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Microencapsulation of ubiquinone-10 in carbohydrate matrices for improved stability

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ABSTRACT

Ubiquinone-10 (CoQ10), a vitamin-like lipophilic compound mainly used in the food and pharmaceutical industries, is vulnerable to light, oxygen and temperature. Microencapsulation of this bioactive material would protect it from such degradative effects. The present study deals with selection of a proper diluent for CoQ10 and emulsion stability followed by microencapsulation using spray drying. Microencapsulation of CoQ10 was performed using different blends of gum arabic, maltodextrin and modified starches as wall materials. The microcapsules were evaluated for the content and storage stability of CoQ10 for 6 weeks. The photostability of encapsulated CoQ10 was evaluated by exposing microcapsules to UV light for 120 min. CoQ10 microcapsules prepared using pure gum arabic as wall material had an enhanced stability at $30 \pm 2\,^{\circ}C$ as well as under UV light as compared to free CoQ10, as seen from the $t_{1/2}$ values of the same.

1. Introduction

CoQ10 is an endogenous antioxidant generally found in the inner mitochondrial membranes and is essential for the production of cellular energy in the form of ATP (Kommuru, Ashraf, Khan, & Reddy, 1999). CoQ10 mediates electron transfer between the flavoproteins and cytochromes in the mitochondrial respiratory chain (Ernster & Dallner, 1995). Ubiquinol, a fully reduced form of CoQ10, inhibits lipid peroxidation in biological membranes and protects mitochondrial proteins and DNA from oxidative damage (Ernster & Dallner, 1995). This bioactive compound has been reported to be a potential candidate for the treatment of various diseases such as cardiovascular disease (Greenberg & Frishman, 1990; Rosenfeldt et al., 2005; Sarter, 2002), Parkinson's disease (Shults, Beal, Song, & Fontaine, 2004) and Huntington's disease (The Huntington Study Group, 2001). Biosynthesized CoQ10 is concentrated in the heart, kidneys, liver, muscle, pancreas, and thyroid glands. A decrease in CoQ10 level in different organs with age is well known (Kalen, Appelkvist, & Dallner, 1989). Weber, Bystead, and Holmer (1996) reported that CoQ10 can be obtained from foods such as meat and fish but its concentration is very low. Hence, some nutritionists have considered CoQ10 suitable as a dietary supplement.

The reasons for human CoQ10 deficiency are mutations in CoQ biosynthetic enzymes and/or mutations indirectly leading to low CoQ10 levels. This has been associated with cases of encephalomyopathy, cerebellar ataxia and Leigh syndrome (Mollet et al., 2004; Quinzii et al., 2006). Some of these CoQ10 deficient patients showed clinical improvements following CoQ10 supplementation treatments (Artuch et al., 2006; Quinzii et al., 2006). The use of CoQ10 is also gaining popularity in the cosmetic industry owing to its antioxidant properties (Hoppe et al., 1999). Many reports suggest external supplementation of CoQ10 to support and stabilize mitochondrial oxidative phosphorylation (Yen et al., 2005), function as a gene regulator (Groneberg et al., 2005), and protect certain cell types (Matthews, Yang, Browne, Baik, & Beal, 1998; Sharma, El Refaey, & Ebadi, 2006). Reports of these findings on the potential health benefits of CoQ10 have led to an increase in consumer demand for this molecule, which has been paralleled by intensified efforts in the development of bioprocesses for its commercial production and thus development of suitable preparations.

CoQ10 has a high molecular weight, is strongly lipophilic, and is almost insoluble in aqueous solution; it is absorbed slowly and incompletely from the small intestine with poor bioavailability in humans (Johns Cupp & Tracy, 2002; Miles et al., 2002; Siekmann & Westesen, 1995). Numerous formulations of CoQ10 including tablets (chewable and non-chewable), powder filled capsules and soft gels containing an oil suspension (Bhagavan & Chopra, 2007) are available in the market. Approaches such as molecular complexes (Cao et al., 2006; Terao et al., 2006), emulsions (Carli,

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Chiellini, Bellich, Macchiavelli, & Cadelli, 2005), and liposome systems (Xia, Xu, Zhang, & Zhong, 2007) have been used to improve the oral bioavailability of CoQ10.

CoQ10 is vulnerable to heat, light and oxygen. Microencapsulation of this bioactive material would protect it from such degradative effects. Although previous work on emulsion stability (Thanatuksorn, Kawai, Hayakawa, Hayashi, & Kajiwara, 2009) has been reported, surprisingly work on microencapsulation of CoQ10 has not been fully explored. The purpose of this study was to prepare microcapsules of CoQ10 by spray drying using different wall materials, and further to evaluate their stability during storage and UV light exposure. During this study, selection of a suitable diluent based on CoQ10 solubility therein was also undertaken.

2. Materials and methods

2.1. Materials

CoQ10 was procured from Balaji Amines Pvt. Ltd., Secunderabad, India. Sodium stearoyl lactylate (SSL) was a gift from Ricinash Oil Mill Ltd., Mumbai, India. Gum Arabic (GA), glycerol monostearate (GMS), lecithin (LC), tween 80 (TW), *n*-hexane, ethanol and 1,4-dioxane were procured from S.D. Fine Chemicals Pvt. Ltd., Mumbai, India. Modified starch (MS) i.e. *n*-OSA starch (CLEARGUM CO 01) and maltodextrin (MD) (GLUCIDEX D17) were gifts from Roquette, Mumbai, India. Saffola oil, safflower oil, olive oil, rice bran oil, coconut oil, and flaxseed oil were procured from a local market, Mumbai, India. All other chemicals used in this study were of AR grade.

2.2. Determination of the solubility of CoQ10 in different oils

Each oil (1 mL) was pipetted into amber coloured or opaque tube at 37 °C. CoQ10 was added to the tubes up to saturated concentration with stirring at 200 rpm in an incubator shaker (Remi Pvt. Ltd., Mumbai, India) at 37 °C. The mixtures were then equilibrated for 24 h and centrifuged at 7000 × *g* for 1 min to separate the insoluble CoQ10. Aliquots of the supernatant (5 μl) were diluted with *n*-hexane, and the total volume of the solutions was adjusted to 25 mL. In order to determine the CoQ10 concentration of the solutions, optical density at 270 nm was determined at 37 °C using UV-spectroscopy (Helios, Themo Scientific, Mumbai, India). The oil-*n*-hexane solutions without CoQ10 were used as blanks. A standard graph was plotted for CoQ10 with the same set of experimental conditions and using a molar absorptivity of 13.359 mol/l/cm for CoQ10 (Thanatuksorn et al., 2009). Statistical analysis of experimental data was performed using a *t*-test (using Microsoft Excel). Statistical significance was expressed at the $P < 0.05$ level.

2.3. Preparation of different oil emulsions

Different oils (olive oil, safflower oil, coconut oil, saffola oil, flaxseed oil and rice bran oil) were taken at concentrations of 10, 20 and 30% (w/w) of GA dispersion medium which was prepared at 20% (w/v) in water. In each oil, CoQ10 was added at 200 mg/mL, four surfactants *viz.* SSL, LC, GMS and TW were added individually at 1.0% (w/v of GA solution) and test emulsions (10 mL) were prepared using a ultra-sonifier (ANALOG UNITS Model S250A, Branson Ultrasonics Corporation, Danbury, CT, USA) at 200 W for 5 min with an intermittent cycle of 1 min on/off in an ice bath.

2.4. Characterization of emulsions

2.4.1. Evaluation of stability of emulsions

The stability of each emulsion system was evaluated visually (keeping emulsions at room temperature, 30 ± 2 °C for 24 h) and by determining droplet size. Emulsion droplet size was measured using a BioVis Image analyzer plus (Expert Vision Labs Pvt. Ltd., Mumbai, India). It consists of a compound microscope, a digital camera and image analyzing software. A fine drop of emulsion was transferred to a microslide, smeared and then covered with a cover slip. This formed a very thin layer of emulsion on the surface of the microslide. The slide was then kept on the stage of the compound microscope, and 10 snaps were taken which were analyzed in terms of droplet size distribution, maximum, minimum and mean aspect (spheroidal) and minor and major axes (elliptical) of the droplets. For the present study, we took the mean aspect of the particle as a parameter for determining the droplet size, considering the droplets to be spheroidal. A representative image of droplet size determination study of emulsion is shown in Fig. 1.

2.4.2. Determination of viscosity of the emulsion

Viscosity of emulsions was determined using a Haake Rotoviscometer (VT550) with a sensor system MV1 (cup) and MV standard (rotor). The viscosity was measured at a constant shear rate of 245.0 s^{-1}. A time period of 1 min was maintained for all viscometric measurements. All measurements were performed in triplicates.

2.5. Optimization of oil loading to prepare dried microcapsules

The oil loading was optimized for two oils (coconut oil and flaxseed oil in which CoQ10 had the highest solubility and emulsion stability) by taking GA dispersion medium (carrier material) and SSL as emulsifier. The oil concentrations used were 10, 15, 20, 40 and 60% (w/w) of GA prepared as dispersion medium. Emulsions of the above mentioned oil loading were prepared using a homogenizer (Model type SPM-9, Indofrench Industries Engineers, Mumbai, India) at 3000 rpm for 10 min, spray dried and the microcapsules thus obtained were analyzed for their encapsulation and entrapment efficiencies. The droplet size of emulsions was comparatively similar as that of emulsions prepared by ultra-sonifier. Hence for ease of handling larger volumes, a homogenizer was used in these set of experiments. The loading that gave the highest encapsulation and entrapment efficiency was considered as the optimized oil loading. Further experimental runs were carried out using this optimized oil loading.

2.6. Experimental design of different batches

An augmented simplex-centroid mixture design was chosen to evaluate the blends of GA, MS and MD as wall material. Experiments on blends of wall materials are a special case of response-surface

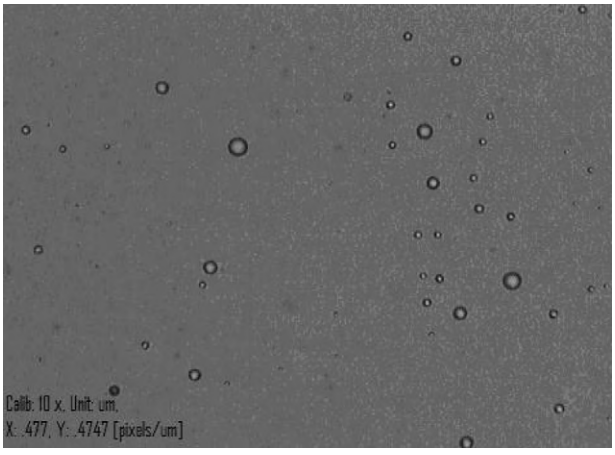

Fig. 1. Representative image of droplet size determination study of emulsion.

experiments in which the response depends only on the proportion of the various components rather than on their absolute amounts.

If x_1, x_2, and x_k are the variables representing the proportion of the k ingredients or components of the mixture,

$$\sum_{i=1}^{k} x_i = x_1 + x_2 + \cdots + x_k = 1 \quad (1)$$

The values of x_i are constrained such that, $(0, x_i, 1)$ with $i = 1, 2, k$, and the proportions of the k ingredients in the mixture sum to unity.

In addition, many mixtures require some or all components to be present in at least minimum proportions. Lower bounds L_i on component proportions impose the constraint $(0, L_i, x_i, 1)$. To simplify the construction of the design coordinates, a set of pseudo-components are constructed by coding the original component variables to a simplex coordinate system for the pseudo-component's variable X_i, with constraint $(0, X_i, 1)$. If the lower bound for component i is L_i and $L = L_i$, then the pseudo-component X_i is computed as:

$$X_i = \frac{X_i - L_i}{1 - L} \quad (2)$$

The proportions of the original components required for mixtures in the experiment can be derived by the reverse transformation once the L boundaries have been established.

$$x_i = L_i + X_i(1 - L_i) \quad (3)$$

Since one of the objectives of this study was to investigate a potential replacement of GA with other carriers, a lower bound $L = 0\%$ was established for GA (0–100%), whereas MS and MD were given a lower bound $L = 0\%$ (0–100% range). Experimental designs (Table 4) were generated and analyzed by using the statistical software package Design-Expert 6.0.10 Stat-Ease Inc., Minneapolis, MN, USA.

2.7. Preparation of microcapsules by spray drying

The stable emulsions (as indicated in Sections 2.5 and 2.6) prepared by using a homogenizer were spray dried in JISL, LSD-48 mini spray drier (Mumbai, India) (inside chamber dimension: 100 cm height, 60 cm diameter) equipped with 0.5 mm diameter nozzle. The pressure of compressed air for the flow of the spray was adjusted to 2 bar. The inlet and outlet temperatures were maintained at 120 and 85 °C, respectively. A peristaltic pump was used to feed the spray drier at 400 mL/h. The microcapsules so prepared were collected from the collecting chamber.

2.8. Stability of CoQ10 microcapsules

2.8.1. Storage stability of CoQ10 microcapsules

The free and CoQ10 microcapsules obtained after simplex-centroid study were analyzed for CoQ10 content during 6 weeks storage at 30 ± 2 °C for %CoQ10 retention in the microcapsules. A semi-log graph of % retention of CoQ10 vs. time was plotted to obtain the rate constant (k) as the slope of the graph. The half-life, $t_{1/2}$, for the retention of CoQ10 was calculated from the rate constant as $0.693/k$ (Sinko, 2005). The half-life is the time required for the reduction of a value to 50% of its original concentration.

2.8.2. Storage stability of CoQ10 microcapsules under UV light

Stability of free and microencapsulated CoQ10 was studied under UV light over a period of 2 h as reported by Fir, Smidovnik, Milivojevic, Zmitek, and Prosek (2009). The wavelength of UV light was 254 nm and distance between sample and UV lamp was 15 cm. Half-life for the retention of CoQ10 under UV light was calculated.

2.9. Determination of total CoQ10 concentration and surface concentration of microcapsules by HPLC

200 mg of CoQ10 microcapsules were dissolved in 20 mL hexane and sonicated in a tub-sonicator (Biosystem Pvt. Ltd., Mumbai, India) for 10 min. The hexane layer was removed using centrifugation and residual microcapsules were added to 15 mL water and sonicated in a tub-sonicator containing cold water (~15 °C) for 10 min. Twenty millilitres of hexane was added in the mixture and it was again sonicated for 10 min. The hexane layer was then separated using a separating funnel. For determination of surface concentration of CoQ10, 200 mg of microcapsules were dissolved in hexane for 10 min and the hexane layer was collected for analysis. The hexane layer so obtained was evaporated to dryness on a rotavapor (Buchi Rotavapor R-124, Flawil, Switzerland). CoQ10 samples were dissolved in 1,4-dioxane and analyzed by HPLC (Jasco, Japan) equipped with a Sperisorb C18 column. Elution was carried out with 100% ethanol at a flow rate of 1 mL/min (Lee, Her, Kim, & Seo, 2004). Detection was carried out with a UV detector at 275 nm. The amount of CoQ10 was estimated from the calibration curve obtained with an authentic sample.

2.10. Determination of encapsulation efficiency

Encapsulation efficiency indicates the efficiency of the process to encapsulate CoQ10 in different carrier materials and is calculated as the ratio between the mass of CoQ10 present in the microcapsules and the mass of CoQ10 added during the time of emulsion formation before spray drying. The encapsulation efficiency was calculated using the formula:

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Total CoQ10 by experimental determination (g/g powder)}}{\text{Theoretical loading (g/g powder)}} \times 100 \quad (4)$$

2.11. Determination of entrapment efficiency

The entrapment efficiency was calculated according to Lin, Lin, and Hwang (1995) as follows:

$$\text{Entrapment efficiency (\%)} = \frac{\text{Total CoQ10 (g/g of powder)} - \text{Surface CoQ10 (g/g of powder)}}{\text{Total CoQ10 (g/g of powder)}} \times 100 \quad (5)$$

3. Results and discussion

3.1. Solubility of CoQ10 in different oils

Fig. 2 shows the solubility of CoQ10 in the tested oils at 37 °C. The solubility of CoQ10 depended on the type of oil used and was maximum in coconut and flaxseed oils. It should be noted that coconut oil showed significantly higher solubility of CoQ10 than the other oils used in the study. Solubility differences could be due to temperature dependence and the effect of specific fatty acids present in the oils. In the present study, we had investigated the solubility of CoQ10 in different oils. The solubility of CoQ10 was observed to be in the order of coconut > flaxseed > safflower > saffola > olive > rice bran at 37 °C. Kommuru, Gurely, Khan, and Reddy (2001) investigated the solubility of CoQ10 in various fats at 37 °C and demonstrated that fats having medium-chain-length fatty acids provided higher solubility than those consisting of long-chain fatty acids. It is known that olive oil, safflower oil and saffola oil have long-chain fatty acids (stearic acid, oleic acid, linoleic acid, and/or

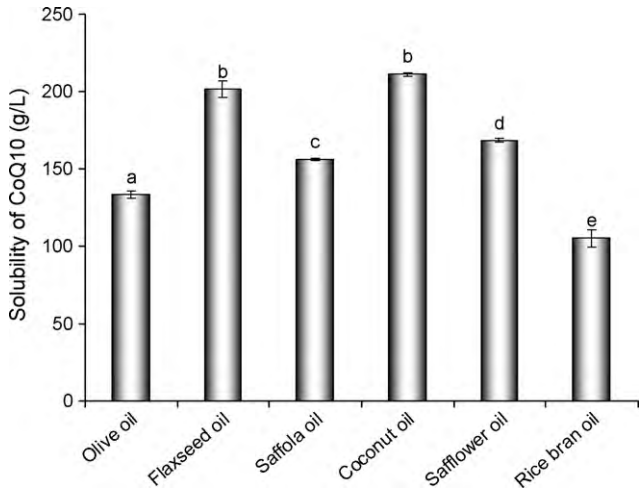

Fig. 2. Solubility of CoQ10 in different oils. All the results were mean ± SD of three individual determinations. Figure showing same alphabets are not significantly different ($P < 0.05$).

palmitic acid) as major components. Coconut oil, on the other hand, has a fatty acid of medium-chain length (lauric acid) as a major component.

3.2. Emulsion stability of different oils

The stability of emulsions was evaluated on the basis of the droplet size and visual inspection after 24 h. The results of this study with coconut and flaxseed oil are listed in Table 1. It was observed that oil droplet size of stable emulsions increased negligibly. The stability of the emulsions depended mainly on the types of emulsifier used for the study. The emulsions prepared from both the oils with SSL showed maximum stability as compared to other emulsifiers. With SSL as an emulsifier, emulsions containing up to 30 g/100 g (w/w) oil were stable during storage, while those with GMS emulsions were partially stable. The stability of other oils (safflower, saffola, olive and rice bran) was also performed (data not shown) and a similar trend as that of coconut and flaxseed oil was observed. The droplet size of the emulsion increased with an increase in the oil loading (see Tables 2 and 3). Emulsifiers with medium HLB (SSL, HLB = 7–9) were observed to be more effective than those with low (GMS and LC, HLB = 3–4) and very high (TW, HLB = 14–15) HLB. From the solubility and stability data, coconut and flaxseed oils with SSL as an emulsifier were taken for further studies.

3.3. Determination of coconut and flaxseed oil loading capacity in emulsions

The oil loading was optimized by taking 20% GA and 1% (w/v) SSL as the standard carrier material and emulsifier, respectively. Tables 2 and 3 document the results for determination of maximum oil loading with coconut and flaxseed oils, respectively. In the case of both the oils, emulsions were stable for 24 h with all oil loading tested (up to 60% (w/w) of GA). It was observed that an increase in oil loading (up to 60%, w/w) resulted in exponential increase of viscosity with both the oils. This could be due to the properties of the oil itself. The mean droplet sizes of the emulsions also increased with an increase in oil loading, probably due to the greater tendency of the oil to form agglomerates at higher oil levels, which is not desirable. The oil droplet size of flaxseed oil was slightly higher than that of coconut oil at respective levels of oil loading.

Other parameters for deciding the optimized oil loading were encapsulation and entrapment efficiencies. It was observed that 15% oil loading gave the best encapsulation and entrapment efficiencies (see Tables 2 and 3), and hence was considered as the optimized loading for further experimental batches. It has been suggested that high solids in the dryer feed increase the retention during spray drying by reducing the time required in forming a semi-permeable membrane at the drying particle surface, thus increasing the encapsulation efficiency (Reineccius & Coulter, 1969). Considering the solubility of CoQ10, entrapment and encap-

Table 1
The average droplet size and stability of emulsions after storage.

Oil	Emulsifier	Amount of oil (g/100 g)	Emulsion droplet size[a] (μm)		Stability
			0 h	24 h	
Coconut oil	SSL	10	6.79 ± 0.08	6.84 ± 0.01	+ +
		20	6.98 ± 0.13	7.15 ± 0.02	+ +
		30	7.00 ± 0.06	7.25 ± 0.02	+ +
	GMS	10	7.20 ± 0.04	7.25 ± 0.01	+ –
		20	7.40 ± 0.16	7.41 ± 0.02	+ –
		30	7.41 ± 0.18	7.62 ± 0.05	+ –
	LC	10	7.12 ± 0.22	NA	– –
		20	7.13 ± 0.32	NA	– –
		30	7.26 ± 0.03	NA	– –
	TW	10	7.16 ± 0.32	NA	– –
		20	7.66 ± 0.45	NA	– –
		30	8.10 ± 0.02	NA	– –
Flaxseed oil	SSL	10	6.51 ± 0.08	6.76 ± 0.07	+ +
		20	6.28 ± 0.21	6.82 ± 0.02	+ +
		30	6.49 ± 0.09	6.84 ± 0.07	+ +
	GMS	10	6.51 ± 0.15	5.56 ± 0.03	+ –
		20	6.62 ± 0.07	6.64 ± 0.06	+ –
		30	6.78 ± 0.36	6.85 ± 0.06	+ –
	LC	10	7.13 ± 0.28	NA	– –
		20	7.45 ± 0.06	NA	– –
		30	7.54 ± 0.10	NA	– –
	TW	10	8.46 ± 0.52	NA	– –
		20	9.45 ± 0.42	NA	– –
		30	9.48 ± 0.14	NA	– –

++: stable; + –: partially stable; – –: unstable; NA: not analyzed.
[a] Results are mean ± SD of 10 individual determinations.

Table 2
Viscosity, emulsion droplet size, encapsulation and entrapment efficiencies of the microcapsules at different oil loadings of coconut oil.

GA (in %)	Oil loading (%)	Viscosity (in cP)	Emulsion droplet size[a] (μm)	Emulsion stability	Entrapment efficiency (%)	Encapsulation efficiency (%)
20	10	75	13.24 ± 0.31	++	89.13 ± 0.96	82.15 ± 2.45
20	15	102	13.69 ± 0.42	++	84.77 ± 1.44	78.70 ± 0.78
20	20	127	14.86 ± 0.11	++	68.44 ± 1.23	63.58 ± 3.17
20	40	159	15.10 ± 0.28	++	52.70 ± 2.05	51.54 ± 1.14
20	60	194	16.33 ± 0.15	++	38.24 ± 1.38	45.19 ± 1.63

++: stable emulsion.
[a] Results are mean ± SD of 10 determinations.

Table 3
Viscosity, emulsion droplet size, encapsulation and entrapment efficiencies of the microcapsules at different oil loadings of flaxseed oil.

GA (in %)	Oil loading (%)	Viscosity (in cP)	Emulsion droplet size[a] (μm)	Emulsion stability	Entrapment efficiency (%)	Encapsulation efficiency (%)
20	10	79	13.75 ± 0.08	++	89.32 ± 0.96	87.7 ± 2.45
20	15	105	14.25 ± 0.36	++	85.06 ± 1.44	81.6 ± 0.78
20	20	132	14.94 ± 0.09	++	74.47 ± 1.23	69.1 ± 3.17
20	40	167	15.37 ± 0.31	++	55.51 ± 2.05	64.58 ± 1.14
20	60	210	16.46 ± 0.38	++	40.16 ± 1.38	53.51 ± 1.63

++: stable emulsion.
[a] Results are mean ± SD of 10 determinations.

sulation efficiency and nutraceutical value of flaxseed oil, it was chosen as a better diluent for CoQ10 for further studies. Thus, an attempt was made to spray dry an emulsion containing 20% of GA as dispersion medium, 15% of oil loading (based on GA medium) in flaxseed oil and 1% (w/v) SSL as an emulsifier.

3.4. Emulsion characterization and encapsulation efficiency studies of the different experimental batches designed using simplex-centroid design

The viscosity and mean emulsion droplet size of different emulsions prepared using various dispersion media (consisting of GA/MD/MS and there blends) is shown in Table 4. Emulsions containing higher proportions of GA showed higher viscosities, whereas those having higher proportions of maltodextrin showed lower viscosities. Modified starch imparted intermediate viscosities. 100% GA gave lowest droplet size and good emulsion stability. Also, blends with higher proportions of GA gave good emulsion stabilities. Thus, high proportions of GA in the formulation were preferable (see Table 4).

The encapsulation efficiency of the various GA/MS/MD blends according to simplex-centroid design is shown in Table 4. Pure GA gave the best encapsulation efficiency, although the blend with 67% GA (GA67-MS17-MD17) gave similar results. From Table 4, it was observed that the emulsions with higher GA supported higher encapsulation efficiency. Thus, pure GA had the best encapsulation efficiency and no other wall material (MS, MD or its blends with GA) could replace GA to encapsulate CoQ10. ANOVA (Design-Expert 6.0.10 Stat-Ease Inc., Minneapolis, MN, USA) used for statistical analysis of simplex-centroid mixture design results showed $P < 0.001$. This confirms adequacy of design to select probable wall material for microencapsulation of CoQ10.

3.5. Storage stability of microencapsulated CoQ10

The CoQ10 microcapsules prepared as detailed in Section 2.6 (see Table 4) were subjected to storage stability study for the period of 6 weeks. Free CoQ10 and CoQ10 microcapsules so obtained with different blends were analyzed for CoQ10 retention, stored at 30 ± 2 °C. The emulsion containing 20% GA, 15% (w/w of GA) oil loading and 1% SSL as emulsifier had highest encapsulation efficiency (84.27 ± 2.76%). Microcapsules prepared using 20% GA as sole wall material gave maximum CoQ10 retention of 15.57 mg/g (%CoQ10 retention 63.60) after 6 weeks as compared to that of other blends. Microcapsules prepared using blend of GA67 + MS17 + MD17 showed CoQ10 retention of 13.10 mg/g (%CoQ10 retention 63.33) under the same conditions. The decrease in the content of CoQ10 is attributed to poor stability of CoQ10 to oxidation and temperature (Laplante, Souchet, & Bryl, 2009).

Since GA showed maximum encapsulation efficiency as well as best stability during 6-week storage, the stability of the microcapsules under UV light was done only with microcapsules

Table 4
Simplex-centroid design used for selection of wall material during encapsulation of CoQ10 in flaxseed oil.

Run no.	GA	MS	MD	Droplet size[a] (μm)	Viscosity (cP)	Encapsulation efficiency (%)
1	1.00	0.00	0.00	14.05 ± 0.66	102	84.27 ± 2.76
2	0.00	1.00	0.00	16.99 ± 0.52	58.8	46.22 ± 1.55
3	0.00	0.00	1.00	15.44 ± 0.19	20.7	53.31 ± 3.42
4	0.50	0.50	0.00	14.58 ± 0.21	80.4	49.24 ± 2.61
5	0.50	0.00	0.50	16.81 ± 0.35	52.9	62.16 ± 0.77
6	0.00	0.50	0.50	17.04 ± 0.49	31.2	55.08 ± 1.35
7	0.33	0.33	0.33	18.37 ± 0.04	45.9	54.21 ± 2.16
8	0.67	0.17	0.17	17.46 ± 0.61	85.1	71.53 ± 2.33
9	0.17	0.67	0.17	20.69 ± 0.18	59.7	44.64 ± 1.17
10	0.17	0.17	0.67	16.56 ± 0.24	30.7	49.36 ± 3.15
11	1.00	0.00	0.00	14.12 ± 0.06	102	81.41 ± 3.06
12	0.00	0.00	1.00	15.44 ± 0.19	20.7	52.26 ± 2.51
13	0.00	1.00	0.00	16.99 ± 0.52	58.8	47.77 ± 1.17
14	0.00	0.50	0.50	17.04 ± 0.49	31.2	56.43 ± 1.44
15	1.00	0.00	0.00	14.05 ± 0.66	102	85.06 ± 1.78

[a] Results are mean ± SD of 10 determinations.

Table 5
Regression analysis of free and microencapsulated CoQ_{10} stored at ambient temperature (30 ± 2 °C).

Wall material (GA, MD, MS)	Regression equations for storage stability	Half-life, $t_{1/2}$, 30 ± 2 °C (weeks)
(1.00, 0.00, 0.00)	ln(%CoQ10 retention) = −0.078 time + 4.6316 (R^2 = 0.9868)	8.88
(0.00, 1.00, 0.00)	ln(%CoQ10 retention) = −0.0945 time + 3.977 (R^2 = 0.9911)	7.37
(0.00, 0.00, 1.00)	ln(%CoQ10 retention) = −0.1274 time + 4.092 (R^2 = 0.9802)	5.46
(0.50, 0.50, 0.00)	ln(%CoQ10 retention) = −0.15 time + 4.04 (R^2 = 0.9891)	4.62
(0.50, 0.00, 0.50)	ln(%CoQ10 retention) = −0.124 time + 4.2961 (R^2 = 0.9943)	5.58
(0.00, 0.50, 0.50)	ln(%CoQ10 retention) = −0.2176 time + 4.15 (R^2 = 0.9767)	3.19
(0.33, 0.33, 0.33)	ln(%CoQ10 retention) = −0.1798 time + 4.147 (R^2 = 0.9735)	3.87
(0.67, 0.17, 0.17)	ln(%CoQ10 retention) = −0.0762 time + 4.421 (R^2 = 0.9905)	9.09
(0.17, 0.67, 0.17)	ln(%CoQ10 retention) = −0.1694 time + 3.946 (R^2 = 0.9847)	4.09
(0.17, 0.17, 0.67)	ln(%CoQ10 retention) = −0.1928 time + 3.8626 (R^2 = 0.9902)	3.61
Free CoQ_{10}	ln(%CoQ10 retention) = −0.3837 time + 4.7817 (R^2 = 0.9653)	1.81

encapsulated in 20% GA alone as wall material. In this case also, less degradation of CoQ10 was observed with microencapsulated CoQ10 as compared to free CoQ10 (data not shown). Microcapsules prepared using 20% GA as wall material gave CoQ10 retention of 21.60 ± 0.18 mg/g (%CoQ10 retention 88.27) after 120 min. In contrast, the retention of free CoQ10 at the end of 120 min exposure to UV was a very low 4.51 mg of 10 mg taken (45.08% CoQ10 retention). This confirms microencapsulation to protect CoQ10 against UV light.

A semi-log plot of %CoQ10 retention vs. storage time for free as well as CoQ10 microcapsules of different blends stored at ambient temperature and under UV light, showed a linear nature of the graph (Figs. 3 and 4) indicating the decrease in the CoQ10 content to follow first-order kinetics. Table 5 shows regression analysis of the graphs, from which the half-life of %CoQ10 retention in all the blends was calculated. Microcapsules having GA as major wall material showed highest half-life (100% GA had a half-life of 8.88 weeks and GA67+ MS17+ MD17 had a half-life of 9.09 weeks). In contrast, free CoQ10 showed a half-life of 1.81 weeks at 30 ± 2 °C. This indicates free CoQ10 to be more susceptible to temperature and oxidation as compared to the microencapsulated CoQ10.

CoQ10 microcapsules prepared using 20% GA as wall material showed a maximum half-life of 693 min as opposed to 111.17 min for free CoQ10 under UV light. The reasons for this stability of CoQ10 under UV light are as yet unclear and need further investigation. GA as a carrier material is known to provide greater protection as compared to that of MD and MS (Reineccius, 1989). This explains the use of GA by flavour industries as a fixative in spray drying applications, wherein the gum encapsulates the flavour compound and protects it from oxidation and volatilization (Qi & Xu, 1999).

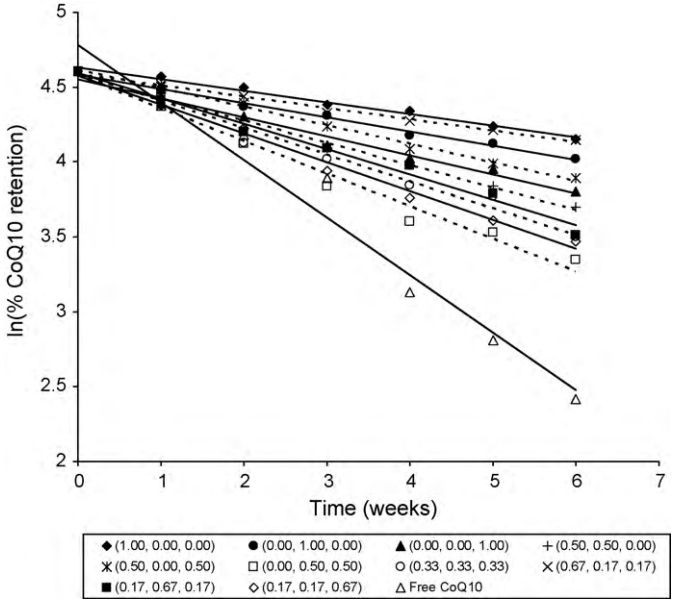

Fig. 3. Stability of CoQ_{10} encapsulated with different blends stored at room temperature (30 ± 2 °C).

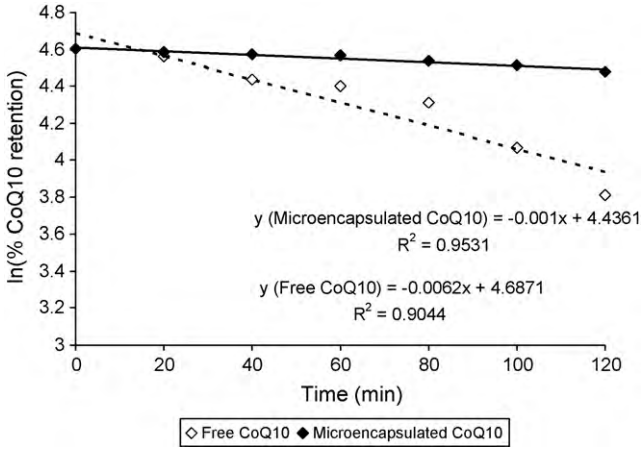

Fig. 4. Photostability of free and microencapsulated CoQ10 exposed to UV light.

4. Conclusions

CoQ10 showed better solubility in coconut and flaxseed oils than other oils used in the study. An emulsion of CoQ10 in flaxseed oil using 1% (w/v) SSL as an emulsifier at 15% loading of a dispersion medium containing 20% (w/v) GA gave microcapsules of CoQ10 on spray drying with best encapsulation and entrapment efficiency. Evaluation of other wall materials such as maltodextrin and modified starch, alone and in combination with GA for preparation of microcapsules of CoQ10 confirmed 20% GA to give maximum encapsulation and entrapment efficiency of CoQ10 during spray drying microencapsulation. Although a blend of GA67 + MS17 + MD17 gave similar retention of CoQ10 as GA alone during subsequent storage, it had a comparatively lower encapsulation and entrapment efficiency than GA alone. Encapsulated CoQ10 was significantly stable at 30 ± 2 °C as well as under UV light as compared to free CoQ10 as seen from the $t_{1/2}$ values of the same.

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References

Artuch, R., Brea-Calvo, G., Briones, P., Aracil, A., Galván, M., Espinós, C., et al. (2006). Cerebellar ataxia with coenzyme Q10 deficiency: Diagnosis and follow-up after coenzyme Q10 supplementation. *Journal of the Neurological Science*, *246*, 153–158.

Bhagavan, H. N., & Chopra, R. J. (2007). Plasma coenzyme Q10 response to oral ingestion of coenzyme Q10 formulations. *Mitochondrion*, *7*, S78–S88.

Cao, X.-L., Xu, Y.-T., Zhang, G.-M., Xie, S.-M., Dong, Y.-M., & Ito, Y. (2006). Purification of coenzyme Q10 from fermentation extract: High speed counter-current chromatography versus silica gel column chromatography. *Journal of Chromatography A*, *1127*(1–2), 92–96.

Carli, F., Chiellini, E. E., Bellich, B., Macchiavelli, S., & Cadelli, G. (2005). Ubidecarenone nanoemulsified composite systems. *International Journal of Pharmaceutics*, *291*(1–2), 113–118.

Ernster, L., & Dallner, G. (1995). Biochemical, physiological and medical aspects of ubiquinone function. *Biochimica et Biophysica Acta*, *1271*, 195–204.

Fir, M. M., Smidovnik, A., Milivojevic, L., Zmitek, J., & Prosek, M. (2009). Studies of CoQ10 and cyclodextrin complexes: Solubility, thermo- and photo-stability. *Journal of Inclusion Phenomena and Macrocyclic Chemistry*, *64*(3–4), 225–232.

Greenberg, S, & Frishman, W. H. (1990). Co-enzyme Q10: A new drug for cardiovascular disease. *Journal of Clinical Pharmacology*, *30*, 596–608.

Groneberg, D. A., Kindermann, B., Althammer, M., Klapper, M., Vormann, J., Littarru, G. P., et al. (2005). Coenzyme Q10 affects expression of genes involved in cell signalling, metabolism and transport in human CaCo-2 cells. *International Journal of Biochemistry and Cell Biology*, *37*, 1208–1218.

Hoppe, U., Bergemann, J., Diembeck, W., Ennen, J., Gohla, S., Harris, I., et al. (1999). Coenzyme Q10, a cutaneous antioxidant and energizer. *Biofactors*, *9*, 371–378.

Johns Cupp, M., & Tracy, T. S. (2002). Coenzyme Q10 (Ubiquinone, Ubidecarenone). In M. Johns Cupp, & T. S. Tracy (Eds.), *Dietary supplements, toxicology and clinical pharmacology* (p. 74). Totowa, NJ: Humana Press.

Kalen, A., Appelkvist, E. L., & Dallner, G. (1989). Age-related changes in the lipid compositions of rat and human tissues. *Lipids*, *24*, 579–584.

Kommuru, T. R., Ashraf, M., Khan, M. A., & Reddy, I. K. (1999). Stability and bioequivalence studies of two marketed formulations of coenzyme Q10 in beagle dogs. *Chemical and Pharmaceutical Bulletin (Tokyo)*, *47*(7), 1024–1028.

Kommuru, T. R., Gurley, B., Khan, M. A., & Reddy, I. K. (2001). Self-emulsifying drug delivery systems (SEDDS) of coenzyme Q10: Formulation development and bioavailability assessment. *International Journal of Pharmaceutics*, *212*, 233–246.

Laplante, S., Souchet, N., & Bryl, P. (2009). Comparison of low-temperature processes for oil and coenzyme Q10 extraction from mackerel and herring. *European Journal of Lipid Science and Technology*, *111*, 1–7.

Lee, J. K., Her, G., Kim, S. Y., & Seo, J. H. (2004). Cloning and functional expression of the *dps* gene encoding decaprenyl diphosphate synthase from *Agrobacterium tumefaciens*. *Biotechnology Progress*, *20*, 51–56.

Lin, C. C., Lin, S. Y., & Hwang, L. S. (1995). Microencapsulation of squid oil with hydrophilic macromolecules for oxidative and thermal stabilization. *Journal of Food Science*, *60*, 36–39.

Matthews, R. T., Yang, L., Browne, S., Baik, M., & Beal, M. F. (1998). Coenzyme Q10 administration increases brain mitochondrial concentrations and exerts neuroprotective effects. *Proceedings of the National Academy of Science of the United States of America*, *95*, 8892–8897.

Miles, M. V., Horn, P. S., Miles, L., Tang, P., Steele, P., & DeGrauw, T. (2002). Bioequivalence of coenzyme Q10 from over-the-counter supplements. *Nutrition Research*, *22*, 919–929.

Mollet, J., Giurgea, I., Schlemmer, D., Dallner, G., Chretien, D., Delahodde, A., et al. (2004). The bioavailability of coenzyme Q10 supplements available in New Zealand differs markedly. *New Zeland Medicine Journal*, *117*, U1108.

Qi, Z. H., & Xu, A. (1999). Starch based ingredients for flavor encapsulation. *Cereal Food World*, *44*(7), 460–465.

Quinzii, C., Naini, A., Salviati, L., Trevisson, E., Navas, P., DiMauro, S., et al. (2006). A mutation in para-hydroxybenzoate-polyprenyl transferase (*COQ2*) causes primary coenzyme Q10 deficiency. *American Journal of Human Genetics*, *78*, 345–349.

Reineccius, G. A. (1989). Carbohydrate for flavour encapsulation. *Food Technology*, *45*, 144–149.

Reineccius, G. A., & Coulter, S. T. (1969). Flavour retention during drying. *Journal of Dairy Science*, *52*(8), 1219–1222.

Rosenfeldt, F., Marasco, S., Lyon, W., Wowk, M., Sheeran, F., Bailey, M., et al. (2005). Coenzyme Q10 therapy before cardiac surgery improves mitochondrial function and invitro contractility of the myocardial tissues. *The Journal of Thoracic and Cardiovascular Surgery*, *129*, 25–32.

Sarter, B. (2002). Coenzyme Q10 and cardiovascular disease: A review. *Journal of Cardiovascular Nursing*, *16*(4), 9–20.

Sharma, S. K., El Refaey, H., & Ebadi, M. (2006). Complex-1 activity and 18F-DOPA uptake in genetically engineered mouse model of Parkinson's disease and the neuroprotective role of coenzyme Q10. *Brain Research Bulletin*, *70*, 22–32.

Shults, C. W., Beal, M. F., Song, D., & Fontaine, D. (2004). Pilot trial of high dosages of coenzyme Q10 in patients with Parkinson's disease. *Experimental Neurology*, *188*, 491–494.

Siekmann, B., & Westesen, K. (1995). Preparation and physicochemical characterization of aqueous dispersions of coenzyme Q10 nanoparticles. *Pharmaceutical Research*, *12*(2), 201–208.

Sinko, P. J. (2005). *Martin's physical pharmacy and pharmaceutical sciences*. New York: Cambridge University Press., pp. 111–112.

Terao, K., Nakata, D., Fukumi, H., Schmid, G., Arima, H., Hirayama, F., et al. (2006). Enhancement of oral bioavailability of coenzyme Q10 by complexation with γ-cyclodextrin in healthy adults. *Nutrition Research*, *26*(10), 503–508.

Thanatuksorn, P., Kawai, K., Hayakawa, M., Hayashi, M., & Kajiwara, K. (2009). Improvement of the oral bioavailability of coenzyme Q10 by emulsification with fats and emulsifiers used in the food industry. *LWT-Food Science and Technology*, *42*, 385–390.

The Huntington Study Group. (2001). A randomized, placebo-controlled trails of coenzyme Q10 and remacemide in Huntington's disease. *Neurology*, *57*, 397–404.

Weber, C., Bystead, A., & Holmer, G. (1996). The coenzyme Q10 content of the average Danish diet. *International Journal of Vitamin and Nutrition Research*, *67*, 123–129.

Xia, S., Xu, S., Zhang, X., & Zhong, F. (2007). Effect of coenzyme Q10 incorporation on the characteristics of nanoliposomes. *Journal of Physical Chemistry B*, *111*(9), 2200–2207.

Yen, D. H., Chan, J. Y., Huang, C. I., Lee, C. H., Chan, S. H., & Chang, A. Y. (2005). Coenzyme Q10 confers cardiovascular protection against acute mevinphos intoxication by ameliorating bioenergetic failure and hypoxia in the rostral ventrolateral medulla of the rat. *Shock*, *23*, 353–359.