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Synthesis and Properties of Chitosan-Pectin Microspheres

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Chitosan-pectin (CS-PT) microspheres were prepared by inverse phase suspension method, with liquid paraffin and Span 80 as the oil phase, chitosan-pectin acetic acid as aqueous solution, glutaraldehyde as cross-linker. Based on the theory of inverse emulsion polymerization, the optimal conditions were studied by single-factor test: the reaction temperature was 60 °C, chitosan-pectin solution of acetic acid was 0.025 g/mL, m_{CS}:m_{PT} = 4:1, the oil were liquid paraffin and a mixture of Span 80, the volume ratio of oil phase to aqueous was 2:1, the dosage of cross-linker was 0.30 mL, the dosage of Span 80 was 0.6 g, the time of reacting was 3 h. The synthesized chitosan-pectin microspheres are dark yellow and have smoother appearance and the diameter is about 50 microns. The structure of microspheres were characterized by FT-IR, Bio-optical microscope and X-ray diffraction studies. The adsorption of chitosan-pectin microspheres was good in the solution of methylene blue.

Keywords: Chitosan, Pectin, Microspheres, Methylene blue.

INTRODUCTION

Chiosan, 1 → 4 linked 2-amino, 2-deoxy, β-D-glucan (Fig. 1), is the only amino polysaccharide distributed in large amounts in nature[1]. It is the deacetylated derivative of chitin[2-5], the most abundant natural polymer on earth after cellulose[6-8], obtained from crustaceans[9-12], such as shrimps, squids and crabs.

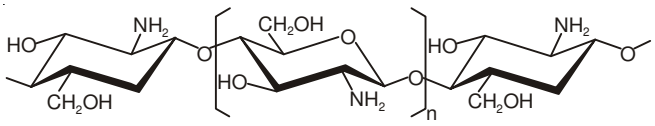

Fig. 1. Chemical structure of chitosan

Chitosan is the only basic polysaccharide in nature. It is greatly dominant to be used as a drug carrier due to the non-toxicity[13]. Chitosan with good biocompatibility and biodegradation has widely application in many fields, such as wastewater treatment, medicine, food, drug, environmental protection, light industry and agriculture[14-19]. Since pectin (Fig. 2) itself is nontoxic, biodegradable and biocompatible[20-22], several biological applications have been reported for pectin, including site-specific drug delivery systems and a drug carrier. Pectin is a non-toxic water soluble gel-forming polysaccharide containing carboxylic acid groups. It is extracted from different sources, *e.g.* apple or citrus. Combination of different polymers with unlike mechanism has been studied previously[23].

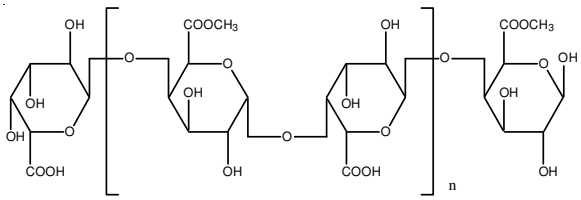

Fig. 2. Chemical structure of pectin

In the appropriate system, chitosan-pectin(CS-PT) composite gel will be prepared on accout of the -NH_2 in chitosan molecules and -COOH in pectin molecule. This step is effective because the chemical groups of chitson involved in the ionic interactions with pectin differ from those forming covalent bonds with glutaraldehyde. The structure of CS-PT composite gel was response as the change of environment pH and ionic strength, so they are commonly used to preparation with pH sensitive membrane and microspheres[24].

The present work focused on studying the effects of reaction temperature, concentration of the acetic acid, the mass ratio of chitson to pectin, the volume ratio of oil phase to aqueous, the dosage of cross-linker, the dosage of Span 80, reaction time. CS-PT microspheres were prepared by using glutaraldehyde for cross-linking agent, with inverse emulsion polymerization method in the chitson and pectin acetum. The CS-PT microspheres were estimated by Fourier-transform infrared (FT-IR), bio-optical microscope and X-ray diffraction spectra. The adsorptive property of the CS-PT microspheres was also studied by the methylene blue solution.

This work also aimed at studying the microspheres of CS-PT with the view to further exploring the microspheres properties and its applications, such as adsorption, colon targeted drug delivery and so on. This provides compelling support for using biopolymers from renewable resources to synthesize strong microspheres with desirable properties.

EXPERIMENTAL

Materials used included chitson (CS, degree of deacetylation 90 %, viscosity 90 m Pa), which was kindly provided as a gift sample by Indian Research Products. Pectin (PT, 95.5 wt. %) was purchased from Fuda pectin chemical Co., in China. Glutaraldehyde (50 wt. %, analytically pure) and Methylene blue were purchased from Tianjin jia xing chemical glassware industry & trade co., in China. Span 80 was purchased from Recovery of Tianjin institute of fine chemicals, in China. All commercially solvents and reagents were used without further purification. All other chemicals were of analytical grade.

Preparation of CS-PT acetic acid: A specific amount of chitson powder was added into 200 mL acetic acid (v/v, 2 %) and dispersion was stirred by a magnetic stirrer for 1 h. A specific amount of pectin was gently added with continuous stirring to the dispersion of chitson solution. The total quantity of chitosan and pectin powder is 5 g. The stirring will not stop until the chitson and pectin completely dissolved. After still standing for 3 h later, the CS-PT acetum was prepared.

Preparation of CS-PT microspheres: Oil phase was prepared by mixing liquid paraffin (30 mL) and Span 80 (0.6 g) in 50 mL beaker. A specific volume of CS-PT acetum was tenderly added into the oil phase with vigorous stirring(1000 rpm) by adding into 0.3 mL glutaraldehyde as cross-linking agent, heating to a constant temperature. After reacting for several hours, the oil phase was completely uniform.

By petroleum ether to remove oil phase and then 2 % sodium bisulfite to remove residues of glutaraldehyde, CS-PT composite microspheres which was deep yellow were prepared after dehydration 2-3 times with acetone, filtering and placing in a temperature of 40 °C drying in vacuum oven for 24 h.

Visualization of films structures

Biological microscope analysis: Ether solution was prepared by dispersing CS-PT microspheres into the solution, which was absorbed and dropped on the slide with glue dropper. Morphology of the microspheres was characterized by the trinocular biological microscope.

Fourier-transform infraredanalysis (FT-IR): FT-IR spectra were recorded using a Spectrum One spectrophotometer (NICOLET 200 SXV FT-IR, Perkin Elmer, USA) equipped with an Universal Attenuated Total Reflectance (UATR) device for tablet analysis in the spectral region (4500-500 cm^{-1}).

X-ray diffraction (XRD)L: The diffraction patterns of chitosan, pectin and CS-PT microspheres samples were recorded using a Siemens D-5000 diffractometer with a cobalt cathode operating in reflectance mode at wavelength of 1.79019 Å. The degree of order (DO) was expressed as I/Dh, where I is the intensity of diffraction maxima and Dh is the width at half-peaks.

Adsorption performance analysis: CS-PT microspheres were added into methylene blue solution (certain concentration), sampling 5 mL above solution at regular intervals and measuring absorbance value, the adsorption performance was measured according to the change of methylene blue solution absorbance.

RESULTS AND DISCUSSION

Influence of synthesis conditions: According to the mechanism of inverse emulsion polymerization, factors influencing the synthesis of CS-PT microspheres are mainly the quality of chitosan and pectin, proportion of oil and water phase volume, the temperature, crosslinking agent, dosage of emulsifier, reaction time and stirring speed, *etc*. This study focused on the influence of the quality of chitosan and pectin, temperature and reaction time on microspheres morphology.

Effect of the quantity of chitosan and pectin: By changing the quantity of chitosan and pectin, other reaction system conditions unchanged (liquid paraffin 30 mL, Span-80 0.6 g, glutaraldehyde 0.3 mL, temperature 60 °C, reaction time 3 h), the influence on the quality of chitosan and pectin was studied in Table-1 and the microspheres morphology are shown in Fig. 3.

All the profiles obtained in Table-1 and Fig. 3 show that the best spherical CS-PT microspheres were prepared on condition that the quantity of chitosan and pectin was 4: 1 with well homodisperse and dark yellow.

Effect of the reaction temperature: By changing the reaction temperature, other reaction system conditions unchanged

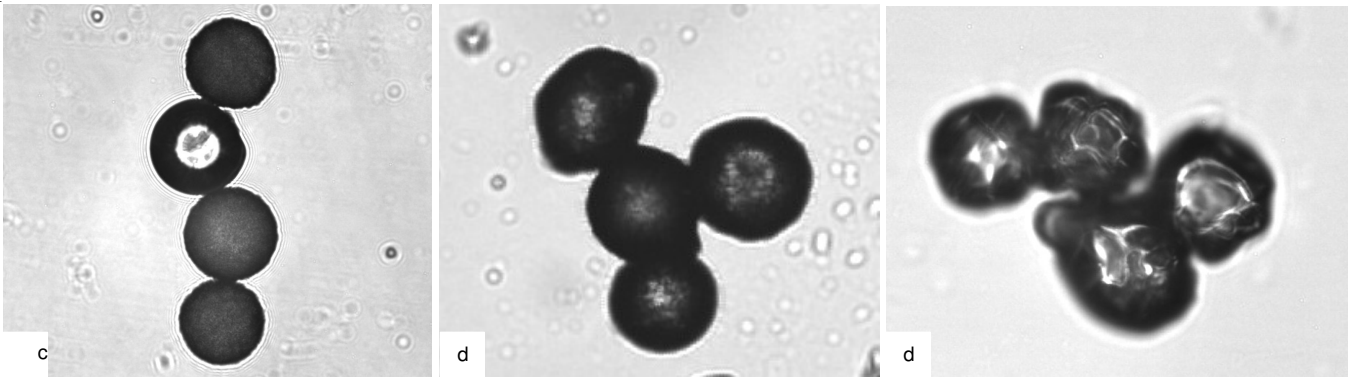

Fig. 3. Microspheres morphology of sample c, sample d and sample e. (× 400)

TABLE-1
EFFECT OF THE QUALITY OF CHITOSAN AND PECTIN

Samples	m_{CS}:m_{PT}	Microspheres morphology
a	7:1	Non-spherical, yellow
b	6:1	Non-spherical, yellow
c	5:1	Spherical, homodisperse, dark yellow
d	4:1	Spherical well, homodisperse well, dark yellow
e	3:1	Non-spherical, heterogeneous dispersion, bonding, yellow
f	2:1	Non-spherical, bonding, pale yellow
g	1:1	Non-spherical, bonding, pale yellow

(liquid paraffin 30 mL, Span 80 0.6 g, glutaraldehyde 0.3 mL, the quantity of chitosan and pectin 4:1, reaction time 3 h), the influence of the reaction temperature was studied in Table-2 and the microspheres morphology are shown in Fig. 4.

TABLE-2
EFFECT OF THE REACTION TEMPERATURE

Sample	Temperature (°C)	Microspheres morphology
α	40	Spherical, bonding, pale yellow
β	50	Spherical, bonding, yellow
γ	60	Spherical well, homodisperse well, dark yellow
δ	70	Spherical, homodisperse well, dark yellow

As shown by Table-2 and Fig. 4, the influence of the reaction temperature was important because that the reaction rate was faster by increasing the temperature. Surface activity of the microspheres was decreased by increasing the temperature. The number of the particles will be reduced by stirring, may also cause many side effects which may influence on the structure and molecular weight distribution of CS-PT microspheres, such as emulsion condensation and demulsification, *etc*.

Effect of the reaction time: Changing the reaction time, other reaction system conditions unchanged (liquid paraffin 30 mL, Span 80 0.6 g, glutaraldehyde 0.3 mL, the quantity of chitosan and pectin 4:1, reaction temperature 60 °C), the influence of the reaction time was studied in Table-3 and the morphology of the microspheres are shown in Fig. 5.

As shown by Table-3 and Fig. 5, the longer the reaction time was, cross-linking effect was better, the morphology of the microspheres was more neatly. When the reaction time was 3-4 h, the microsphere morphology was the best, So the optimal reaction time was 3-4 h.

TABLE-3
EFFECT OF THE REACTION TIME

Sample	Time (h)	Microspheres morphology
I	1	Spherical, bonding
II	2	Spherical, bonding
III	3	Spherical well, homodisperse well, yellow
IV	4	Spherical, homodisperse, yellow

Structural analysis: FT-IR spectra of chitson, pectin and CS-PT microspheres was shown in Fig. 6. FT-IR spectroscopy was used to ensure that the crosslinking reaction between the chitosan and pectin had occurred. From the FT-IR spectral interpretation the following result were obtained. The FT-IR of chitosan show intense band at 1597 cm^{-1} correspongding to the functional group -NH_2 bending. And as the FT-IR of pectin shown, the band at 1747 cm^{-1} belongs to the -COOH stretching vibration peak.

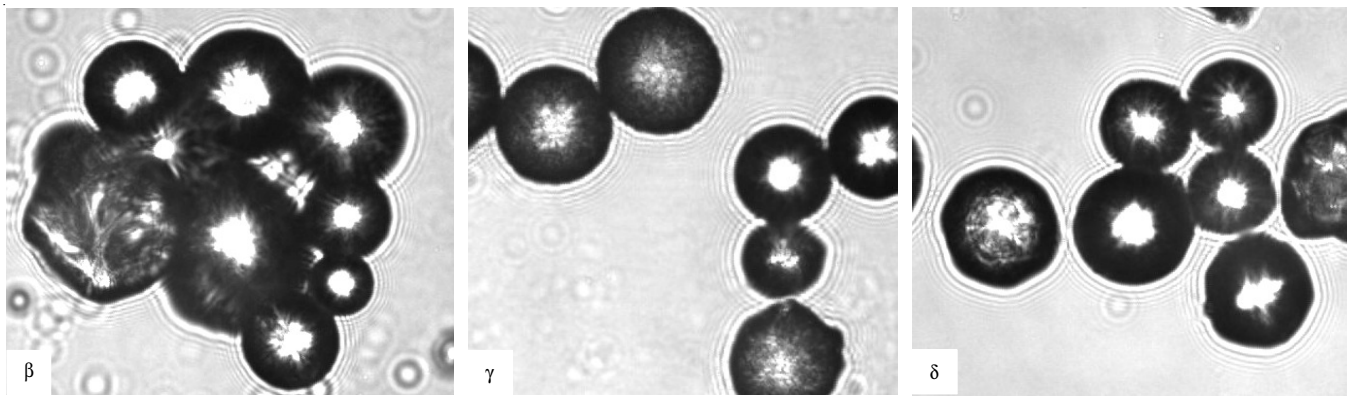

Fig. 4. Microspheres morphology of sample β, sample γ and sample δ. (× 400)

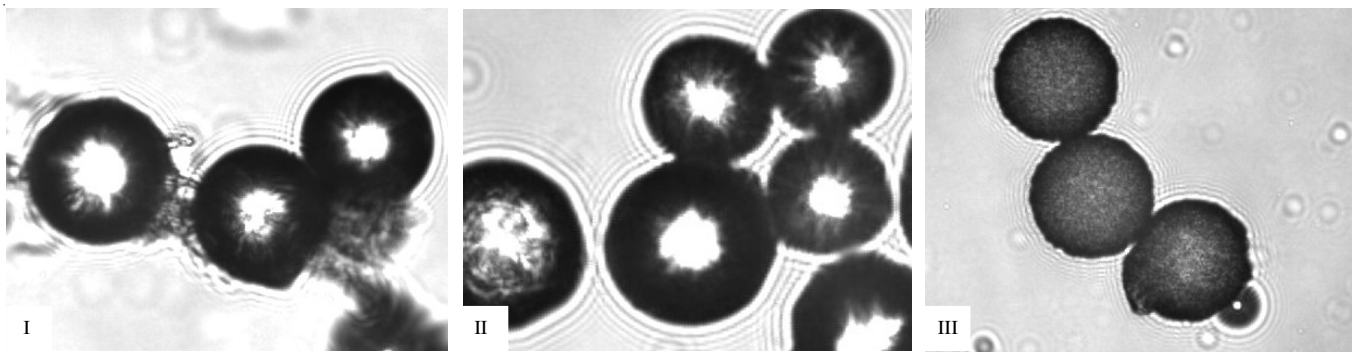

Fig. 5. Microspheres morphology of sample I, sample II and sample III. (× 400)

In contrast, after crosslinking, the vibrational band corresponding to primary amino groups at 1597 cm^{-1} was weaken and the vibrational band at 1747 cm^{-1} disappeared (Fig. 6), which prominent bands at 1630 cm^{-1} was ascribed to -NH_3^+-COO^-, bands at 1410 cm^{-1} was ascribed to -COO- symmetric stretching vibration peak. These results clearly confirmed that the crosslinking reaction had occured between the polymers.

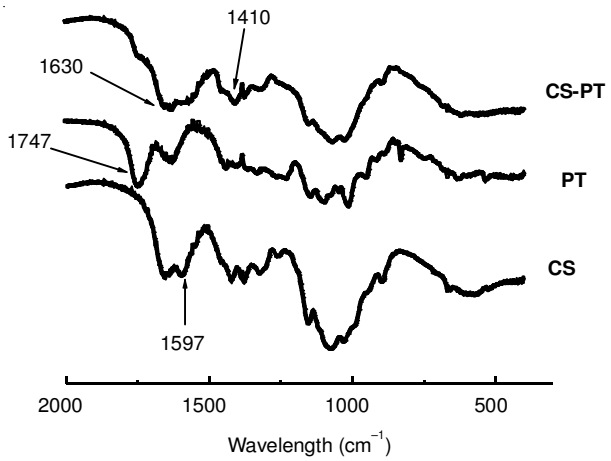

Fig. 6. FT-IR spectra of chitson, pectin and CS-PT microspheres

X-ray diffraction analysis: X-ray diffraction of chitosan, pectin and CS-PT microsphere was shown in Fig. 7. XRD data indicated that the diffraction peaks at 10.6°, 19.8° and 29.3° were assigned to chitosan and the diffraction peaks at 11.2°, 20.5°, 28.7°, 31.2° and 33.8° were assigned to pectin. However, the diffraction peaks above were obviously decreased or disappeared.

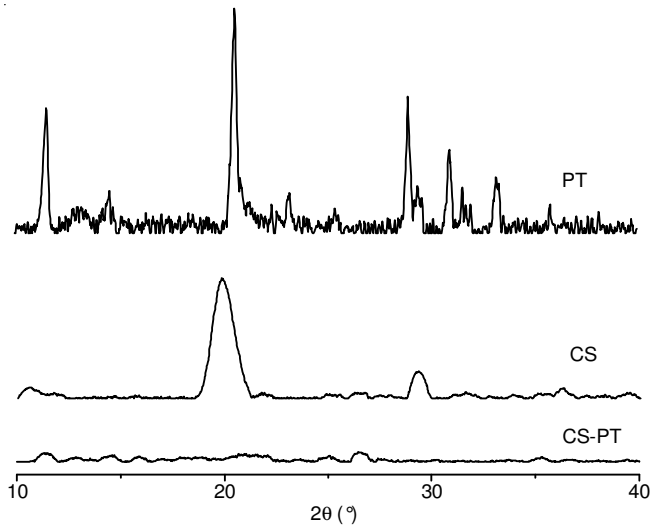

Fig. 7. X-ray diffraction of chitosan, pectin and CS-PT microsphere

CS-PT composite limited the freedom of movement of polymer chain, damaged the orderly structure of CS and PT, weaken the hydrogen bonding interaction between the molecules, resulted in the decrease of crystallization ability, which was conducive to interact with adsorption mass transfer and adsorption.

Adsorption of methylene blue analysis: CS-PT composite microspheres absorption curve of methylene blue solution was shown in Fig. 8. Seen from the absorbance changes of methylene blue, with the increase of time, absorption rate of methylene blue was accelerated, the adsorption quantity increased and the solution was near to colorless. If the increase of adsorption time, composite microspheres of methylene blue absorption curve will be straight.

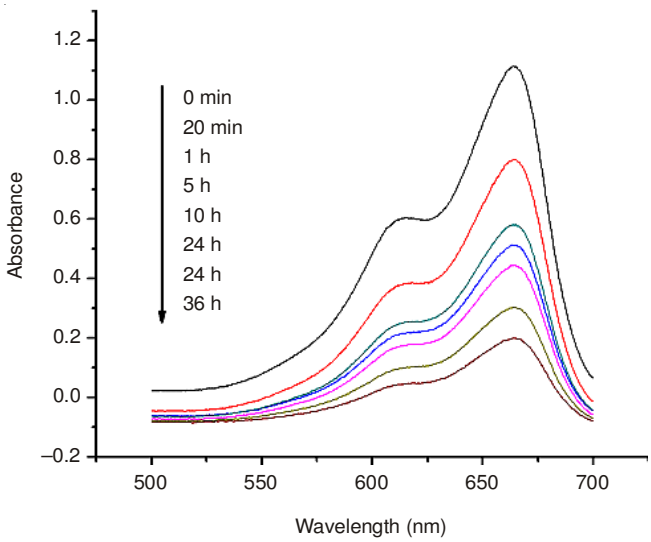

Fig. 8. Absorption curve of methylene blue solution with CS-PT composite microspheres

Experimental results showed that CS-PT microspheres had good adsorption effect to the methylene blue, which was a kind of application prospect of dye waste water treatment agent. Also they can be used in absorbing Ca^{2+}, Cd^{2+}, Mo^{6+}, Bi^{3+}, Cu^{2+}, which was existed in industrial wastewater.

Conclusion

The chitosan-pectin composite microspheres were synthesized and the optimum reaction conditions was as follows: reaction temperature was 60 °C, with 0.025 g/mL of chitosan-pectin acetic acid solution for the water phase, the quantity of chitosan and pectin was 4:1, the amount of crosslinking agent glutaraldehyde was 0.30 mL, the amount of Span 80 was 0.6 g, reaction time was 3 h. The microspheres were dark yellow and which size was about 50 μm.

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