Liposomal Encapsulation of α-lipoic Acid as a Food Supplement

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***Abstract*—α-Lipoic acid (6, 8-dithiooctanoic acid) has been attributed with many salubrious benefits such as aid in reduction of oxidative stress, reducing hypertension, improving lipid profile and averting risks of diabetes. However, the biomolecule being vulnerable to environmental oxidation is required to be encapsulated. Nanoliposomal encapsulation of α-lipoic acid was conducted in the present study using solvent evaporation followed by probe sonication with the objective of enhancing the stability of the biomolecule. The encapsulation was accomplished with optimized process parameters that were 3 g lipid phase (soya phosphatidylcholine: Tween 80= 1:1) with 150 mg lipoic acid; with the aim to achieve the recommended dietary supplementation of the biomolecule conveniently through the consumption of the liposome. The encapsulated α-lipoic acid was assessed for parameters such as encapsulation efficiency, antioxidant activity, zeta potential, and FE-SEM analyses. The data revealed successful entrapment of the biomolecule with 97% of encapsulation efficiency and desired attributes that indicate liposomal entrapment to be a suitable technique for encapsulation of α-lipoic acid.**

***Index Terms*—nanoliposome, probe sonication, lipoic acid, antioxidant potency**

I. Introduction

α-lipoic acid (ALA), chemically 1,2-dithiolane-3-pentanoic acid, has nowadays become very important constituent in dietary supplements. The biomolecule that is a natural dithiol, is synthesized in the body inside the mitochondria from octanoic acid. The biomolecule has been attributed with many benefits that include scavenging of free radicals (antioxidant activity), anti-diabetic potency, remedial action in peripheral neuropathy, aid in dermal care and as an important geriatric supplement (by restoring depleting glutathione levels) [1]-[3].

ALA plays an important role as a cofactor in mitochondrial metabolism as well (tricarboxylic acid cycle), and it has been argued that the molecule supports diverse patho-physiological conditions [3]. ALA is commonly derived from natural sources such as muscle based meats, and to a certain extent from fruits and vegetables. A dietary supplementation of ALA is often recommended since its source from nature as such is not very high. Being highly necessary supplement, ALA is readily absorbed in skeletal muscles, heart and liver. ALA has also been attributed with the potency of inhibiting diabetic micro and macrovascular disorders, and also in diabetic neuropathy [4], [5].

Being a medically and nutritionally valued supplemental biomolecule, its pre-delivery sustenance and site specific delivery in the body are important aspects. These types of medically important biomolecules are by nature vulnerable to environmental stresses, and there is also a challenge in achieving target oriented delivery, post administration. Encapsulation of biomolecules is thus an important measure that ensures meeting both the objectives [6]. It has been quite some time that encapsulation of valued biomolecules has been in practice, and with good reason. Many researchers have also previously attempted encapsulating ALA, either singly, or in combination with coat material. The authors have employed numerous techniques for achieving the entrapment that have ranged from using electrospray [7], solid-lipid nanoparticles [8], to hot high pressure homogenization [9], interfacial polymer deposition [10], and liposomal entrapment of ALA and coenzyme Q10 [11]. These authors have received success in their respective endeavors. However, a convenient, ALA targeted entrapment, along with average technological intervention during preparation is yet awaited.

The present research aims to develop a nanoliposome based encapsulate of ALA that would supplement the recommended levels of the biomolecule conveniently as well as effectively. The study focuses probing on all important aspects of such an objective by optimizing the

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formulation for liposome preparation, analysis of encapsulation efficiency, assessment of zeta potential, image analysis of the encapsulate and antioxidant potency of the ALA entrapped.

II. Materials and Methods

A. Materials

α-lipoic acid, Tween 80, soya phosphatidylcholine and dialysis membrane (cellulose membrane, molecular weight cut off 12,000 Da) were procured from Himedia, India. All chemicals used in this work were of AR grade.

B. Formulation of α-Lipoic Acid Nanoliposome

Encapsulation of ALA was conducted by probe sonication method according to the method reported by Dutta and Bhattacharjee [6] with slight modification. Briefly, for preparation of nanoliposome, lipid phase was formulated with soya phosphatidylcholine (S) and Tween 80 (T) using ethanol. Different amount of total lipid (S+T) along with different ratios of S and T were used for optimization of process to encapsulate the recommended daily dosage (RDD) of lipoic acid (100-200 mg).

To formulate nanoliposome, ALA (150 mg) was dissolved in the lipid phase and ethanol from the solution was evaporated by purging a gentle stream of nitrogen, to obtain a thin film of the lipid phase. This lipid film was rehydrated with 20 mL phosphate buffer saline (PBS, 0.01 M, pH 7.2) and stirred vigorously for 30 min to obtain multilamellar liposomal suspension. This suspension was subjected to probe sonication (VCX ultrasound processor, Sonics, New York, USA) using 19 mm probe and 80% amplitude at 20 kHz for 30 min (3 min sonication, 2 min interval for 6 times) for reduction of size and lamellarity.

After liposome preparation, the solution containing both entrapped and non-encapsulated ALA was subjected to dialysis using phosphate buffer (pH 7.0, 0.05 M) at 4 ± 1 ℃, for 24 h, to separate the non-encapsulated ALA. For that, the dialysis bag was soaked in water for at least 1 h and then 2 mL nanoliposome was placed in the bag. Then 100 mL buffer was used to dip the dialysis bag that contained the liposomal solution and the set was kept at 4 ± 1 ℃ with continuous gentle stirring for 24 h. The buffer was replaced by fresh buffer at predetermined time intervals (2, 4 and 6 h). After 24 h, nanoliposomes were recovered from the dialysis bag and stored in amber colored screw capped vials, in an inert atmosphere of nitrogen at 4±1 ℃ in the dark, until further analyses.

C. Estimation of Encapsulation Efficiency of Nanoliposomes

Encapsulation efficiency (%EE) of nanoliposome was estimated by quantifying the amount of ALA entrapped in the nanoliposome. The liposome recovered after dialysis was treated with methanol (liposome: methanol=1:2) to disrupt the lipid bilayer according to the method reported by Laouini et al. [12]. Then the encapsulated ALA was quantified by UV-Vis spectrophotometer (multimode microplate reader, SpectraMax iD3, Molecular Devices, US) at 330 nm using the standard curve of ALA. The encapsulation efficiency was calculated by the following equation (1).

$$\%EE = \frac{ALA\ encapsulated\ in\ liposome}{Initial\ ALA\ used\ for\ preparation\ of\ lipsome} \times 100 \quad (1)$$

D. Characterization of Nanoliposome

1) Field emission scanning electron microscopy (FE-SEM)

ALA prepared in optimized conditions was subjected to FE-SEM for analysis of morphology of the nanoliposome. For this, 10 μL of liposome was placed on a cover slip and dried in a desiccator. The sample was subsequently coated with gold by Autofine Coater (JFC-1600, JEOL, Japan) and analyzed by FE-SEM (JSM-6700F, JEOL, Japan) at 4 kV at working distance of 3.3 mm.

2) Transmission electron microscopy (TEM)

Nanoliposome was further analyzed by TEM using 20 times dilution of the same. The diluted liposome was placed on a carbon coated copper grid (300 mesh, Electron Microscopy Sciences, US), dried overnight and analyzed by TEM (JEM 2100, JEOL, Japan), with the instrument being operated at 200 kV, at 23 ± 2 ℃.

3) Size distribution and zeta potential of nanoliposomes

ALA nanoliposome was diluted 10 times for analysis of size distribution by dynamic light scattering (DLS) method, at a scattering angle of 90 °, using Zetasizer Nano ZS90 (Malvern Instruments Pvt. Ltd., UK) at 23 ± 2 ℃. Estimation of zeta potential was also conducted using the same instrument to analyze the surface charge of liposome.

4) Antioxidant properties of nanoliposomes

Nanoliposome prepared at the optimized conditions was treated with methanol was analyzed for the antioxidant potency by estimating the DPPH (2,2-Diphenyl-1-picrylhydrazyl) radical scavenging activity. This assay was done in accordance with the method reported by Aiyegoro and Okoh [13] and data was expressed as IC_{50} values (mg/mL).

E. Statistical Analysis

All experiments were conducted in triplicate and the data are expressed as means ± SD of three independent experimental runs. Duncan's multiple range tests with *P* value<0.05 was performed with IBM SPSS Statistics software version 20 (IBM, USA) to verify the significance of all tests. Student's t-test, one-way ANOVA, Regression analysis, predicted vs. observed efficiency and similar evaluations were performed with STATISTICA 8.0 software (Statsoft, OK, USA).

III. Results and Discussion

A. Optimization of Parameters for Formulation of Nanoliposome

To optimize the encapsulation process, preliminary study was conducted with 2 and 4 g of lipid (S+T) keeping the ratio of S:T=1:2, which showed decreased %EE with increased (4 g) lipid phase.

Therefore, optimization of S:T was conducted with 3 g lipid. Nanoliposomes were formulated with different S:T ratios (1:1, 1:2, 1:3) to optimize the same. Amount of ALA in the initial solution was also varied (150, 300 mg) to estimate the maximum amount of ALA that can be encapsulated in nanoliposome (Table I) (Fig. 1). It was found that an increase in amount of ALA in the primary solution for preparation of nanoliposome decreased the %EE significantly. Decrease in the lipid phase (2 g) also decreased the %EE. Maximum EE (97%) was observed for the liposome prepared with 3 g lipid (S+T), S:T= 1:1, and 150 mg ALA. Therefore, the process was optimized at the above mentioned conditions. The nanoliposomes prepared at the optimized conditions were subjected to further analyses. The encapsulation efficiency at the optimized conditions has been highlighted in bold (Table I).

TABLE I. OPTIMIZATION OF S:T FOR FORMULATION OF ALA NANOLIPOSOME

Run No.	Lipid (S+T) (g)	Ratio (S:T)	Lipoic acid (mg)	EE (%)*
1	3	1:1	150	**90.3 ±4^{d}**
2	3	1:2	150	60.0 ±3^{c}
3	3	1:3	150	56.8 ±3^{b}
4	2	1:1	150	55.6 ±2^{b}
5	3	1:2	300	29.3 ±1^{a}
6	3	1:3	300	31.6 ±2^{a}

*Different letters in a column indicate significant differences at P < 0.05.

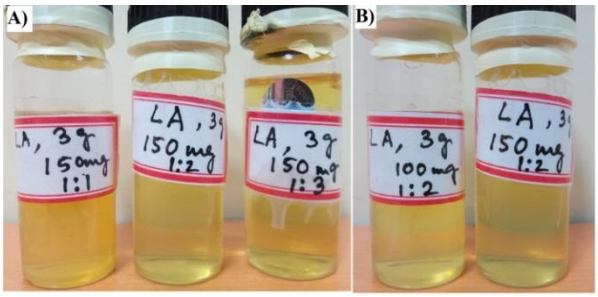

Figure 1. ALA nanoliposome at different conditions. A) With 3 g lipid and different S:T, B) With different amount of ALA

The data were congruent with statistical prediciton as well. The graph of predicted vs. observed efficiency showed a close fit (Fig. 2). Also, by t-test, it was found that each of the three indepndedent variables had a significant effect p=0.0000 on encapsulation efficiency. This reflected that by changing the compsiotion of the liposome, the efficiency could be varied.

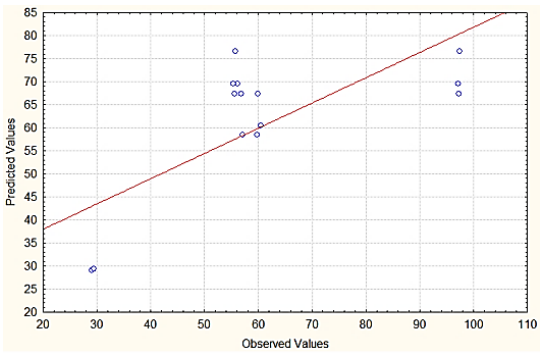

Figure 2. Predicted vs. observed encapsulation efficiency values

In fact, the relation of the independent variables on efficiency was depicted by the regression equations for the process (2) , and the 3-D surface plots (Fig. 3, 4) and corresponding equations for effects of independent variables on encapsulation efficiency (equations 3 and 4, respectively). ANOVA data has been shown in Table II.

$$\%EE = 1258.5+35.7x-29090y-0.30z \quad (2)$$

where, x is (S+T), y is (S:T) and z is lipoic acid (mg)

$$\%EE=26169.93-3.92x-1.18y-0.01x^2+1.34y^2+100.73xy \quad (3)$$

where x is lipoic acid (mg) and y is (S:T),

$$\%EE=166.02+50.22x-1.63y-0.54x^2+0.04y^2-0.21xy \quad (4)$$

where, x is lipid (S+T), y is lipoic acid (mg)

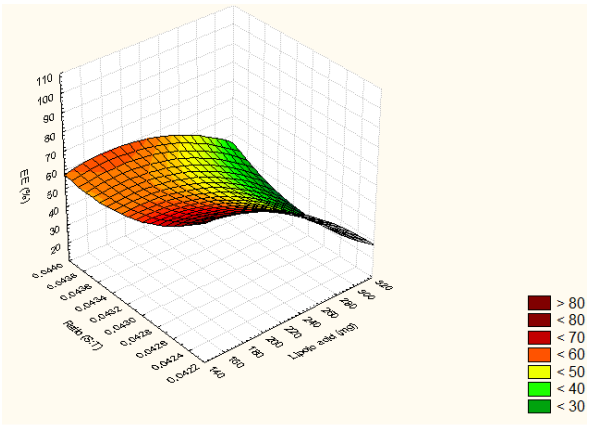

Figure 3. Surface plot (3D) of effect of lipoic acid and ratio of S:T on % encapsulation efficinecy

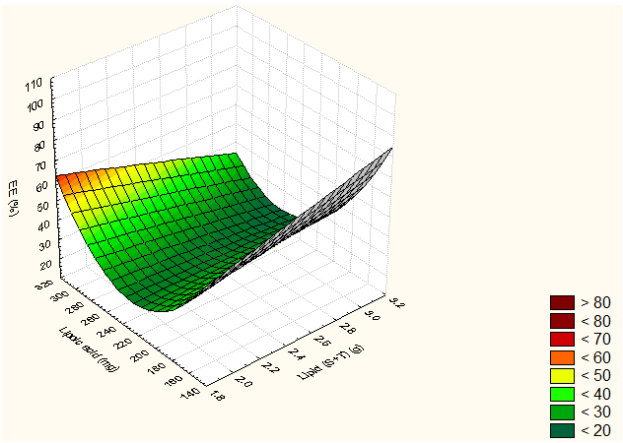

Figure 4. Surface plot (3D) of effect of lipid content and lipoic acid on % encapsulation efficiency

TABLE II. ANOVA OF EFFECT OF PROCESS VARIABLES

Effects	SS	MS	df	F
Regress.	6520.59	2173.53	3	40.84
Residual	585.34	53.22	11	
Total	7105.98			

SS-sum of squares, MS- mean of squares, df- degree of freedom, F- Fisher's index

The statistical predictions corroborated the experimental findings and provided a means to avoid experimental bias, as well as provided a feasibility analysis for a futuristic scale up of this laboratory based process.

B. Morphology Study of Nanoliposome

FE-SEM analysis of ALA nanoliposome revealed formation of spherical uniform nano vesicles, having size in the range of 17-24 nm (average particle size 20.4±5 nm) (Fig. 5A). Similar visible distinct nano structure was also obtained from TEM analysis of the liposome (Fig. 5B).

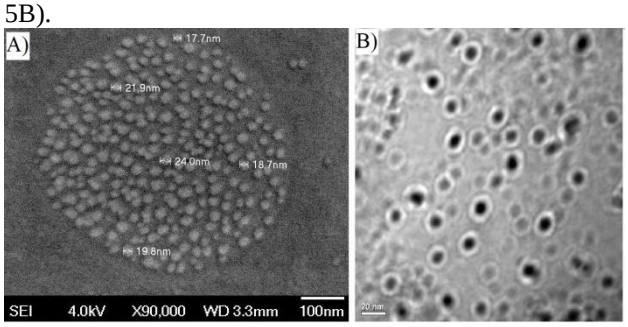

Figure 5. Morphology of nanoliposome. A) FE-SEM image, B) TEM image.

C. Size Distribution and Zeta Potential Analyses of Nanoliposomes

Size distribution and polydispersity index (PDI) of ALA nanoliposome exhibited an average size of 33.31 nm from the distribution curve; along with 0.211 PDI, that indicated a good monodispersity of nanoliposomes (Fig. 6A). Similar result has also been obtained by Dutta and Bhattacharjee [6] for encapsulation of black pepper extract.

Zeta potential of the nanoliposome was found to be -15.8 mV which reflected negative charge of liposomes, with moderate electrostatic repulsion (>15 mV shows strong repulsion [14]), among particles that prevent aggregation of nanoliposomes (Fig. 6B). This is also necessary for the stability of the same.

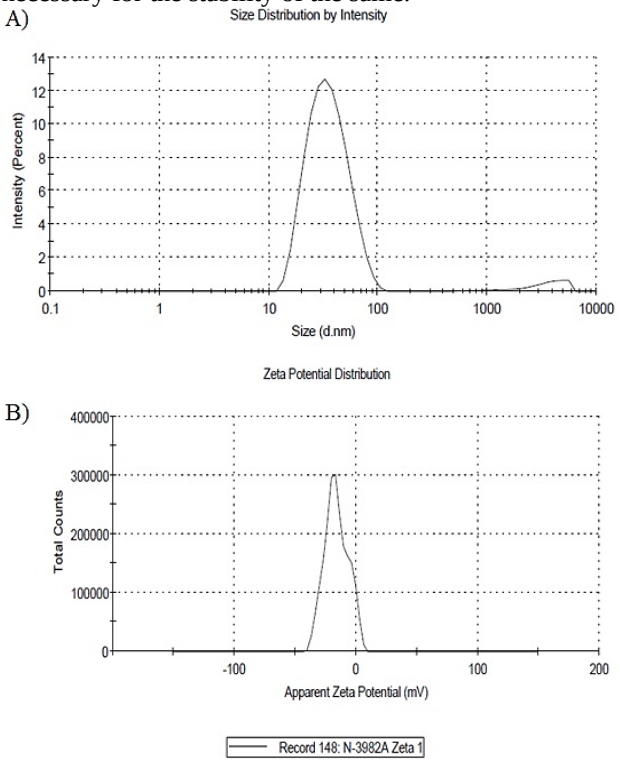

Figure 6. A) Size distribution and B) Zeta potential of nanoliposome.

D. Antioxidant Potentials of Nanoliposomes

The DPPH radical scavenging potency of optimized ALA liposome was found to be 0.168 mg/mL (IC_{50}).

IV. Conclusion

ALA has successfully been encapsulated as nanoliposome by probe sonication method, and with convenience. The optimized condition for the process was found to be 3 g of lipid (S+T) and S:T = 1:1 with 150 mg of ALA. The average particle size was obtained as 20.4 nm with moderate repulsion among the particles and good monodispersity among them. Since the liposome has good antioxidant potency, it can be used as supplement for antioxidant in different food and/or pharmaceutical applications.

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