Extraction of lutein ester from marigold petals and its clathration with β-cyclodextrin

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Abstract— *Lutein ester was extracted from marigold petals under hexane reflux at 60°C. For protection from oxidation, the as-obtained lutein ester was clathrated with β-cyclodextrin (β-CD) using two methods, viz. grinding lutein ester with β-CD in the presence of ethanol and through the mixing of an ethanol solution of lutein ester with an aqueous solution of β-CD. The results of DSC, IR, H1NMR analyses suggest successful clathration of lutein ester in β-CD. In terms of inclusion efficiency, the former is better than the latter to achieve clathration. We studied factors that can affect the stability of lutein ester such as light, air and temperature change, and the results confirm good stability of the clathrated lutein ester. The toxicity and pharmacokinetic tests on mice showed that the lutein-β-cyclodextrin complex is non-toxic and has an efficient release rate that matches body physiology.*

Keywords— Lutein ester; Marigold petals; β-Cyclodextrin; Clathrated compound; Toxicity test.

I. INTRODUCTION

Lutein is a natural carotenoid widely exists in fruits and vegetables. It is known to be rich in marigold flowers (*Tagetes erecta*).[1] Carotenoids are fat-soluble vitamins and show high biological activity in fat. Lutein ester hydrolyses naturally in body to free lutein, and it is known that the absorption rate of lutein esters in fat is higher than that of lutein by 60%.[2-3]

Lutein is brilliantly yellow and is a natural pigment. Being a natural antioxidant and non-toxic, it is used to enhance nutritional value of food.[4] It is known to protect vision and reduce the incidence of cataract.[4-7] Furthermore, it can slow down the hardening of arteries and prevent age-related macular degeneration.[6,8] It is used to improve body immunity for anti-cancer and anti-radiation purposes as well as for skin nourishment and anti-ageing.[5-11] Lutein ester is widely used in industries such as medicine, health, food, tobacco, cosmetics, as well as animal and poultry feed.[11] As an approved food supplement by the U.S. food and drug administration, it has wide application, and is named "plant gold" because it has the price of gold.

β-Cyclodextrin (β-CD) is non-toxic[12] and rapidly hydrolyzed to glucose. Because of its unique "hydrophilic and hydrophobic" structure, it is used to Clathrate a wide variety of objects.[13-15] Once clathrated, a compound changes in bioavailability due to the change of solubility and dissolution rate.[15,16] For example, liquid medicines are clathrated to prevent volatile components from being lost. It is common to find medicines clathrated by β-CD for the purpose of stability enhancement, odor containment and reduction of ill side effects.[14-16] In this article, we describe the clathration of lutein ester using β-CD. To the best of our knowledge, this kind of lutein ester clathrate has never been reported before.

II. EXPERIMENTAL

2.1 Instruments and reagents

The rotary evaporation apparatus (RE-52 c) was from Gongyi City, China Instrument Company, Ltd. The constant temperature heating magnetic stirrer (DF-101-s) was from Meiyukou Instrument Factory, China. The Fourier infrared spectrometer (AVATAR type 370) was from Nicolet, and the UV-vis spectrophotometer (UV–2400) from Shinadzu. The nuclear magnetic resonance instrument was performed over a BRUKER AV400 equipment. Thermal analysis was conducted over a DSC-Q10 (TA Company) analyzer. The digital melting point instrument (QB - 2) was from Shanghai ShenGuang Instrument and Meter Plant.

In this study, AR reagents were used. n-Hexane was from Tianjin Chemical Company, Ltd., China. Anhydrous ethanol was from New Chemical Plant of Harbin, China. n-Butyl alcohol, methylene chloride and sodium hydroxide were from Tianjin

Wind Ship Chemical Technology Company, Ltd., China, while β-CD was from Recovery of Tianjin Institute of Fine Chemicals, China.

2.2 Extraction of lutein ester

Marigold petals (10 g) and hexane (150 ml) were put inside a 300 ml three-neck round bottom flask, and the mixture was subject to reflux at 60°C for 5 h. After being cooled to room temperature, hexane was removed using a rotary evaporation apparatus. The residue was immerged in 50 ml of hot ethanol. After suction filtration, the filter paper as well as the yellow oily liquid was cooled in ice water for half an hour and then vacuum dried for 24 h. The results of H^1MNR and IR analysis indicated that the yellow oily liquid is lutein ester. From 10.0 g of marigold petals, 5.63 g of lutein ester was extracted using this method.[17]

2.3 Clathration of lutein ester using β-CD

2.3.1 By grinding

A mixture made up of 2.00 g of lutein ester, 2.17 g of β-CD (mole ratio = 1:1), and 5 ml of ethanol was ground in an agate mortar at room temperature for 4 h. During the grinding process, ethanol was added to keep the mixture in the form of a paste. The as-obtained product was vacuum dried at 45°C in an oven for 8 h. The amount of lutein-B-CD clathrate obtained was 4.19 g. Hereinafter the as-obtained sample is denoted as DHA-g.

2.3.2 By mixing lutein ester/ethanol solution with β-CD/water solution

Through a constant-pressure drop funnel, an aqueous solution of B-CD (2.45 g made up to 20 ml in water) was added to a 10 ml ethanol solution that contained 2.00 g of lutein ester at 60°C. The mixture was vigorously stirred for 48 h. Then the upper orange oily solid flake was separated, and the upper pale yellow turbid liquid was subject to vacuum suction filtration. The as-obtained solid light yellow in color was vacuum dried at 45°C for 8 h. The amount of lutein- B-CD clathrate obtained was 2.79 g. Hereinafter the as-obtained sample is denoted as DHA-m.

2.4 Toxicity and pharmacokinetic tests

The tests were performed at Xiangtan Hospital, Hunan, China.

2.4.1 Ingestion pathway[18]

Fifteen mice were divided into three groups. For the first group, DHA-g was taken in orally, while the second by injection. The third group acted as control. For the first group, the digestion of lutein ester was monitored. For the second group, the amount of white blood cells in blood was examined. The results were compared with those of the control group. The daily dosage was 0.2-0.4 g/kg, and the period of test was 30 days.

2.4.2 Heart beat[4,19]

We tested 20 mice with irregular heart-beat problem. Ten of them were subject to oral intake of DHA-g (daily dosage of 0.2-0.4 g/kg). The heart beat and cardiac ejection function of the two groups were monitored for a period of 30 days.

2.4.3 Liver[12,15]

A group of 10 mice with liver problem was tested. Another group of 10 normal mice were taken as control. They were subject to oral intake of DHA-g (daily dosage of 0.2-0.4 g/kg). The endogenous creatinine clearance rate (Ccr) of all the mice was monitored for 30 days.

2.4.4 Excretion pathway[16]

For the two groups of mice subject to Ccr monitoring, urine samples were taken and analyzed.

III. RESULTS AND DISCUSSION

3.1 DSC

Shown in Figs. 1 and 2 are the DSC spectra of DHA-g and DHA-m. They were compared with the standard DSC spectra of B-CD. The heating rate was 10°C and the temperature range was 150-400°C.

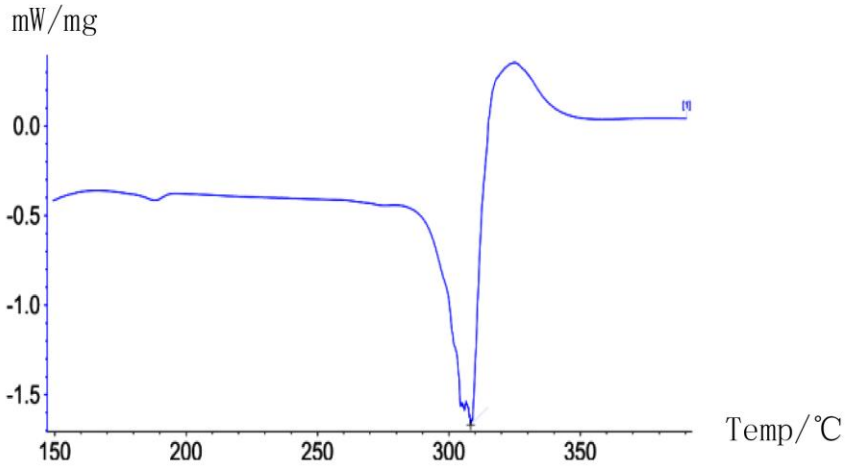

FIGURE 1 DSC SPECTRUM OF DHA-g

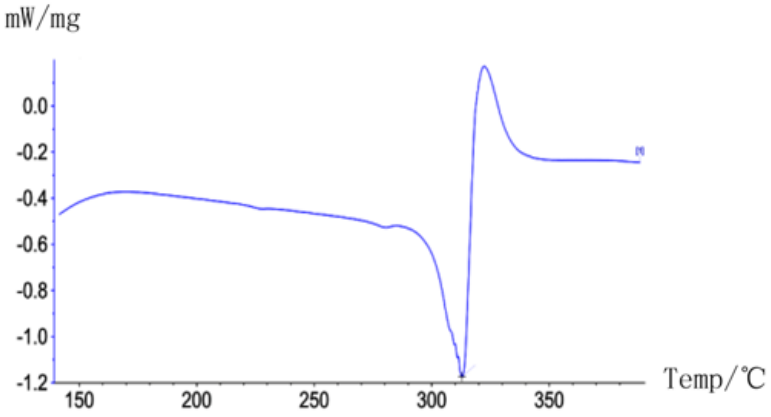

FIGURE 2 DSC SPECTRUM OF DHA-m

As reported in the literatures,[15] lutein ester melts at about 190°C, and β-CD starts to melt at about 300°C. In the DSC spectra of DHA-g and DHA-m, we do not see any signal ascribable to the melting of lutein ester at around 190°C, but there is a signal stretching from 280 to 308.3 or 312.8°C (Figs. 1 & 2) attributable to the melting of β-CD. It is deduced that there is tight integration of lutein ester inside the β-CD cavity. It is plausible that the water molecules inside the β-CD cavity are replaced by lutein ester, and there is the formation of lutein ester clathrate.

3.2 IR

The infrared spectra of β-CD and DHA-g are shown in Figs. 3 and 4, respectively

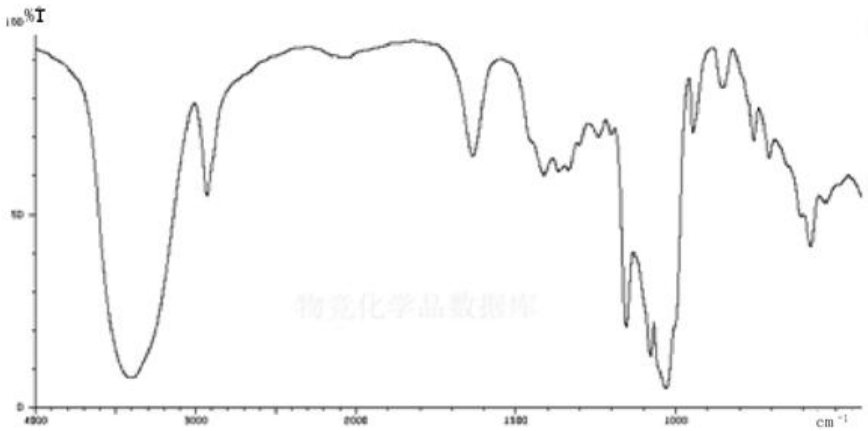

FIGURE 3 IR SPECTRUM OF β-CD

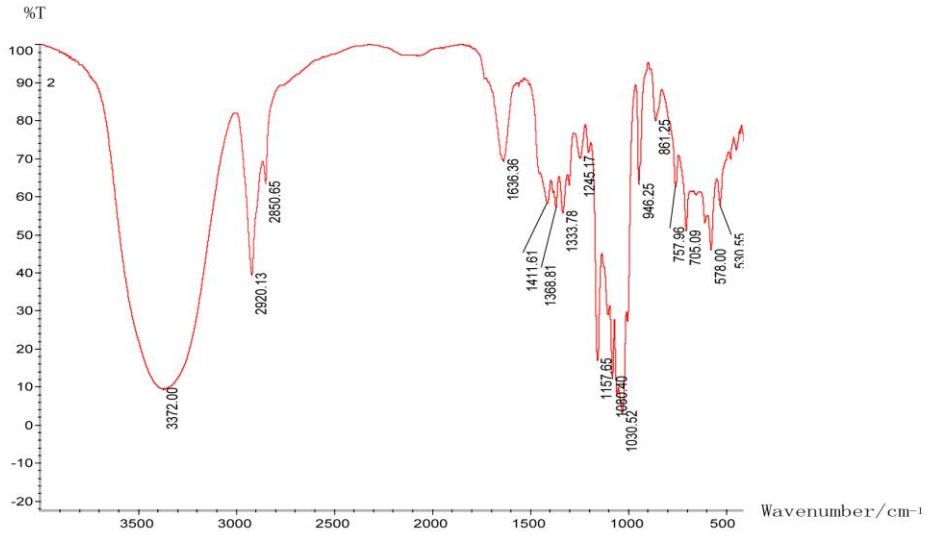

FIGURE 4 IR SPECTRUM OF DHA-g

As indicated in the literatures,[15] the ester materials show strong features of carbonyl stretching vibration next to those of the C-O-C asymmetric and symmetric stretching vibration. The latter vibration peaks can clearly distinguish the esters from the other carbonyl compounds. The main composition of lutein ester is palmitate and nutmeg acid ester. The asymmetric and symmetric stretching vibrations of the C-O-C base units show absorption at 1171 and 1196 cm^{-1}, respectively. The signal at 1741 cm^{-1} is the characteristic vibration peak of carbonyl (C=O). The one at around 1461 cm^{-1} is attributable to the bending vibration of methylene, and that at around 1370 cm^{-1} is due to methyl bending vibration. Compared with the data available in the literatures, the IR data of our lutein ester sample shows high similarity except that in our case there is a bimodal signal near 1460 cm^{-1} ascribable to nutmeg acid ester.

Shown in Fig. 3 is the infrared spectrum of β-CD. There is a strong signal attributable to hydroxyl stretching vibration at 3356 cm^{-1}. The peak at 2923 cm^{-1} is ascribable to C-H stretching vibration. The peak at 1413 cm^{-1} is due to the scissor vibration of methylene. The 1158 and 1081 cm^{-1} signals are due to the C-O and C-OH vibration of β-CD. The peak between 1158 and 1081 cm^{-1} is the para hydroxy C-O stretching vibration of the belt.

Compared with the IR spectrum of β-CD, there is the absence of para hydroxy C-O peak between 1158 and 1081 cm^{-1} in the case of DHA-g. It is understandable because with the inclusion of the lutein ester molecules, the water molecules inside β-CD are displaced by the lutein ester molecules. It is hence difficult to have formate-hydroxyl hydrogen bonding, and the hydroxyl peak shifts to the takanami position at 3372 cm^{-1}, and looks sharper and smaller. Furthermore, the IR spectrum of DHA-g does not show the characteristic absorption peaks of lutein ester at 1742 cm^{-1}. The overall IR results indicate that there is a change in the micro-environment of lutein ester and β-CD as a result of the inclusion of the former in the latter.

3.3 H^1NMR

The H^1NMR spectra of β-CD and DHA-g are shown in Figs. 5 and 6. The β-CD clathrate shows main peaks at 0.000, 1.225, 2.490, 3.316, 3.626, 4.422, 4.822, and 5.645 ppm

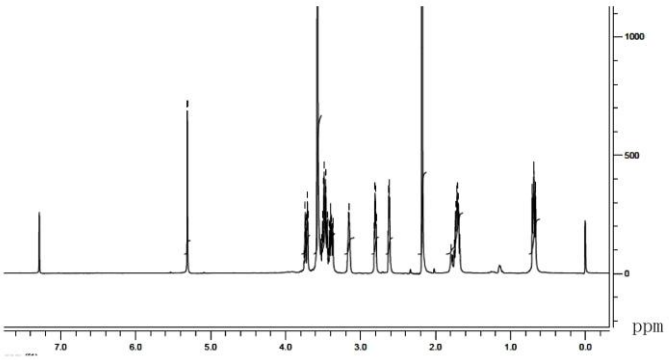

FIGURE 5 H^1NMR SPECTRUM OF β-CD

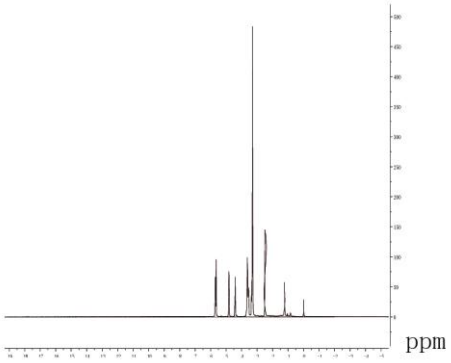

FIGURE 6 H^1NMR SPECTRUM OF DHA-g

Comparing the NMR spectrum of DHA-g with that of β-CD, there is no chemical shift of lutein ester. It is because the lutein ester of DHA-g is shielded by β-CD. The result suggests that the lutein ester is in the inner chamber of β-CD molecules.

3.4 Stability studies

We dissolved 0.50 g of lutein esters and DHA-m separately in ethyl acetate and made up the solution to 25.0 ml in the absence of direct sunlight. The two solutions were subject to tests to find out the effect of light, air and temperature change on the stability of free and included lutein ester.

3.4.1 Effect of light

The absorbance values of the two under 454 nm light irradiation were recorded at time intervals within a period of 12 h. The results are shown in Fig. 7

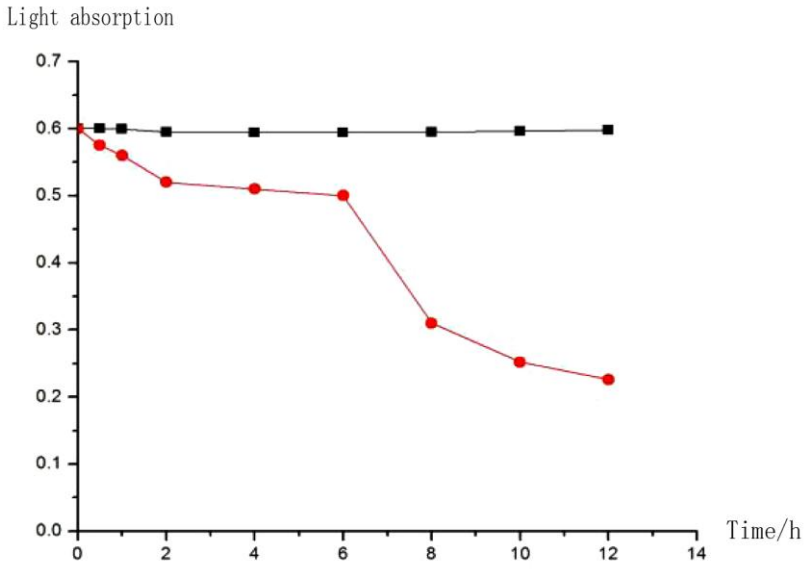

FIGURE 7 EFFECT OF LIGHT (■—DHA-g, ●-- lutein ester)

From Fig. 7, one can see that there is no change of color intensity with the DHA-g solution whereas there is gradual decline of color intensity with the solution of lutein ester. It is apparent that the lutein ester without the protection of β-CD is unstable under light irradiation due to photon-induced degradation.

3.4.2 Effect of air

We tested the effect of air on lutein ester and DHA-g in ambient environment, and the results are shown in Fig. 8

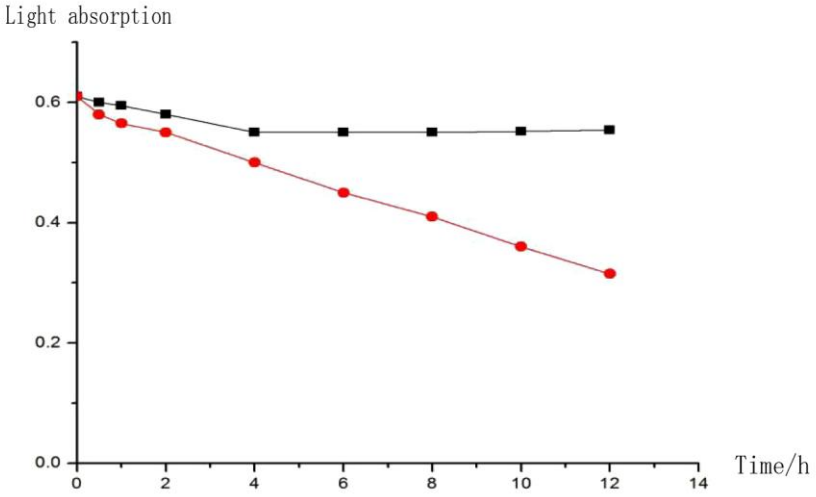

FIGURE 8 EFFECT OF AIR (■—DHA-g, ●-- lutein ester)

From Fig. 8, one can see that with the protection of β-CD, the lutein ester is more stable in air. It is apparent that when exposed to air, lutein ester undergoes oxidation and becomes degraded.

3.4.3 Effect of temperature change

We tested the effect of temperature rise on lutein ester and DHA-g under an atmosphere of high-purity nitrogen. The results are depicted in Fig. 9.

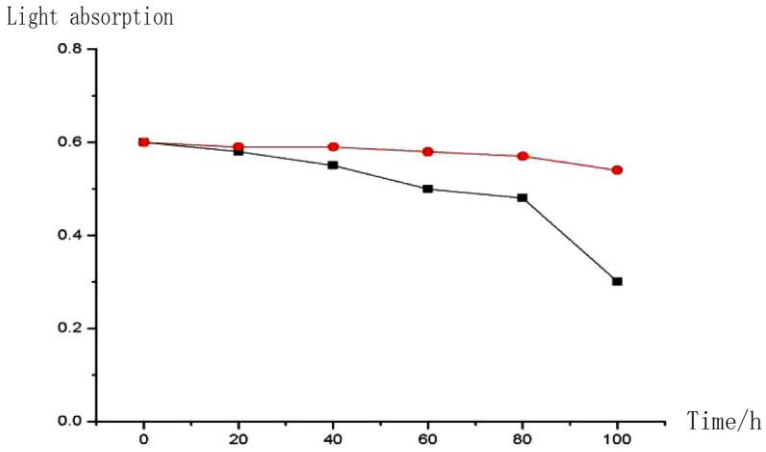

FIGURE 9 EFFECT OF TEMPERATURE CHANGE (■— lutein ester, ●--DHA-g)

From Fig. 9, one can see that with the protection of β-CD, the lutein ester becomes more stable with the rise of temperature. From the results of Figs. 7-9, it is clear that once clathrated in β-CD, lutein ester becomes more stable in ambient environment. Consequently, the scope of lutein ester application can be greatly extended.

3.5 Toxicity and pharmacokinetic test

DHA-g shows a constant release rate of lutein ester in 24 h. According to the membrane push-pull osmotic pump control principle, the release rate of lutein ester is zero-order. The rate is not affected by gastriointestinal peristalsis and pH value. After digestion the inactive ingredients of β-CD go through the gastriointestinal tract and pass out as insoluble shell in feces.

Absorption: Lutein ester is completely absorbed by the body. Due to gut first-pass metabolism, the biological availability of immediate-release lutein ester reaches 45-56%, and at the steady state, reaches 75-92%. The intake of lutein ester together with food only affects the gut first-pass metabolism slightly and has no effect on the biological of the released lutein ester.

Bioconversion: Lutein ester mainly undergoes intestine and liver metabolism. The metabolites leave the body mainly through renal excretion, while about 2-10% through bilirary excretion. Only less than 0.1% is found in urine.

IV. CONCLUSION

We demonstrated that the extraction yield of lutein ester from marigold petals can be 56.3% at 60°C by hexane solvent extraction. Through the simple grinding of lutein ester with β-CD in the presence of ethanol, there is efficient clathration of lutein ester in β-CD as confirmed by the results of DSC, IR and H^1NMR analysis. It was observed that the clathrated lutein ester is more stable that the free counterpart in ambient environment. The results of toxicity test of mice indicate that the lutein-β-CD complex is non-toxic, and has a release rate of lutein agreeable to body physiology.

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