

The incorporation of water-soluble Hydroxypropyl- β -Cyclodextrin-Lycopene inclusion complex in biopolymer chitosan nanoparticles: Physicochemical characteristics and in-vitro release

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ABSTRACT

Lycopene (Lyc) from Gac fruit is a potent antioxidant, but its poor water solubility limits its applications. To address this, Lyc was complexed with Hydroxypropyl- β -cyclodextrin (HPCD) and encapsulated in chitosan nanoparticles via ionic gelation. The optimal 1:1 Lyc-HPCD mole ratio achieved $63.0 \pm 3.1\%$ encapsulation, while chitosan-loaded complexes (127.6 nm , $+21.38 \text{ mV}$) reached $68.79 \pm 3.75\%$. Characterization by UV-Vis, FTIR, DSC, and SEM confirmed the structure, and the system showed sustained release over 24 hours at varying pH. This nanocarrier offers an efficient platform for lycopene delivery in food and cosmetic applications.

Keywords: Lycopene; Hydroxypropyl- β -cyclodextrin; Chitosan nanoparticles; Antioxidant.

1. INTRODUCTION

Lycopene, a carotenoid found in tomato, watermelon, carrot, and Gac fruit, is a potent antioxidant with 11 conjugated double bonds that quench singlet oxygen and scavenge free radicals [1]. It protects aging skin, reverses insulin resistance, promotes neovascularization [2], inhibits pro-inflammatory cytokines (IL-1 β , IL-6, IL-8, TNF- α), and reduces cardiovascular risk by lowering cholesterol and triglycerides [3]. However, lycopene suffers from poor water solubility and instability under heat, light, and oxygen [4], necessitating carriers for protection and controlled release [5]. Cyclodextrins (CDs) are widely used for this purpose. CDs are cyclic oligosaccharides (5–7 glucose units) with hydrophobic cavities and hydrophilic surfaces [6]. They improve the solubility of hydrophobic molecules [7]. Hydroxypropyl- β -cyclodextrin (HPCD) is favored for higher solubility and lower toxicity compared to β -CD and methyl- β -CD [8]. Its cavity (6–6.5 Å, 265 Å³) suits molecules of 200–800 g/mol [9, 10]. Applications span food, cosmetics, and materials. For instance, β -carotene solubility improved via co-precipitation with β -CD [11], dual encapsulation with chitosan for 3D printing [12], and ternary lycopene/HPCD/PEG6000 systems via hot melt extrusion, achieving 32-fold solubility increase over lycopene/ β -CD [13].

Chitosan (CS), a biocompatible, biodegradable cationic polymer of D-glucosamine and N-acetyl-D-glucosamine, is a versatile nanocarrier [14]. It enhances mucoadhesion, permeation, and controlled release in oral delivery [15]. CS combined with HPCD improved mesalazine delivery, yielding nanoparticles (~90 nm, 33.8 mV) with superior inhibition of inflammatory mediators and sustained release compared to free mesalazine [8].

This study hypothesizes embedding lycopene in HPCD to enhance solubility, with CS as a nanocarrier for controlled release of Lyc-HPCD. Sodium tripolyphosphate (TPP) was used as a crosslinker to form CS nanoparticles. Characterization employed UV-Vis, FTIR, DSC, DLS, zeta potential, and SEM. This strategy offers a promising platform for oral or topical delivery of lycopene and other carotenoids in food supplements and cosmetics.

2. EXPERIMENTAL

2.1. Material

Lycopene was received from the Vietnam Academy of Science and Technology, extracted from

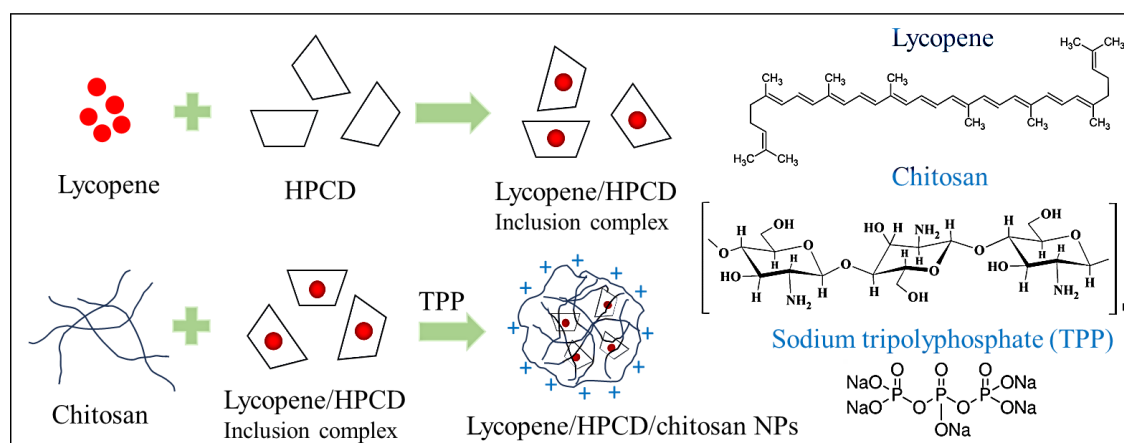
Gac fruit (*Momordica cochinchinensis Spreng*), and preserved at -8 °C [16]. Besides, (2-Hydroxypropyl)- β -cyclodextrin from Sigma Aldrich, chitosan (Sigma Aldrich, low molecular weight), TPP (sodium tripolyphosphate from Sigma Aldrich, technical grade 85 %). The solvents such as dichloromethane (DCM), acetone, methanol, absolute ethanol, and doubly distilled water are used without further purification.

From previously reported research, the extraction of lycopene from Gac aril was conducted by dichloromethane or chloroform, resulting in the content ranging from 0.28% to 0.46% with the purity more than 90%.

2.2. Experiment preparation

2.2.1. Preparation of lycopene/HPCD inclusion complex and chitosan-loaded inclusion complex

The inclusion complex of lycopene and HPCD was prepared by freeze-drying after sonication in acetone and mixing with HPCD solutions at varying mole ratios [9]. The mixture was vortexed for 24 h in the dark, centrifuged, filtered to remove free lycopene, and lyophilized for storage at -20 °C. A physical mixture of lycopene and HPCD was also prepared for comparison. For chitosan nanoparticles, filtrates containing Lyc HPCD were mixed with TPP and CS solution, producing a turbid red suspension after 24 h. Nanoparticles were recovered by centrifugation, washed, lyophilized, and characterized with controls prepared without Lyc HPCD. The process was summarized in Scheme 1.



Scheme 1. Formation of Lycopene-HPCD inclusion complex and ionic gelation-assisted chitosan nanoparticles containing Lycopene-HPCD.

2.2.2. Characterization methods

UV-Vis spectra were recorded for Lyc-HPCD complexes at different mole ratios, along with standard lycopene and HPCD. The size and zeta potential of Lyc-HPCD-chitosan nanoparticles were measured after redispersion and sonication. FTIR and DSC analyses were performed on lycopene, HPCD, physical mixtures, inclusion complexes, CS nanoparticles, and Lyc-HPCD-CS samples. SEM imaging further revealed the morphology of lycopene, HPCD, inclusion complex, and Lyc-HPCD-CS nanoparticles.

2.2.3. Determine the encapsulation rate

To determine the inclusion efficiency, the procedure was conducted as described [17]. For Lyc-HPCD, filtrates were diluted with ethanol (1 mL), vortexed, then mixed with CH_2Cl_2 (2 mL) to separate layers. The lower red layer was diluted with ethanol, and absorbance was measured at 472 nm.

$$\text{Entrapment efficiency (\%)} = \frac{\text{amount of lycopene recorded}}{\text{the initial amount of lycopene}} \times 100$$

Unlike Lyc-HPCD, the encapsulation efficiency of Lyc-HPCD-Chitosan was determined indirectly from the supernatant after centrifugation. A small volume of supernatant was mixed with 1 mL of ethanol, vortexed, and then combined with 2 mL CH₂Cl₂ to form two layers. The lower orange layer was diluted with ethanol to quantify untrapped lycopene.

$$\text{Entrapment efficiency (\%)} = \frac{\text{total lycopene} - \text{untrapped lycopene}}{\text{total lycopene}} \times 100$$

Standard lycopene solutions in CH₂Cl₂ diluted with ethanol were prepared, absorbance was measured at 472 nm, and a calibration curve was established to calculate efficiencies. All of the experiments were performed in triplicate, and the average value \pm SD.

2.2.4. *In vitro* release

A drug release experiment was conducted by using a dialysis membrane (molecular weight cut off 3.5 kDa) in the medium of phosphate buffers, such as pH 2 and 7, which are close to gastric and intestinal conditions [18]. The dialysis membrane was pretreated overnight with release medium before testing. Lycopene samples (pure, Lyc-HPCD, and Lyc-HPCD-chitosan) were placed in dialysis bags and immersed in release medium at 37 °C with stirring, with aliquots withdrawn at intervals and replaced to maintain volume. Extracted lycopenes were analyzed at 472 nm using a calibration curve to quantify released lycopene.

3. RESULTS AND DISCUSSION

3.1. The formation of an inclusion complex of lycopene

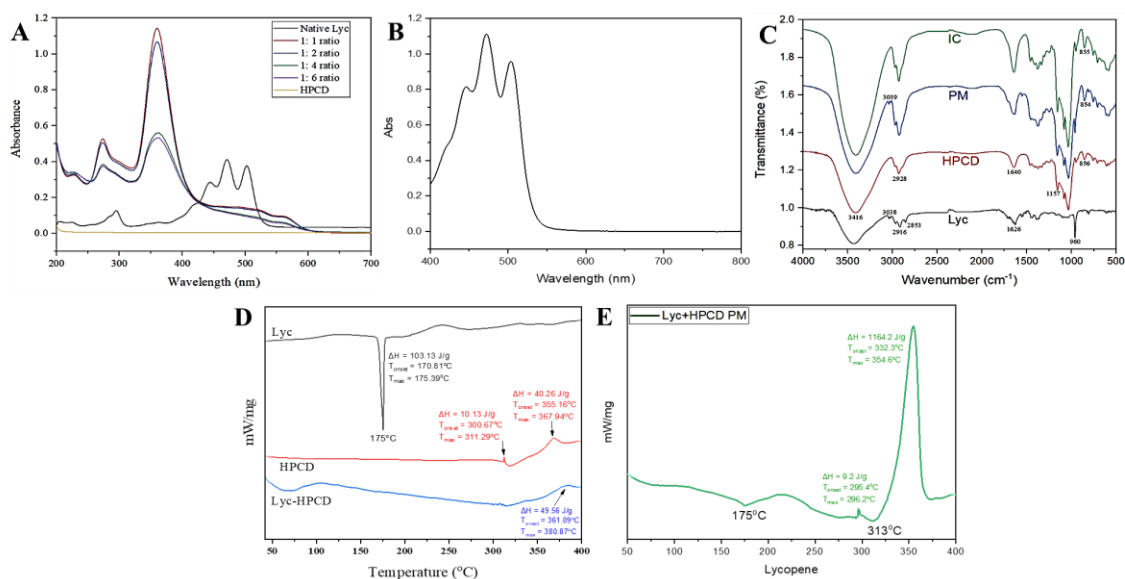


Figure 1. (A) UV-Vis spectra of lycopene (Lyc), HPCD, and Lyc-HPCD complexes at mole ratios, with (B) additional spectra of lycopene extracted from the complex. (C) FTIR analysis compared lycopene, HPCD, the physical mixture, and the inclusion complex, while (D) DSC curves characterized lycopene, HPCD, and their complex. (E) A separate DSC curve of the physical mixture highlighted differences from the inclusion complex.

Instead of phase solubility, encapsulation efficiency, and complex type were investigated at different lycopene/HPCD mole ratios. UV-Vis spectra (Figure 1A) showed lycopene peaks at 443, 472, and 502 nm, which disappeared and shifted after interaction with HPCD, confirming inclusion complexation rather than non-inclusion [10, 17, 19]. Recovery with ethanol/DCM (Figure 1B) yielded signals similar to native lycopene, with $63.0 \pm 3.1\%$ encapsulation at a 1:1 ratio. Higher

HPCD concentrations reduced lycopene loading due to self-aggregation of HPCD or guest/host interactions-induced aggregates [20]. FTIR spectra (Figure 1C) revealed characteristic HPCD peaks (3416, 2970, 2928, 1640, 1157, 856 cm^{-1}) [21, 22] and lycopene peaks (1626, 2853, 2916, 3038, 960 cm^{-1}) [12, 13]. The disappearance of the latter two in the complex indicated that lycopene was shielded by HPCD. Raman studies elsewhere confirmed hydrophobic interactions and C=C band shifts consistent with host-guest binding [23]. DSC analysis (Figure 1D–E) showed lycopene melting at 175 °C ($\Delta H = 103.13 \text{ J/g}$) with decomposition at 225–250 °C [13, 22, 24], while HPCD decomposed at 311 and 367 °C. In the complex, lycopene’s endothermic peak vanished, replaced by a broad peak at 380.87 °C ($\Delta H = 49.56 \text{ J/g}$), reflecting water displacement in the cavity and reduced crystallinity to an amorphous state [13, 17]. In contrast, the physical mixture retained distinct lycopene and HPCD peaks (175 °C, 354.6 °C), strongly supporting successful inclusion complex formation.

3.2. The formation of medicated chitosan nanoparticles

After enhancing lycopene solubility with HPCD, the inclusion complex was transferred into chitosan nanoparticles for sustained release. Table 1 summarizes the effects of chitosan/TPP mass ratio, chitosan concentration, and pH on particle size and polydispersity index (PDI). Nanoparticle formation occurred spontaneously via ionic gelation between polycationic chitosan and polyanionic TPP [25]. Increasing chitosan concentration enlarged particle size, but excessive amounts promoted aggregation due to reduced intermolecular spacing [26]. pH also strongly influenced nanoparticle properties: at low pH (<3.5), protonation of amine groups prevented particle formation, yielding transparent solutions; at high pH (>6.3), reduced protonation led to turbidity and aggregation from diminished electrostatic repulsion [8]. Under optimized conditions, the smallest Lyc-HPCD-Chitosan nanoparticles measured 127.6 nm with zeta potential +21.38 mV and PDI 0.207, confirming stability and moderate dispersity. Encapsulation efficiency reached $68.79 \pm 3.75\%$, demonstrating effective loading of the inclusion complex into chitosan carriers.

Table 1. The impact of CS/TPP mass ratio, the chitosan concentration, and pH on the size and zeta potential of chitosan nanoparticles containing Lyc-HPCD.

pH of chitosan	TPP (mg)	Chitosan (mg/mL)	Lyc-HPCD (mL)	Size (nm)	PDI	Zeta (mV)
4.70	2.4	0.75	3	218.2	0.320	+ 28.94
4.70	3.4	0.75	3	201.3	0.318	+ 29.03
4.70	4.3	0.75	3	175	0.283	+ 27.05
4.70	5.6	0.75	3	144.9	0.235	+ 21.31
4.70	6.2	0.75	3	127.6	0.207	+ 21.38
4.70	6.2	1.05	3	218.8	0.364	+ 32.57
4.70	6.2	1.35	3	315.1	0.356	+ 37.00
4.70	6.2	1.65	3	402.4	0.294	+ 39.27
4.70	6.2	1.95	3	581.7	0.275	+ 40.92
5.24	6.2	0.75	3	167.6	0.243	+ 32.37
5.62	6.2	0.75	3	164.7	0.291	+33.27
3.38	6.2	0.75	3	130.9	0.377	+23.68
4.26	6.2	0.75	3	135.3	0.331	+27.14

Table 2 compares our formulation with other reports. Although encapsulation efficiency was moderate, our nanoparticles exhibited the smallest size among comparable systems and a positive zeta potential, which is favorable for absorption. This highlights chitosan as a safe, multifunctional carrier with potential in food and cosmetics. Physicochemical characterization is critical in drug delivery, ensuring stability, safety, and efficacy by revealing drug-carrier interactions and predicting bioavailability and toxicity.

Table 2. The comparison of lycopene encapsulation efficiency (EE %) in some research.

Method	Usage	EE (%)	Appearance	Ref.
Kneading method with β -CD and thin-layer encapsulation by liposome	1:4 molar ratio	78.9 ± 3	A formulation with a size of 255.15 ± 3 nm, zeta potential -32.6 mV, showing a sustained release up to 49.5% after 12 hours.	[22]
Co-precipitation with β -CD	1:1 molar ratio	71.8	Rod shape, $2.5\mu\text{m}$ in length and $1\mu\text{m}$ in width.	[10]
Nanoliposome	4 mg/mL	71.9	Only 5% of lycopene was released from the nanoliposomes after 2 h, and about 50% after a 12 h period.	[27]
Chitosan contains lycopene-Hydroxypropyl- β -cyclodextrin inclusion complex.	CS/TPP 2.4, 0.75 mg/mL CS, pH 4.7	68.79 ± 3.75	The nanoparticles were 127.6 nm in diameter, with a zeta potential of $+21.38$ mV.	This study

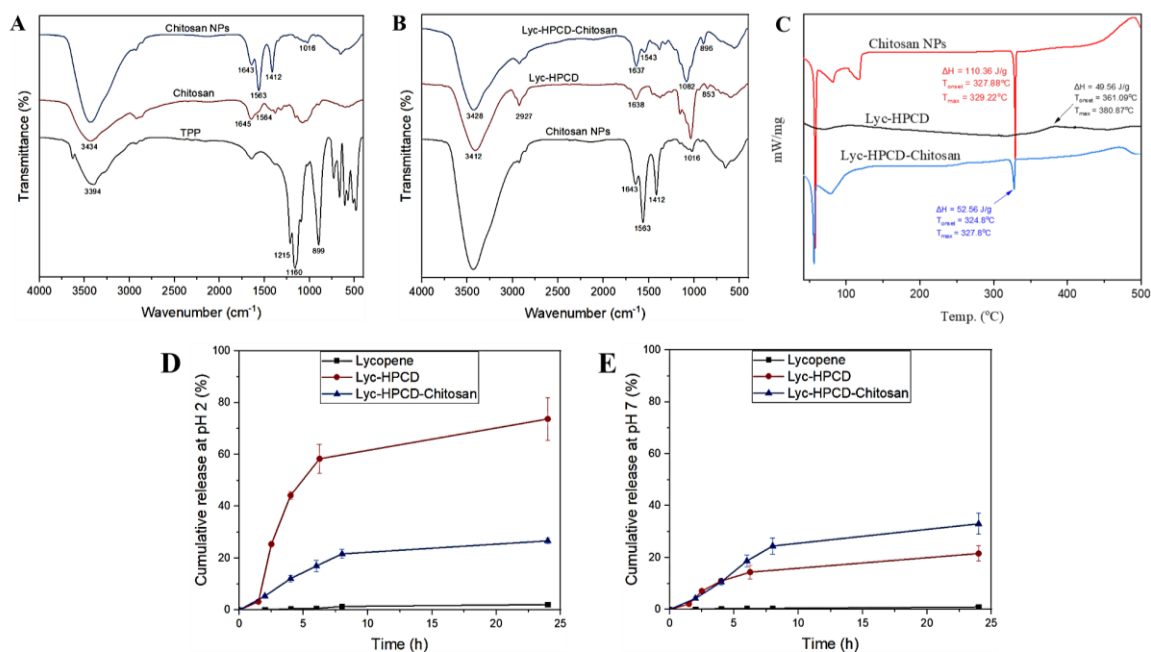


Figure 2. (A) FTIR spectra were recorded for TPP, chitosan, and chitosan nanoparticles, while additional FTIR (B) and DSC graphs (C) compared chitosan nanoparticles, Lyc-HPCD complexes, and Lyc-HPCD-chitosan nanoparticles. Drug release profiles at pH 2 (D) and pH 7 (E) further illustrated the release behavior of lycopene, Lyc-HPCD, and Lyc-HPCD-chitosan over time.

FTIR spectra (Figure 2A) confirmed chitosan nanoparticle formation: TPP peaks at 1215, 1161, 1092 cm^{-1} (P=O, PO₂, PO₃ stretching) and 899 cm^{-1} (P–O–P) [26–28]; chitosan peaks at 3435 (OH), 2927/2862 (C–H), 1646 (C=O), and 1565 cm^{-1} (N–H) [28]. Shifts to 1643 and 1564 cm^{-1} in nanoparticles indicated NH₃⁺–phosphate interactions, with additional C–N and P=O bands at 1413 and 1016 cm^{-1} [29]. In Figure 2B, further shifts (1638 and 1543 cm^{-1}) confirmed incorporation of Lyc-HPCD into chitosan. DSC (Figure 2C) showed moisture loss at 60–120 °C; chitosan decomposed at 329 °C ($\Delta H = 110.36$ J/g), but this peak weakened (327.8 °C, $\Delta H = 52.56$ J/g) after

complexation [25]. The main peak of Lyc-HPCD at 380.87 °C disappeared in the composite, confirming encapsulation. Release studies (Figures 2D–E) at pH 2 and 7 demonstrated the role of HPCD in solubility and of chitosan in sustained release. At pH 2, Lyc-HPCD showed burst release (~60% at 6 h, 70% at 24 h), while Lyc-HPCD-chitosan released only 20–24%. At pH 7, release from Lyc-HPCD decreased (19% at 24 h), whereas Lyc-HPCD-chitosan improved to 33%. This behavior reflects chitosan’s pH-dependent amino groups: acidic conditions dissolve the matrix, enhancing release, while neutral pH reduces protonation, weakens crosslinking, and increases porosity, allowing controlled liberation [14, 30]. The morphologies of native lycopene, HPCD, Lyc-HPCD, and Lyc-HPCD-Chitosan are presented in Figure 3. There are changes in the form of the original ingredients. Pure lycopene has a crystalline structure with various sizes, while HPCD is porous and spherical with cavities [9]. Then, the inclusion complex shows a change in morphology compared with the ingredients; porous and spherical shapes were replaced by irregular amorphous lumps, which indicated the formation of a new solid phase. Finally, the Lyc-HPCD-Chitosan gave smaller particles, though they were sticky and aggregated after free-drying due to the bio-adhesion of natural polysaccharides [31]. Therefore, mannitol could be applied to reduce the mechanical stress during the freeze-drying process to prevent aggregation and will be further investigated in the future to get non-sticky, monodisperse nanoparticles for the oral or transdermal route.

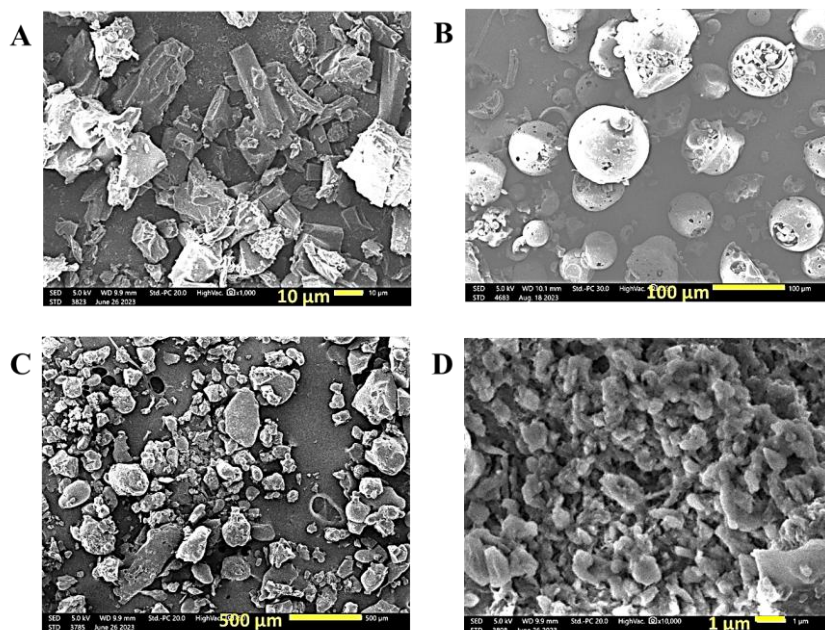


Figure 3. SEM pictures of (A) lycopene, (B) HPCD, (C) Lyc-HPCD, and (D) Lyc-HPCD-Chitosan.

4. CONCLUSIONS

Lycopene’s water solubility was enhanced through complexation with Hydroxypropyl- β -cyclodextrin and encapsulation in chitosan nanoparticles. The inclusion complex was characterized using UV–Vis, FTIR, DSC, and SEM to reveal its physicochemical and structural properties. Chitosan nanoparticles acted as efficient carriers, with a size of 127.6 nm and zeta potential of +21.38 mV. They achieved $68.79 \pm 3.75\%$ active loading and enabled sustained release for 24 hours. These formulations show promise for functional food and cosmetic applications due to their safety and efficacy. However, further studies are needed to assess stability and gastrointestinal behavior in vivo.

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TÓM TẮT

Tổng hợp nano chitosan mang phức hợp tan trong nước của Hydroxypropyl- β -Cyclodextrin-Lycopene: Đặc tính hóa lý và khả năng giải phóng in-vitro

Lycopene (Lyc), có nguồn gốc từ quả gấc (*Momordica cochinchinensis Spreng*), là một chất chống oxy hóa mạnh mẽ đối với sức khỏe con người, nhưng khả năng tan trong nước của nó là một vấn đề quan trọng đối với các ứng dụng thực tiễn. Trong nghiên cứu này, lycopene được tạo phức với Hydroxypropyl- β -cyclodextrin (Lyc-HPCD), và kỹ thuật tạo gel ion được sử dụng để hình thành các hạt nano chitosan mang phức bao. Đối với Lyc-HPCD, tỷ lệ mol là 1:1, hiệu suất bao gói khoảng $63,0 \pm 3,1\%$. Đối với nano chitosan mang phức bao (Lyc-HPCD-Chitosan), các hạt nano có đường kính 127,6 nm, thế zeta +21.38 mV, với hiệu suất bao gói khoảng $68,79 \pm 3,75\%$. Chúng được đặc trưng bằng các phương pháp UV-Vis, FTIR, DSC và SEM, và cho thấy khả năng giải phóng kéo dài trong 24 giờ ở các giá trị pH khác nhau. Hệ chất mang nano này thúc đẩy khai thác lycopene trong nhiều ứng dụng khác nhau, chẳng hạn như thực phẩm bổ sung hoặc mỹ phẩm.

Từ khóa: Lycopene; Hydroxypropyl- β -cyclodextrin; Nano chitosan; Chống oxy hóa.