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Preparation and characterization of pH sensitive comb-shaped chitosan material for the controlled release of coenzyme A

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Abstract A novel kind of pH sensitive comb-shaped copolymer P(CS-Ma-PEGMA) was synthesized with chitosan (CS), maleic anhydride (Ma) and Poly (ethylene glycol) methacrylate (PEGMA) by grafting and co-polymerization. The structure of P(CS-Ma-PEGMA) was characterized by FT-IR and ^{1}H-NMR, and it was found that PEGMA was grafted onto CS and PEGMAylated chitosan was soluble. The co-polymer was subjected to coenzyme A adsorption study in order to assess its application in biomedical area. The factors affecting release behavior, such as concentration and pH were discussed in this paper. The higher concentration of the copolymer showed higher absorbance peak than the lower one. The pH of the solution also had significant impact on the release of coenzyme A, and the mechanism of adsorption was suggested. The results suggested that the novel copolymer could be used as drug delivery carrier.

1 Introduction

Polymers and their complexes, because of the presence of certain functional groups along the polymer chain, are often sensitive to the conditions of the surrounding environment, such as temperature [1], pH [2], the ionic strength [3] of the solution, the presence of a magnetic field [4] and ultraviolet light [5], which are referred to as ''intelligent materials''. However, biological applications need materials with good biocompatibility [6, 7]. Chitosan (CS) is naturally originated and characterized as non-toxic biomaterials with good biocompatibility [8]. It has been used widely as blood plasma-bulking agent and medical accessory material. Chitosan contains a large number of hydroxyl and amino groups, and can be modified by various chemical reactions to prepare series of chitosan derivatives [9], which have attracted much interest in various applications [10–12], such as wound healing, anti microbial agent, drug delivery carrier, blood vessel, metal chelating agent, immobilization of enzymes and food processing technology, etc.

Poly (ethylene glycol) methacrylate (PEGMA) was functional hydrophilic macromonomer [13]. P(CS-Ma-PEGMA) is comb-shaped copolymer and PEGMA along the CS main chains are as long side chains in copolymer P(CS-Ma-PEGMA).

In the past 20 years, drug delivery system has become an important research field of medicine. Self-correcting drug release system can control the drug release by the information feedback from the body without the outside intervener. The drug release rate was affected by the changes of pH or temperature [14], which are one of the important ways. Chitosan with good compatibility and biodegradation was always used as drug carrier. To combine the advantage of synthetic and natural polymers and at the same time maintain the favorite property of natural polymers such as biodegradation and bioactivity, in this work, a novel comb-shaped copolymer was prepared by the

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reaction of CS with Ma and PEGMA. Here maleic anhydride (Ma) took the effect of its carboxyl groups. The copolymer P(CS-Ma-PEGMA) contained amino and carboxyl groups, so it has pH sensitivity in the solution. Its controlled release of coenzyme A at different concentrations and pH values solution was studied, and the mechanism of adsorption was also proposed. The novel copolymer P(CS-Ma-PEGMA) with pH sensitivity seems to be of great promise in drug delivery systems.

2 Experimental part

2.1 Materials

Chitosan was obtained from Tokyo Kasei Kogyo Co., Ltd. and the degree of deacetylation was 0.85. PEGMA and coenzyme A (Co A) were all purchased from Aldrich. $(NH_4)_2S_2O_8$ was obtained from Peking chemical industry, China, and was recrystallized before use. All other chemicals used were of analytical grade, without further purification.

2.2 Synthesis of CS-Ma

Chitosan (2 g) was dissolved in 0.1 M acetic acid aqueous solution (100 ml). After stirring for 24 h, the solution was filtered through a medium pore sintered glass to remove insoluble substance. Then the solution was poured into a Teflon-coated mold. Solvent was allowed to evaporate in air for 4 days. Then the film was washed with 0.1 M NaOH/methanol (v1:1) and methanol/water (v1:1) to neutralize the acid. The final drying step was carried out under vacuum. Film thickness was between 40 and 100 μm.

The synthesis of CS-Ma was carried out in solution. 0.5 g CS films and 9.13 g Ma were put into the dry flask. Then 0.298 g dicyclohexyl carboimide (DCC) as water condenser and 100 ml CH_3OH were added into the flask. After stirring for 24 h, the film was washed in methanol for 3 times and dried in vacuum. The grafting percentage was estimated from the mass of the polymer before and after grafting using the relationship $\%\text{Grafting} = (W_g - W_0)/W_0 \times 100\%$, where W_g and W_0 are the masses of the grafted copolymer and of the chitosan, respectively. Here the grafting percentage of the copolymer was 42.4%.

2.3 Synthesis of P(CS-Ma-PEGMA)

About 0.2 g CS-Ma was dissolved in 15 ml 2% HCl aqueous solution at 75°C for 2 h. Then 0.8 ml PEGMA and 10 mg $(NH_4)_2S_2O_8$ were added into the solution at room temperature. Then the mixture was incubated at 75°C for 6 h. Extra CH_3OH was added to the solution as a precipitator. The copolymer was isolated by filtration, reprecipitated and dissolved for 3 times, and then dried in vacuum oven at 40°C.

3 Results and discussion

3.1 Analysis of IR spectra of the copolymer

IR was used to characterize the functional groups on the chitosan before and after modification. All IR spectra were collected at a resolution of 4 cm^{-1} by using AVATAR-360FT-IR. The IR spectra of CS, CS-Ma and P(CS-Ma-PEGMA) were shown in Fig. 1 (a: CS; b: CS-Ma; c: P(CS-Ma-PEGMA)). In curve a, the non-modified chitosan films showed signals at 1647 and 1590 cm^{-1} for the C–O stretching (amide) and N–H bending (amine), respectively. The spectra of modified CS films (curve b) is similar to that of the original chitosan (curve a), while a new peak appeared at 1705 cm^{-1} which is assigned to the carbonyl groups on the side chains. Compared to the IR spectra of CS (a), CS-Ma (b), the IR spectra of P(CS-Ma-PEGMA)(curve c) has a new peak appearing around 1726 cm^{-1}, corresponding to the ester on the PEGMA side chains. Curve c also showed the presence of a $-CH_3$ at 1454 cm^{-1}, and the peak intensity was greatly strengthened. The peak around 1096 cm^{-1} was attributed to the characteristic absorption of $-CH_2-O-CH_2-$ on the side chains. All the above characterization showed that we synthesized the copolymer.

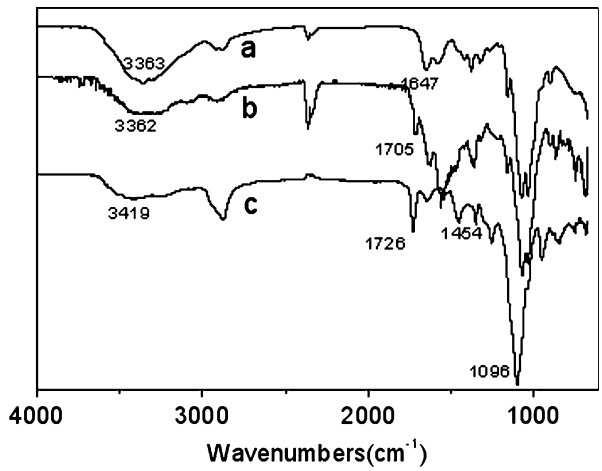

Fig. 1 IR spectra of chitosan (CS) (**a**), CS-maleic anhydride (Ma) (**b**) and P [CS-Ma-Poly (ethylene glycol) methacrylate (PEGMA)](**c**)

3.2 The analysis of ^{1}H-NMR spectra of the copolymer

In order to further determine the structure of the copolymer, the copolymer was analyzed by a nuclear magnetic resonance spectrometer (EW360L, 400 MHz) by using D_2O as solvent. The structural formula of the copolymer is shown in Fig. 2. All chemical shifts are given in ppm. The chemical shifts of H atom in the copolymer were as follows: the chemical shifts of H in the CS: δ_1 = 4.46 (s, 1 H, CH_2OH*), δ_2 = 3.26–3.28 (d, J = 5.7 Hz, 2 H, CH*$_2$OH), δ_3 = 3.51–3.54 (t, J = 9.5 Hz, 1 H, CH*), δ_4 = 3.21–3.23 (d, J = 9.5 Hz, 1 H, CH*), δ_5 = 3.30 (d, J = 9.0 Hz, 1 H, CH*OH), δ_6 = 4.91 (s, 1 H, CHOH*),δ_7 = 3.25 (d, J = 7.5 Hz, 1 H, CH*NH), δ_8 = 4.97–4.99 (d, J = 7.8 Hz, 1 H, CH*), and the chemical shifts of H in the side chains: δ_9 = 8.57 (s, 1 H, NH*),δ_{10} = 2.15–2.17 (d, J = 6.9 Hz, 2 H, CH_2*), δ_{11} = 2.50 (t, J = 7.4 Hz, 1 H, CH*),δ_{12} = 1.21–1.23 (d, J = 7.6 Hz, 2 H, CH_2*), δ_{13} = 1.17 (s, 3 H, CH_3*),δ_{14} = 4.30–4.33 (t, J = 6.9 Hz, 2 H, CH_2*), δ_{15} = 3.74 (s, 1 H, CH_2OH*). The chemical shifts of H atom were well accordance with literature. All the above data showed that P(CS-Ma-PEGMA) was the target copolymer.

3.3 The effect of P(CS-Ma-PEGMA) on the controlled release of coenzyme A

In order to assess the copolymer's use in biomedical application, we use the copolymer to control the release of coenzyme A at different experimental conditions. The UV spectrum of the copolymer solution was recorded with a UV–visible spectrophotometer (UV-540, US). According to the changes of height of characteristic absorption peak of coenzyme A at 260 nm in the copolymer solution, we determine the release or control on coenzyme A.

3.3.1 Effect of concentration of the copolymer on the controlled release of coenzyme A

The UV spectrum of coenzyme A in aqueous solution at pH 6.8 under room temperature was shown in Fig. 3.

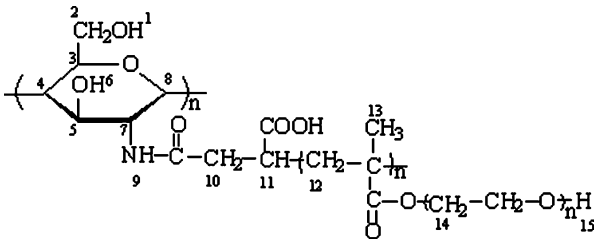

Fig. 2 The structural formula of the copolymer

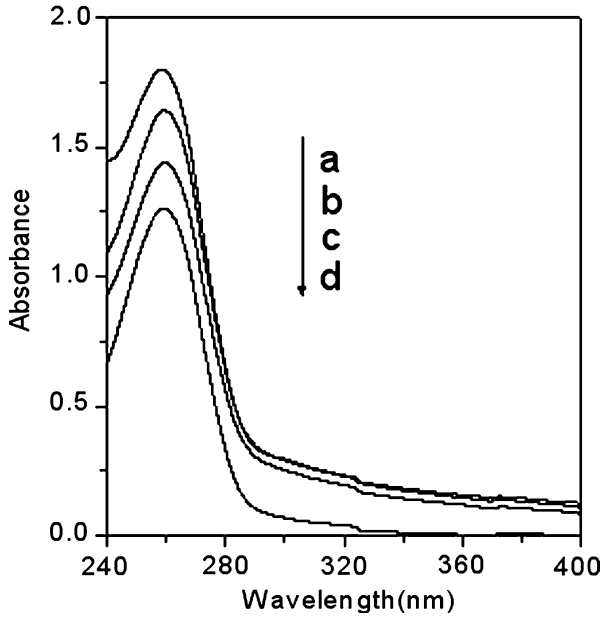

Fig. 3 UV absorbance of coenzyme A controlled delivery at different concentrations of copolymer aqueous solution. pH = 6.8, [Co A] = 0.5%, Concentration of copolymer (wt%): a, 0.1; b, 0.05; c, 0.01; d, 0.0

The concentration of Co A was 0.5wt% and the concentration of P(CS-Ma-PEGMA) was 0.1, 0.05, 0.01 and 0 wt% as shown in Fig. 3 as curves a, b, c and d, respectively. It was found that the characteristic absorption peak of coenzyme A in the copolymer solution is higher than that in water, and increased with the concentration of P(CS-Ma-PEGMA) increasing. This indicated that the copolymer could adsorb coenzyme A at different concentrations. This is probably due to –OH, –NH_2, –COOH and C–O–C groups in the copolymer combined with the phosphoric group of coenzyme A in the form of H-bond, which reduced the phosphoric group combining with the N atom in pyridine. The electron supplying capacity of pyridine increased, so the absorbance peak increased. We can determine proper concentration according to the height of the peak when the copolymer was used as drug carrier.

3.3.2 Effect of pH in the copolymer solution on the controlled release of coenzyme A

To determine the effect of pH in the copolymer solution on the controlled release of coenzyme A, buffers with the same ionic strength (I = 0.5) and various pH values were used in this work. In Fig. 4, curves a, b and c were the absorbance of coenzyme A in the copolymer solution at different pH values (a: pH = 9; b: pH = 3.7; c: pH = 6.8, the copolymer

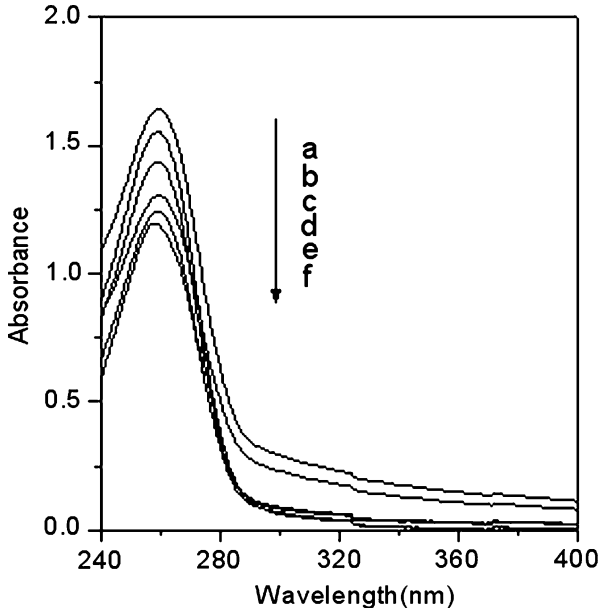

Fig. 4 UV absorbance of coenzyme A controlled release at different pH values copolymer solution, [Co A] = 0.5%. Curve a (pH = 9, [P(CS-Ma-PEGMA)] = 0.01%), d (pH = 9, no polymer), Curve b (pH = 3.7, [P(CS-Ma-PEGMA)] = 0.01%), f (pH = 3.7, no polymer), Curve c (pH = 6.8, [P(CS-Ma-PEGMA)] = 0.01%), e (pH = 6.8, no polymer)

concentration was 0.01 wt% and [Co A] = 0.5%). Curves d, f and e were absorbance of coenzyme A in no copolymer solution at different pH values in water (d, pH = 9; f, pH = 3.7; e, pH = 6.8). According to Fig. 4, the characteristic absorption peak of coenzyme A in the copolymer solution is higher than that of the same pH value in water. That is to say the copolymer can adsorb coenzyme A at all pH values. Among the curves a, b and c in Fig. 4, the absorbance peak of coenzyme A is the lowest when pH is 6.8, and while pH is 9, the peak is the highest. This is different from that of the same pH value solution without the copolymer, i.e. the effect of pH in the copolymer solution on the controlled release of coenzyme A is considerable. This can be explained by the different conformation of the macromolecular and the effect between the macromolecular and coenzyme A at different experimental conditions. In the acid condition (pH = 3.7), residual amido $-NH_2$ on the CS chains was protonized to NH_3^+. On one hand, amine salt $-NH_3^+$ on the main chain leads to electrostatic repulsion between the main chains and it makes the chain expand. On the other hand, there is great deal of –OH on the main chains and –COOH, –OH and CH_2–O–CH_2 groups on the branch chains. Large numbers of H-bond form between them and the effect of H-bond makes the macromolecule compact. As a result of the two effects, the space between the main chains is near. The opportunity of H-bond forming between macromolecules is bigger than that between macromolecular and the phosphoric group of coenzyme A. In the alkaline condition (pH = 9), –COOH on the branch chains changes into $-COO^-$. The electrostatic repulsion in the alkaline condition between branch chains is stronger than that between main chains in the acid condition. So the whole macromolecule is relative stretched. The chance of function group of macromolecular combining with the phosphoric group of coenzyme A is increasing. When pH is 6.8, all the function groups in the macromolecule can form H-bond between intra-molecule and inter-molecule, the effect of a great deal of H-bond make the macromolecule be like physically cross-linked state. The space between macromolecule is the smallest. The chance of all groups forming H-bond between them greatly increased, and the opportunity of combination with phosphoric group of coenzyme A enormously decreased. As what is analyzed above, the order of the chance of the copolymer's combining with coenzyme A at different buffers solution was: basic > acidic > neutral. So in basic solution, the adsorption of coenzyme A is the most and UV characteristic absorption peak of coenzyme A is the highest. So we can use the copolymer to control the release of coenzyme A at different pH values.

4 Conclusion

In this paper, novel comb-shaped copolymer P(CS-Ma-PEGMA) was prepared by using functional macromonomer PEGMA and chitosan, and the structure of the copolymer was characterized. It can be used to control the release of coenzyme A at different concentrations and at different pH values, and the conformation of the macromolecule was suggested based on the changes observed by UV spectroscopy.

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