Characteristics and Bioactivities of Carrageenan/Chitosan Microparticles Loading A-mangostin

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CHARACTERISTICS AND BIOACTIVITIES OF CARRAGEENAN/CHITOSAN MICROPARTICLES LOADING α-MANGOSTIN

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Abstract

This study attempted to develop carrageenan/chitosan based microparticles loading α-mangostin which was extracted from Vietnamese mangosteen skin. The carrageenan/chitosan/α-mangostin microparticles were prepared by ionic gelation method by mixing chitosan, carrageenan with α-mangostin and subsequently cross-linking the mixtures with sodium tripolyphosphate crosslinking agent. The content of α-mangostin in microparticles was changed to evaluate the effect of α-mangostin content on physical, morphological properties, particles size and bioactivities of the

carrageenan/chitosan/α-mangostin microparticles. The obtained results showed that carrageenan, chitosan was interacted together and with α-mangostin. The presence of polymers matrix improved the release ability of α-mangostin into ethanol/pH buffer solutions. The carrageenan/chitosan/ α-mangostin microparticles have antibacterial (gram (+) strains) and anti-oxidant activities. The results suggested that combination of chitosan and carrageenan in the microparticles can enhance the control release of α-mangostin into solutions as well as keep the bioactivities of α-mangostin.
Keywords. α-Mangostin, carrageenan/chitosan drug delivery, solubility, anti-oxidant, antimicrobial activity.

1. INTRODUCTION

The α-mangostin (MGS) is a xanthone derivative compound extracted from the pericarps of Mangosteen – a tropical fruit which is mainly found in the Southest Asia countries like Vietnam, Thailand and Malaysia. This xanthone derivative is found to have a variety of bioactivities such as antibacterial [1, 2], anti-inflammatory [3-5], antioxidant [6,7], anticancer activities [8-10] as well as antifungal [11] and anti-allergic [12]. Therefore, the application of this compound in the pharmaceutical industry is promising and worth investing. In fact, mangosteen skins have usually eliminated directly to environment without treatment. It can cause the pollution environment as well as waste many valuable organic compounds in it. Therefore, the extraction of organic compounds from mangosteen skins, particularly, α-mangostin that have great bioactivities is a good way to re-use of mangosteen skins. However, direct consumption of mangostins is ineffective because of its hydrophobic characteristic. The α- mangostin has low solubility in water with only 2.03×10^{-4} $mg.L^{-1}$ at room temperature. Thus, there have been many studies to solve this disadvantage, including co - solvation, structure modification, complex formation [13-15] and micro/nanoparticle drug delivery systems [16-18]. Among the above methods, using micro/nanoparticles to increase the solubility of MGS is our focus in this work because they can help to improve the solubility and therapeutic index of the drug compounds [19, 20].

One of widely used nanoparticle drug delivery systems is polymeric drug delivery system, in which, polymers are natural or synthesis polymers having

biocompatibility and biodegradability [20, 21]. In our study, chitosan and carrageenan have been chosen for loading MGS to improve the solubility and bioavailability of MGS in use. Chitosan is a biopolymer can be obtained through the processing of seafood waste (crabs shells, lobsters shells, shrimps shells or krill shells) [21, 22]. It is found to be biodegradable, non-toxic and have good biocompatibility [23-25], and it can be used for safe drugs and bioactive compounds delivery. Additionally, chitosan can be adsorbed to the mucus membrane along the gastrointestinal tract thanks to its mucoadhesive property, hence it is usually applied to carry colon-targeted drugs [26-29]. Carrageenan which can be extracted from various types of reg algae like Agardhiella, Encheuma, Furcellaria, Gigartina and Hypnea, etc. are linear sulfated polysaccharides [30-32]. These polysaccharides also show myriads of bioactive properties such as antioxidant [33, 34], anticoagulant [35, 36], antiviral [37-39], antibacterial [40] and antitumor [41, 42]. Thus, it is a potential agent to be used in the biomedical fields to treat various diseases. Pacheco-Quito E-M *et al.* has made a schematic layout of carrageenan applications in various pharmaceutical formulations, including tablets, pellets, films, suppositories, inhalable systems, micro particles and nanoparticles [43].

For preparation of α–mangostin carrying polymeric system, the α-mangostin has been loaded by β-cyclodextrin [8, 44], PLGA [15, 45], chitosan [46], chitosan/alginate [47, 48], chitosan and Eudragit S100 [49]. The combination of chitosan and carrageenan as a carrier for α–mangostin has been limited in publication. Therefore, this study focuses on fabrication and characterization of the carrageenan/chitosan microparticles loading α–mangostin. In addition, the release ability and kinetic of α–mangostin from carrageenan/chitosan/α–mangostin microparticles in simulated body fluids as well as anti-oxidation and antimicrobial activity of α–mangostin and carrageenan/chitosan/α–mangostin microparticles will be evaluated and discussed.

2. EXPERIMENTAL

2.1. Materials

The main materials and chemicals used for the study are α-mangostin (MGS, powdery, extracted from the skin of mangosteen, purity of 90%, Vietnam),

carrageenan (powdery, κ-carrageenan is predominant, Sigma Aldrich), chitosan (powdery, M_w of $1.61x10^5$ Da, DDA ~ 75-85 %, Sigma Aldrich), sodium tripolyphosphate (STPP, powdery, Sigma Aldrich), ethanol (99.7 %, Vietnam), acetic acid (99.5 %, China), etc.

2.2. Preparation of carrageenan/chitosan/α–mangostin microparticles

The procedure for preparation of carrageenan/chitosan/α–mangostin microparticles is following:

Preparation of carrageenan solution: 50 mg of carrageenan was added into 100 mL of distilled water. The mixture was stirred on the magnetic stirrer at 80°C for 15 minutes to completely dissolve the carrageenan to form a transparent solution. Next, the carrageenan solution was stirred and cooled to 50°C before slowly adding the KCl solution (5 mg KCl/5 mL distilled water). The solution was continuously stirred for 15 minutes to obtain a transparent carrageenan solution (solution A).

Preparation of chitosan solution: 100 mg of CS was added into 100 mL of 1% acetic acid solution. The mixture was stirred on the magnetic stirrer for 30 minutes to obtain chitosan solution (solution B).

Preparation of MGS solution: An accurately amount of MGS was weighted and added to 20 mL of ethanol to get a transparent yellow MGS solution (solution C).

Preparation of STPP solution: 20 mg of STPP was dissolved in 2 mL of distilled water (solution D).

The solution A was cooled to 40°C before adding slowly the solution B and ultra - sonication at 10.000 rpm to obtain solution AB. Next, solution C was dropped at a rate of 3 mL/min to the solution AB in ultrasonic stirring. Then, solution D was added slowly to the solution AB to cross-link polymer in solution. After that, the solution was maintained in ultrasonic stirring for 5 minutes to obtain a homogeneous solution. Finally, the solution was iced in salt-ice-water mixture for 2 hours before centrifuging at 6000 rpm to obtain the solid part. The solid part was freeze-dried, finely grounded and stored in PE tubes at room temperature until use. The ratio of components and designation of carrageenan/chitosan/α–mangostin samples were presented in Table I.

Table I

2.3. Characterization

The morphology of the CCG microparticles was evaluated using field emission scanning electron microscope (FESEM) (Hitachi S-4800, Japan). The size distribution of the CCG microparticles was assessed using dynamic light scattering (DLS) method (SZ100, Horiba, Japan). The thermal behavior of the CCG microparticles was determined using differential scanning calorimetric (DSC) (DSC204F1, Netzsch, Germany).

2.4. Drug release investigation

2.4.1. Setting up calibration equation of MGS in different pH buffer solutions

When taken orally, MGS and CCG microparticles will be taken in the digestive system with different pH environments. Therefore, investigation of MGS release will be done in different pH buffer solutions (pH 1.2, pH 4.5, pH 6.8, pH 7.4), which simulated the body fluids.

To determine the amount of MGS released from CCG microparticles, it is necessary to determine calibration equation of MGS in pH solutions with above list of pHs. Due to the poor solubility of MGS in buffer solutions, ethanol was mixed with buffer solution (50/50 v/v) to evaluate more accuracy the release of MGS [48].

The calibration equation of MGS in pH solutions was built by diluting method from solution having standard concentration. 10 mg of MGS was added to 200 mL of buffer/ethanol solution. The mixture was stirred continuously for 8 hours until MGS was dissolved completely. Next, this solution was withdrawn and diluted to certain concentrations before taking ultraviolet and visible (UV-Vis) spectroscopy (S80 Libra, Biochrom, UK). Excel software was used to build the calibration equation of MGS based on the obtained optical density values and to calculate the regression coefficient (R^2).

2.4.2. Drug release analysis

10 mg of CCG microparticles was added in 200 mL of buffer solution. The mixture was stirred continuously for 360 minutes at 37°C. During the first hour, for every 20 minute and then every hour after that, exactly 5 mL of the solution was withdrawn and 5 mL of fresh buffer solution was added to maintain volume of solution. Next, the withdrawn solution was measured UV-Vis spectra at the maximum

wavelength. The amount of MGS released from CCG microparticles is calculated based on the calibration equation and the measured optical density value. The experiment was done in triplicate and the value is mean value.

The percentage of MGS released is calculated using the formula:

$$\% \text{ MGS released} = \frac{C_t}{C_0} \times 100 \quad (1)$$

Where: C_0 and C_t are initial carried MGS and released MGS at time t, respectively.

2.5. Bioactivities of MGS and CCG microparticles

2.5.1. Antibacterial activity testing method

This is a method to test the antibacterial activity in order to evaluate the level of strong antimicrobial strength of test samples through turbidity of the culture medium. The values for showing activity are IC_{50} (50% Inhibitor Concentration), MIC (Minimum Inhibitor Concentration), MBC (Minimum Bactericidal Concentration). and MFC (Minimum Fungicidal Concentration). Typical bacteria and fungi such as: *Bacillus subtilis* (ATCC 6633): are gram (+) bacilli, spore-forming, usually not pathogenic; *Staphylococcus aureus* (ATCC 13709): gram (+) cocci, causing purulent wounds, burns, sore throat, purulent infections on the skin and internal organs; *Lactobacillus fermentum* (N4): gram (+) bacteria, which are beneficial fermented stomach bacteria, are often present in the digestive system of humans and animals; *Escherichia coli* (ATCC 25922): gram (-) bacteria, causing some digestive tract diseases such as gastritis, colitis, enteritis, bacillary dysentery; *Pseudomonas aeruginosa* (ATCC 15442): gram (-) bacteria, green pus bacillus, causing sepsis, infections of the skin and mucous membranes, causing inflammation of the urinary tract, meningitis, endocarditis, enteritis; *Salmonella enterica*: gram (-) bacteria, bacteria that cause typhoid, intestinal infections in humans and animals; *Candida albicans* (ATCC 10231): yeast, which often causes thrush in children and gynecological diseases. Growth testing medium: MHB (Mueller-Hinton Broth), MHA (Mueller-Hinton Agar); TSB (Tryptic Soy Broth); TSA (Tryptic Soy Agar) for bacteria; SDB (Sabourand-2% dextrose broth) and SA (Sabourand- 4% dextrose agar) for fungi.

Testing procedure was indicated as follow:

* *Test samples dilution:*

The original sample is diluted with 2 steps, firstly in 100% DMSO then distilled water into a series of 4-10 concentrations. The highest test concentration was 256 µg/mL with the extract and 128 µg/mLof the clean matter. In special cases, samples are mixed as required.

** Activity testing:*

- The test microorganisms are kept at -80ºC. Before the experiment, the test microorganisms are activated in the culture medium so that the concentration of bacteria reaches $5x10^5$ CFU/mL; Fungi concentration reached $1x10^3$ CFU/mL.

- 10µL of sample solution at different concentrations was added to 96-well plate, then 190 µL of active microorganism solution was added, incubated at 37°C for 16-24 hours.

** Result analysis:*

- MIC value was determined at the well which has the lowest concentration of sample inhibited completely the growth of microorganism.

- IC_{50} was calculated through the microorganism inhibition percentage by Rawdata software.

$$\% \text{ inhibition} = (OD_{control\ (+)} - OD_{sample})/(OD_{control\ (+)} - OD_{control\ (-)}) \times 100 \quad (2)$$

$$IC_{50} = High_{Conc} - \frac{(High_{Inh\%} - 50) \times (High_{Conc} - Low_{Conc})}{High_{Inh\%} - Low_{Inh\%}} \quad (3)$$

Where, $High_{Conc}$/Low_{Conc}: the sample at the high/low concentration; $High_{Inh\%}$/$Low_{Inh\%}$: inhibition percentage at high/low concentration).

** Reference substance*

- Ampicillin, cefotaxim, nystatin.

2.5.2. Anti-oxidant activity testing method

Analysis of the ability to trap free radicals generated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) is an approved method for rapid determination of antioxidant activity of samples. The sample was dissolved in dimethyl sulfoxide (DMSO 100%) and DPPH was diluted in 96% ethanol. The absorption of DPPH at λ = 515 nm (Infinite F50, Tecan, Switzerland) was determined after dropping DPPH to the test sample solution on a 96-well microplate and incubating at 37 ° C for 30 minutes. The

results of the tests were expressed as the mean of at least 3 replicate tests ± standard deviation ($p \leq 0.05$). Flavonoid or ascorbic acid was used as positive control.

The mean value of scavenging capacity (SC, %) at the sample concentrations was entered into an Excel data processing program by the following formula:

$$SC(\%) = \left[100 \times \frac{OD_{sample} - OD_{DMSO}}{OD_{control\ (-)}} \times 100\right] \pm \sigma \quad (4)$$

3. RESULTS AND DISCUSSION

3.1. Morphology of the carrageenan/chitosan/α-mangostin (CCG) microparticles

FESEM images of MGS and CCG microparticles prepared with different MGS content were shown in Figure I.

Figure I

As observation from Figure I, the MGS had a structure surface more separately than the CCG microparticles. The MGS was in thin sheets stacked together to form blocks (Figure I a, g). The CCG microparticles had a dense structure, chitosan and carrageenan were mixed and bonded together better through a polyelectrolyte complex (PEC) between OSO_3^- of carrageenan and protonated amine (NH_3^+) of chitosan as well as ionic cross-linking of tripolyphosphate anion bridges with NH_3^+ cations of chitosan and NH_3^+ cations of PEC [47]. The cross-linking of polymers in CCG microparticles through tripolyphosphate anion bridges could be also observed on the FESEM images. As loading MGS, the CCG microparticles tend to form smaller particles with less voids on the surface (Figure I i, j, k, l) as compared to the CCG0 sample (Figure I h). The MGS may be filled in the voids between chitosan and carrageenan, entrapped inside [50]. This indicated that MGS could interact effectively with chitosan and carrageenan in our proposed schema (Figure II).

Figure II

3.2. Particle size distribution of CCG microparticles

The CCG microparticles were dispersed in distilled water to record diagrams of their particle size distribution. These diagrams of CCG microparticles were shown in Figure III as well as the size range and average particle size of CCG microparticles

were listed in Table II. It is clear that the CCG0 microparticles had larger Z-average particle size than the CCG5, CCG10, CCG15 and CCG20 samples. This suggested that CCG microparticles loading MGS could be dispersed in water better than the CCG0 sample. From Figure III and Table II, the CCG microparticles had a range of size from 43 to 1106 nm with the various peak sizes depending on MGS content. The Z-average particle size of CCG microparticles is larger than peak size of them showing that a small and a large component in number of particles. The PDI > 0.4 indicated that the CCG microparticles has a broadly polydisperse distribution type. As increasing the MGS content in CCG microparticles, the Z-average size of samples tends an increase. This may be due to the hydrophobic nature of MGS.

Figure III

Table II

3.3. DSC analysis of CCG microparticles

DSC diagrams of the MGS and CCG microparticles were displayed in Figure IV. The melting temperature of MGS was found at 172.8^{o}C with the melting enthalpy or melting energy of 90.33 J/g (Table III) [51]. For carrageenan/chitosan microparticles without MGS, one broad peak appeared at 90.8 ^{o}C with the melting enthalpy of 396.7 J/g could be attributed for melting process of carrageenan and glass transition process of chitosan [52, 53]. The appearance of only one peak in range of 40^{o}C to 150^{o}C indicated that carrageenan was good miscible with chitosan through bonds as presented in Figure II. As loading MGS, the position of this above peak was slightly shifted and the enthalpy was decreased corresponding to the reduction in the crystallization of CCG microparticles (Table III). It may be due to the dispersion and interaction of MGS with polymers leading to the limitation in the molecular movement/mobility of the carrageenan and chitosan chains, and then presenting an amorphous state of the microparticles [48]. Another evidence for the interaction of MGS with polymers is the melting peak of MGS does not be assigned in DSC diagrams of the CCG10 and CCG20 microparticles. As increasing MGS content, the compatibility of MGS and polymers was reduced. This was exhibited a very small peak at around 175^{o}C in the DSC diagram of the CCG20 sample.

Figure IV

Table III

From DSC results, it can be suggested that MGS was interacted with chitosan and carrageenan, leading to the decrease in melting enthalpy of CCG microparticles.

3.4. Release of MGS from CCG composites

3.4.1. Calibration equation of MGS in different ethanol/buffer solutions

The MGS content in the solution is determined by using UV-Vis method. As observation from UV-Vis spectra of MGS in the different solutions (ethanol, ethanol/buffer solutions (50/50 v/v)) in the wavelength range from 200 to 400 nm, it can be seen that the absorption peaks at 244 nm can be found in all UV-Vis spectra (Figure V). Therefore, the maximum wavelength of 244 nm has been chosen to determine the content of MGS in these solutions.

Figure V

The calibration equation of MGS in ethanol/buffer solutions and the corresponding linear regression coefficients (R^2) were shown in Figure VI. These calibration equations have high values of linear regression coefficient ($\geq$ 0.99), therefore they can be used to calculate the amount of MGS released from the CCG microparticles in different ethanol/buffer solutions.

Figure VI

3.4.2. Release amount of MGS from CCG microparticles

The MGS amount released from free MGS and the CCG microparticles in different ethanol/buffer solutions was displayed in Figure VII. It can be seen that the release of MGS from the free MGS and CCG microparticles depends on pH of buffer solution, polymer matrix, testing time and MGS content in the CCG microparticles.

Figure VII

In different ethanol/buffer solutions, the MGS release amount from the free MGS and CCG microparticles was varied and ordered in ethanol/pH 1.2 buffer > ethanol/pH 4.5 buffer ethanol/pH 6.8 buffer > ethanol/pH 7.4 buffer. The better release of MGS in acidic environment may be due to MGS is a weak acid (pKa1 = 3.68 (primary carbonyl)). Moreover, the sulfate groups in carrageenan can react with proton H^+ in acidic environment, leading to the MGS to release more easily. On the

other hand, the degradation of electrostatic interaction of components in the CCG microparticles (Figure II) due to the presence of H+ could cause to the increase in the MGS release from the CCG microparticles [50]. In ethanol/pH 1.2 buffer and ethanol/pH 4.5 buffer solutions, the MGS was released almost completely from the CCG5 sample after 360 minutes of testing. In ethanol/pH 6.8 buffer and ethanol/pH 7.4 buffer solutions, the highest MGS release amount from the CCG10 sample after 360 minutes of testing is 87.63 and 74.42 %, respectively.

From Figure VII, it can be recognized that carrageenan/chitosan matrix had a strong effect on the release of MGS [46, 51, 54]. The difference in MGS release amount from the free MGS and CCG microparticles suggested that MGS was loaded by carrageenan/chitosan microparticles and MGS and polymer matrix was interacted together as aforementioned.

The MGS was distributed in both surface and inside of microparticles, therefore, an initial burst effect could be observed for first 120 minutes of testing because of the release of MGS on the surface of the CCG microparticles and then, the release rate of MGS became slowly due to the release of MGS linked with polymer matrix in the CCG microparticles [51, 54].

The release of MGS from the CCG microparticles was also affected by the content of MGS in the CCG microparticles. The MGS amount released from the CCG20 sample in all tested solutions was much lower than that from others. This can be due to the less compatibility of MGS with polymer matrix as mentioned in DSC analysis subsection. In acidic environment, the MGS release amount from the CCG5 sample was higher than that of the CCG10 and CCG15 samples while in alkaline environment, the MGS release amount from CCG10 sample was higher. This difference may be explained by the dissimilar interaction ability of drug – polymers, polymers – solutions, drug – solution.

3.4.3. MGS release kinetic

The kinetic models expressing release mechanisms of a drug from a certain matrix such as zero-order (ZO), first-order (FO), Higuchi (HG), Hixson–Crowell (HC) and Korsmeyer-Peppas (KMP) are typical [46, 51, 55]. To study the release mechanism of MGS from the CCG microparticles in two stage, fast release for first 60

minutes of testing and slow release in following minutes, the release patterns were fitted to those five models based on the R^2 value (Table IV).

Table IV

In first stage (0-120 min of testing), *in vitro* release of MGS from the free MGS followed the KMP model in all tested solution while it followed the KMP model when formulated into chitosan/carrageenan microparticles in ethanol/pH 1.2 buffer solution, ethanol/pH 6.8 buffer and ethanol/pH 7.4 buffer solutions. In ethanol/pH 4.5 buffer solution, the release of MGS from the CCG5, CCG10, CCG15 and CCG20 was complied with the KMP, FO, ZO and KMP models. In general, the release of MGS in fast release stage was complex [55]. This process was combined by various mechanisms such as swelling of polymers, dissolution of polymers, diffusion of MGS, dissolution of MGS in solvents, etc.

In second stage, most of the MGS release processes from the free MGS and CCG microparticles were fitted well with ZO or FO models depending on pH of buffer solution and MGS content in CCG microparticles. This indicated that the release of MGS was concentration-independent or mainly controlled by drug concentration [51]. The diffusion constants obtained from the KMP model reflecting MGS release mechanism from the free MGS and CCG microparticles are different suggesting that the release of MGS can follow or un-follow Fick's law of diffusion depending on investigation conditions.

3.5. Antibacterial activity of CCG microparticles

The results of Antibacterial activity testing in Table V indicated that MGS and CCG microparticles can inhibit Gram (+) strains (*Staphylococcus aureus, Bacillus subtilis, Lactobacillus fermentum*) and cannot inhibit Gram (-) strains (*Salmonella enterica, Escherichia coli, Pseudomonas aeruginosa*) and yeast (*Candida albican*) at tetsted concentration. The MGS has a great antibacterial activity [2]. The IC_{50} and MIC of CCG microparticles were higher than that of MGS showing to a less antibacteria activity of CCG microparticles as compared to MGS. This may be due to the low content of MGS in CCG microparticles (5-20 wt.%) and low antimicrobial activity of polymer matrix [56, 57]. The MGS and CCG microparticles can inhibit *Staphylococcus aureus* better than *Bacillus subtilis, Lactobacillus fermentum*. As

increasing the MGS in samples, the antibacterial activity of CCG microparticles became stronger.

Table V

3.6. Anti-oxidant activity of CCG microparticles

The anti-oxidant activity of the MGS and CCG microparticles was listed in Table VI. The MGS was known as a good anti-oxidant substance [6, 7]. It can be seen that MGS has better anti-oxidant activity than the CCG microparticles. As increasing the MGS content in CCG microparticles, their anti-oxidant activity was increased.

Table VI

CONCLUSIONS

In this study, the carrageenan/chitosan microparticles loading α-mangostin (extracted from the skin of Vietnamese mangosteen) were prepared successfully by ionic gelation method. Chitosan and carrageenan can be mixed well together owing to the formation of chitosan-carrageenan polyelectrolyte complex. The α-mangostin was embedded in carrageenan/chitosan microparticles thanks to the ionic cross-linking and interaction of components in the carrageenan/chitosan/α-mangostin microparticles. These microparticles loading α-mangostin had a more compact structure and smaller particle size than the carrageenan/chitosan microparticles. The carrageenan/chitosan matrix can improve the release ability of α-mangostin in ethanol/buffer solutions. The release of α-mangostin from the carrageenan/chitosan microparticles in different ethanol/buffer solutions is a complex process and depends on pH of buffer solution, testing time and α-mangostin content in the microparticles. The carrageenan/chitosan microparticles loading α-mangostin can inhibit gram (+) strains and adequate anti-oxidant activity. This is promising for application of carrageenan/chitosan/α-mangostin microparticles in food and beverages fields.

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FIGURE CAPTIONS

Figure I. FESEM images of MGS and CCG microparticles at different magnifications: MGS (a, g), CCG0 (b, h), CCG5 (c, i), CCG10 (d, j), CCG15 (e, k) and CCG20 (f, l)

Figure II. Illustration of the ionically crosslinked chitosan–tripolyphosphate and chitosan–carrageenan polyelectrolyte complex in the chitosan–carrageenan microparticles

Figure III. Diagrams of particle size distribution of CCG microparticles

Figure IV. DSC diagrams of CCG microparticles

Figure V. UV-Vis spectra of MGS in different solutions

Figure VI. Calibration equations of MGS in different ethanol/buffer solutions

Figure VII. Released MGS amount from free MGS and CCG microparticles in different ethanol/buffer solutions, (a) EtOH/pH 1.2, (b) EtOH/pH 4.5, (c) EtOH/pH 6.8, and (d) EtOH/pH 7.4

Figures

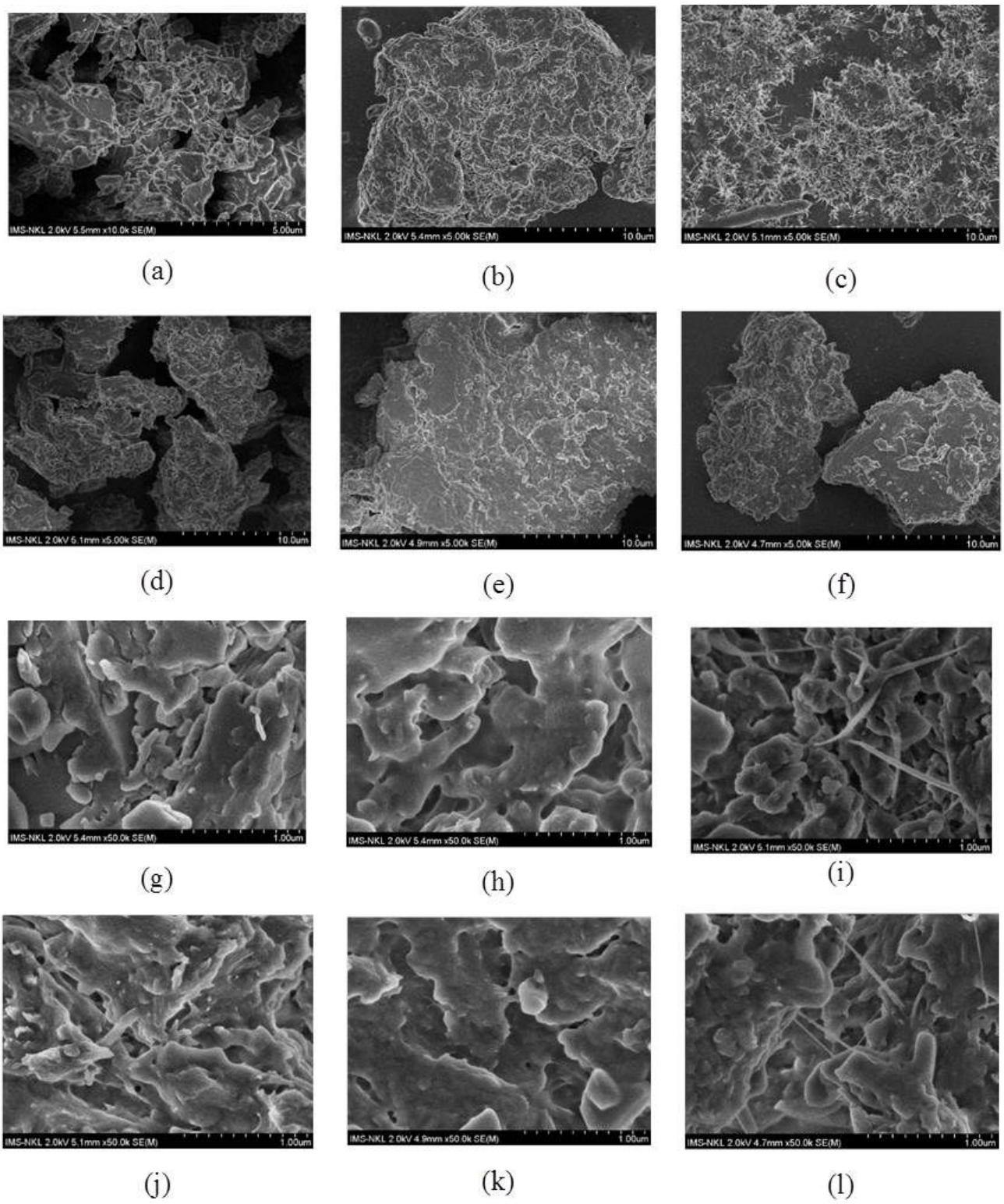

Figure 1

FESEM images of MGS and CCG microparticles at different magnifications: MGS (a, g), CCG0 (b, h), CCG5 (c, i), CCG10 (d, j), CCG15 (e, k) and CCG20 (f, l)

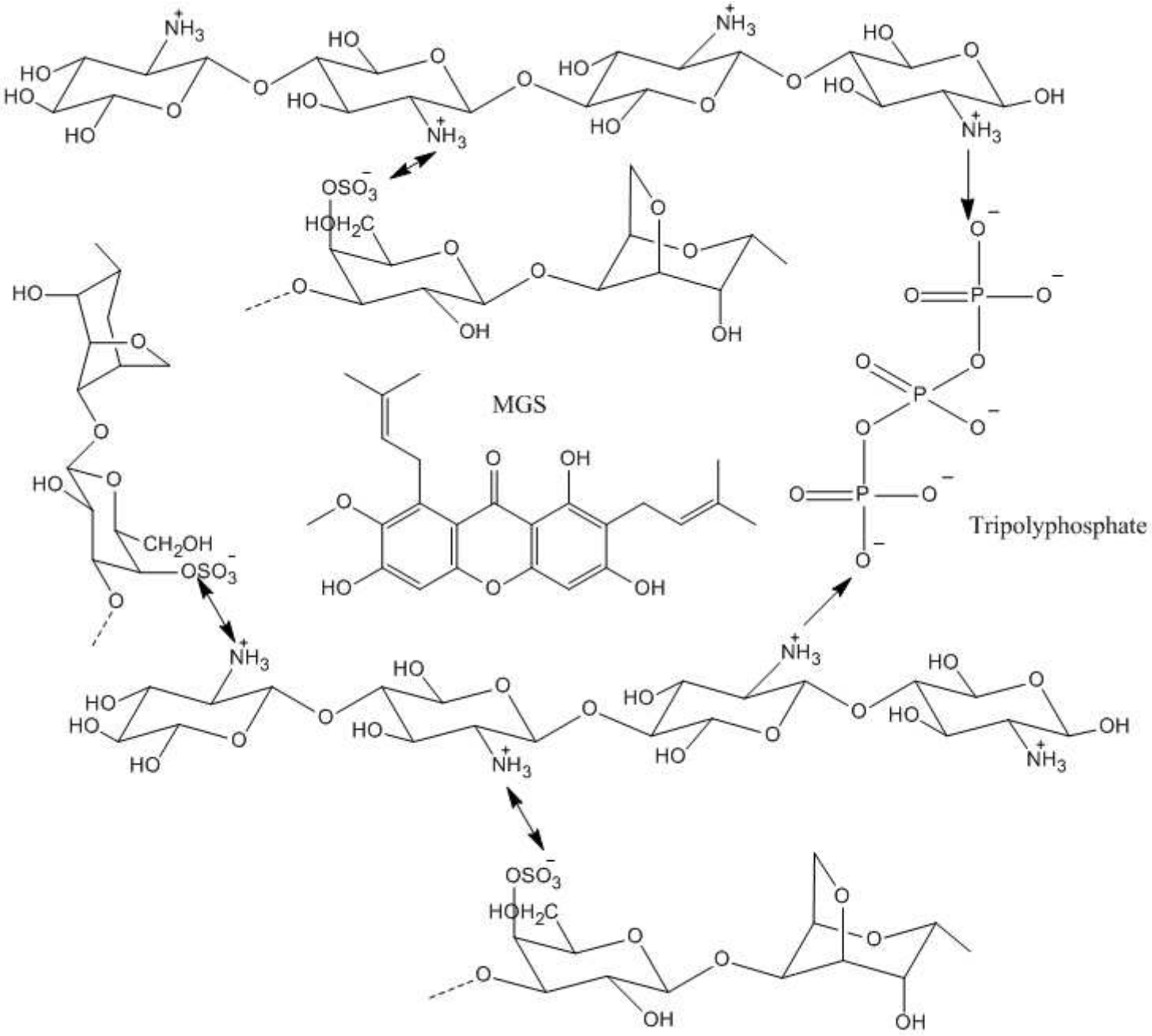

Figure 2

Illustration of the ionically crosslinked chitosan–tripolyphosphate and chitosan–carrageenan polyelectrolyte complex in the chitosan–carrageenan microparticles

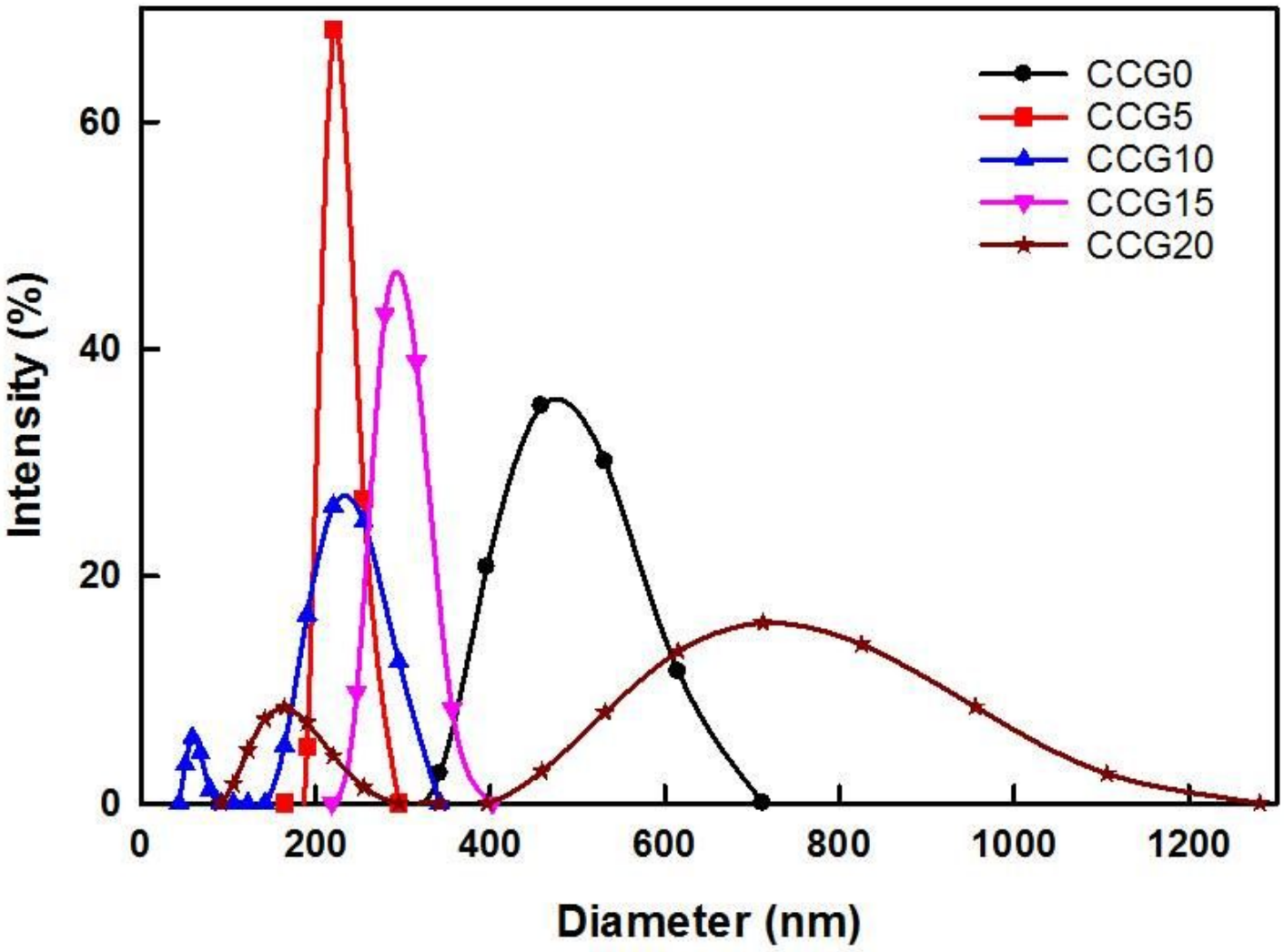

Figure 3

Diagrams of particle size distribution of CCG microparticles

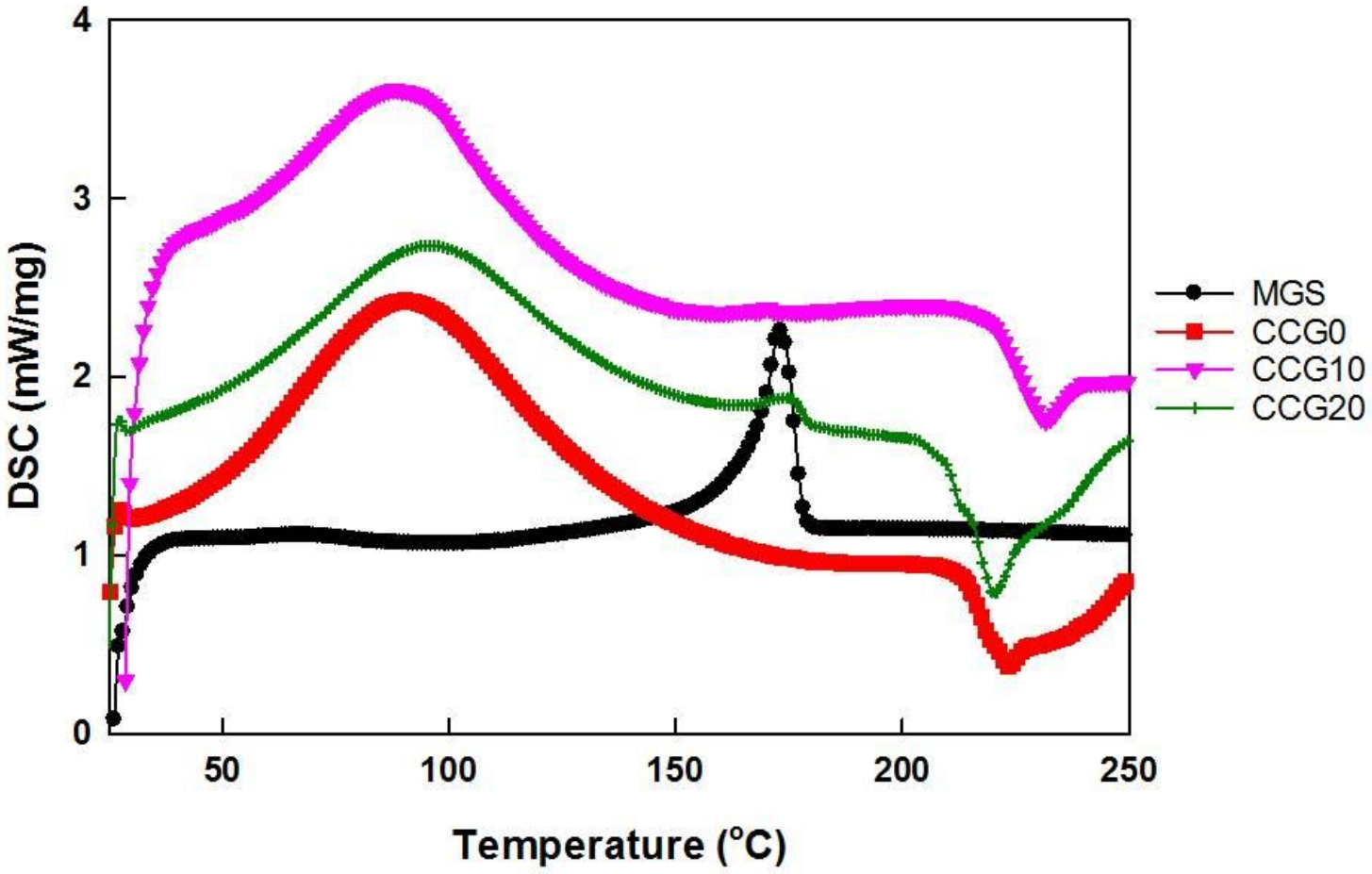

Figure 4

DSC diagrams of CCG microparticles

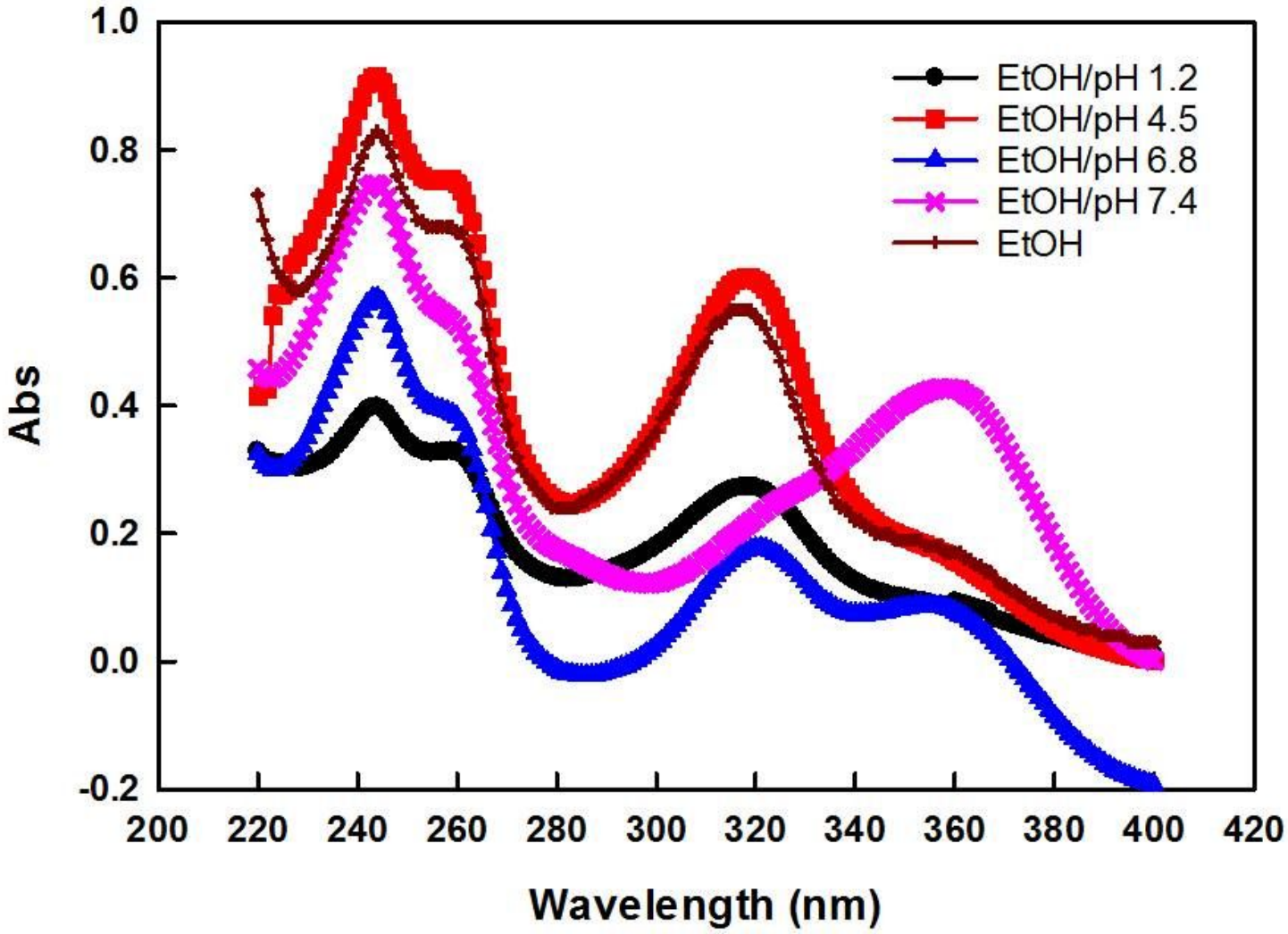

Figure 5

UV-Vis spectra of MGS in different solutions

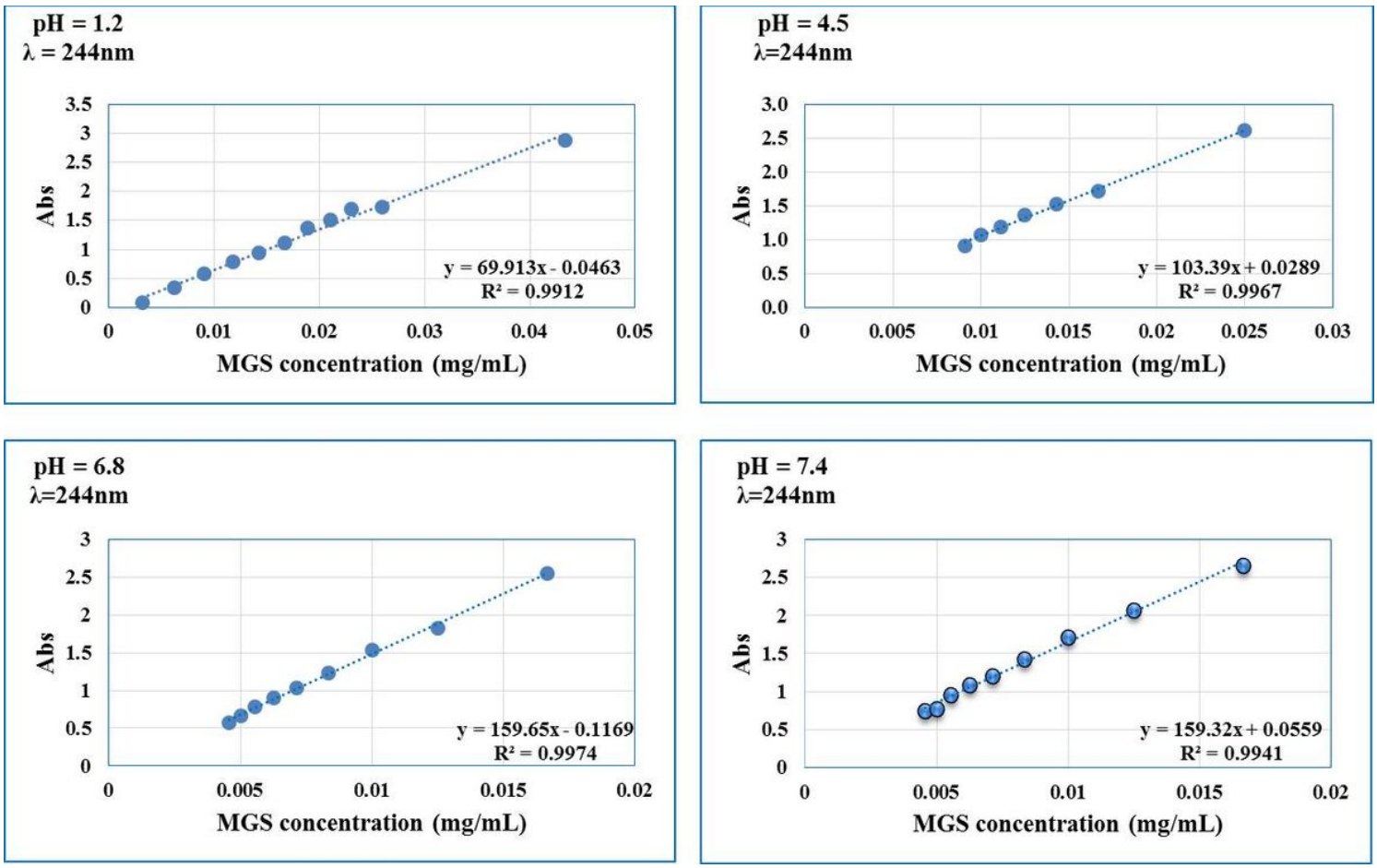

Figure 6

Calibration equations of MGS in different ethanol/buffer solutions

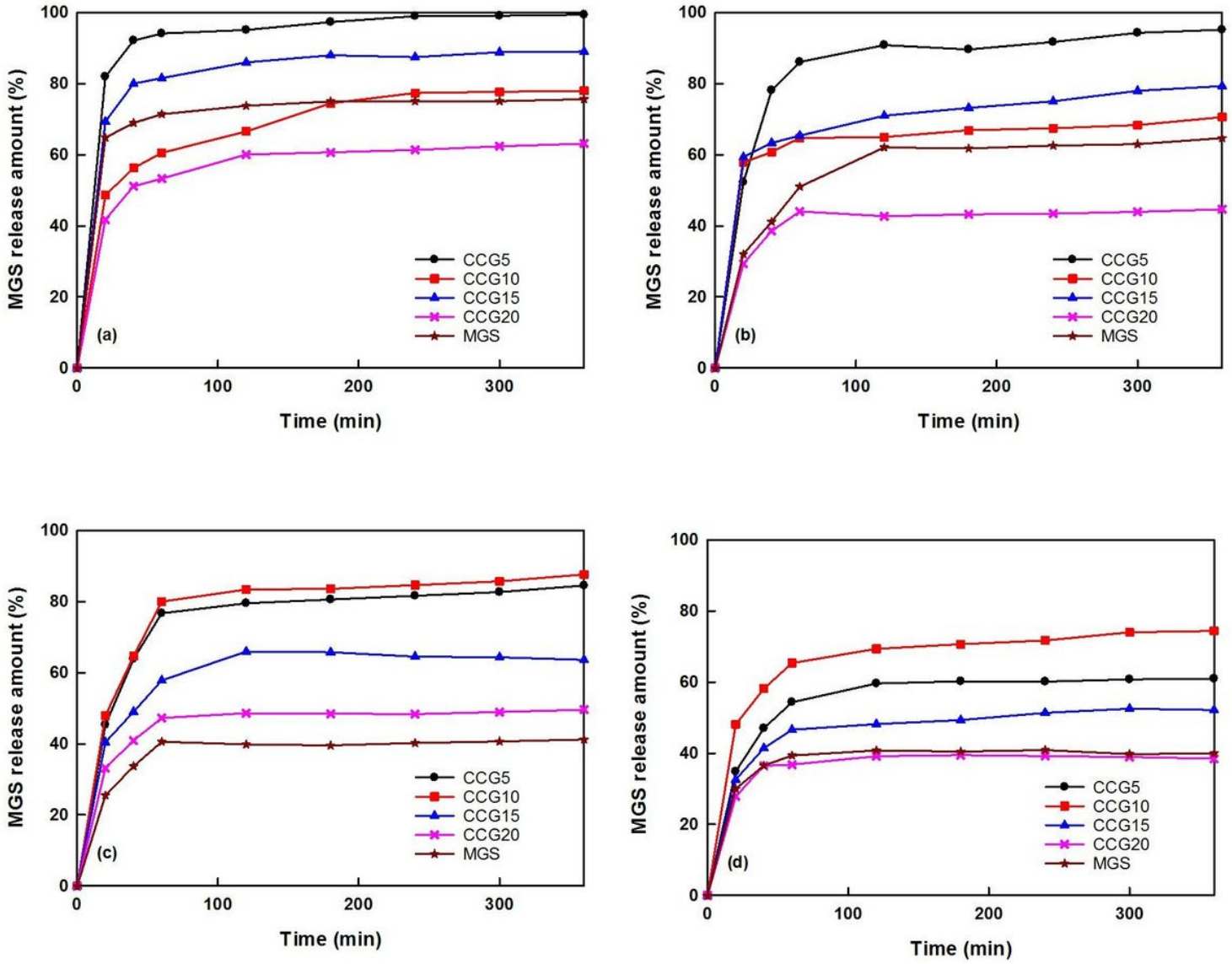

Figure 7

Released MGS amount from free MGS and CCG microparticles in different ethanol/buffer solutions, (a) EtOH/pH 1.2, (b) EtOH/pH 4.5, (c) EtOH/pH 6.8, and (d) EtOH/pH 7.4