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Preparation, characterization, and *in vitro* release of carboxymethyl starch/β-cyclodextrin microgel–ascorbic acid inclusion complexes

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Carboxymethyl starch (CMS)/β-cyclodextrin (β-CD) microgels have been synthesized. The percentages of effective β-CD in the microgels have been determined by measuring the amount of iodine retained in its hydrophobic cavity. A microgel–ascorbic acid inclusion complex has been prepared and characterized by Fourier-transform infrared (FTIR) spectroscopy and differential scanning calorimetry (DSC). *In vitro* release of ascorbic acid from the microgel has been investigated. Most of the microgel particles had diameters distributed between 10 and 25 μm. The effective β-CD contents in microgels with weight ratios $R_{\beta\text{-CD/CMS}}$ of 0.05, 0.1, 0.2, and 0.4 were 1.04, 2.27, 3.96, and 4.12%, respectively. The ascorbic acid loading of the microgels increased as ascorbic acid concentration was increased, but the encapsulation efficiencies of the microgels decreased with increasing its concentration. FTIR and DSC data demonstrated the formation of a microgel–ascorbic acid inclusion complex. *In vitro* release results indicated that the CMS/β-CD microgels may potentially be applied as a carrier system to prevent the early release of ascorbic acid in the stomach and target its delivery to the intestine.

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1. Introduction

Ascorbic acid, a soluble vitamin, is an essential nutrient for humans. In living organisms, ascorbic acid is an antioxidant that can protect the body against oxidative stress.[1] However, it is highly sensitive to heat, alkali, oxygen, light, and also to contact with traces of copper and iron.[2] This instability of ascorbic acid represents an inconvenience for its preservation and application. Cyclodextrins (CDs) are capable of including guest molecules in their hydrophobic internal cavity, thus protecting them from the influence of external factors.[3] The inclusion of ascorbic acid in β-CD in an acidic medium increases its stability to oxidation. However, no effect is observed with α-CD.[4]

Microgels are commonly meant hydrogels with an average diameter ranging between 50 nm and 100 μm.[5] Potent microgels explored for pharmaceutical and biological applications are largely synthetic and seldom of natural origin. Examples of widely studied synthetic hydrophilic polymers include poly(ethylene glycol) (PEG),[6] poly(vinyl alcohol) (PVA),[7] poly(acrylic acid) (PAA),[8] polyacrylamide (PAM),[9] and poly(methyl methacrylate) (PMA).[10] Examples of the few natural polymers studied in this context are alginic acid,[11] pectin,[12] chitin and chitosan,[13] dextran,[14] agarose,[15] starch,[16] and chitin.[17] Comparatively high toxicity and lower biodegradability and bioactivity of synthetic polymers have often compelled scientists to take interest in natural/biopolymers as better alternatives. Indeed, they show excellent biocompatibility and biodegradability, and are natural carriers of more biologically recognizable moieties that support good cellular activities.

A suitable microgel contains a molecular inclusion component such as β-CD and a pH-sensitive component such as CMS. Due to the unique molecular recognition ability of cyclodextrin and environmental stimuli-sensitive nature of microgels, the combination of microgels with cyclodextrin is becoming increasingly attractive.[18,19] The microgels obtained may not only possess the function of including organic compounds, but may also sensitively respond to external stimuli, such as pH and ionic strength. Furthermore, such dual-functionalities may be effectively applied in many industries to develop new functional products. In our previous work, we synthesized pH-responsive carboxymethyl starch microgels, which showed shrinkage at low pH due to protonation of their carboxylic groups and expanded at neutral pH due to the dissociation of these groups.[20] In this study, carboxymethyl starch/β-cyclodextrin microgels have been synthesized by chemical crosslinking with sodium trimetaphosphate (STMP). A microgel–ascorbic acid inclusion complex has been prepared and characterized by Fourier-transform infrared (FTIR) spectroscopy. *In vitro* release of ascorbic acid from the microgel has also been evaluated.

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2. Materials and methods

2.1 Materials

CMS, with a degree of substitution of 0.35, was purchased from Puluoxing Starch Co., Ltd (Hangzhou, China) and the moisture of CMS was 10.5%. Sodium trimetaphosphate (STMP) was purchased from Sigma-Aldrich Trading Co. Ltd (Shanghai, China) and was of analytical grade. β-CD was purchased from TCI, Japan. All other chemicals and reagents were purchased from Sinopharm Chemical Reagent Co., Ltd (Suzhou, China) and were of analytical grade.

2.2 Preparation of CMS/β-CD microgels

The microgels were prepared by crosslinking CMS and β-CD using STMP as the crosslinking agent according to the methods described in previous reports with slight modification.[20] First, CMS polymer (10 g) and the different requisite masses of β-CD were dissolved in deionized water (50 mL) with stirring using a glass bar at 25 °C, which required around 30 min. The cross-linker STMP (2 g) and sodium hydroxide (0.67 g) were then added to the polymer solution. The mixture was thoroughly stirred with the glass bar, and then heated at 40 °C for 1 h without stirring, which led to gel formation. The weight ratios of β-CD to CMS ($R_{\beta\text{-CD/CMS}}$) were 0.05, 0.1, 0.2, and 0.4. After the gel had formed, it was kept overnight in a cold room at 4 °C. The whole gel was ground and passed through a sieve (1 mm) covered with a nylon cloth of 200 mesh to obtain reasonably uniform microgel particles. The gel was then washed at least three times with deionized water through the nylon cover on the sieve to remove salts until the electrical conductivity of the washings was equal to that of deionized water. Thereafter, the microgel particles were washed for a further three times with 100% ethanol to remove water, and then three times with 100% acetone to remove the ethanol and traces of water. Finally, the microgel particles were dried overnight in an oven at 40 °C. The dried microgel powder was again ground to obtain small and homogeneous particles that passed through a 200-mesh sieve.

2.3 Size distribution of microgel particles

The size distributions of the microgel particles with different $R_{\beta\text{-CD/CMS}}$ in suspensions were determined using a Malvern MasterSizer 2000 (Malvern Instruments, Ltd, UK). The sample concentration was within the range of the instrument specifications. Prior to measurement, suspensions were sonicated for 15 min to obtain finely dispersed gel particles.

2.4 Field emission scanning electron microscopy (FE-SEM)

Samples of the microgel particles with different $R_{\beta\text{-CD/CMS}}$ were fixed on aluminum specimen stubs with double-sided adhesive tape, coated with a thin film of gold (10 nm), and placed in a vacuum evaporator. The specimens were observed in a FE-SEM (Hitachi S-4800; Hitachi Company, Japan) at an accelerating voltage of 5 kV.

2.5 β-Cyclodextrin determination in the microgels

The β-cyclodextrin in the microgels was determined by iodine absorption according to a previously reported method.[21] Microgel (100 mg) was soaked in 10 mL of 0.1 N iodine solution in a 10% solution of potassium iodide (KI) for 24 h under gentle stirring. Thereafter, 5 mL of the iodine solution was removed and assayed for iodine content by titration with 0.1 N $Na_2S_2O_3$ in the presence of starch solution (1%) as an indicator. In parallel, blank samples of the CMS microgels were checked for iodine retention. The amount of effective β-CD in the microgels was determined as follows:

$$\beta\text{-CD}\ (\%) = (I_A - I_B) \times M_{w\beta\text{-CD}}/M_{w\text{Iodine}}$$

where I_A is % iodine retained by the CMS/β-CD microgel, I_B is % iodine retained by the CMS microgel, and $M_{w\beta\text{-CD}}$ and $M_{w\text{Iodine}}$ are the molecular weights of β-CD and iodine, respectively.

2.6 Preparation of microgel–ascorbic acid inclusion complexes

The concentration dependence of ascorbic acid uptake by the microgels was determined. Dry microgels with different $R_{\beta\text{-CD/CMS}}$ (10 mg) were suspended in 25 mL ascorbic acid solutions of different concentrations (0.1, 1, 10, 20, 50, and 100 mg mL^{-1}) and the mixtures were sonicated for 1 h. The samples were subsequently centrifuged at 5000×g for 10 min, and the ascorbic acid concentration in the supernatant was measured according to the previous method.[22] The ascorbic acid loading Γ (mg ascorbic acid per mg dry gel) and encapsulation efficiency (EE%) in each microgel were calculated from the mass balance given by:

$$\Gamma = \frac{C_{add} \times V_{add} - C_s \times V_s}{m_{gel}} \quad (1)$$

$$EE = \frac{C_{add} \times V_{add} - C_s \times V_s}{C_{add} \times V_{add}} \times 100 \quad (2)$$

where C_{add} is the ascorbic acid concentration added, V_{add} is the volume of the ascorbic acid solution added, V_s is the volume of the supernatant after centrifugation, C_s is the ascorbic acid concentration in the supernatant, and m_{gel} is the weight of added dry microgel.

2.7 Fourier-transform infrared (FTIR) spectroscopy

Samples of ascorbic acid, microgels, a physical mixture of ascorbic acid and microgel, and an ascorbic acid–microgel inclusion complex were blended with KBr powder and then pressed into tablets before measurement. FTIR spectra were recorded between 2000 and 900 cm^{-1} using an FTIR spectrometer (5DXC FTIR, Nicolet Co., USA).

2.8 Differential scanning calorimetry (DSC)

The thermal properties of ascorbic acid, microgels, the physical mixture of ascorbic acid and microgels, and the microgels–ascorbic acid inclusion complex samples were determined using a DSC7000 instrument (Seiko Instruments Inc., Chiba,

Japan) under ultrahigh-purity nitrogen atmosphere. All samples were sealed in an aluminum pan and then scanned from 20 °C to 300 °C at a heating rate of 10 °C min^{-1}.

2.9 *In vitro* release of ascorbic acid from the microgels

Simulated gastrointestinal fluid was prepared according to a previous method.[23] The simulated stomach fluid with enzyme at pH 2 consisted of 1 L of aqueous solution containing pepsin (0.26 g) and 10% (w/w) hydrochloric acid (16.4 mL). Likewise, intestinal fluid at pH 6.8 was simulated by dissolving potassium dihydrogen phosphate (6.8 g) in water (500 mL). The solution was adjusted to pH 6.8 with a 0.1 M solution of sodium hydroxide. Pancreatin (10 g) was added to the above solution, and the resulting mixture was diluted to 1 L with distilled water.

Samples of microgel–ascorbic acid complexes (the sediments after centrifugation) were prepared under the same conditions with an ascorbic acid concentration of 50 mg mL^{-1}, and these were added to 30 mL incubation fluid with continuous agitation by a magnetic stirrer bar (at 100 rpm) at 37 °C.[24] This concentration was set as 100%, and the concentrations of all other samples were related to this value. Following standard pharmacopoeia methods,[25] the compounds were firstly incubated for 2 h in simulated gastric fluid at 37 °C; the percentage of released ascorbic acid was measured at times 0, 15, 30, 60, 80, 100, and 120 min. After incubation in the stomach-mimicking medium, the microgel–ascorbic acid complexes were separated and transferred to simulated intestinal fluid for continuous release for 3 h. The percentage of released ascorbic acid was measured at times 0, 15, 30, 60, 90, 120, and 180 min in the stimulated intestinal fluid. At periodic intervals, samples (1 mL) were withdrawn and centrifuged, and the ascorbic acid content of the supernatant was determined according to previously reported methods.[22] An equal volume of medium was added to the release mixture after each sampling to maintain a constant volume. These studies were carried out in triplicate. The quoted data represent average values from three independent experiments.

2.10 Statistical analysis

The samples were analyzed in triplicate and standard deviations were evaluated. The means were compared by a Tukey's test (to a 5% level of significance) using analysis of variance (ANOVA).

3. Results and discussion

3.1 Size distribution of microgel particles

Fig. 1 shows the number of carboxymethyl starch/β-cyclodextrin microgel particles *versus* particle size (volume-weighted mean diameter in μm) for microgels with varying $R_{\beta\text{-CD/CMS}}$ in water. The microgel particle size exhibited a relatively wide distribution, although it was mostly concentrated in the range 10–25 μm. The size distributions of the microgels slightly shifted to lower values as $R_{\beta\text{-CD/CMS}}$ increased, which could be attributed to smaller amounts of carboxyl groups in the microgels with high $R_{\beta\text{-CD/CMS}}$.

3.2 Surface morphology of microgels

The surface morphology of microgels with different $R_{\beta\text{-CD/CMS}}$ was characterized by SEM. Similar surface morphology was observed for all microgels (Fig. 2). There was aggregation behavior of microgels resulted from drying methods. The size of microgels was smaller than the result determined using a Malvern MasterSizer 2000 for the same sample, which was attributed to their swelling capacity in the deionized water.

3.3 β-Cyclodextrin determination in microgels

The effective β-CD in microgels at various $R_{\beta\text{-CD/CMS}}$ is presented in Fig. 3. The effective β-CD content in the microgels increased with increasing $R_{\beta\text{-CD/CMS}}$. According to the literature, STMP reacts with two alcohol groups belonging to two different polymer chains, thus forming an intermolecular linkage.[26] A higher weight ratio of β-CD to CMS meant a larger number of hydroxyl groups available for reaction and therefore a higher probability of linking in the polymer network. The effective β-CD contents in the microgels with $R_{\beta\text{-CD/CMS}}$ of 0.05, 0.1, 0.2, and 0.4 were 1.04, 2.27, 3.96, and 4.12%, respectively.

3.4 Microgel–ascorbic acid inclusion complexes

As can be seen in Table 1, the ascorbic acid loading of the microgel with $R_{\beta\text{-CD/CMS}}$ 0.05 significantly increased from 0.101 to 1.883 mg mg^{-1} as the concentration of ascorbic acid was increased from 0.1 to 50 mg mL^{-1}, but thereafter remained constant. However, the encapsulation efficiency of the microgel with $R_{\beta\text{-CD/CMS}}$ 0.05 (Table 2) decreased when the concentration of ascorbic acid was increased. The results could be explained by the fact that loading of ascorbic acid reached its saturation at 50 mg mL^{-1}, such that a further increase in the ascorbic acid concentration had little effect on the amount loaded, but significantly decreased the efficiency of its encapsulation. Furthermore, increasing $R_{\beta\text{-CD/CMS}}$ resulted in a relatively higher ascorbic acid loading ratio and encapsulation efficiency. The results were positively correlative with the estimated effective β-CD contents in the microgels.

3.5 Fourier-transform infrared (FTIR) spectroscopy

Fig. 4 shows the infrared spectra of (a) ascorbic acid, (b) the microgel with $R_{\beta\text{-CD/CMS}}$ 0.2, (c) a microgel–ascorbic acid inclusion complex, and (d) a microgel/ascorbic acid physical mixture. The characteristic bands of ascorbic acid were found at $\nu = 1755$ (C═O), 1500 (C═C), and 1117 cm^{-1} (C–O–C),[27] which were not superimposed by bands of the microgel. Differences between the spectrum of ascorbic acid and those of the mixed systems were found in these regions. In the spectrum of the microgel–ascorbic acid inclusion complex, the band of the carbonyl group of ascorbic acid at $\nu = 1755$ cm^{-1} was shifted to $\nu = 1770$ cm^{-1} and was decreased in intensity, whereas the bands corresponding to

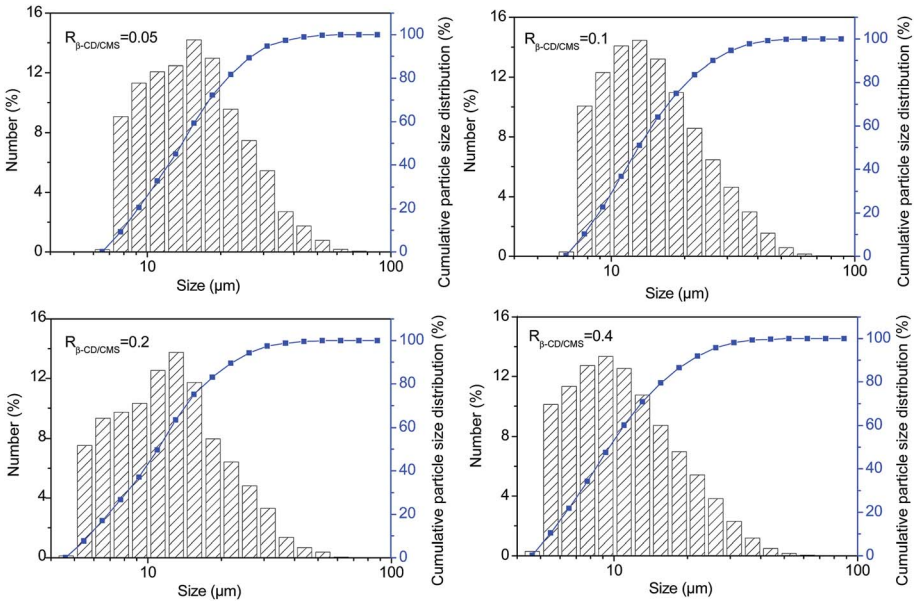

Fig. 1 Number of carboxymethyl starch/β-cyclodextrin microgel particles *vs.* particle size (volume-weighted mean diameter in μm) for microgels with varying $R_{\beta\text{-CD/CMS}}$ in water.

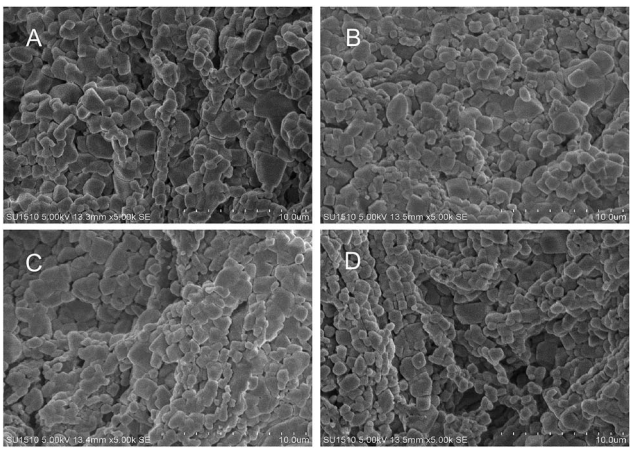

Fig. 2 SEM micrographs of microgels with different $R_{\beta\text{-CD/CMS}}$ ((A) $R_{\beta\text{-CD/CMS}}$ 0.05, (B) $R_{\beta\text{-CD/CMS}}$ 0.1, (C) $R_{\beta\text{-CD/CMS}}$ 0.2, (D) $R_{\beta\text{-CD/CMS}}$ 0.4).

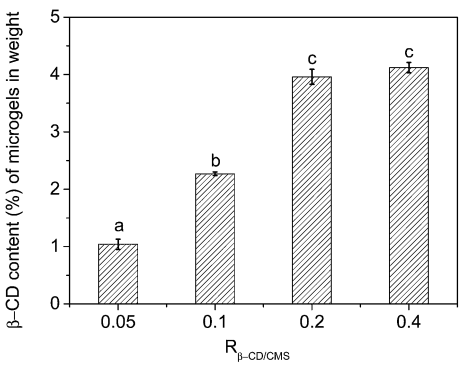

Fig. 3 Estimated amounts of β-cyclodextrin in microgels with different $R_{\beta\text{-CD/CMS}}$ in weight.

C=C and C–O–C (at $\nu = 1500$ and 1117 cm^{-1}, respectively) were shifted to 1490 and 1159 cm^{-1}, respectively. These observations could be attributed to the formation of hydrogen bonds between the encapsulated ascorbic acid and the microgel, suggesting that the ring moiety of the ascorbic acid molecule interacts with the microgel. However, the FTIR spectrum of the physical mixture showed approximate superimposition of the individual spectra of ascorbic acid and the microgel. These results indicated that a microgel–ascorbic acid inclusion complex had been obtained.

3.6 DSC

Fig. 5 shows DSC curves of microgels with $R_{\beta\text{-CD/CMS}}$ 0.2, ascorbic acid, the microgel/ascorbic acid physical mixture, and the microgel–ascorbic acid inclusion complex. The thermogram of ascorbic acid presented an endothermic peak at about 197 °C, which was consistent with previous reports.[22] The thermogram of microgel showed a wide endothermic peak at around 135 °C. The DSC curve of the microgel/ascorbic acid physical mixture showed a superimposition of the two individual components, which was ascribed to both of peaks at about 135 °C and 197 °C observed in the thermogram. However, the thermogram of microgel–ascorbic acid inclusion complex presented an endothermic peak at about 144 °C, which was attributed to the fact that the endothermic peak of microgel was slightly shifted to a higher temperature. This single peak indicated an interaction between ascorbic acid and microgels. The endothermic peak of ascorbic acid at 197 °C was not observed in the DSC curve of the microgel–ascorbic acid inclusion complex, indicating that the microgel–ascorbic acid inclusion complex had been formed.

3.7 *In vitro* release of ascorbic acid from microgels

To evaluate the effectiveness of the microgel as an intestine-targeted delivery system, release experiments were performed

Table 1 Amount loading of ascorbic acid (mg mg^{-1}) in the microgels with different $R_{\beta\text{-CD/CMS}}$[a]

	Ascorbic acid concentration (mg mL^{-1})					
$R_{\beta\text{-CD/CMS}}$	0.1	1	10	20	50	100
0.05	0.101 ± 0.004 a A	0.572 ± 0.009 a B	0.975 ± 0.013 a C	1.524 ± 0.011 a D	1.883 ± 0.024 a F	1.892 ± 0.014 a F
0.1	0.125 ± 0.003 b A	0.597 ± 0.01 b B	0.991 ± 0.017 a C	1.593 ± 0.009 b D	1.909 ± 0.015 ab F	1.908 ± 0.016 a F
0.2	0.138 ± 0.005 c A	0.621 ± 0.008 c B	1.026 ± 0.005 b C	1.642 ± 0.007 c D	1.937 ± 0.011 b F	1.942 ± 0.012 b F
0.4	0.139 ± 0.007 c A	0.624 ± 0.014 c B	1.029 ± 0.013 b C	1.648 ± 0.012 c D	1.941 ± 0.012 b F	1.949 ± 0.009 b F

[a] Values are means ± SD. Values with the same lowercase superscript letters in the same column are not significantly different ($p > 0.05$); numbers in the same row followed by the same uppercase superscript letters are not significantly different ($p > 0.05$).

Table 2 Ascorbic acid encapsulation efficiency (%) in the microgels with different $R_{\beta\text{-CD/CMS}}$[a]

	Ascorbic acid concentration (mg mL^{-1})					
$R_{\beta\text{-CD/CMS}}$	0.1	1	10	20	50	100
0.05	50.5 ± 2 a A	28.6 ± 0.45 a B	4.875 ± 0.065 a C	3.81 ± 0.028 a D	1.883 ± 0.024 a F	0.946 ± 0.007 a G
0.1	62.5 ± 1.5 b A	29.85 ± 0.5 b B	4.955 ± 0.085 a C	3.983 ± 0.023 b D	1.909 ± 0.015 ab F	0.954 ± 0.008 a G
0.2	69 ± 2.5 c A	31.05 ± 0.4 c B	5.13 ± 0.025 b C	4.105 ± 0.018 c D	1.937 ± 0.011 b F	0.971 ± 0.006 b G
0.4	69.5 ± 3.5 c A	31.2 ± 0.7 c B	5.145 ± 0.065 b C	4.12 ± 0.03 c D	1.941 ± 0.012 b F	0.975 ± 0.005 b G

[a] Values are means ± SD. Values with the same lowercase superscript letters in the same column are not significantly different ($p > 0.05$); numbers in the same row followed by the same uppercase superscript letters are not significantly different ($p > 0.05$).

in vitro in simulated physiological gastric and intestinal fluids. Fig. 6 shows the percentages of ascorbic acid released from microgels during incubation in the simulated gastric fluid. The pH of simulated gastric fluid was about 2 with enzyme. It was found that the release rates of ascorbic acid from the microgels decreased with increasing $R_{\beta\text{-CD/CMS}}$. For example, the cumulative amounts of ascorbic acid released from the microgels with $R_{\beta\text{-CD/CMS}}$ 0.05, 0.1, 0.2, and 0.4 were 15.4, 13.46, 12.38, and 12.13% after 120 min, respectively. The mechanism of ascorbic acid release after the initial boost could be mainly attributed to its diffusion from the surface and inclusion in the cavities of β-CD. Thus, ascorbic acid may also be adsorbed on the surface of the microgel. This surface-adsorbed ascorbic acid would account for the observed release in the simulated gastric fluid.

After incubation in the stomach-mimicking medium, the microgels were separated and transferred to simulated intestinal fluid. The difference between the stomach and the small intestine is that the pH and ionic strength in the latter are substantially higher. The percentages of ascorbic acid released from the microgels during incubation in simulated intestinal fluid were plotted in Fig. 7. The release rates of ascorbic acid from the microgels were faster in simulated intestinal fluid than those in simulated gastric fluid. This might be attributed to a higher swelling degree of the microgels in simulated intestinal fluid as a result of the ionization of carboxylic groups, leading to a stronger electrostatic repulsion. The pore size of the microgels became larger, resulting in a rapid release of ascorbic acid. Furthermore, the increase in salt concentration in the simulated gastric fluid could also promote the release of ascorbic acid.[25] The results indicated that such microgels could be useful

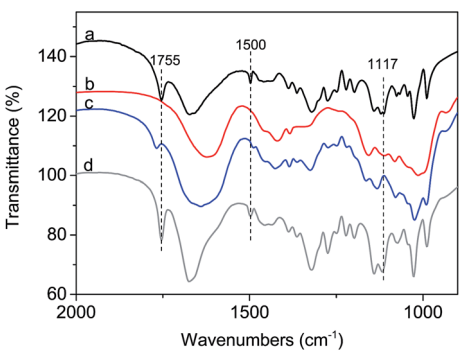

Fig. 4 Infrared spectra of (a) ascorbic acid, (b) microgel with $R_{\beta\text{-CD/CMS}}$ = 0.2, (c) microgel–ascorbic acid inclusion complex, and (d) microgel/ascorbic acid physical mixture.

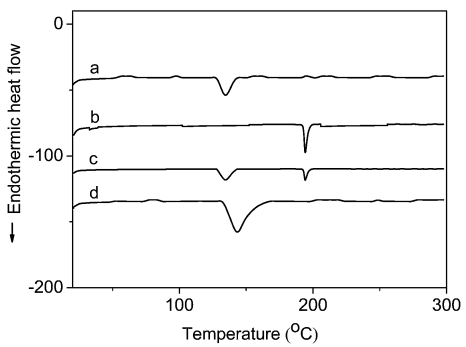

Fig. 5 DSC curves of (a) microgels with $R_{\beta\text{-CD/CMS}}$ 0.2, (b) ascorbic acid, (c) the microgel/ascorbic acid physical mixture, and (d) the microgel–ascorbic acid inclusion complex.

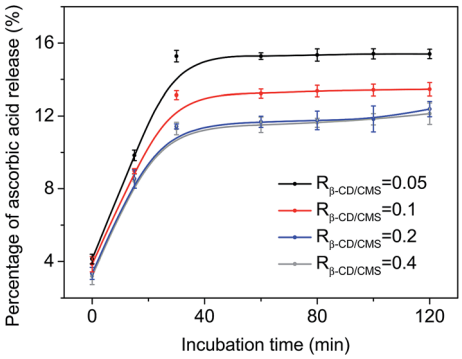

Fig. 6 Percentages of ascorbic acid released from microgels during incubation in simulated gastric fluid. The pH of the simulated gastric fluid was about 2 with enzyme.

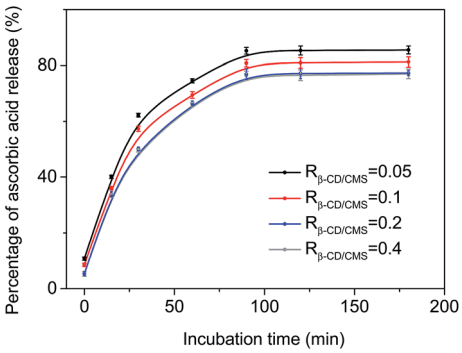

Fig. 7 Percentages of ascorbic acid released from microgels during incubation in simulated intestinal fluid. The pH of the simulated intestinal fluid was about 6.8 with pancreatic enzyme.

in the design and development of novel controlled delivery systems.

4. Conclusions

In this study, β-CD-based microgels with CMS have been synthesized by chemical crosslinking. It was found that the encapsulation efficiency was increased with increasing the weight ratio of β-CD to CMS. Formation of a microgel–ascorbic acid inclusion complex has been proven by FTIR and DSC analyses. The results clearly indicated that as carrier materials, the β-CD-based microgels indeed possessed unique release characteristics. This could be useful in the design and development of novel controlled delivery systems. The CMS/β-CD microgels would seem to be a good starting point for developing an intestinal-targeted delivery system for sensitive functional ingredients.

Acknowledgements

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