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THE ENHANCED STABILITY OF β-CAROTENE BY ENCAPSULATION INTO HOLLOW MESOPOROUS SILICA NANOPARTICLES

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Abstract

Carotenoids are widely-distributed botanical pigments which are closely involved in photosynthesis and present multiple bioactivities in human health. β-carotene (β-car) is one of the most extensively investigated carotenoids because of its provitamin A activity and many biological activities. However, its instability and highly hydrophobicity limit its applications in food processing and decrease its bioavailability. Although many efforts had been made by encapsulating β-car into different materials to solve these problems, these systems cannot satisfy the requirements to the varied and complex application of β-car in food and medicine field. Therefore, development of alternative new carriers may provide diversified carrier systems for β-car that best fit the practical usage of β-car in different processing operations. Considering the widely-accepted advantages of hollow mesoporous silica nanoparticles (HMSN) in the drug delivery system research, this study aimed to encapsulate β-car into HMSN in order to improve its instability and poor aqueous dispersibility. HMSN with average particle size about 230 nm was successfully prepared. The BET surface area, pore volume, and average pore size were calculated as 423.3 m^2/g, 0.456 cm^3/g and 3.95 nm, respectively. β-car was encapsulated into HMSN via solvent impregnation method to form a nanocomposites (HMSN@β-car) which could be well dispersed in water. The drug loading rate was calculated as 17.5%. And then, the stability of β-car in HMSN@β-car and its free forms against temperature, pH and light irradiation was compared. The results showed that encapsulation of β-car in HMSN enhanced its stability against temperature, pH and light irradiation. Besides, DPPH and ABTS assays showed that HMSN@β-car reduced the loss of antioxidant activity caused by temperature. In conclusion, encapsulation of β-car into HMSN could enhance its stability, decrease the loss of its antioxidant activity, and improve its aqueous dispersivity. Our results indicated that HMSN could serve as a candidate system for β-car carrier when we choose the system that best fit the practical usage of β-car in different processing operations.

Key words: Carotenoids, β-carotene, Hollow mesoporous silica nanoparticles, Stability, Antioxidant activity.

Introduction

Carotenoids are widely-distributed pigments in natural plants including some fruits, vegetables, and leaves (Hofmann *et al*., 1996; Wihong *et al*., 2017; Nanta *et al*., 2020). It plays an essential role in photosynthesis. Also, their antioxidant activities allow them to protect photosynthetic organisms from the damage of ROS or excess light exposure (Hofmann *et al*., 1996; Cerullo *et al*., 2002; Hashimoto *et al*., 2016; Zakar *et al*., 2016). Carotenoids are not only increasingly popular natural color additives because of its good biosafety and rich sources (Molina *et al*., 2023), but also have multiple bioactivities, such as antioxidant, antidiabetic, anticancer, anti-inflammatory, cardio-protective, neuroprotective, hepato-protective, anti-aging and osteo-protective (Stahl & Sies, 2005; Brazionis *et al*., 2008). Therefore, carotenoids were widely used in food industry to improve sensory quality, or prevent and cure many diseases in pharmaceutical field. However, instability and water insolubility are the main obstacles to limit the wide application of carotenoids (Soukoulis & Bohn, 2018; Rodriguez-Amaya, 2018; Albuquerque *et al*., 2020).

B-carotene (β-car) is one of the most extensively investigated carotenoids because of its provitamin A activity and many biological activities (Wang *et al*., 2014; Johra *et al*., 2020; Anand *et al*., 2022; Yi *et al*., 2023). Like most other carotenoids, β-car is a highly unstable substance which is vulnerable to light, heat, and oxygen, prone to isomerization and oxidative degradation, and thereby resulting in a loss of biological activities and a color fading (Colle *et al*., 2016; Syamila *et al*., 2019; Borba *et al*., 2019; Lavelli & Sereikaitė, 2022). Besides, β-car is a highly hydrophobic substance which limits its applications in food processing and decreases its bioavailability. Therefore, for the sake of its extensive use, many efforts had been made by encapsulating β-car into different materials to enhance its stability, bioaccessibility and bioavailability, and aqueous dispersivity. In hence, many nanostructured delivery systems were used to improving the bioaccessibility and bioavailability of β-car (Kohno *et al*., 2016; Rostamabadi *et al*., 2019; Martínez *et al*., 2020; Sridhar *et al*., 2021; Zare *et al*., 2021; Wahdan *et al*., 2022; Molteni *et al*., 2022; Jalali-Jivan *et al*., 2022). Among them, lipid-based delivery systems and biopolymeric nanocarriers were widely reported because the components of these systems come from edible organisms and were widely proved to be safe. However, these systems were usually formed by noncovalent bonds and unstable in the practical production and processing process (Rostamabadi *et al*., 2019; Sridhar *et al*., 2021; Zare *et al*., 2021).

Mesoporous silica nanocarriers possessed relatively stable structure, large surface areas, tunable pore sizes, varied morphology, and well-defined surface properties, convenient synthesis, and low cost. Therefore, mesoporous silica nanocarriers were widely applied in the field of biomedicine researches as carriers for small drug molecules, proteins and nucleic acids (Sun *et al*., 2015; Zhang *et al*., 2022; Vallet-Regí*et al*., 2022; Djayanti *et al*., 2023). Besides, it has been approved by the FDA as a new biocompatible

material (Tang *et al*., 2012). Many works attempted to investigate the encapsulation of β-car in mesoporous silica nanocarriers (Kohno *et al*., 2016; Martínez *et al*., 2020; Wahdan *et al*., 2022). Martíne, *et al* successfully incorporated β-car into a silica matrix by adding β-car into the condensation process of silica precursor and the obtained nanocomposites enhanced the stability of β-car against heat (Martínez *et al*., 2020). Similarly, Wahdan, *et al* prepared β-car-incorporated silica nanoparticles via an in situ-loading method with high entrapment efficiency (86%), and proved that the nanocomposites could enhance the stability and bioavailability of β-car (Wahdan *et al*., 2022).

Although hollow mesoporous silica nanoparticles (HMSNs) belong to mesoporous silica nanocarriers, their internal hollow structures bring greater encapsulation capacity as compared with other mesoporous silica nanocarriers. Therefore, HMSNs are always one of the hot focuses in the researches on drug delivery systems (Yang *et al*., 2016; Sharma & Polizos, 2020; Li & Zhou, 2023). However, HMSNs have not yet been reported to be used as the carrier of β-car. In this study, HMSNs were firstly prepared and characterized. And then β-car was encapsulated into HMSNs by solvent impregnation method. At last, the effect of heat, pH and light on the stability of β-car in HMSNs was investigated. Also, DPPH and ABTS assays were conducted to evaluate the antioxidant activity of β-car in HMSNs.

Material and Methods

β-car (99%), tetraethyl orthosilicate (TEOS), triethylamine (99%), cetyltrimethylammonium bromide (CTAB) (99%) and ammonia water (AR, 25-28%) were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. 1-Diphenyl-2-bitter hydrazine radical (DPPH radical) (≥97%) and 2, 2'-diazobis (3-ethylbenzothiazoline-6-sulfuric acid) diamine salt (ABTS radical) (96%) were purchased from Shanghai Macklin Biochemical Co., Ltd. Other chemicals and solvents were of analytical grade.

Synthesis of hollow mesoporous silica nanoparticles: The HMSNs were prepared based on the Stöber method with some modification (Fang *et al*., 2011). Firstly, 2 g CTAB was dissolved into 100 mL H_2O to obtained solution A. Then, 5 mL of deionized water, 2 mL of ammonia solution, and 60 mL of ethanol were mixed at 30 ℃ with magnetic stirring for 10 min. 1.8 mL of TEOS was added into the mixed solution and stirred for 2 h to obtained solution B. The solution B was then added into solution A with continuously magnetic stirring for 30 min. When the temperature was increased to 70 ℃, 0.6 mL of TEOS was added into the reaction system, and the reaction was kept for 2 hours. The monodisperse silica NPs were obtained by 12000 rpm for 10 min. The precipitates were resuspended in 200 mL of deionized water and kept magnetic stirring. When the temperature was increased to 50℃, 8 g of Na_2CO_3 was added to the suspension and kept for stirring for 120 min. At last, HMSNs were obtained by 12000 rpm for 10 min. The etched HMSNs were washed with DI water and then resuspended in 500 mL HCl/ethanol solution (10:90, v/v), and refluxed for 6 hours to remove CTAB.

Characterization of hollow mesoporous silica nanoparticles: The morphologies and hollow mesoporous structure of the samples were determined via SEM (JEOL, Kyoto, Japan, JSM7800F) and TEM (JEOL, Kyoto, Japan, 2100F, 200KV). The zeta potential and size distribution of the samples were analyzed via ZS90 Nanosizer (Malvern Instruments Ltd., Malvern, UK) and laser Particle Size Analyzer (Malvern, UK, Mastersizer 3000). The FTIR was analyzed using an FTIR spectrometer (Bruker Optics Inc., German, Tensor27). The specific surface area and pore size distribution were determined via specific surface area and porosity analyzer (Micromeritics, USA, ASAP2460). The XRD patterns were analyzed via polycrystalline powder diffractometer (Rigaku, Japan, Ultima IV).

Encapsulation of β-carotene with hollow mesoporous silica nanoparticles: The loading of β-car into HMSNs was realized by solvent impregnation method. First, 100 mg β-car was dissolved in 10 mL dichloromethane. 100 mg HMSNs was weighed and resuspended into the β-car dichloromethane solution with the aid of ultrasound. And then the mixed system was sealed and kept shaking for 24 hours in dark place. After centrifugation, the supernatant solution was carefully collected. And then the content of β-car in supernatant solution was measured (M_{sup}). At last, the precipitate was lyophilized to obtain HMSN@β-car. The loading capacity of HMSN@β-car was calculated via the following formula:

$$\text{The loading capacity} = (100\text{-}M_{sup}) \times 100\% / (200\text{-}M_{sup})$$

Study on the stability of β-car in hollow mesoporous silica nanoparticles: Lyophilizated HMSN@β-car were accurately weighed and resuspended into deionized water to obtain a uniformly dispersed solution with β-car being 0.1 mg/mL. And then, 1 mL of the solution was equally distributed into 2.0 mL EP tubes and sealed. As for the free β-car groups, β-car was dissolved in DMSO with β-car being 0.1 mg/mL. Similarly, l mL of the β-car solution was equally distributed into 2.0 mL centrifuge tubes.

As for HMSN@β-car, the control groups for the temperature and light experiments were placed in 4 ℃ and kept in dark. The other samples were placed in 30 ℃, 60℃, 80℃ and 100℃ for 3 h, respectively. Meanwhile, the influence of light irradiation on the stability of β-car was conducted by placing the samples under ultraviolet lamp (20 W), outdoor sunlight and indoor lighting (18 W) for 3 h, respectively.

The effect of pH on the stability of β-car was conducted by mixing 0.1 mL DMSO, 0.1 mL hydrofluoric acid or 0.1 mL triethylamine with 1 mL suspension of HMSN@β-car in 2.0 mL EP tubes. And then the mixed solution was sealed and kept in dark under room temperature for 60 min. At last, all the samples were collected and centrifuged. After discarding the supernatant, 1 mL DMSO was added into each EP tubes to resuspend the precipitate with the aid of ultrasound. After centrifugation, the absorptions of β-car in the control group (OD_{con}) and the other experimental samples (OD_{exp}) were measured and the retention rate of β-car was calculated as follow:

$$\text{The retention rate} = OD_{exp} \times 100\%/OD_{con}$$

As for the free β-car group, when the action of light, heat, hydrofluoric acid or triethylamine finished, all the samples were collected and the absorption of β-car were measured directly. The other experimental conditions and the calculation of retention rate were the same as the HMSN@β-car groups.

Study on the antioxidant activity of β-car: DPPH and ABTS assays were used to evaluate the influence of temperature on the antioxidant activity of β-car. At first, the samples of β-car and β-car @HMSNs were pretreated under 30℃ for 3 hours. The control groups for β-car and β-car@HMSNs were placed in 4℃ and kept in dark. As for HMSN@β-car group, the samples were collected and centrifuged after the heat-treatment. After the supernatant was discarded, 1mL DMSO was added into each EP tubes to resuspend the precipitate with the aid of ultrasound. After centrifugation, the supernatant was collected for the antioxidant analysis.

DPPH radical scavenging activity: 1 mg DPPH was dissolved in 24 mL DMSO and stored in dark under 4℃. And then 1 mL of DPPH solution was added into each of the six EP tubes (10 mL). 0.5 mL DMSO was added into one EP tubes and the OD at 519 nm was measured as A_0. 0.5 mL of the other prepared samples was fetched and added into other EP tubes, respectively. All the samples were sealed and well-mixed and kept away from light for 1 h. The OD_{519nm} was measured as A_{sam}. Each sample was tested at least four times. The DPPH radical scavenging rate (%) was calculated as follows.

$$\text{DPPH scavenging activity (\%)} = (A_0 - A_{sam}) \times 100\%/A_0$$

ABTS radical scavenging activity: Solutions A were firstly prepared by mixing equal volumes of 7 mM ABTS solution and 2.45 mM $K_2S_2O_8$. The solutions A was kept away from light for 12 h to form ABTS radicals ($ABTS^{\bullet+}$). And then, 1 mL of the stock solution was mixed with 49 mL DMSO and the OD of mixed solution at 734 nm was around 0.7. Subsequently, 2 mL diluted $ABTS^{\bullet+}$ solution was added into each of 6 EP tubes. 0.5 mL DMSO was added into one EP tubes and the OD_{734nm} was measured as B_0. 0.5 mL of the other prepared samples was fetched and added into other EP tubes, respectively. All the samples were sealed and well-mixed and kept away from light for 2 h. The $OD_{734\ nm}$ was measured as B_{sam}. Each sample was tested four times. The ABTS radical scavenging rate (%) was calculated as follows.

$$ABTS^{\bullet+}\text{ scavenging activity (\%)} = (B_0 - B_{sam}) \times 100\%/B_0$$

Results

Synthesis of hollow mesoporous silica nanoparticles: As shown in Fig. 1A, the TEM images showed that the prepared nanoparticles had a spherical morphology and well-defined hollow structure, and the diameter of the hollow part was around 200 nm. The particle size distribution and zeta potential of prepared HMSNs in ddH_2O were also measured as 330 ±27 nm (with the PDI being 0.16) and -21.1±1.08 mV, respectively (Fig. 1B and C).

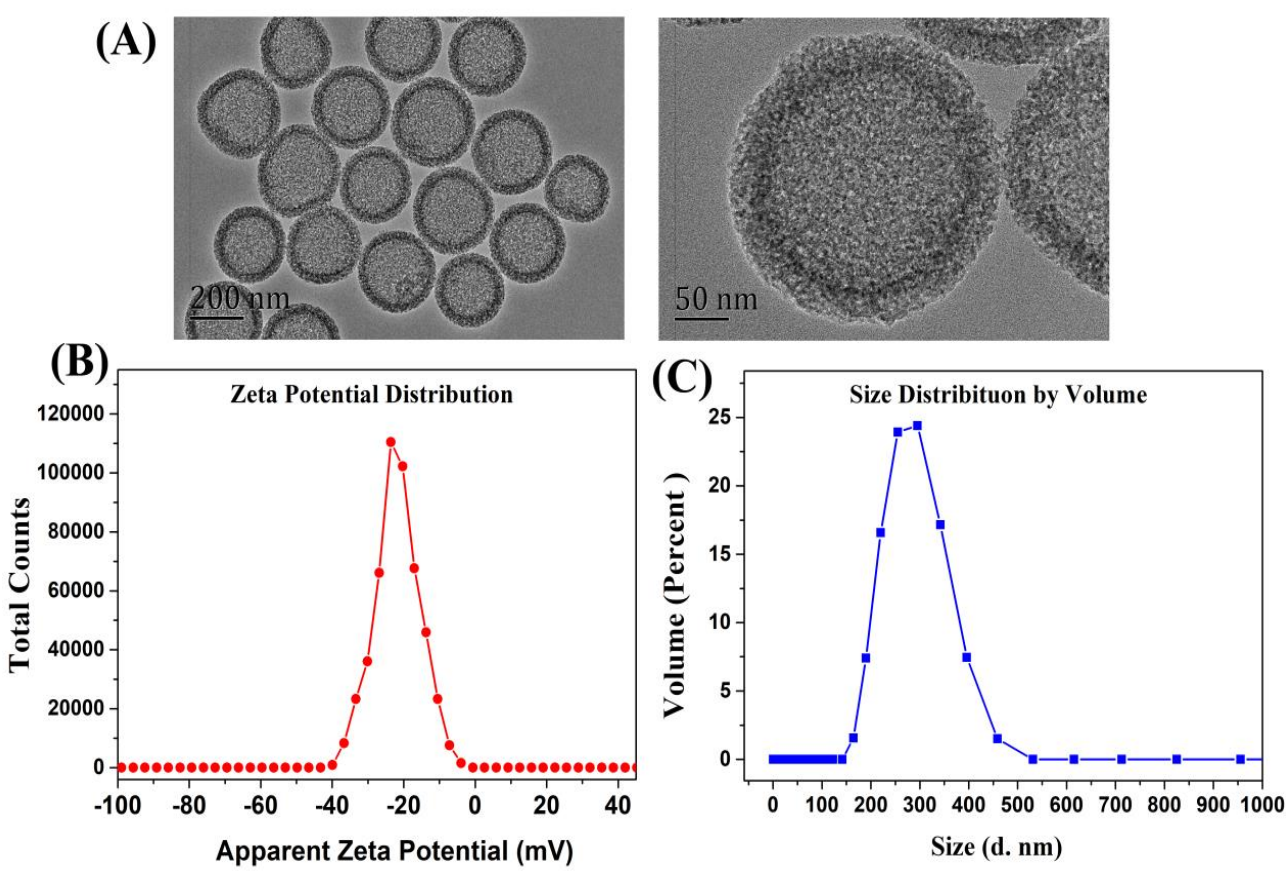

Fig. 1. The TEM (A), zeta potential (B) and particle size distribution (C) of HMSNs.

Nitrogen adsorption-desorption isotherms were measured in order to elucidate more detailed information about the HMSNs. As shown in Fig. 2A, HMSNs possessed the typical Langmuir IV hysteresis loops, which suggested that there existed a mesopores structure and the BET adsorption average pore diameter about 3.95 nm (Fig. 2B). The BET surface area and pore volume were calculated as 423.3 m^2/g and 0.456370 cm^3/g, respectively.

Encapsulation of β-carotene with hollow mesoporous silica nanoparticles: The loading of β-car into HMNS was realized by solvent impregnation method. The drug loading was calculated as 17.5%. The successful encapsulation of β-car could be confirmed from the results of infrared absorption spectra (Fig. 3A). The infrared spectrum of β-car presented a characteristic peak around 1725cm^{-1} which could be attributed to the C=C bond (stretching, non-conjugated) of β-car. Meanwhile, the peaks from 1335 to 1478cm^{-1} could be attributed to the CH_2/CH_3 scissoring of β-car (Christidis & Kosiari., 2003; Escobar-Puentes *et al*., 2022). The characterized peaks for β-car could be observed in the FTIR spectra of HMSN@β-car rather than HMSN, which suggested that β-car was successfully encapsulated into HMSN.

As shown in Fig. 3B, the XRD patterns of β-car exhibited its characteristic crystalline peaks at 2θ from 13° to 26°. The crystalline peaks centered at 11.7, 14.3, 14.7, 16.5, 18.6, 21.6, and 24.4 were in line with the previous reports for β-car (Pan *et al*., 2007; Rocha *et al*., 2018), which suggested that free β-car existed in a crystalline state. Meanwhile, it could be observed from the XRD patterns of HMSNs that the amorphous nature of silica particles had a characteristic broad peak band from 15 to 30°. The XRD patterns of HMSN@β-car showed the similar XRD patterns with HMSNs. The characteristic crystalline peaks for β-car could not be observed in the XRD patterns of HMSN@β-car, but could be observed in that of the physical mixture of HMSN and β-car. It could be inferred that the encapsulation of β-car into HMSNs might lead to the disappearance of crystalline peaks of β-car, which was in consistent with the encapsulation of β-car in the aerogel (Zhang *et al*., 2023). Also, it could be also explained that the encapsulation of β-car into HMSNs might lead to the amorphization of β-car in HMSN just like in the nanofibers (Yildiz *et al*., 2023). The obtained HMSN@β-car could be easily dispersed in water. As shown in Fig. 3C, after stewing for 12 hours, aqueous suspension of HMSN@β-car had better dispersion state as compared with that for β-car alone. The result showed that encapsulation of β-car in HMSN could enhance its dispersity in water phase.

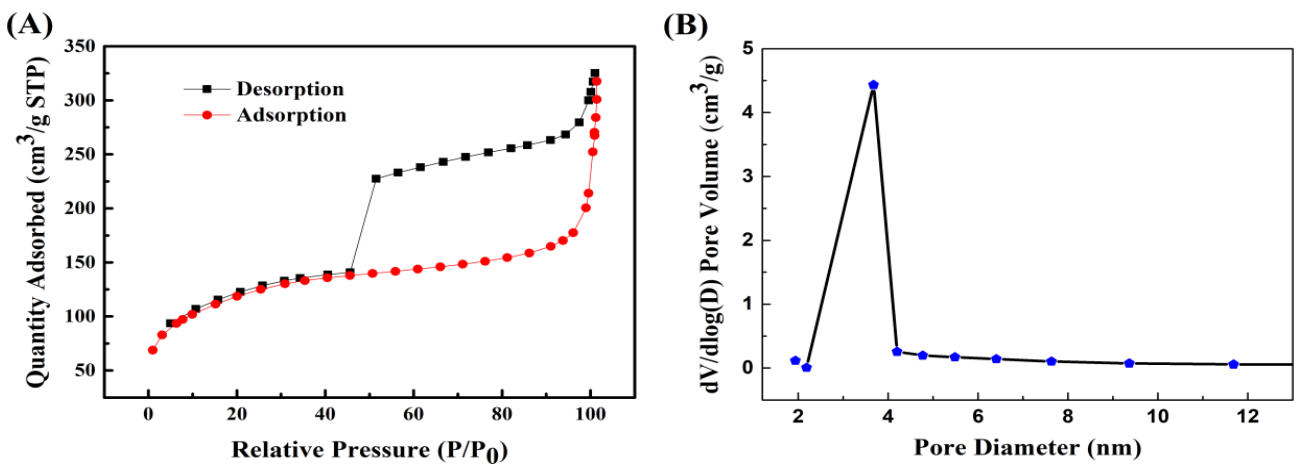

Fig. 2. N_2 adsorption-desorption isotherms (A) and pore size distribution (B) of HMSNs.

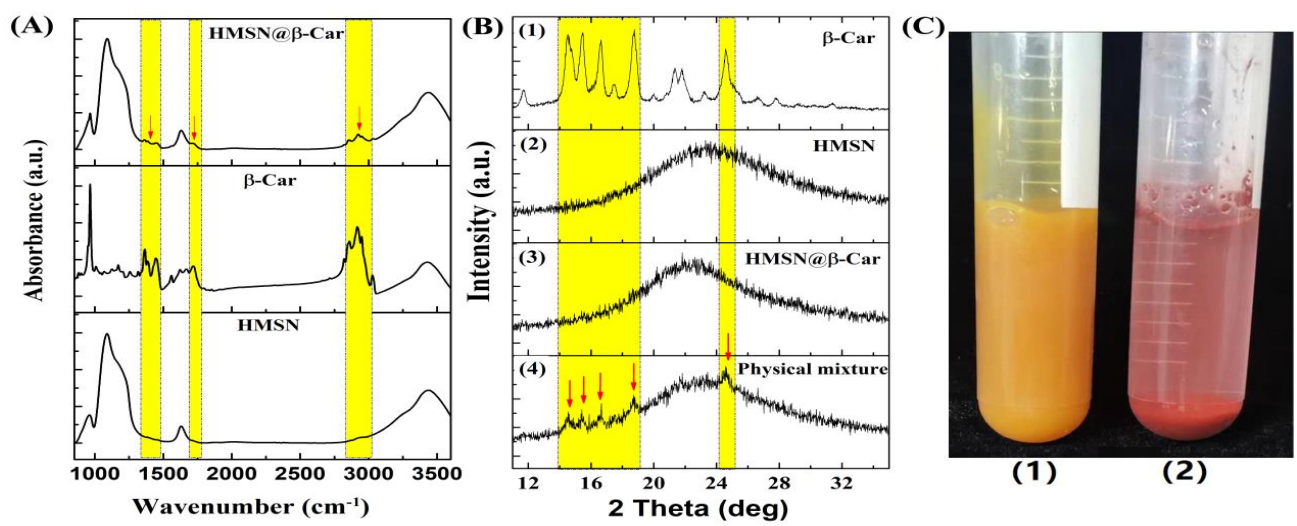

Fig. 3. **(A)** FTIR spectra of HMSN@β-car, β-car and HMSN, **(B)** XRD patterns of β-car, HMSN, HMSN@β-car and the physical mixture of HMSN and β-car, (C) the dispersion of HMSN@β-car (1) and β-car (2) in ddH_2O.

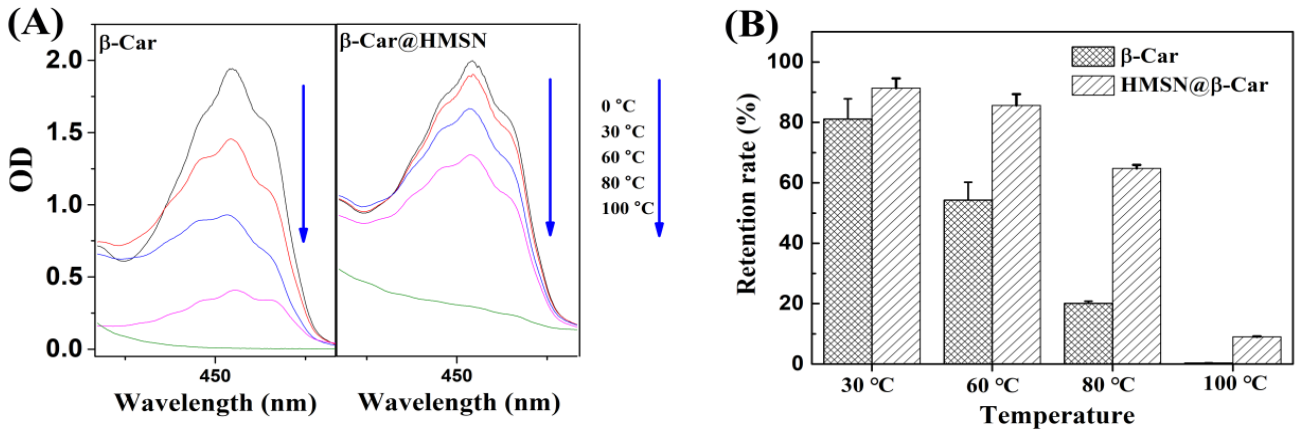

Fig. 4. **(A)** the influence of temperature on the absorption spectra around 460 nm for β-car and HMSN@β-car. **(B)** The influence of temperature on the retention rate of β-car in β-car group and HMSN@β-car group, respectively.

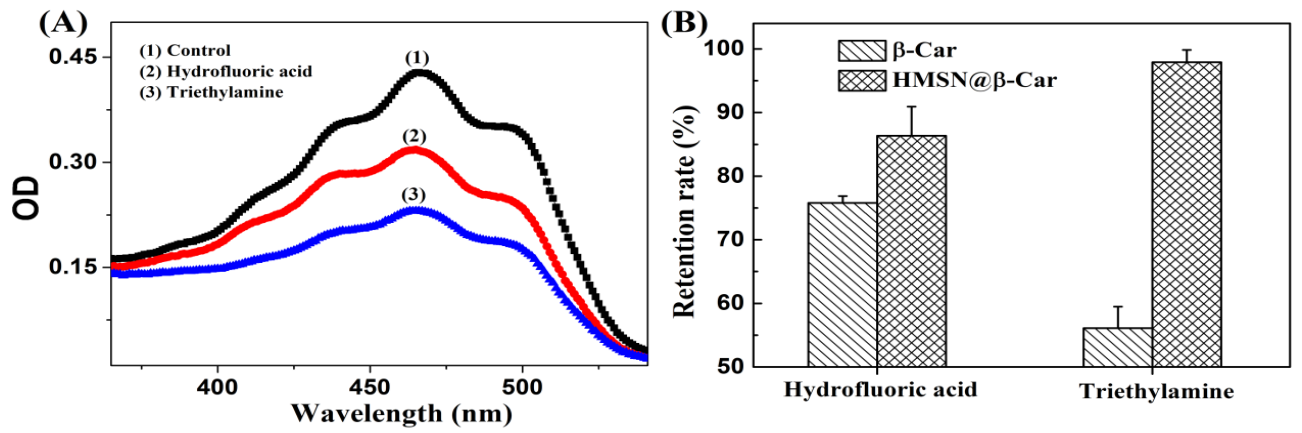

Fig. 5. **(A)** the influence of hydrofluoric acid and triethylamine on the absorption spectra of β-car around 460 nm. **(B)** The influence of hydrofluoric acid and triethylamine on the retention rate of β-car in β-car group and HMSN@β-car group, respectively.

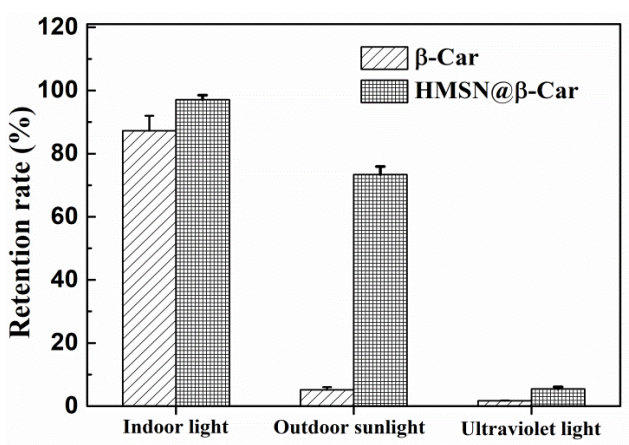

Fig. 6. The influence of light irradiation on the retention rate of β-car in β-car group and HMSN@β-car group, respectively.

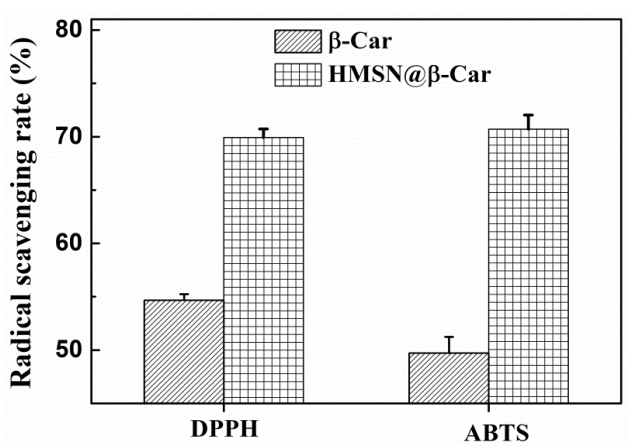

Fig. 7. The scavenging rates of DPPH radical and $ABTS^{\bullet+}$ by β-car in β-car group and HMSN@β-car group after the samples were pretreated by 30°C for 3 hours.

The enhanced stability of β-car in HMSNs: It was widely-known that β-car was a highly unstable substance and vulnerable to light, heat, pH and oxygen (Colle *et al*., 2016; Lavelli & Sereikaitė, 2022). Therefore, whether a carrier could enhance the stability of β-car should be the first consideration when a new candidate carrier for β-car is designed. The influence of light, heat and pH on the stability of β-car in HMSN@β-car or its free form was investigated and compared.

The characteristic absorption around 460 nm decreased as the temperature increased from 4 °C to 100 °C no matter whether β-car was encapsulated in HMSNs or in its free forms (Fig. 4). It was suggested that heat could lead to the degradation of β-car. However, the retention rates for β-car

groups at different temperatures were obviously smaller than that for HMSN@β-car groups at corresponding temperatures, especially at 60 and 80°C. It could be concluded that encapsulation of β-car in HMSNs could enhance its stability against heat. It was reported that the interaction of β-car with silica was strong and it was their strong interaction that led to a remarkable thermostability when it was embedded in a silica matrix (Martínez *et al*., 2020).

Many references had mentioned that β-car was unstable under extreme pH conditions (Meléndez-Martínez *et al*., 2010; Borba *et al*., 2019; Chandra *et al*., 2021; Molteni *et al*., 2022; Jalali-Jivan *et al*., 2022). Therefore, this study also studied the effect of extreme pH conditions on the stability of β-car. It is difficult for common water-soluble acid or base to reach β-car because β-car is a highly hydrophobic substance. Therefore, hydrofluoric acid and triethylamine were used in this work to create extreme pH conditions because they could dissolve in organic and aqueous solution systems. As shown in Fig. 5A, both hydrofluoric acid and triethylamine could decrease the absorbance of β-car around 460 nm. Triethylamine could result in more obvious decrease in the characteristic absorbance of β-car. It could be concluded that β-car might undergo degradation or transformation into other substances in the presence of hydrofluoric acid or triethylamine. The retention rates for β-car in free β-car group and HMSN@β-car group under the extreme pH conditions were then compared. The results showed that HMSN@β-car groups showed higher β-car retention rate than the free β-car groups both in the acid and base conditions, especially in the presence of triethylamine.

Light irradiation is a sensitive factor that can easily lead to β-car degradation (Syamila *et al*., 2019; Borba *et al*., 2019). Herein, indoor lighting, outdoor sunlight and the UV light were designed to study the degradation of β-car in its free form and encapsulated form. As shown in Fig. 6, light irradiation especially for outdoor sunlight and the UV light could lead to remarkable β-car degradation. As compared with the free β-car groups, the encapsulated β-car presented remarkable higher retention rate, especially under the irradiation of outdoor sunlight.

In this study, temperature, pH and light irradiation were designed according to the reported factors that could influence the stability of β-car. Our results showed that the three factors herein could all cause the degradation of β-car. Based on these models, the retention rates of β-car in HMSN@β-car and its free form were compared. In sum, encapsulation of β-car in HMSNs could enhance its stability against temperature, pH and light irradiation.

The antioxidant activity of β-car: The antioxidant activity is one of the most distinctive biological activities for β-car. Therefore, it is necessary to investigate the change of its antioxidant activity when a new encapsulation system for β-car is designed. DPPH and ABTS assays are the two most widely used methods to evaluate the antioxidant activity. In this study, HMSN@β-car and its free forms were pretreated by heat. And then, the antioxidant capacity for HMSN@β-car and β-car were calculated and compared. As show in Fig. 7A, the rate of scavenging DPPH free radical for heat-pretreated β-car was 57.95% which was obviously lower than that for heat-pretreated HMSN@β-car (89.89%).

The antioxidant capacity by ABTS assay presented a similar profile with the results from DPPH assay. The $ABTS^{\bullet+}$ scavenging activity for the HMSN@β-car group was 90.23% which was remarkable higher than that for β-car group (47.85%). It could be concluded from DPPH and ABTS assays that encapsulation of β-car in HMSN could reduce the loss of its antioxidant activity caused by temperature.

Discussion

Carotenoids are ubiquitous secondary plant compounds, which not only play indispensable roles in plant photosynthesis, but also have great potential in human health promotion. However, their bioavailability is often limited by the inefficient release from the food matrix, poor water solubility and instability in processing and storage processes. Encapsulation of carotenoids has been proved to be a very effective strategy to solve these problems (Soukoulis & Bohn, 2018). Encapsulation involves a process to entrap an active molecule into a structurally engineered system which provides a barrier against destructive conditions, such as temperature, oxygen, light, or pH (Soukoulis & Bohn, 2018; Albuquerque *et al*., 2020).

Many nanocarriers were designed including lipid-based nanoparticles, biopolymeric-based nanoparticles in order to enhance the bioavailability and stability of β-car (Sridhar *et al*., 2021, Molteni *et al*., 2022; Jalali-Jivan *et al*., 2022). Lipid-based nanoparticles are the most widely-used carrier systems for β-car because its composites have the same molecule basis with human being and show comparatively well biosafety. The encapsulation of β-car into SLN and liposomes could also lead to higher stability, water-dispersibility and bioavailability (Rostamabadi *et al*., 2019, Zare *et al*., 2021). Other materials such as biopolymeric-based nanoparticles, electrospun nanofibers, different gel systems, were also employed to encapsulate β-car (Pan *et al*., 2007; Yi *et al*., 2015; Rocha *et al*., 2018; Lino *et al*., 2022; Drosou *et al*., 2022; Yildiz *et al*., 2023; Zhang *et al*., 2023). Although these carriers possessed differential advantages for loading β-car, they also faced many limitations in the practical operations, such as the unstable carrier system, low loading capacity, high cost (Sridhar *et al*., 2021; Jalali-Jivan *et al*., 2022). In order to diversify the candidate carrier systems for β-car, silica materials had been employed to encapsulate β-car. Silica-base systems show stable and controllable morphological structure and acceptable biosafety (Tang *et al*., 2012; Sun *et al*., 2015; Zhang *et al*., 2022; Vallet-Regí*et al*., 2022; Djayanti *et al*., 2023). The embedment of β-car in mesoporous silica could enhance its stability against light and heat (Kohno *et al*., 2016; Martínez *et al*., 2020; Wahdan *et al*., 2022). The loading of β-car in mesoporous silica was accomplished through an in situ-loading method during the sol–gel process which could bring about higher encapsulation efficiency (Martínez *et al*., 2020; Wahdan *et al*., 2022). However, the addition of β-car into the formation process of silica materials posed great heterogeneity or irregularity to the final material morphology. In this work, the prepared HMSNs had regular morphology, well-defined hollow structure, and uniform particle size distribution. Subsequently, the loading of β-car

into HMSNs was realized by solvent impregnation method and the loading rate was 17.5%. Regretfully, solvent impregnation method usually resulted in lower encapsulation efficiency. However, this problem may be solved by recycling unpackaged β-car in organic solvent.

Generally speaking, the encapsulation of β-car into the inside of carriers could enhance the stability of β-car via providing a physical barrier to protect β-car from many destroying factors such as light, temperature or oxygen (Lino *et al*., 2022; Drosou *et al*., 2022; Yildiz *et al*., 2023; Zhang *et al*., 2023). Besides, the interaction of β-car with the groups on the carriers could also enhance its stability against temperature (Kohno *et al*., 2016; Martínez *et al*., 2020). HMSNs has plenty of Si-OH which could interact with β-car, thereby improving its stability against temperature. As shown in Fig. 1, the prepared HMSNs herein had a well-defined hollow structure. After β-car was encapsulated into HMSN, the shell structure of HMSNs might provide a barrier for β-car away from the destroying factors outsides, which might account for the enhanced β-car retention rate in HMSN@β-car as compared with free β-car under the action of pH and light irradiation.

Conclusion

In this study, HMSNs with a good morphology and structure were successfully prepared and characterized. The encapsulation of β-car into HMSNs was realized via solvent impregnation method. The drug loading was calculated as 17.5%. The prepared HMSN@β-car could be well dispersed in water. Besides, the encapsulation of β-car could enhance its stability against heat, pH and light irradiation. DPPH and ABTS assays also showed that encapsulation of β-car in HMSNs could decrease the loss of its antioxidant activity caused by heat. The results suggested that HMSN could serve as the carrier of β-car in order to enhance its stability and aqueous dispersivity. Considering that β-car is one of the most representative carotenoids, this work also provides a new available formulation which could allow us to choose the system that best fits the practical usage of carotenoids or other plant pigments in different processing operations.

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