

# Formation of Polyelectrolyte Complex Colloid Particles between Chitosan and Pectin with Different Degree of Esterification

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**Abstract:** The effects of degree of esterification, pectin/chitosan ratio and pH on the formation of polyelectrolyte complex colloid particles between chitosan (CS) and pectin (PE) were investigated. Low methoxyl pectin (LPE) was achieved by de-esterifying high methoxyl pectin (HPE) with pectin methyl esterase. Turbidity titration and colorimetric method was used to determine the stability of complex colloid particles. The structure and morphology of complex particles were characterized by FTIR and TEM. When pectin solution was dropped into chitosan solution, complex colloidal dispersion was stable as PE/CS mass ratio was no more than 3:2. Colloidal particles of HPE-CS complex coagulated at larger ratio of PE/CS than LPE-CS. The maximum complex occurred at pH 6.1 for HPE-CS and pH 5.7 for LPE-CS, and decreasing pH led to the dissociation of complex particles. Electrostatic interactions between carboxyl groups on pectin and amino groups on chitosan were confirmed by FTIR. Colloidal particle sizes ranged from about 100 nm to 400 nm with spherical shape.

## 1. Introduction

Electrostatic interactions occurring between polyanions and polycations result in polyelectrolyte complex (PEC) or forming an insoluble solid. A series of special surface properties, excellent emulsifying properties or different scales of PEC can be obtained based on the different reaction degree of materials [1]. The encapsulation characters of PECs directly promote research and development of controlled release system. PECs have been applied in the field of agriculture, biotechnology, food flavoring, biomedicine, cosmetics and textiles [2]. PEC reaction degree can be controlled by different reaction conditions, such as pH, ionic strength, the ratio of polyanion and polycation, temperature, etc [3]. If core materials exist in the system, different sizes of encapsulating particles, such as microcapsules or nanocapsules are formed. Nanocapsules with small particle size are easy to disperse and suspend in water to form a stable colloidal solution with unique properties, and thus have more wide applications [4].

PECs of natural polysaccharides are excellent controlled release carrier due to rich material source, security and stability, mild reaction conditions, being able to maintain stability and activity of embedding materials, being degradable or soluble under normal physiological conditions, environmentally responsibility [5]. Pectin is composed of partly methylated galacturonic acid with good film-forming property. According to degree of esterification (DE), pectin can be divided into low methoxyl pectin (LPE, DE < 50%) and high methoxyl pectin (HPE, DE > 50%). Pectin extracted from fruit peel is mostly HPE, and LPE is mainly prepared from de-esterification from HPE. Enzymatic preparation of LMP is by removing methyl ester groups from galacturonic acid of HPE backbone



through pectin methyl esterase (PME). Chitosan and pectin can form complex by electrostatic attraction, which is often applied in the controlled release of active materials and in the preparation of membranes and microspheres with pH sensitive properties. Charge density in macromolecules significantly affects the final complexes, therefore pectin molecular chain with different DE affect complexation with chitosan due to different number of negative ions [6].

This paper studied the preparation of complex colloidal particles by chitosan and pectin which was extracted from banana peel and de-esterified to form LPE by PME. The effects of DE of pectin, PE/CS ratio and pH value on the colloid particles were examined. The study is intended to provide foundation for encapsulation of active material by complex colloidal particles in the next step.

## 2. Materials and Methods

### 2.1. Materials

Banana peel was from Brazil banana in Sanya farm (Hainan province, China). Novoshapes pectin methyl esterase was obtained from Novozymes China. Chitosan (degree of acetylation of more than 95%) was obtained from Qiyun Biotechnology Co., Ltd (Guangdong Province, China) with molecular weight of  $4 \times 10^5$  by viscosity method. Other reagents were analytical grade.

### 2.2. Experimentals

**2.2.1. Preparation and Purification of Pectin.** Pectin was extracted from banana peel by acid method after pretreatment of peel. The extraction solution was centrifuged and the supernatant was divided into two parts, one of which was recorded pectin solution 1. The other part was diluted with equal volume of water followed by adjusting pH to 4.5 and PME was added into the solution. The solution was mixed at 45 °C for 70 min followed by quickly inactivation of enzyme by boiling solution for 3 min, and cooled. The solution was recorded as pectin solution 2. Two pectin solutions were concentrated, decolorized by macroporous resin, subjected to protein removal by Sevag method, then dialyzed with deionized water in dialysis bag with molecular weight cut-off of 12 kDa for 24 hours, precipitated with ethanol, dried to achieve pectin 1 and pectin 2 respectively.

**2.2.2. Determination of DE, Intrinsic Viscosity  $[\eta]$  and Viscosity Average Molecular Weight  $M_\eta$  of pectin.** DE of pectin samples was determined by titration method.

Measurement of  $M_\eta$ : 5 mg/mL pectin solution was prepared with 0.1 M phosphate buffer solution as solvent. 10 mL pectin solution was pipetted to Ostwald U-tube viscometer in water bath at 30°C. The flow times of pectin solution with different concentrations were recorded by a stopwatch.  $M_\eta$  was calculated by the following relationships: Relative viscosity ( $\eta_r$ ) =  $t/t_0$ , Specific viscosity ( $\eta_{sp}$ ) =  $\eta_r - 1$ , Reduced viscosity ( $\eta_{red}$ ) =  $\eta_{sp}/C$ , Huggins equation:  $\eta_{sp}/C = [\eta] + k'[\eta]C$ , Intrinsic viscosity  $[\eta] = \eta_{sp}/C$  (when  $C \rightarrow 0$ ),  $M_\eta$ : Mark-Houwink-Sakurada relationship  $[\eta] = kM_\eta^a$  (where  $k = 2.34 \times 10^{-5}$ ,  $a = 0.8224$ ).

**2.2.3. Effect of PE/CS Ratio on the Formation of Stable Complex Colloidal Dispersion.** The pectin solution (1 mg/ml) and 1 mg/mL chitosan solution of acetic acid (1% (w/v)) were prepared and pH was adjusted to 5.5. Pectin solution was added to chitosan solution while stirring at room temperature, and the changes of the system in the process of complex formation were observed. The conditions to form a stable colloidal dispersion were determined.

The complex dispersions with different PE/CS ratio were centrifuged and settled down. The supernatants were used to determine the content of chitosan by colorimetric method [19]. Chitosan in supernatant did not form colloidal particles with pectin.

**2.2.4. The Effect of pH on the Formation of Stable Complex Colloidal Dispersion by Turbidity Titration.** The pectin solution (1 mg/ml) and chitosan solution of acetic acid (1 mg/ml) were mixed, pH of the mixture was adjusted to 11-12. The absorbance of the solution was measured at 500 nm by

UV-VIS spectrophotometer with the change of pH. No absorbance of pectin and chitosan solutions was recorded at this wavelength.

**2.2.5. Particle Size Measurement.** Particle size was determined by using a Malvern Mastersizer 2000, equipped with Malvern application software version 4.00 (Mastersizer 2000, Malvern Instruments Ltd., UK). The instrument is based on the principle of laser light-scattering and capable of measuring size down to 0.01  $\mu\text{m}$ .

**2.2.6. FT-IR Measurement.** Dried samples were ground with KBr in 1:20 ratio, pressed into pellets and measured by FTIR spectrometer (IRAffinity-1, Japan). The samples were scanned over a range of 400  $\text{cm}^{-1}$  - 4000  $\text{cm}^{-1}$  (30 scans).

**2.2.7. Transmission Electron Microscopy.** A small amount of colloid dispersion was placed in copper grid directly and dyed with phosphotungstic acid, blotted with filter paper. The image was acquired using transmission electron microscope (HitachiH - 600) at 200 kV.

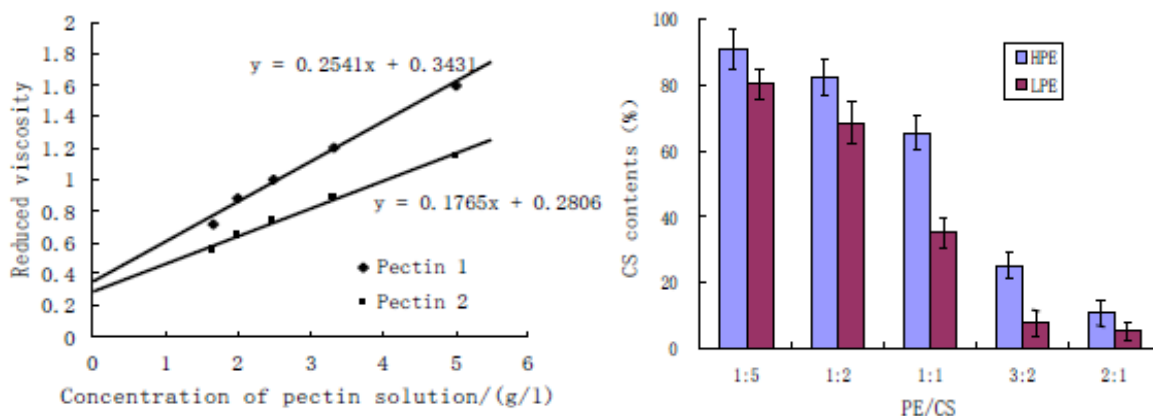
### 3. Results and Discussion

#### 3.1. DE, intrinsic viscosity $[\eta]$ and $M_\eta$ of Pectin

Table 1 shows DE, intrinsic viscosity  $[\eta]$  and  $M_\eta$  of two types of pectin. Pectin 1 was extracted from banana peel with acid method and DE was 64.5%, Pectin 2 was the de-esterification product of pectin 1 and DE was 42.2%. The results demonstrate that banana peel pectin was HPE and LPE was achieved by PME hydrolysis.

**Table 1.** The characteristics of two types of pectin.

Pectin	DE (%)	$[\eta]$ (l/g)	$M_\eta$
Pectin 1 (HPE)	64.51 $\pm$ 4.19	0.3431	116680
Pectin 2 (LPE)	42.27 $\pm$ 2.97	0.2806	91159



**Figure 1.** Linear relationship between reduced viscosity and concentration of pectin

**Figure 2.** The CS contents in supernatant after centrifugation of PE-CS complex solution.

Figure 1 is Huggins fitting line for pectin 1 and pectin 2. Intrinsic viscosity  $[\eta]$  can be obtained by plotting reduced viscosity against polymer concentration on extrapolation to infinite dilution.  $[\eta]$  is the eigenvalues of the polymer and indirectly reflects the shape and size of the polymer. When the testing conditions are fixed,  $[\eta]$  values are only determined by the molecular weight of the sample.  $M_\eta$  of the pectin is obtained by Mark-Houwink-Sakurada equation (see table 1). The results show that  $M_\eta$  of

banana peel HPE is  $1.17 \times 10^5$  and LPE is  $9.0 \times 10^4$ , less than HPE. This may be due to elimination reaction during enzymatic hydrolysis.

### 3.2. The effect of PE/CS Ratio on the Stability of Complex Colloid Dispersion

Stable colloidal dispersion of pectin and chitosan complex can be formed when their concentrations are less than 5 mg/mL, otherwise complex colloid is easy to coagulate. Table 2 presents the change of complex system when pectin was dropped into chitosan solution. It can be seen that stable colloidal dispersion was formed in certain mass ratio range of PE/CS, beyond this range, colloidal dispersion coagulated. This is because the negatively charged carboxylic groups on pectin molecular chains interacted with positively charged amino groups of chitosan, hydrophobic polyelectrolyte complexes were generated. When less pectin was added, too much unreacted amino groups surrounded outside the core of complex to form hydrophilic shell, since like charges repel each other, coagulation of complex particles was hampered and stable colloidal dispersion was formed. When pectin content was gradually increasing, unreacted amino groups decreased and positive charges outside colloid particles decreased, thus phase separation occurred at certain PE/CS ratio. Colloid particles coagulated at 2:1 of HPE/CS, while coagulated at 3:2 of LPE/CS. The results show that different charge density in pectin molecular chain affects the formation of polyelectrolyte complexes, because the LPE molecules have more carboxylic groups than HPE, less LPE were needed to completely interact with chitosan.

**Table 2.** The effect of PE/CS ratio on the stability of complex system.

PE/CS	1:10	1:5	1:2	1:1	3:2	2:1
HPE	√	√	√	√	√	×
LPE	√	√	√	√	×	×

√ indicates stable complex colloid dispersion;

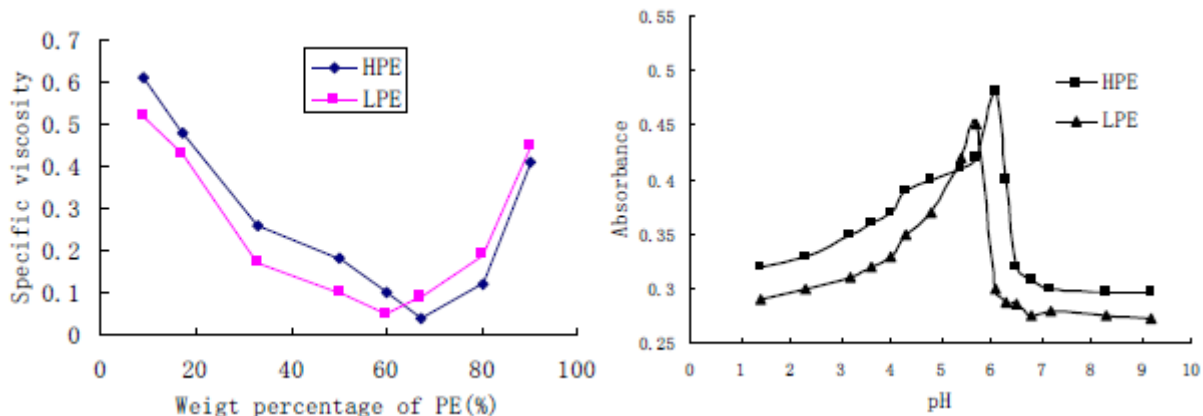
× indicates coagulation of complex colloid

The CS contents in supernatant were determined after centrifugation of PE-CS complex solution to settle down colloid particles. The results are shown in Figure 2. The CS contents in the supernatant decreased gradually with the increase of PE, which means that reacted chitosan increased. Complex degree of LPE was larger than that of HPE at the same PE/CS ratio. Almost all of CS was present in the solution at 1:5 of PE/CS since very few complex particles formed. The unreacted CS in the supernatant was much less at 3:2 of LPE/CS, reacted CS percentage reached 92.51%, and reacted CS percentage at 2:1 of HPE/CS was 89.23%.

Figure 3 show the relationship between the specific viscosity of supernatant solutions of PE-CS complex and the weight percentage of pectin in pH 5.5. The specific viscosity of the supernatant solutions continuously decreased to a minimum value with increase in the weight percentage of pectin in the complex and then increased as the weight percentage of pectin increased. The weight percentage of pectin at minimum value of specific viscosity for HPE-CS was larger than LPE-CS, which means there were more HPE complexed with CS than LPH. The results coincided with Figure 2.

### 3.3. The Effects of pH on the Stability of Complex Colloid Dispersion.

Figure 4 is the absorbance values of solution varying with the change of pH at 3:2 of PE/CS. It shows that the absorbance of system had almost no change in alkaline solution, the absorbance increased sharply when pH decreased to a certain value. The maximum absorbance for HPE was at pH 6.1, and for LPE was at pH 5.7. This is because protonation of CS amino groups increased when pH decreased, and the electrostatic interaction between amino groups and carboxylic acid became more and more strong, finally achieved full complexation. With pH further decreased, dissociation of carboxylic acid decreased, charge density decreased, which led to partial dissociation of complex particles and absorbance of solution decreased. The pH value at the maximum absorbance for HPE was larger than LPE.



**Figure 3.** Specific viscosity of supernatant solutions of PE-CS complex in pH 5.5.

**Figure 4.** The effect of pH on absorbance of complex solution.

### 3.4. The Effects of NaCl Concentration on Complex Colloid Particle Size.

There are two cases for polyelectrolyte complexes to the response of the ionic strength, coagulation and dissolved, depended on the structure of the polyelectrolyte. 10% NaCl solution was added to the complex colloid dispersion (The weight ratio of PE/CS was 3:2) and the particle size of the colloid were measures after half hour stirring. The results are shown in Table 3. It can be seen that the particle size of LPE-CS was larger than HPE-CS and partile size of both complex decreased with the increase of NaCl concentration. The HPE-CS particles dissolved when NaCl concentration was 60 mg/mL and the system became clear solution and LPE-CS particles dissolved at 70 mg/mL of NaCl concentration. Ionic strength has more effect on HPE-CS colloid dispersion than on LPE-CS.

**Table 3.** The effect of NaCl concentration on PE-CS complex particle size with NaCl 10%.

NaCl (mg/ml)		0	10	20	30	40	50	60	70
Particle size (nm)	LPE-CS	432	440	426	395	347	284	206	Particles dissolved
	HPE-CS	323	351	324	286	237	198	Particles dissolved	Particles dissolved

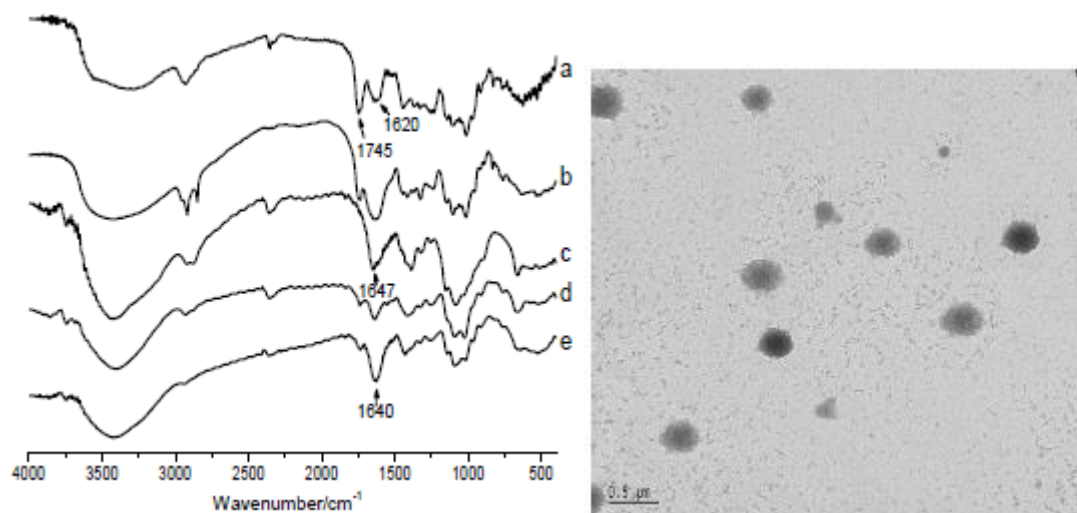
### 3.5. FT-IR Analysis of PE/CS Complex.

A general review of the FTIR spectra of HPE, LPE, CS, HPE-CS and LPE-CS are shown in Figure 5. The obtained spectral data were analyzed by comparing the following characteristic regions: O-H stretching ( $3100 \sim 3600 \text{ cm}^{-1}$ ), C-H stretching ( $2800 \sim 3000 \text{ cm}^{-1}$ ), carboxylic group stretching region ( $1200 \sim 1800 \text{ cm}^{-1}$ ) and C-O stretching ( $1000 \sim 1300 \text{ cm}^{-1}$ ), which reflects the monosaccharide composition of the pectin. Two peaks at  $1745$  and  $1620 \text{ cm}^{-1}$  in the carboxylic groups region assigned to the C=O stretching of methylated carboxyl groups, and to anti-symmetric stretching modes of  $\text{COO}^-$ , respectively. The spectrum of HPE and LPE reveal that a decrease of  $1745 \text{ cm}^{-1}$  band and increase of  $1620 \text{ cm}^{-1}$  band were related to the decrease of pectin DE, thus the area ratio of these two absorption peaks can indicate the DE. The infrared analysis of the PE-CS complex samples revealed some changes occurred. N-H stretching of CS was in  $1647 \text{ cm}^{-1}$ , and it shifted to lower wavenumber to  $1640 \text{ cm}^{-1}$  in PE-CS. a decrease of  $1745 \text{ cm}^{-1}$  band and disappear of  $1620 \text{ cm}^{-1}$  band demonstrate that electrostatic interactions occurred in PE-CS complex [20].



### 3.6. Morphology of Complex Colloid Particles.

The TEM images of LPE-CS particles in stable colloid are depicted Figure 6. It is clear that the colloid particles were monodispersed and the majority of particles were basically spherical. The particles had a wide size distribution with a diameter range of 100 nm ~ 400 nm. There was no agglomeration of particles, which means that the system was stable. There is no much difference between the morphology of LPE-CS and HPE-CS colloid particles.



**Figure 5.** FTIR spectrum of a) HPE, b) LPE, c) CS, d) HPE-CS, e) LPE-CS

**Figure 6.** TEM image of LPE-CS complex colloid (LPE/CS ratio 3:2, pH5.5).

## 4. Conclusion

DE of pectin, solution concentration, ratio of PE/CS and pH value have effect on complex reaction of negatively charged pectin and positively charged chitosan. The formation of stable complex colloidal dispersion is due to the charges on the particle surface. Particle surface charges were neutralized when PE and CS thoroughly complexed, and phase separation occurred. DE of pectin reflects the number of carboxylic acid on the molecule. LPE has more carboxylic acid groups than HPE, and has more negative charges in solution after dissociation. HPE reacted with more chitosan than LPE when they are same amount and HPE reached maximum complexation with chitosan in higher pH than LPE. Complexation between pectin and chitosan was mainly via electrostatic interaction, colloid particles were basically spherical and particle sizes were about 100 nm to 400 nm.

## 5. Acknowledgements

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## 6. References

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