mL of 40 mM HCl. TPTZ reagent was prepared by mixing 2.5 mL of TPTZ solution and 2.5 mL of $FeCl_3$ solution with 20 mL of 0.03 M sodium acetate buffer (pH 3.6). Standards were prepared mixing different volumes of 0.1 mM Fe_2SO_4 solution (0–800 μL) with 2.5 mL TPTZ reagent and making the total volume to 25 mL using sodium acetate buffer to obtain 0–80 nM Fe(II). Two millilitres of each standard was mixed with 0.2 mL of sodium acetate buffer. The absorbance was measured at 593 nm in UV–vis spectrophotometer. α-Lipoic acid (0.01 g) was dissolved in 50 mL of absolute ethanol. Both placebo chitosan microspheres and α-lipoic acid encapsulated chitosan microspheres (0.02 g) were mixed separately with 50 mL of absolute

ethanol and stirred for 1 h. Two hundred microlitres of samples were mixed with 2 mL of TPTZ reagent and measured the absorbance at 593 nm. The reducing capacity was obtained from the ratio of the slope of the standard curve (= ΔA per mM) and expressed as mM equivalent of the Fe(II) in 1 g of powder.

2.5. Analysis of encapsulation efficiency

The total amount of LA-encapsulated into the microspheres was obtained by incubating/extracting the microsphere samples in a mixture of 2% aqueous acetic acid and absolute ethanol (1:1). The LA-loaded microsphere samples were also incubated/extracted using absolute ethanol. The samples in absolute ethanol were filtered (0.45 μm Millipore) prior to analysis due to the presence of a solid residue. The lipoic acid in the ethanolic extracts was measured using HPLC method adapted from Bernkop-Schnurch et al. (2004). The HPLC system consisted of a HP-1050 series quaternary HPLC pump, degasser, autosampler and diode array detector (DAD). ChemStation software (LC-3D Rev A.09.03) was used for data acquisition and instrument control. The lipoic acid samples were analysed by reversed-phase HPLC on an Alltima RP-C18 column [150 mm × 4.6 mm I.D. (5 μm), Alltech]. A ternary solvent gradient (A: H_3PO_4 (20 mM) in 10% acetonitrile; B: 10% acetonitrile; C: acetonitrile) with a flow rate of 0.8 mL/min was employed to separate the lipoic acid from other components in the samples. The lipoic acid was detected at 200 nm with the DAD.

3. Results and discussion

3.1. Scanning electron microscopy

Fig. 1a and b show the SEM pictures of placebo and LA-encapsulated chitosan microspheres. They were found to have a spherical geometry. The placebo microspheres exhibit a characteristic wrinkled surface morphology whereas the surface of the LA-loaded microspheres was found to be smooth. Similar effects were observed in our previous studies on placebo and olive leaf extract encapsulated chitosan microspheres (Kosaraju et al., 2006). The placebo and drug-loaded chitosan-gelatin and chitosan-pluronic microspheres (Huang et al., 2003) have also shown similar surface morphologies. These studies indicate that lipoic acid had a significant influence on the loaded microspheres which alters the surface morphology. The final surface area of the particles plays a crucial role in the controlled release behaviour of the delivery system (Engstrom et al., 2007).

3.2. Particle size distribution analysis

PSD analysis of placebo and lipoic acid loaded chitosan microspheres is shown in Fig. 2a and b. The volume distribution [$d(0.5)$] i.e., below 50% of placebo microspheres was found to be 3.53 μm and that of LA-encapsulated microspheres was about 7.89 μm.

The larger particle size of the LA-loaded microspheres was also evidenced from optical microscopy (results not shown).

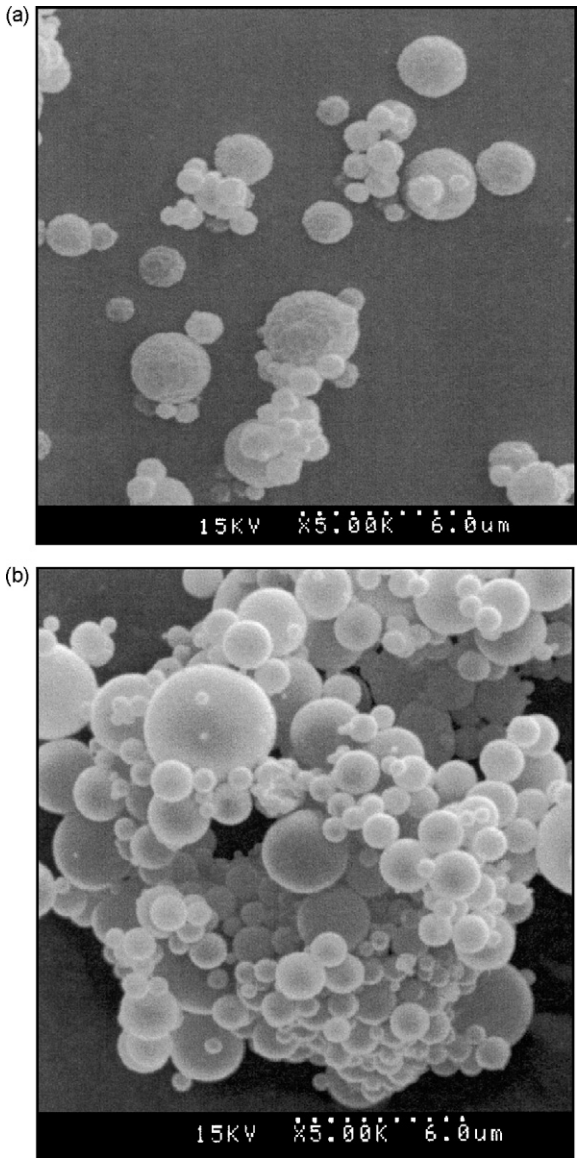

Fig. 1. (a) SEM of placebo chitosan microspheres taken at – X5.00K magnification. (b) SEM of lipoic acid encapsulated chitosan microspheres.

This may be due to the formation of aggregates due to interactions between lipoic acid and chitosan matrix.

3.3. FTIR spectroscopy

FTIR spectra of placebo and LA-loaded chitosan microspheres are shown in Fig. 3. The absorbance at 3488 cm^{-1} (–OH and –NH stretching) indicates that there is a considerable change in the peak intensities between placebo and LA-loaded microspheres. This change may be attributed to interactions between hydroxyl/amino groups of chitosan and carboxylic acid groups of lipoic acid. A small shoulder peak at 1670 cm^{-1} appeared in the spectrum of the LA-loaded sample. This may be due to overlapping of the carboxylic acid group in LA with the amide group in chitosan. The absorbance at 1375 cm^{-1} also increased in the LA-loaded sample when compared to the placebo. This may be due to the carboxylate ion stretching vibration.

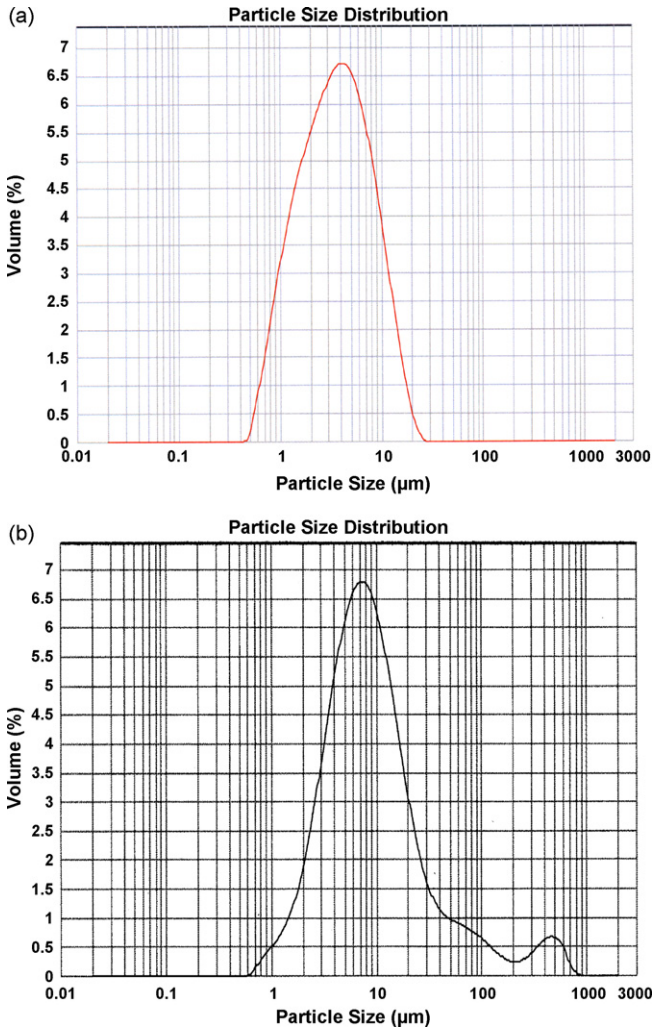

Fig. 2. (a) Particle size distribution of placebo chitosan microspheres. (b) Particle size distribution of lipoic acid encapsulated chitosan microspheres.

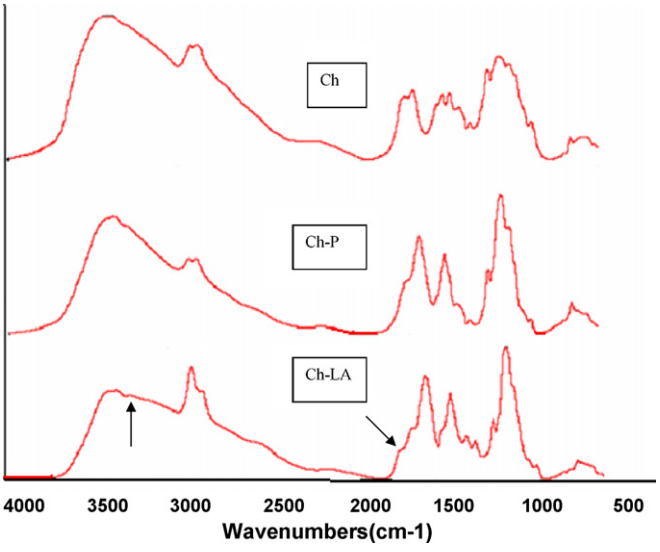

Fig. 3. FTIR of chitosan (Ch), placebo chitosan microspheres (Ch-p) and lipoic acid encapsulated chitosan microspheres (Ch-LA).

These modifications to the peak intensities indicate that there are minor interactions between the chitosan matrix and LA after encapsulation. Further studies on encapsulation efficiency are required to understand the availability of free lipoic acid after encapsulation.

3.4. Differential scanning calorimetry

DSC of placebo chitosan microspheres showed an endothermic transition at 155 °C and a clear endothermic peak at 175 °C, whereas Ch-LA microspheres displayed a broad endothermic transition ranging between 170 and 200 °C (Fig. 4). There was a second endothermic transition initiated at 200 °C, with a peak at 223 °C. Pure lipoic acid shows a melting peak at 63 °C. The first endothermic transition in Ch-LA may be attributed to the effect of structural interactions between chitosan and lipoic acid that has altered the melting of chitosan polymer. Earlier studies on thermal analysis of LA reported melting temperatures of 286 °C, along with clear-cut endothermic melting peak observed within the temperature range of 59.5–62 °C (Lvova et al., 1993). The presence of a second peak on the melting curve has been explained based on the polymerization of lipoic acid upon melting. The second endothermic transition with the peak at 223 °C in Ch-LA may be attributed to the influence of encapsulated lipoic acid which has undergone structural interaction with the chitosan matrix during the spray-drying process. The formation of a polymeric product with chitosan may also hinder the determination of the melting temperature of the un-interacted LA and may require special heating conditions as reported earlier (USSR Pharmacopia, 1987).

3.5. Antioxidant activity

In a recent study, the antioxidant activity of several phytochemicals were compared using two different assays: the oxygen radical absorbance capacity (ORAC) and the total oxyradical

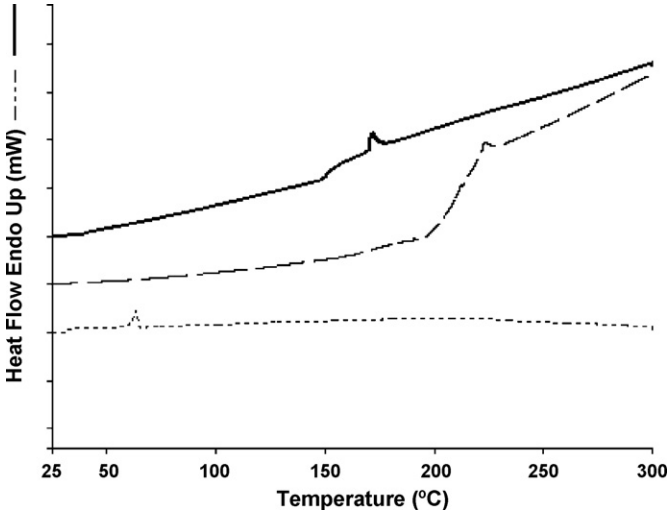

Fig. 4. DSC analysis of placebo chitosan microspheres (solid line); lipoic acid encapsulated chitosan microspheres (dashed line) and pure lipoic acid (dotted line).

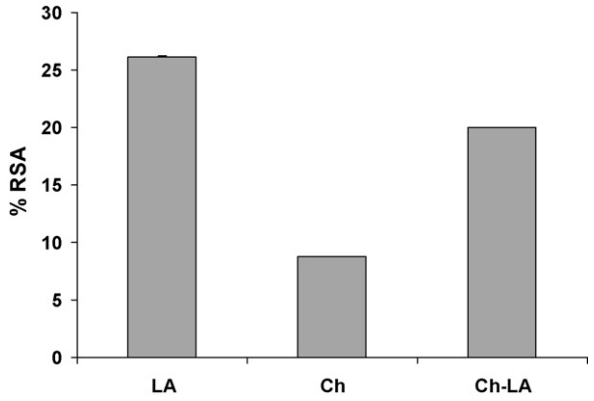

Fig. 5. Antioxidant activity by DPPH assay of free lipoic acid (LA), placebo chitosan microspheres (Ch) and lipoic acid encapsulated microspheres (Ch-LA).

scavenging capacity (TOSC). The results obtained indicate that rutin and α-lipoic acid (LA) had low ORAC values but high TOSC values (Tomer et al., 2007). LA with its antioxidant and oxidant scavenging properties may be of potential therapeutic value in protecting the liver against oxidative injury due to ischemia-reperfusion (Dulundu et al., 2007). Fluorinated amphiphilic LA derivatives were found to have antioxidant activity in both in-vitro and in-vivo studies (Ortial et al., 2006). The scavenging ability of LA towards superoxide anions, hydroxyl radicals and hydrogen peroxide was evaluated by chemiluminescence. This study demonstrates that LA scavenges superoxide anions effectively in neutral or acidic environment and is concentration dependent (Li et al., 2004).

The antioxidant activity of free and encapsulated LA was studied using the free-radical scavenging activity of DPPH radicals (Fig. 5). The assay is based on the measurement of the reducing ability of antioxidants towards DPPH free radical. Free LA exhibited a maximum of 26% of radical scavenging activity as tested in this study. Placebo chitosan microspheres had about 8% and LA-loaded chitosan microspheres showed about 20% of free-radical scavenging effect. Incomplete extraction of the encapsulated LA due to structural interactions may contribute to the lower antioxidant activity when compared to the free lipoic acid. The radical scavenging activity of placebo chitosan microspheres indicates the added advantage of the matrix effect in enhancing the antioxidant activity of encapsulated LA. Chitosan has been documented extensively for its antioxidant properties in many previous studies (Xie et al., 2001; Tharanathan and Kittur, 2003). It was reported that there was a 99% reduction in the lipid oxidation product (hexanal) in meat after treatment with *N*-carboxymethyl chitosan. *N*,*O*-carboxymethyl chitosan and its lactate and acetate salts were similarly effective in controlling the oxidation and flavour deterioration of cooked meat over a 9-day storage period at refrigerated temperatures (St. Angelo and Vercellotti, 1989). The mechanism by which this inhibition takes place is thought to be related to the chelation of iron, which is released from hemoproteins of meat during heat processing. This would, in turn, inhibit the catalytic activity of ferric ions on lipid oxidation.

FRAP assay was developed by Benzie and Strain (1996) to measure reducing power in plasma, but the assay has been adapted to measure antioxidant activity of several botanical compounds (Gil, 2000). The antioxidant activity of lipoic acid encapsulated in chitosan micropsheres was found to be lower than the same concentration of free lipoic acid (Fig. 6). When encapsulated lipoic acid was extracted with 2% acetic acid and absolute ethanol (1:1) the antioxidant activity obtained was found to be higher {Ch-La(b)} than ethanol extracted lipoic acid {Ch-La(a)}. This indicates the structural interaction between chitosan matrix and lipoic acid as discussed earlier. The antioxidant activity of placebo chitosan microspheres was found to be negligible by FRAP as compared to the DPPH assay.

3.6. Encapsulation efficiency of LA

Encapsulation efficiency of LA-chitosan microspheres was 50.6% (S.D. ± 0.77) using absolute ethanol as the extraction solvent. With the 2% aqueous acetic acid and absolute ethanol (1:1) solvent mixture, the encapsulation efficiency increased to 55.2% (S.D. ± 0.57). Increasing the extraction time from 1 to 2 h did not improve the extraction efficiency in both solvent systems. The amount of LA extracted with the 2% aqueous acetic acid and absolute ethanol (1:1) solvent mixture did not increase, even after 48 h.

The remaining 45% of LA in the LA-chitosan microspheres was either still bound to the chitosan matrix or was polymerised during the spray-drying process. However, in a previous study, it was reported that high levels of acetic acid [Chitosan:acetic acid (1:1)] were required to release all the bound LA from LA-chitosan tablets (Bernkop-Schnurch et al.,2004). Lipoic acid polymerisation would not have occurred in these LA-chitosan tablets as they were prepared at low temperature (37 °C). This suggests that the missing lipoic acid in the LA-encapsulated chitosan microspheres was still bound to the chitosan after extraction with the ethanol/acetic acid solvent mixture.

From existing knowledge on the enzymatic degradation of chitosan in the gastrointestinal tract (Nordtveit et al., 1996) and the ionic interactions with bile salts, most of the lipoic acid in the

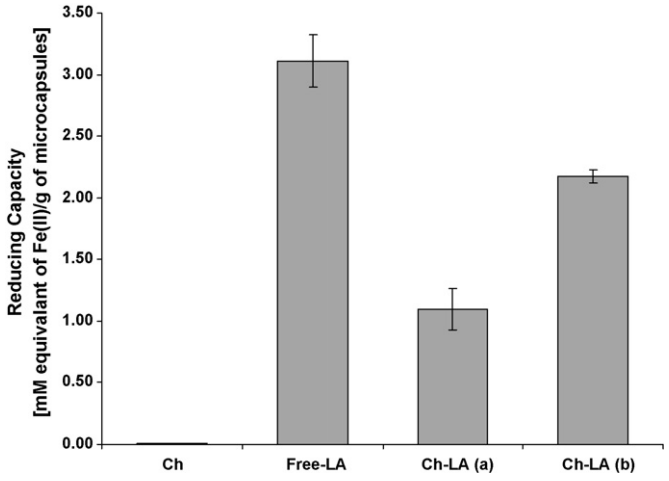

Fig. 6. Antioxidant activity by FRAP assay of free lipoic acid (LA), placebo chitosan microspheres (Ch), lipoic acid encapsulated microspheres {Ch-LA (a)} and lipoic acid encapsulated microspheres {Ch-LA (b)}.

LA-encapsulated chitosan microspheres may be slowly released during digestion.

4. Conclusion

Encapsulation of LA using chitosan as encapsulant material was carried out by a spray-drying process. Both the placebo and LA-encapsulated particles exhibited spherical geometry. LA-loaded microspheres had smooth surface morphology. Particle size distribution of placebo and LA-loaded microspheres indicated that 50% of the particles were less than 3.53 and 7.89 μm diameter, respectively. Studies on antioxidant activity of encapsulated LA demonstrated a significant level of retention of activity when compared to free lipoic acid. FTIR and DSC analyses revealed minor interactions with the chitosan matrix after encapsulation of LA. Encapsulation efficiency of LA was about 55% as determined by the extraction method. The retention of the non-extractable LA in the chitosan matrix may provide a sustained release of the antioxidant for an extended period of time.

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References

Benzie, I.F.F., Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal. Biochem. 239, 70–76.

Bernkop-Schnurch, A., Schuhbauer, H., Clausen, A.E., Hanel, R., 2004. Development of sustained release dosage form for α-lipoic acid. I. Design and in vitro evaluation. Drug Dev. Ind. Pharm. 30, 27–34.

Byrd, E.A., 2003. Controlled release lipoic acid. US Patent US 6,572,888 B2, 3 Jun.

Byrd, E.A., Janjikhel, R., 2001a. Method for reducing serum glucose levels. US Patent US 6,191,162 B1, 20 Feb.

Byrd, E.A., Janjikhel, R., 2001b. Controlled release lipoic acid. US Patent US 6,197,340 B1, 6 Mar.

Dulundu, E., Ozel, Y., Topaloglu, U., Sehirli, O., Ercan, F., Gedik, N., Sener, G., 2007. Alpha-lipoic acid protects against hepatic ischemia-reperfusion injury in rats. Pharmacology 79, 163–170.

Engstrom, J.D., Simpson, D.T., Lai, E.S., Williams III, R.O., Johnston, K.P., 2007. Morphology of protein particles produced by spray freezing of concentrated solutions. Eur. J. Pharm. Biopharm. 65, 149–162.

Genta, I., Pavanetto, F., Conti, B., Glunchedi, P., Conte, U., 1995. Improvement of dexamethasone dissolution rate from spray-dried chitosan microspheres. S.T.P. Pharm. Sci. 5, 202–207.

Gil, M.I., 2000. Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. J. Agric. Food Chem. 48, 4581–4589.

Huang, Y.-C., Chiang, C.-H., Yeh, M.-K., 2003. Optimizing formulation factors in preparing chitosan microparticles by spray-drying method. J Microencapsul. 20, 247–260.

Huang, D., Ou, B., Prior, R.L., 2005. The chemistry behind antioxidant assays. J. Agric. Food Chem. 53, 1841–1856.

Kosaraju, S.L., D'ath, L., Lawrence, A., 2006. Preparation and characterisation of chitosan microspheres for antioxidant delivery. Carbohydr. Polym. 64, 163–167.

Li, Y., Zhao, Y., Yu, W., Jiang, S., 2004. Scavenging ability on ROS of alpha-lipoic acid (ALA). Food Chem. 84, 563–567.

Lvova, M.Sh., Kozlov, E.I., Wishnevezkaya, I.A., 1993. Thermal analysis in the quality control and standardization of some drugs. J. Thermal Anal. 40, 405–411.

Nordtveit, R.J., Varum, K.M., Smidsrod, O., 1996. Degradation of partially *N*-acetylated chitosans with hen egg white and human lysozyme. Carbohydr. Polym. 29, 163–167.

Ortial, S., Durand, G., Poeggeler, B., Polidori, A., Pappolla, M.A., Boker, J., Hardeland, R., Pucci, B., 2006. Fluorinated amphiphilic amino acid derivatives as antioxidant carriers: a new class of protective agents. J. Med. Chem. 49, 2812–2820.

Pekkarinen, S.S., Stockmann, H., Schwarz, K., Heinonen, I.M., Hopia, A.I., 1999. Antioxidant activity and partitioning of phenolic acids in bulk and emulsified methyl linoleate. J. Agric. Food Chem. 47, 3036–3043.

Reuscher, H., Bauer, M., 2006. Process for preparing an alpha-lipoic acid/cyclodextrin complex and product prepared. United States Patent Application Publication US 2006/0135475 A1, 22 Jun.

Scott, B.C., Aruoma, O.I., Evans, P.J., O'Neil, C., Van der Vliet, A., Cross, C.E., Tritschler, H., Halliwell, B., 1994. Lipoic and dihydrolipoic acids as antioxidants, a critical evaluation. Free Radic. Res. 20, 119–133.

Souto, E.B., Muller, R.H., Gohla, S., 2005. A novel approach based on lipid nanoparticles (SLN^R) for topical delivery of α-lipoic acid. J. Microencapsul. 22, 581–592.

St. Angelo, A.J.,Vercellotti, J.R., 1989. Inhibition of Warmed-Over Flavour and Preserving of Uncured Meat Containing Materials. US patent, 4, 871,556. 3 Oct.

Teichert, J., Kern, J., Tritschler, H.-J., Ulrich, H., Preib, R., 1998. Investigations on the pharmacokinetics of α-lipoic acid in healthy volunteers. Int. J. Clin. Pharm Th. 36, 625–628.

Tharanathan, R.N., Kittur, F.S., 2003. Crit. Rev. Food Sci. Nutr. 43, 61–87.

Tomer, D.P., Mcleman, L.D., Ohmine, S., Scherer, P.M., Murray, B.K., O'Neill, K.L., 2007. Comparison of the total oxyradical scavenging capacity and oxygen radical absorbance capacity antioxidant assays. J. Med. Food 10, 337–344.

The USSR State Pharmacoepia, 11th ed., 1, 1987, pp. 16.

Xie, W., Xu, P., Liu, Q., 2001. Antioxidant activity of water-soluble chitosan derivatives. Bioorg. Med. Chem. Lett. 11, 1699–1701.