Ionic Self-Assembly and Characterization of a Polysaccharide-Based Polyelectrolyte Complex of Maleic Starch Half-Ester Acid with Chitosan

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ABSTRACT: A novel polysaccharide-based polyelectrolyte complex was formed via ionic self-assembly (ISA) of a carboxylic derivative of starch, maleic starch half-ester acid (MSA), with chitosan (CS) and precipitated from aqueous solution. Both Fourier transform infrared (FTIR) spectroscopy and elementary analysis results showed that there was CS in the complex. Thermogravimetric analysis (TGA) showed that the thermal resistance of the complex was higher than that of two components and the corresponding blend. X-ray diffraction (XRD) analysis result revealed that the complex was amorphous, whereas its components were semi-crystalline. In addition, the drug release behavior of the complex that contains 5-fluorouracil behaved pH-responsive. All the experimental results verified the complex was composed of MSA and CS, and also indicated that the driving force for the self-assembly of the complex was predominantly the electrostatic interactions between two oppositely charged polyelectrolytes, cationic CS, and the anionic MSA. © 2009 Wiley Periodicals, Inc. J Appl Polym Sci 112: 2255–2260, 2009

Key words: polyelectrolyte; complex; starch; chitosan; ionic self-assembly

INTRODUCTION

The polyelectrolyte complex (PEC) is of interest due to its facile preparation and responsibility to suitable environmental stimuli. Moreover, using water as a solvent is attractive for biomedical applications.[1,2] A lot of PECs aimed at biomedical applications are obtained via self-assembly in the solution but they are nondegradable,[3] which is not enough to be a good candidate for the delivery of bioactive molecules or tissue engineering. Thus, biodegradable PECs made of polypeptide and/or polysaccharides have been developed in recent years.[4]

Starch and chitosan (CS) are abundant naturally occurring polysaccharide.[5] Both of them are cheap, renewable, nontoxic, and biodegradable. It is well known that CS is a cationic biopolymer[6] and starch is easily to be transferred into an anionic polysaccharide via chemical modification.[7] The combination of hydrogen bonding, opposite charge attraction between CS cations, and negatively charged starch will provide polysaccharide-based PEC excellent performances.[8]

The technology of ionic self-assembly (ISA), forming PEC by electrostatic interactions, has the advantages of low cost, reliability, mild preparation conditions, and the simplicity of synthesis with high purity.[9] Herein, it is adopted as the approach to form a novel polysaccharide-based PEC, MSA-CS. The effects of carboxyl group percentage content of MSA and the stoichiometric relation of two ionic polysaccharides on the formation of the PEC are examined. The driving force of self-assembling MAS-CS will be discussed and presented.

EXPERIMENTAL

Materials

CS (minimum 90% deacetylation) was purchased from Shanghai Chemical Agents (China) and dried before use. The carboxylic derivative of starch, maleic starch half-ester acid (MSA) that contains 7.27 and 14.55% carboxylic groups was prepared according to the literature[10] by using soluble starch and maleic anhydride as starting materials. Acetic acid was analytical grade reagents, purchased from Xilong Chemical Agent Factory (Guangdong Province, China) and used as received; 5-fluorouracil

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(5-Fu) was purchased from Shanghai Biochemical Agents (China).

Ionic self-assembly of MSA-CS

A predetermined amount of MSA and CS was dissolved in distilled water and 5% acetic acid, respectively, to obtain homogeneous aqueous solution. Then 0.025% MSA solution was added into 0.02% CS solution drop by drop at different MSA:CS (wt/wt) ratios. MSA-CS was formed as precipitate. It was removed, rinsed with distilled water, and dried under vacuum at 37°C to constant weight.

Characterization of MSA-CS

MSA-CS was ground into powder, mixed with dry KBr, and compressed into disk. Then, FTIR spectra of the samples were recorded using a Nexus 470 FTIR spectrometer.

The percentage content of nitrogen, oxygen, and hydrogen elements of MSA-CS were analyzed with a Vario EL III elemental analysis instrument. The samples were embedded with a tin and allowed to be completely decomposed under 99.99%-purity nitrogen atmosphere at 1150°C.

X-ray diffraction (XRD) profiles of dried MSA-CS powder were collected with a Bruker D8-Advanced diffractometer using Nickel-filtered Cu Kα radiation ($\lambda = 0.15406$ nm) and scanned from 2 to 60° at a scan speed of 2°/min.

Thermogravimetric analysis (TGA) was carried out with a TA Instruments SDT 2960 Simultaneous DSC-TGA analyzer. MSA-CS samples were heated from 0 to 500°C at 20°C/min to record TGA profiles under nitrogen atmosphere.

In vitro release of 5-Fu-loaded MSA-CS

A predetermined amount of 5-Fu was dissolved in distilled water and mixed with MSA solution. Then, the CS solution was added into the 5-Fu-MSA solution in drops. The precipitates were centrifuged, rinsed with distilled water two times, and dried under vacuum at 37°C to constant weight. To obtain the loading amount, 5-Fu-loaded MSA-CS samples were weighed, ground into powder, soaked in distilled water, and measured with a Shimadzu UV2450 UV-visible spectrophotometer at 266 nm until 5-Fu release completely.

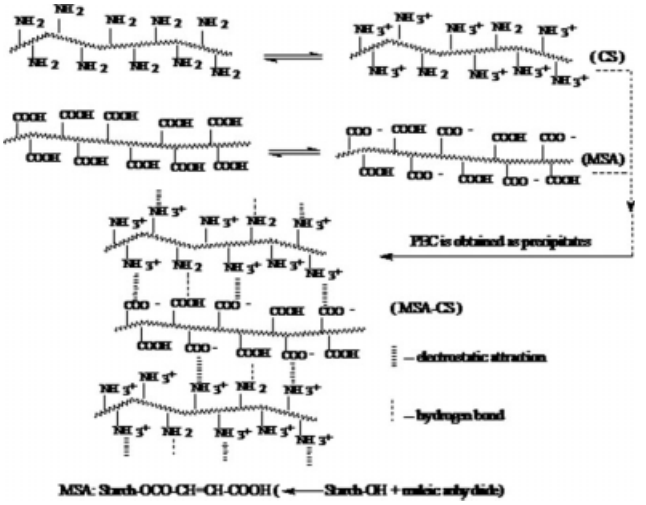
To examine its release behavior, 5-Fu-loaded MSA-CS samples were weighed and placed in vials that contains 50 mL hydrochloric acid (HCl, 0.1*M*, pH 1.2), phosphate-buffer saline (PBS, 0.1*M*, pH 7.4), and Na_2HPO_4-NaOH (0.1*M*, pH 12), respectively, and maintained at 37°C ± 0.5°C. At timed intervals, 5 mL sample liquid was removed to be analyzed with the same UV-visible spectrophotometer at 266 nm, and 5 mL fresh buffer solution was added in the meantime.

RESULTS AND DISCUSSION

Ionic self-assembly of MSA-CS

CS is obtained by alkaline deacetylation from chitin, and most of the moieties on the adopted CS backbone are 2-amino-2-deoxy-D-glucopyranose as its degree of deacetylation is higher than 90%. MSA is the carboxylic derivate of starch, which is prepared via the esterification reaction of starch with maleic anhydride. CS and MSA are soluble in water and dilute acetic acid, respectively. The amino and carboxyl groups of these two polysaccharide-based polyelectrolytes are ionizable groups bearing opposite charges. PKa of CS is about 6.3 and CS is a weak base.[11,12] Most of the amino groups of CS exist as $-NH_3^+$ and some of them are still in the form of $-NH_2$ in dilute acetic acid solution. On the other hand, both acetic acid and MSA are weak acid. Some carboxyl groups of MSA are ionized as $-COO^-$ anion whereas some of them keep their original $-COOH$. It is comprehensible that two interactions will occur between MSA and CS, which lead to the formation of MSA-CS complex. The electrostatic attractions of oppositely charged groups on the MSA and CS chains take place and act as ionic crosslinks. Secondary interaction, hydrogen bonding (>NH···O or N···HO—) between $-NH_2$ of CS and —COOH or —OH on MSA molecules, enhances the combination of MSA and CS. Because electrostatic interaction is considerably stronger than hydrogen bonding,[13] it can be regarded as the main driving force for the formation of MSA-CS.[6] As a result, ISA of MSA-CS is performed as represented in Scheme 1.

Evidently, the ratio between amino and carboxyl groups will be an important factor for the formation of MSA-CS. Considering the occurrence of batch-to-batch variation of natural polymers, three parameters such as the mass ratio instead of molar ratio, degree of deacetylation of CS, and carboxyl content of MSA are adopted to quantitatively describe the ratio between carboxyl and amino groups. As shown in Figure 1, MSA-CS precipitate can be harvested within a wide MSA:CS (wt/wt) feeding ratios when the carboxyl groups of MSA was 14.55 and 7.27%, respectively. In whole, the weight of the precipitate increases with increasing the amount of the polycation till the CS:MSA ratios is 12 : 1 and 17 : 1 for MSA containing 14.55 and 7.27% carboxyl groups, respectively. Because the amount of ionizable groups on equal-mass MSA and CS is unequal, the maximum of precipitate is not obtained around the



Scheme 1 Ionic self-assembly of MSA-CS.

MSA:CS ratio of 5 : 5. Noting that the MSA-CS is precipitate instead of something else such as micell that is difficult to harvest directly, increasing the concentration of cationic CS will benefit to combine MSA completely. Thus, the more CS added, the more MSA-CS is obtained. On the other hand, higher carboxyl group percentage content will offer more sites to bind with the ionic groups on CS, and form more crosslink points between MSA and CS chains. In other words, MSA that containing higher carboxyl group percentage content is easier to form insoluble PEC complex and will consume more CS. However, this will result in some MSA added lately is still remained in the solution and the amount of precipitate is reduced. In addition, both a few or a great deal cationic CS, such as at the CS:MSA ratio as low as 1 : 9 or as high as 15 : 1, can lead the MSA solution to precipitating. These results suggest that ionic self-assembling between MSA and CS is carried out and depends predominantly on the ionic interactions of oppositely charged polyelectrolyte chains.

TABLE I
The Elemental Percentage Content of MSA-CS

	Content (%)	
Elements	MSA7-CS[a]	MSA14-CS[b]
N	0.9755	1.263
C	38.675	39.465
H	7.8755	7.2375
O	52.474	52.0345

MSA:CS = 1 : 9, wt/wt.
[a] The carboxyl percentage content of MSA is 7.27%.
[b] The carboxyl percentage content of MSA is 14.55%.

Characterization of MSA-CS

Elemental analysis is used to examine whether the precipitate consists of CS.[14] Table I show that there is nitrogen element in two randomly selected MSA-CS samples. As MSA is a weak acid and CS is soluble in dilute acidic solution, this analysis result confirms that the product harvested is the polyelectrolyte complex of CS and MSA. The percentage contents of nitrogen element in MSA-CS obtained from MSA of 7.27 and 14.55% carboxyl are 0.976 and 1.263%, respectively, which also indicates that the MSA of higher carboxyl group content binds more CS to form insoluble complex.

MSA-CS is also verified with FTIR spectroscopy. It is found that the characteristic peaks of carbonyl and ester groups appear at 1720 and 1367/cm^{-1} on the FTIR spectra of MSA, and the characteristic

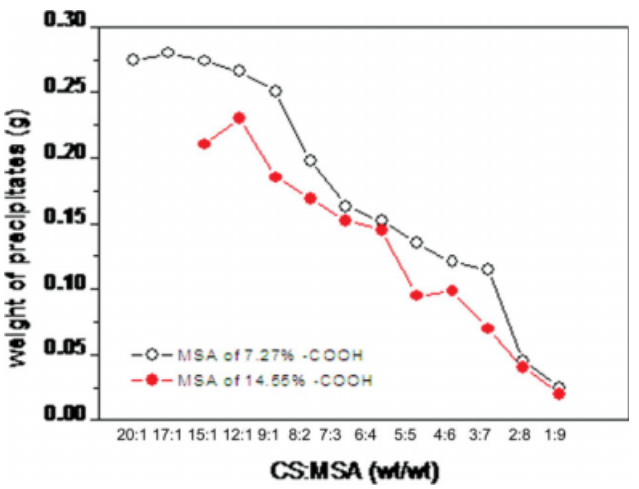


Figure 1 The effect of feeding ratio on the formation of MSA-CS. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

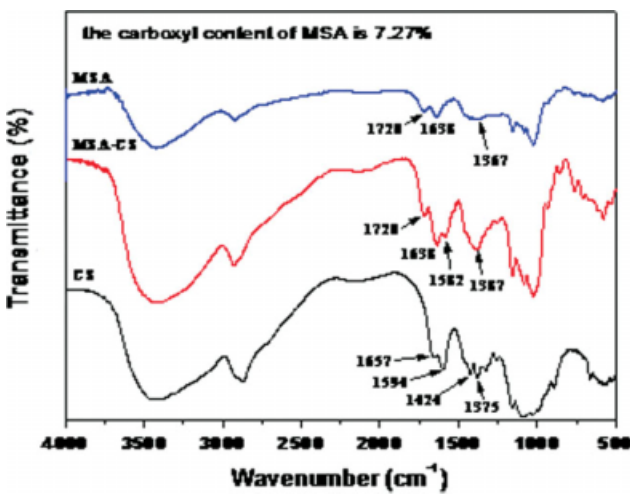


Figure 2 The FTIR spectra of MSA-CS, MSA, and CS. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

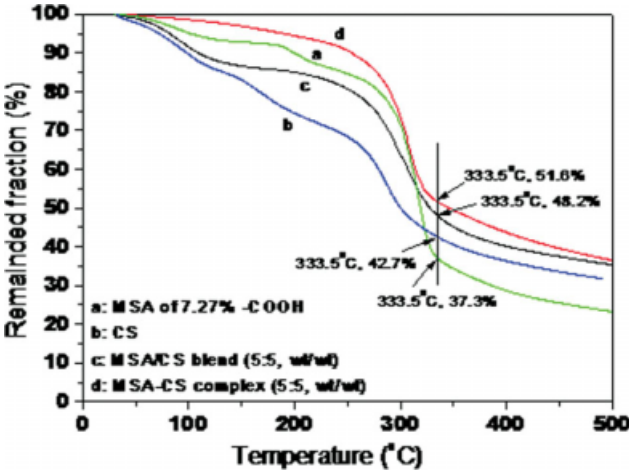


Figure 3 Thermogravimetric analysis profiles of MSA-CS, MSA/CS, MSA, and CS. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

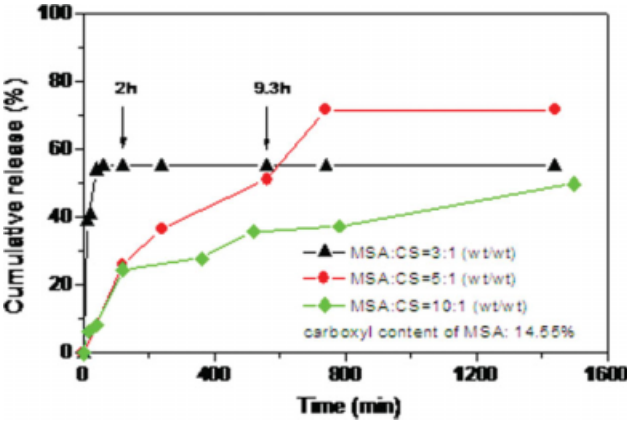


Figure 5 The effect of feeding ratio on *in vitro* release behavior of 5-Fu-loaded MSA-CS (MSA of 14.55% carboxyl groups) in PBS (0.1*M*, pH 7.4) at 37°C. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

absorption bands of CS exhibit at 1657 and 1594/cm^{-1}. The absorption bands at 1720 and 1582/cm^{-1} are observed on the FTIR spectra of MSA-CS (Fig. 2), which are attributed to carbonyl of —COO$^-$ and —NH$_3^+$ groups, respectively.[15] These results indicate that the MSA-CS is the complex of CS binding MSA.

There are electrostatic interactions and hydrogen bonding among the chains of MSA-CS, whereas no ionic attractions exist in MSA or CS alone. As a result, thermal resistance of MSA-CS is higher than that of MSA or CS. The remained fractions of MSA-CS, MSA, and CS are 51.6, 37.3, and 42.7% at 333.5°C, respectively, (Fig. 3). A MSA/CS blend is prepared by solution-casting at the same MSA:CS ratio as a control. It is of interest to note that the decomposition rate of the blend is faster than that of the complex and the remained fraction of MSA/CS at 333.5°C is 48.2%, also lower than that of MSA-CS. During the aggregation procedure of the blend, the oppositely charged polyelectrolyte chains are difficult to diffuse homogeneously and their combination is not so well as that in MSA-CS. Therefore, the difference of thermal stability between MSA-CS and MSA/CS can be attributed to that the ionic interaction plays predominant role in the formation of the complex.

There are two peaks which exhibit at 10.5 and 19.9°, 10.5 and 18.8°on the XRD profiles of MSA and

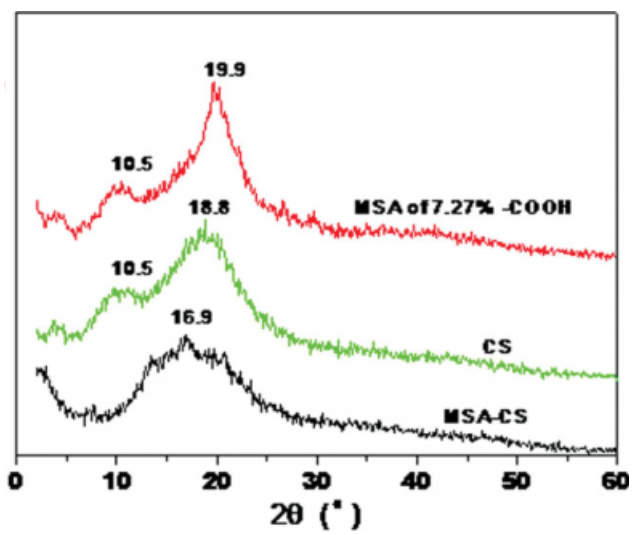


Figure 4 The WAXD curves of MSA-CS, MSA, and CS powder. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

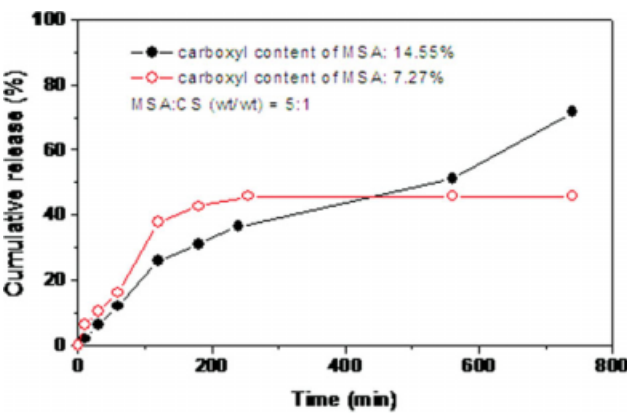


Figure 6 The effect of carboxyl content on *in vitro* release behavior of 5-Fu-loaded MSA-CS (MSA:CS = 5 : 1, wt/wt) in PBS (0.1*M*, pH 7.4) at 37°C. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

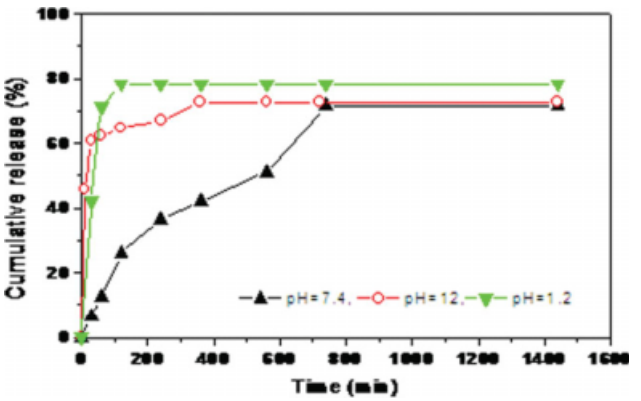


Figure 7 pH-responsive release behavior of 5-Fu-loaded MSA-CS (MSA:CS = 5 : 1, wt/wt; MSA of 14.55% carboxyl groups). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

CS, respectively (Fig. 4), which indicates that both MSA and CS are semi-crystalline. In contrast to that, only one wide peak appears at 16.9° on the XRD pattern of MSA-CS. This result shows that MSA-CS is amorphous, just like the other complexes formed between oppositely charged polyelectrolytes.[16]

In vitro release of 5-Fu-loaded MSA-CS

As mentioned above, higher carboxyl group content provides more ionic crosslinking sites and makes MSA and CS ready to self-assemble insoluble complex by the incorporation of polyelectrolyte chains. In other words, MSA-CS will be more compact by increasing the carboxyl group content of MSA when the concentration of CS is fixed. This assumption is verified with the *in vitro* release behavior of 5-Fu-loaded MSA-CS. It is found that the cumulative release of 5-Fu is adjustable by changing the feeding ratio MSA:CS or the carboxyl group content. As shown in Figure 5, the more MSA added, the lower is the initial release rate of 5-Fu. The cumulative release percentages of 5-Fu from 5-Fu-loaded MSA-CS obtained with the MSA:CS ratio of 10 : 1, 5 : 1, and 3 : 1 are 24.7, 26.1, and 55.1%, respectively, after soaking 2 h. Evidently, slower release rate can be attributed to the more compact interior morphology of MSA-CS. Such tendency has been lasting for ca. 9 h. Thereafter, the release of 5-Fu from the swollen complex is too free to be matrix-controlled. Similarly, it is found that the initial release rate of 5-Fu from 5-Fu-loaded MSA-CS obtained with MSA containing higher carboxyl percentage content and at the same MSA:CS ratio is slower (Fig. 6). The cumulative release percentages of 5-Fu from 5-Fu-loaded MSA-CS prepared with MSA containing 7.27 and 14.55% carboxyl groups are 37.9 and 26.1%, respectively, after soaking in PBS for 2 h. Such a matrix-controlled release behavior can be maintained for 7 h. In addition, more compact MSA-CS can load more 5-Fu and sustain the release of 5-Fu longer.

Supramolecular systems usually exhibit fast responses to external stimuli.[17] As the charge balance inside PECs, and therefore, the degree of interaction between two polyelectrolytes will be changed with the pH change of medium, PEC exhibit pH-sensitive swelling.[6] The experimental results show that the release rate of 5-Fu from 5-Fu-loaded MSA-CS depends on the pH value of medium (Fig. 7). The cumulative release percentages of 5-Fu from 5-Fu-loaded MSA-CS are 78.3, 64.7, and 26.1% after soaking 2 h in HCl (pH 1.2), Na_2HPO_4-NaOH (pH 12), and PBS (pH 7.4), respectively. In acidic or basic medium, free charges appear inside MSA-CS. The electrostatic repulsion causes MSA-CS swelling[6] and becoming loose. Consequently, both the cumulative release percentage of 5-Fu in HCl or Na_2HPO_4-NaOH are higher than that in PBS. The pH-responsive release behavior of 5-Fu-loaded MSA-CS indicates that MSA-CS is formed mainly by ionic interactions once more.

CONCLUSIONS

A novel polysaccharide-based polyelectrolyte complex, MSA-CS, has been prepared via the ISA of CS and a carboxylic derivative of starch by using water as solvent. MSA-CS is confirmed with elemental analysis, FTIR spectroscopy, XRD, TGA, and pH-responsive release behavior of MSA-CS. The determination results indicate that the complex is mainly formed via ISA. Meanwhile, hydrogen bonding as the secondary driving forces is involved.

The carboxyl group content of MSA is a key factor for the self-assembly of MSA-CS. MSA containing higher carboxyl group content is more convenient to control the formation of MSA-CS and its properties. In view of the excellent properties of MSA and CS, MSA-CS can be considered as a candidate for drug controlled release or other biomedical applications.

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