Chitosan-Based Nanoparticles for Controlled-Release Delivery of α–Mangostin

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***Abstract*—The aim of this present work was to prepare chitosan-based nanoparticles for controlled-release delivery of orally administered α–mangostin. Chitosan/alginate (CS/ALG) and thiolated chitosan/alginate (TCS/ALG) nanoparticles loaded α–mangostin were prepared by ionotropic gelation method, following by crosslinking with genipin (GP) at the various times in order to achieve the α–mangostin release in a controllable way. Nanoparticles were characterized in terms of particle size, size distribution and surface potential by a dynamic light scattering, while α–mangostin encapsulation, entrapment efficiency and capacity were also determined. After characterizing the nanoparticles, the α–mangostin release pattern was studied in simulated gastrointestinal conditions. The particle size analysis showed that CS-based particles exhibited a nanoscale in the aqueous solution, but dilated to the larger particle size when crosslinked with GP. However, the surface potential was decreased with the increasing the crosslinking time. The TCS-based nanoparticles provided a slight smaller particle than that of CS. In addition, a high α–mangostin loading was found with the long-time crosslinking reaction. The α–mangostin release pattern demonstrated that the crosslinking times affected the α–mangostin release patterns. The CS/ALG and TCS/ALG nanoparticles, following by crosslinking with GP exhibited a controlled release profile. The results followed Higuchi's model that explained the main mechanism of α–mangostin release was diffusion. These results demonstrated that nanoparticles constructed with CS and TCS, following by GP crosslinking can be a promising controlled-release drug delivery system for natural or hydrophobic compounds such as α–mangostin.**

***Index Terms*—chitosan, thiolated chitosan, nanopartices, α–mangostin**

I. Introduction

The controlled-release formulations are designed and developed for a particular purpose, as for instance increasing the release duration, achieving a particular kinetic mechanism or delaying the release initiation [1]. For the oral controlled-release formulations is mainly passed the gastrointestinal conditions that varies and hence the number of barriers to reach the drug at active site or to be absorbed. The pH environment of stomach to colon is ranged from 1 to 7.5, also induces the oxidation, deamination and hydrolysis of drug. In addition, the colon is the main site of absorption for the controlled-release delivery systems, because they are designed to release the drug over a long period of time (up to 24 h). The small intestine is not long enough to ensure a complete absorption of the drug. Thus, the controlled-release systems need to be stable and protect the drug from the conditions of gastrointestinal tract. The carrier system such as polymeric nanoparticles (NPs), micro/nanoemulsions, liposomes, micelles offer the major improvements in physical and chemical stability and the efficient delivery of drug [2]. NPs, particles with a diameter lower than 1 μm are able to overcome several barriers of oral drug delivery. They are stable in the gastrointestinal tract and protect the encapsulated drug from the pH and enzyme degradation [3]. Moreover, NPs can avoid the release of drug in the upper gastrointestinal tract. Polymeric NPs can be prepared from the natural and synthetic polymers. Chitosan (CS) and its derivatives such thiolated chitosan (TCS), a natural-based polymer obtained by alkaline deacetylation of chitin have the properties to be a promising material for NPs drug delivery. CS-based NPs are generated by auto-aggregation between CS and macromolecules of opposite charge, or when ionic crosslinking agent exists [4]. Genipin (GP), a natural crosslinking agent reacts with primary amine groups of CS, thus producing the rigid NPs structure and the slow degradation rate to control the swelling, degradation and drug release rates. The α-mangostin, a natural compound shows the pharmacological activities. It reported to have a great variety of activities including antioxidant, antibacterial, cytotoxic, anti-inflammatory, anti-HIV activity. Furthermore, α-mangostin is insoluble in water, has low oral bioavailability and light instability. Thus, CS-based NPs may be used for the delivery of α-mangostin in the controllable way and protected the stability of α-mangostin. The aim of this present work was to prepare

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CS and TCS-based NPs for controlled-release delivery of orally administered α–mangostin. The characteristics of NPs was evaluated such as particle size, surface potential, entrapment efficiency and the α–mangostin release at gastrointestinal condition.

II. Materials and Methods

A. Materials

The α-mangostin was purified from a pericarp of mangosteen, a gift from Kaewmungkorn Co., Ltd., Thailand. CS (degree of deacetylation, 0.85; MW, 110 kDa), ALG, alginic acid sodium salt from brown algae, cysteine hydrochloride, 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC) and 5, 5'-dithio-bis-(2-nitrobenzoic acid) (DTNB) were purchased from Sigma, St. Louis, MO, USA. GP, as crystal-like powders, reagent grade was obtained from Challenge Bioproducts Co., Ltd. (Taichung, Taiwan). All other reagents and solvents were commercially available and were of analytical grade.

B. Methods

Preparation of GP-crosslinked α–mangostin-loaded CS-based NPs: CS, low molecular weight and degree of deacethylation of 0.85 and the synthesized TCS were used to prepare α-mangostin-loaded CS and TCS-based NPs. TCS was synthesized following as the preparation procedures [5]. Briefly, the carboxylic acid groups of cysteine were activated with EDAC and followed by adding into the CS solution. The mixture was stirred for 6 h and then the TCS was isolated in the dark by dialysis. The amount of thiol group was determined with Ellman's reagent.

The α-mangostin-loaded CS and TCS-based NPs were prepared using ionotropic gelation method. The α-mangostin was dissolved in ethanol and then was slowly added into 0.025 % w/v ALG solution, and followed by dropwise into 0.05 % w/v CS or TCS solution (in 2 %v/v acetic acid) using a 27-gauge, stainless steel needle. The mixture was stirred under mechanical stirring at 1400 rpm for 30 minutes. The GP crosslinking of NPs was conducted by the addition 0.0125 % w/v of GP into the suspension of NPs. The mixture was stirred under mechanical stirring for 2, 4 and 6 hours and then washed twice with deionized water to remove the excess α–mangostin and GP. The non-crosslinked NPs were also investigated.

Evaluations of GP-crosslinked α–mangostin-loaded CS-based NPs: The mean particle size and surface zeta potential of NPs were characterized by a dynamic light scattering using a Zetasizer Nano ZS (Malvern Instruments, Malvern, UK).

The amount of α–mangostin in the CS and TCS-based NPs was analyzed using high-performance liquid chromatography (HPLC) with a C18 column. The HPLC analysis was performed, as previous described [6]. The each NPs formulation was accurately weighed, dispersed in methanol and centrifuged at 14000 rpm for 30 min to break the NPs. The supernatant was collected and filtered through a 0.45 μm nylon membrane filter before quantifying the amount of α–mangostin using HPLC. The entrapment efficiency (EE) and entrapment capacity (EC) were determined according to equation (1) and (2), respectively.

$$EE\ (\%) = \frac{amount\ of\ \alpha\text{–}mangostin\ in\ nanoparticles}{initial\ of\ amount\ \alpha\text{–}mangostin\ used} \times 100 \quad (1)$$

$$\text{EC}\ (\%) = \frac{amount\ of\ \alpha\text{–}mangostin\ in\ nanoparticles}{amount\ of\ nanoparticles\ and\ \alpha\text{–}mangostin} \times 100 \quad (2)$$

The release characteristics of α–mangostin from the NPs were evaluated using a dialysis method in three conditions according to the gastrointestinal condition. The NPs was transferred to a dialysis bag and then immersed into 0.1 N HCl (pH 1.2) under the shaken speed of 200 rpm with sink conditions at 37 ± 0.5°C for 2 h. Afterwards, the pH was adjusted to 6.8 for 5 h and followed by pH 7.4 for 8 h. At a given time point, an aliquot of the medium was withdrawn and an equal amount of the fresh medium was refilled. The amount of α-mangostin in sample solutions was analyzed by HPLC. The release kinetics of α–mangostin from the NPs was investigated using a zero-order model, a first-order mode and the Higuchi's model.

C. Statistical Analysis

The experimental measurements were collected in triplicate. Data are represented as the means ± SD.

III. Results and Discussion

A. Particle Size and Surface Zeta Potential of GP-Crosslinked α–Mangostin-Loaded CS-Based NPs

The CS and synthesized TCS were successfully prepared the NPs with ALG. The NPs were obtained spontaneously under the ionotropic gelation method. Their particle sizes and surface zeta potential are present in Table I. The different CS derivatives influenced the size of the NPs. The mean particle size of CS-based NPs (ranged from 412 to 743 nm) was slight bigger than those of TCS (ranged from 365 to 532 nm). TCS exhibited the free thiol groups on the polymeric backbones, resulted in the disulfide bond within their polymeric chain [7]. These might interacted with the TCS-based NPs and decreased in the particle size. The GP crosslinking with CS and TCS-based NPs showed that the mean particle sizes of GP-crosslinked NPs were larger than those of non-crosslinked NPs, because the GP formed the intra and intermolecular covalent bonds with the amine groups of NPs, enlarged the linkages between the CS and TCS with ALG and resulted in the larger particles [8]. Moreover, the long duration of GP crosslinking was obviously seen the increase in the mean particle size. The particle size ranking of the NPs was 6 h > 4 h > 2 h of GP-crosslinked NPs > non-crosslinked NPs. Table I also shows the zeta potential of NPs. The NPs possessed positive charges (51 to 66 mV) on the surface according to the amine groups of CS and TCS. The positive surface charges exhibited the stability of the colloidal systems including the NPs [9]. The TCS-based NPs provided a slightly lower in positive

charges than those of CS which was due to the reduction of free amine groups on the TCS polymeric backbones by thiol substitution [10]. However, the amine groups were not completely substituted and there were some amine groups that provided the positive charge on the surface of TCS-based NPs. The reduced surface charges were obtained due to the neutralization of the positive charge present on the amine group of NPs after GP crosslinking [11].

TABLE I. THE MEAN PARTICLE SIZE AND ZETA POTENTIAL OF THE CS-BASED NPS

NPs	Particle size (nm)		Zeta potential (mV)	
	CS/ALG	TCS/ALG	CS/ALG	TCS/ALG
α-mangostin loaded NPs				
	437.6 ± 50.3	365.9 ± 6.2	66.2 ± 1.2	57.1 ± 2.3
GP crosslinked α-mangostin loaded NNPs				
2 h	715.4 ± 21.7	399.5 ± 1.9	55.6 ± 3.2	58.5 ± 1.0
4 h	412.4 ± 49.0	432.7 ± 7.1	54.7 ± 1.7	60.6 ± 1.7
6 h	743.8 ± 34.0	532.9 ± 15.1	53.2 ± 1.7	51.9 ± 1.8

B. Evaluations of GP-Crosslinked α–Mangostin-Loaded CS-Based NPs

The α–mangostin was incorporated into the NPs. The EE and EC are showed in Table II. The results showed the CS derivatives and GP crosslinking had an influence the EE and EC. The α–mangostin loaded TCS-based NPs showed the higher EE and EC than that those of CS. The hydrophobic α–mangostin was favor incorporated into the hydrophobic CS derivatives [12], resulting in the high EE and EC of TCS-based NPs. Moreover, TCS formed the disulfide bond within their chain and NPs that promoted the α–mangostin encapsulation in the NPs. Increasing the GP crosslinking time (from 2 to 6 h) led to the further increase in EE and EC. This was because the GP formed the covalent bonds, enlarged the linkages and particle size of NPs. There was a more capable space to incorporate the α–mangostin during the crosslinking process [13]. In addition, the high EE and EC led to a large particle size of GP-crosslinked NPs. The long duration time of GP crosslinking was observed the high EE, EC and large particle size of NPs.

TABLE II. THE AMOUNT OF ALPHA–MANGOSTIN IN THE NPS, PRESENTED AS THE EE (%) AND EC (%)

NPs	EE (%)		EC (%)	
	CS/ALG	TCS/ALG	CS/ALG	TCS/ALG
α-mangostin loaded NPs				
	14.6 ± 1.8	38.2 ± 1.7	0.29 ± 0.04	0.76 ± 0.02
GP crosslinked α-mangostin loaded NPs				
2 h	26.8 ± 2.8	53.1 ± 0.5	0.38 ± 0.02	0.79 ± 0.01
4 h	32.2 ± 3.3	54.4 ± 1.0	0.45 ± 0.02	0.80 ± 0.02
6 h	25.7 ± 3.1	57.3 ± 1.5	0.53 ± 0.05	0.85 ± 0.02

Based on the gastrointestinal condition that pH in the stomach is 1 to 2, in the small intestine is 5.1 to 7.5 and in the colon is 7 to 7.5 [14], the release characteristics of α–mangostin from the NPs and free drug was evaluated at these pH environments (pH 1.2, 6.8 and 7.4) in order to mimic the gastrointestinal condition. The time interval was at 2 h in the acidic condition, then 3-5 h in the pH 6.8 and 6-8 h in the pH 7.4. The release patterns in the different condition are demonstrated in Fig. 1. The α–mangostin free drug rapidly and completely released within 2-3 h. The release rate of α–mangostin from the non-crosslinked CS and TCS-based NPs (completely released within 5-6 h) was slower than that of the free drug due to the diffusion of α–mangostin across the tortuous NPs. The TCS-based NPs showed the slight slow release than those of the CS. The TCS exactly contains the thiol substitution, was formed the inter- and intra-disulfide bond and hence resulted in the slow α–mangostin release. Moreover, the release of α–mangostin further decreased according to the GP crosslinking to the NPs. GP conducted the rigid NPs that obstructed the diffusion between the release medium and α–mangostin for the release process [13]. The decrease in α–mangostin release was clearly seen when GP crosslinking for the longer time. The GP-crosslinked NNPs was very stable at all pHs and slowly release the α–mangostin. The 2 h of GP-crosslinked NPs provided a good α–mangostin release pattern, compared to the 4 and 6 h that gave the very low α–mangostin release. These results indicated that the 2 h of GP-crosslinked α–mangostin-loaded CS and TCS-based NPs may be a prospective candidate as controlled-release delivery carrier for the efficient delivery of α–mangostin.

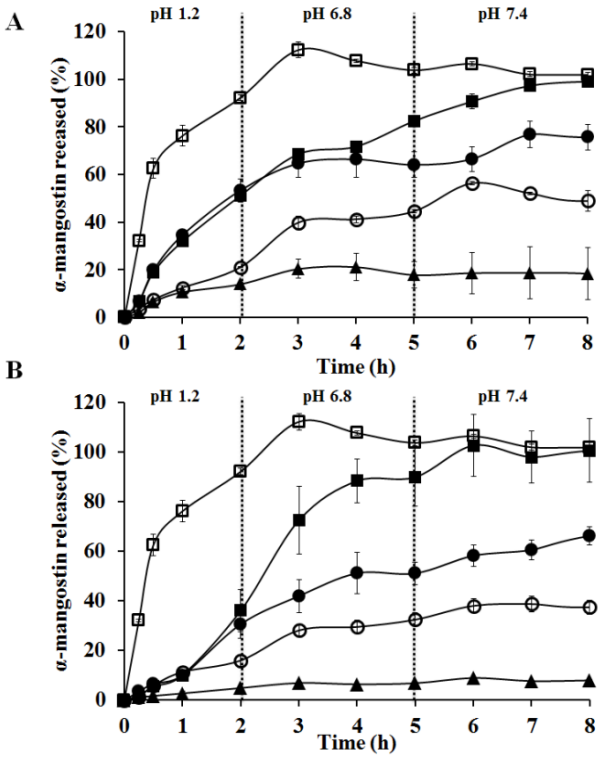

Figure 1. The α-mangostin release profiles from (A) CS and (B) TCS-based NPs at gastrointestinal condition; (■) non-crosslinked NPs, GP-crosslinked NPs for (●) 2 h, (○) 4 h and (▲) 6 h, compared to (□) α–mangostin free drug.

The release kinetics of α–mangostin was further investigated to determine the mechanism of α–mangostin release. Table III presents the R^2 of kinetics models for the α–mangostin release, fitting with zero-order, first-order and Higuchi's models. It was found that the release profiles were best fit by the Higuchi's model with the highest R^2. These indicated that the release kinetics was governed by the Higuchi's model and the main mechanism of the α–mangostin release was diffusion.

TABLE III. THE AMOUNT OF ALPHA–MANGOSTIN IN THE NPS, PRESENTED AS THE EE (%) AND EC (%)

Kinetics	Non-GP crosslinked NPs		GP crosslinked NPs		
			2 h	4 h	6 h
Zero order	CS/ALG	0.9142	0.7854	0.8464	0.4715
	TCS/ALG	0.8894	0.9256	0.9062	0.8322
First order	CS/ALG	0.6921	0.5874	0.7164	0.3372
	TCS/ALG	0.7091	0.7484	0.6456	0.7159
Higuchi's model	CS/ALG	0.9857	0.8737	0.9082	0.5323
	TCS/ALG	0.9399	0.9755	0.9678	0.9159

IV. CONCLUSION

The α–mangostin-loaded CS and TCS-based NPs were successfully prepared by an ionotropic gelation method with GP crosslinking process. The nanosized scale of NPs was obtained with the α–mangostin was incorporated into the NPs. The GP formed the molecular crosslinking with the NPs that provided the controlled-release of α–mangostin at gastrointestinal conditions. Thus, these NPs may have the potential to be a desirable candidate for a controlled release drug delivery system of hydrophobic compounds including α–mangostin.

CONFLICT OF INTEREST

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

AUTHOR CONTRIBUTIONS

Wipada Samprasit conducted the research, analyzed the data and developed the manuscript. Praneet Opanasopit proofed and gave the comment on the manuscript. All authors had approved the final version.

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