Preparation, Characterization and Antioxidative Activity of Anti-aging Nanoemulsion and Lipid Nanoparticles

H. Wang, Q. Xia, J. Tang, J. Wang

School of Biological Science and Medical Engineering, State Key Laboratory of Bioelectronics, Southeast University, Nanjing 210096, China
Suzhou Key Lab of Biomedical Materials and Technology, Suzhou 215123, China
3Suzhou Nanohealth Biotech. Corp. Limited, Suzhou 215123, China
Tel/Fax: +86 512 62867117, Email: xiaq@seu.edu.cn

ABSTRACT

Nanoemulsions (NEs) and Lipid Nanoparticles(LNs) are regarded as suitable nanosized carriers of water-insoluble active components such as Alpha Lipoic Acid (ALA), Coenzyme Q10 (CoQ10) and Vitamin E (VE) to improve the physical and chemical stabilities of active components. Good physical and chemical stability of particles was proved by Photon Correlation Spectroscopy (PCS), Laser Diffraction (LD), viscosity and UV/HPLC with different storage times. UV/HPLC measurements showed that the loading capacity of active after production could reach 10% for ALA, 8% for CoQ10 and 10% for VE. The high entrapment efficiency (nearly 100%) was obtained for all formulations. Through study on inhibition rate of anti-aging active components to free radical, good antioxidant activity of ALA and CoQ10 was determined.

Keywords: Nanemulsion, Lipid Nanoparticle, Preparation, Characterization, Antioxidative Activity

1. INTRODUCTION

Increase in dietary-intake-related illnesses and skin problems such as obesity, cardiovascular disease, hypertension, cancer, wrinkle and stain brought about by skin caducity have led to food and cosmetic industry using bioactive components apriority[1]. However, many bioactive components in food and cosmetic area such as ALA, CoQ10 and VE are highly lipophilic resulting in poor absorption and bioavailability. NEs and LNs are two types of active components loaded carrier systems of 1990s[2, 3] to solve the problem of low capacity, low bioavailability and low stability. Both of them have the advantage of high permeability of the active component through the gut wall and skin[4]. Up to now, their special features such as the improved active components entrapment efficiency[5], the morphological characteri- zation[6], the possibility of topical use[7], the crystal order by differential scanning calorimetry (DSC) and 1HNMR[8] were studied. High pressure homogenization is the main method for LNs preparation. The major advantage of high pressure homogenization (HPH) is that it can be produced on large scale.

2. EXPERIMENT

The main method for anti-aging NEs and LNs is HPH. CoQ10-NLC[9], ALA-LN[10], VE-NE and so on were all prepared by this method.

Study on phase behavior of high temperature micro-emulsion phase diagram is one of the keys for LNs and NEs preparation. Through phase diagram, the ratio among components of emulsifiers, oils and active substances can be determined. Preparation process parameters such as homogenization pressure, cycles, ratio of emulsifier toil phase of NEs and LNs produced by HPH are obtained using single-factor index. Finally, repeating preparation study was taken on mean particle size, Zeta potential and PDI.

Stability study can be divided into physical and chemical stability. Physical stability mainly investigate on particle size, Zeta potential, PDI, viscosity and other parameters in different external conditions. Chemical stability is mainly about influence of light and temperature on the content.

Antioxidant study mainly investigate LNs and NEs' scavenging rate to DPPH[11], hydroxyl radical[12] and superoxide anion radical[13], and contrast antioxidant properties with commercially available products in the same conditions and concentration.

3. RESULTS AND DISCUSSION

3.1 Preparation of Anti-aging NEs and LNs

In the preparation of VE-NEs, ALA-LN and CoQ10-LN, the concentration of 10% (wt/wt) VE, 10% (wt/wt) ALA, 8% (wt/wt) CoQ10 were entrapped. Proportion of oil and emulsifier required were determined by hot temperature pseudoternary phase diagram. Figure 1 is pseudoternary phase diagram of VE-NE system. This phase diagram contains two areas—transparent and non transparent, bioactive component-loaded NEs or LNs had stable structure obtained according to proportion of transparent area. So this part should be chosen to study and preparation by HPH is based on this part. From Figure 1, when SPC: Tween40 was between 0:10 and 4:6, system can be transparent while percentage (wt/wt) for emulsifier needed is different. After homogenous, average particle sizes of 145 nm for CoQ10-NLC, 220 nm for ALA-LN and 45 nm

for VE-NE were obtained, and could be also got prepared repeatedly.

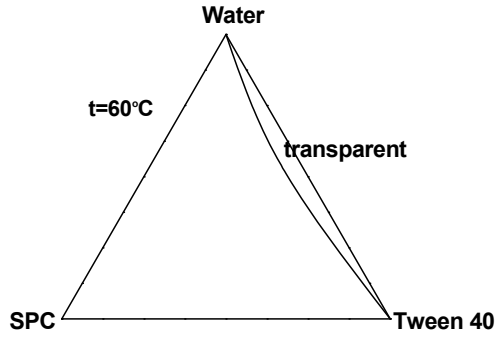

Figure 1: Part of pseudoternary phase diagram of SPC/Tween40/water system

3.2 Characterization of Anti-aging NEs and LNs

After shearing and homogeneous for 7cycles at 600 bar, average size of 145±8 nm was obtained for CoQ10-NLC, and PDI was 0.225±0.1 which showed particle size had good homogeneity. VE-NE and ALA-LN after homogeneous also had respectively appropriate average sizes. All of the NEs and LNs prepared had good physical stability of centrifugation, pH, dilution, standing, and temperature.

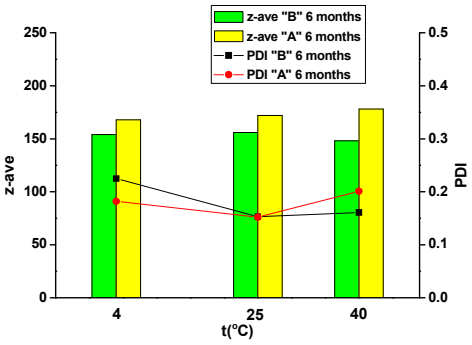

Figure 2: z-ave and PDI of CoQ10-NLC for 6 months at different temperature, “B”means “Before”, ”A” means “After”

Table 1: LD of CoQ10-NLC for 6 months at different temperature

t (°C)	LD10%(μm) 6 months(B/A)		LD50%(μm) 6 months(B/A)		LD90%(μm) 6 months(B/A)	
4	0.139	0.142	0.187	0.194	0.277	0.276
25	0.139	0.139	0.191	0.188	0.253	0.263
40	0.142	0.138	0.185	0.186	0.261	0.259

Figure 2 and Table 1 show the data of standing stability. By PCS, average particle size of CoQ10-NLC had little change after 6 months at 4, 25 and 40 °C, and from Table 1, it is found that there was no big particles existing after 6 months. So CoQ10-NLC has good standing stability, and the best storage temperature is 4 °C.

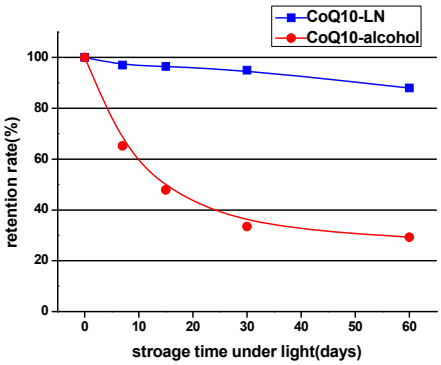

Figure 3: Under natural light irradiation for two months of CoQ10 - NLC and CoQ10 - ethanol solution content retention ratio

From Figure 3, It is easy to find that content retention ratio of CoQ10 is about 82% of NLC, while it is only 28% for CoQ10-alcohol. So after packing active components, chemical stability is improved because of high entrapment efficiency (nearly 100%).

To obtain more information about the particle size and shape, TEM, SEM and AFM analysis were also performed. Figure 4 shows the image of VE-loaded NE. As expected, particles reveal anisometric shape with a size of less than 50 nm, this particle size was coincident with the transparent appearance of V_E-NE. Furthermore, micro-droplet was regular circle globe and presented an good homogeneity. Some report showed that particles from much less pure cosmetic lipid (i.e. chemically polydispersed lipids) show a perfect spherical shape as shown by atomic force microscopy (AFM)[9].

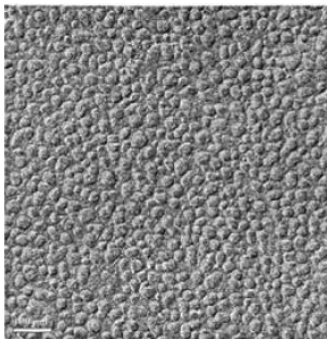

Figure 4: TEM image of VE-loaded NE

3.3 Antioxidative Activity of Anti-aging NEs and LNs

By the chemical experiment of scavenging superoxide anion, DPPH and hydroxyl radical of the same concentration of CoQ10-LN and commercially available CoQ10 products was evaluated and their anti-oxidative activity was compared.

Alkaline environment of pyrogallol solution was guaranteed by pH=8.2 Tris-HCl buffer solution, so pyrogallol occurres auto-oxidation reaction with producing colored substances. And the accumulation of colored substances values have acertain linear relationship with absorbance. Furthermore, with the first 4 min, the absorbance values change rate has a linear relationship with time.

From the following three figures, the standard curve formula of them is:

Y0=0.1307X0+0.0923, Y1=0.0163X1+1.558,

Ycommercial product=0.1149Xcommercial product+0.1411,

The inhibition rate of them can be got by Eq.(1):

$$Y\% = (1 - V_t / V_0) * 100\% \quad \text{Eq.(1)}$$

"Vt" and "V0" mean to the slope of different formulas.

By this Eq., inhibition rate of loading 2% (wt/wt) CoQ10-NLC diluted by 10 times to superoxide anion is 87.5%, while commercial CoQ10 product is only 12.1%. And the concentration of CoQ10-NLC diluted by 10 times was lower than commercial CoQ10 product, but have higher inhibition rate to superoxide anion than commercial CoQ10 product. Similarly, inhibition rate of CoQ10-NLC loading 2% (wt/wt) CoQ10 to DPPH radical and hydroxyl radical are much higher than CoQ10 production on sale.

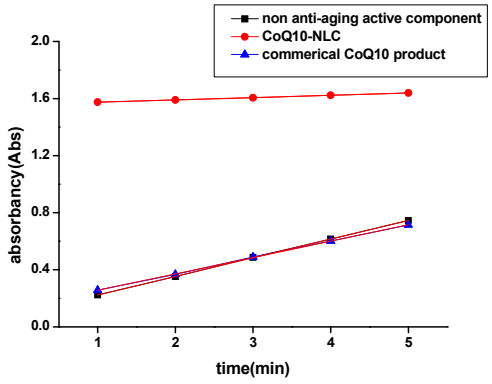

Figure 5: At 25 oC, influence of CoQ10-NLC and Ccommercial CoQ10 product to autoxidation rate of pyrogallol

3.4 INDUSTRIAL PREPARATION

The industrialization of LNs or NEs is one of the most important challenge for the real application of nanoscience and nanotechnology. The good result obtained from the research labs should be extended to the large-scale production or in another words, the products must be produced with the commercial scale and appropriate production cost while the good quality still keeping as it obtained from labs. It is extremely necessary to build or find large-scale production line for the industrialization of functional nanomaterials.

The production line includes the operation module, pre- and post-treatment module as well as assistant module with electric control all the process. The operation module was used to conduct the high pressure homogenization with different cycles. The pre-treatment module was designed to obtain pre-emulsion before formal operation. And the post-treatment module could treat the products further such as filtration. While the temperature control and CIP could be conducted by the assistant module.

Figure 6 Panorama of the production line for functional lipid nanoparticles

Table 2: Mean size results of 5% ALA-NLC products under the 10 KG production.

Batch No.	1	2	3	4	5
ParticleSize/nm	220	219	218	223	228
Mean size/nm			221		
RelativeError/%	-0.45	-0.90	-1.36	0.90	3.17

Table 2 gives the measurement of mean size of 10 KG production. The table was obtained from the complete same production condition of ALA-NLC with 10 KG per batch. It could be seen from the table, the production has very good consistency and also very close to the results obtained from the lab-scale preparation of about 100 grams.

The success of production line have great important influence on the real industrialization of active-NLCs and help the anti-aging NLC go into the cosmetic market finally.

4. CONCLUSION

Some water-insoluble anti-abing bioactive components such as ALA, CoQ10 and VE have low physical and

chemical stability. By HPH, NEs and NLs loading these anti-aging bioactive components with high concentration are obtained with relatively appropriate particle size, and have good phsical and chemical stability. Furthermore, NEs and LNs loading anti-aging active components have higher antioxidative activity than some commerical products. Therefore anti-aging loaded NEs and LNs have very good application prospects in healthy food and cosmetic fields.

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[i] Suzhou Industry Park, Road Ren'ai, NO.150, Room B328, Ph/Fax: (86)0512-62867117, xiaq@seu.edu.cn