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ADSORPTION OF NUCLEASE P1 ON CHITOSAN NANO-PARTICLES

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Abstract - The sorption of nuclease P1 onto chitosan nano-particles is studied in this paper. The effect of some adsorption kinetics factors such as nuclease P1 concentration, chitosan nano-particles solution concentration, adsorption temperature, chitosan nano-particles size, solution pH, etc. is investigated. Adsorption of nuclease P1 onto chitosan nano-particles is fitted into Lagergren first-order equation at initial nuclease P1 concentration of 3.0 mg/mL. The first-order constant for nuclease P1 is 22.98 h^{-1}. When nuclease P1 concentration is controlled into certain region, the adsorption fits into Freundlich isothermal linear equation. A mechanism of adsorption for nuclease P1 is proposed by analyzing IR spectra. The IR spectra shows that the hydrogen bond might be the main force between the hydroxyl group, the NH_2 group and the nuclease P1.
Keywords: Chitosan; Nano-particles; Sorption kinetics; Sorption mechanism.

INTRODUCTION

A biopolymer, chitosan is the deacetylated form of chitin and is composed of glucosamine or (1–4)-2-amino-2-deoxy-d-glucose. Chitosan has three types of reactive functional groups, an amino group and both primary and secondary hydroxyl groups at the C-2, C-3 and C-6 positions, respectively (Fereidoon and Janak et al., 1999). This special structure allows chelation with various metal ions (Boukhlifi and Bencheikh, 2000). Muzzarelli (Muzzarelli, 1986) pointed out that chitosan combines with metal ions in three forms: ion exchange, sorption and chelation. Chitosan has been broadly used for the sorption of heavy metal ions (Coughlin and Deshaies et al., 1990; Udaybhaskar and Iyengar et al., 1990; Jansson and Guibal et al., 1996). Further physical and chemical modifications of chitosan have been made to improve the selectivity and the capacity for metals ions (Kumar and Majeti, 2000; Guibal and Jansson et al., 1995; Piron and Accominoti et al., 1997; Choong and Wolfgang, 2003). Chitosan is also characterized by weak diffusion properties: long contact times are required to reach equilibrium. Sorption capacity can be controlled by sorbent particle size (Guibal and Jansson et al., 1995). Due to the low porosity of chitosan, sorption performances are frequently controlled by mass transfer resistance. To reduce this resistance to mass transfer, chitosan gel beads have been developed to expand the polymer structure and reduce its crystallinity (Guibal and Milot et al., 1998; Alam and Inoue et al., 1998; Hsien and Rorrer, 1997). However, these treatments resulted in either a decrease of the number of available sorption sites, or of the volumetric sorption capacity (Ruiz and Sastre et al., 2003). Controlled drying can increase the volumetric sorption capacity (Ruiz and Sastre et al., 2002). Another possibility for increasing this volumetric sorption capacity is the grafting of supplementary functional groups (Chassary and Vincent et al., 2004).

Nuclease P1 (EC 3.1.30.1), an extra-cellular enzyme was first identified by Kuninaka from

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Penicillium citrinum. This enzyme cleaves 5′-nucleotides successively from the 3′-hydroxy termini of 3′-hydroxy-terminated oligonucleotides originated from RNA. 5′-Nucleotides are known to exhibit enhancing flavor properties in food. When Nuclease P1 is added during the production of bakers yeast, the enzyme hydrolyses the yeast RNA efficiently into 5′-GMP. It has been widely used in pharmaceutical and food industry.

A number of nano-scale inorganic particles offer favorable properties for the selective removal of target contaminants. For example, hydrated Fe (III) oxides particles can selectively adsorb dissolved heavy metals like zinc, copper or metalloids like arsenic oxyacids or oxyanions (Cumbal and Greenleaf et al., 2003). Due to the small size and great surface area of nano-particles, chitosan nano-particles had been synthesized and applied as drug carriers (Xu and Du, 2003). However, to the best of our knowledge, few studies of nuclease P1 immobilized on chitosan nano-particles have been reported. The present work aims to study chitosan nano-particles sorption kinetics and sorption mechanism for nuclease P1.

MATERIALS AND METHODS

Materials

Chitosan was provided by Yuhuan Ocean Biochemical Co. Lit (Zhejiang, China), its molecule weight was 91,000. TPP abbreviated from Sodium polyphosphate was purchased from Dongsheng chemical reagent Factory (Zhejiang, China). All other chemicals were of analytical grade.

Culture Conditions for Nuclease P1 Production

For nuclease P1 production, the bacteria were grown at 30°C in a medium (pH 7.0) consisting of (w/v) 3.0 % sucrose, 0.10 % potassium dihydrogen phosphate, 0.010 % ferrous sulfate, 0.30 % sodium nitrate, 0.050 % magnesium sulfate, 0.050 % potassium chloride, and 20 % potato extract. This 24 h grown mother culture (10 mL) was used to inoculate 50 mL of production medium containing (w/v) 6.0 % glucose, 0.20 % peptone, 0.50 % groundnut meal, 0.030 % zinc sulfate, 0.040 % calcium carbonate, and 0.10 % potassium dihydrogen phosphate. The pH of the medium was adjusted to 5.4 with HCl. Erlenmeyer flasks (500 mL) containing 50 mL of medium were incubated at 28°C in an orbital shaker at 240 rpm for 49 h. The 5 N phosphodiesterase solution was harvested by centrifuging at 4000 rpm at 4°C for 10 min, and the supernatant thus obtained was used as the crude enzyme preparation. The enzyme was purified to homogeneity determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using thermal deactivation, ultrafiltration, $(NH_4)_2SO_4$ precipitation, phenyl Sepharose chromatography, ion-exchange chromatography, and gel filtration. Fig.1 presents the results.

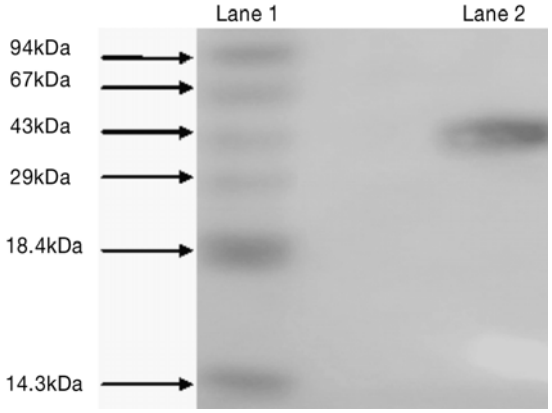

Figure 1: Electrophoretogram of protein after various steps of purification on SDSPAGE. Electrophoresis was carried out using a 15 % crosslinked polyacrylamide. Lane 1: standard molecular weight markers; Lane 2: purified enzyme.

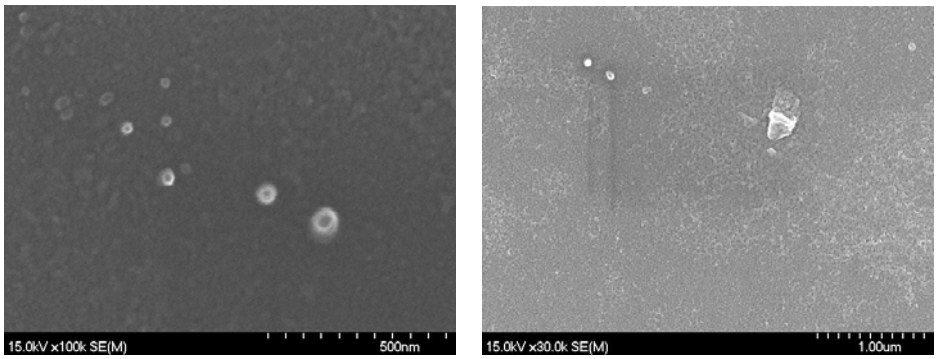

Figure 2: SEM of chitosan nano-particles. The size of chitosan nano-particles (60 nm) can be measured by the ruler in Figure 2.

Preparation of Chitosan Nano-Particles

20 mg chitosan were dissolved in 40 mL of 2.0 % (v/v) acetic acid. 20 mL of 0.75 mg/mL TPP were dropped into the beaker. Then the solution was treated with super-filtration membrane to remove the residual TPP. Chitosan nano-particles were stored up in the distilled water. The morphologic characterization of chitosan nanoparticles was fulfilled by scanning electron microscope. Figure 2 presents the results.

Morphology and Structure Characterization of Chitosan Nano-Particles

Chitosan nano-particles were gold coated using a Hitachi coating unit IB-2 coater under a high vacuum, 0.1 Torr, high voltage, 1.2 kV and 50 mA. Coated samples were examined using a XL30-SEM scanning electron microscope to characterize the morphology and size of nano-particles.

FT-IR of chitosan nano-particles were taken with KBr pellets on Nicolet Nexus 670 Spectrum FT-IR. The samples were chitosan, chitosan nano-particles, chitosan nano-particles with adsorbed nuclease P1 respectively.

Sorption Kinetics Experiments

Batch experiments for determination of kinetics of nuclease P1 on chitosan nano-particles were carried out using a continuously stirred glass vessel. Different concentrations of different mean particle sizes chitosan nano-particles solutions were brought in contact with 1.0 mL nuclease P1 solutions under continuous stirring, at temperature (30 ~ 60°C). The pH was 7.0. The initial concentration of nuclease P1 was varied to investigate its effect on the sorption kinetics. During the kinetic experiments, samples were withdrawn at fixed time intervals, filtered, and analyzed with an UV/visible spectrometer 751 (Shanghai, China).

Equilibrium Experiments

Batch equilibrium experiments were carried out using chitosan nano-particles as sorbent. A series of flasks containing nuclease P1 solutions of varied concentrations prepared from nuclease P1 and a fixed concentration of chitosan nano-particles were agitated in a rotary shaker at room temperature. Nuclease P1 uptake experiments were conducted under pH 7.0. After reaching equilibrium, nuclease P1 solutions were filtered and analyzed. Nuclease P1-free and sorbent-free blanks were used as controls. Amounts of nuclease P1 taken up by the sorbent in each flask were determined by the following mass balance equation:

$$Q = \frac{V\left(C_0 - C_e\right)}{W}$$

where Q is the sorption capacity (mg/g), C_0 and C_e are, respectively, the initial and solution phase nuclease P1 concentration at equilibrium (mg/L), V the solution volume (L), and W the mass of sorbent (g).

RESULTS AND DISCUSSION

Characterization of Chitosan Nano-Particles

a) Size and Morphology of Chitosan Nano-Particles

The preparation of chitosan nano-particles was based on an ionic gelation interaction between

positively-charged chitosan and negatively-charged tri-polyphosphate (Kevin and Marie et al., 2001; Angela and Alejandro, 2001). Chitosan nano-particles prepared in the experiment exhibited white powder shape. Results are shown in Figure 2.

b) Surface Functional Groups of Chitosan Nano-Particles

Spherical chitosan tri-polyphosphate chelating beads have been prepared and applied in the field of metal ions adsorption due to the enhanced intra-particular diffusion and excellent uptake capacity (Lee and Mi et al., 2001). In this work, in order to increase the sorption capacity of chitosan, chitosan nano-particles were prepared by ionic gelation of chitosan and tri-polyphosphate.

FT-IR spectra of chitosan and chitosan nano-particles were analyzed, and results showed that the $CONH_2$ and NH_2 groups of chitosan are both slightly cross-linked with a sodium polyphosphate molecule (Figures 3 and 4) (Qi and Xu et al., 2004). Nuclease P1 adsorbed nano-particles were formed by sorption of nuclease P1. As can be seen from the IR spectrum of chitosan nano-particles, the peak indicating P=O stretching at 1217 cm^{-1} appears (Lee and Mi et al., 2001), but disappears for the nano-particles after adsorbing nuclease P1 due to the hydrogen bond between nuclease P1 and phosphoric groups. The peaks at 1066 cm^{-1} (OH) in the spectrum of nuclease P1 adsorbed nano-particles are sharper in Figure 5. This behavior reflects the interaction between the amino groups and nuclease P1. Therefore, chitosan nano-particles provided sorption sites for nuclease P1 except the amino and hydroxyl groups.

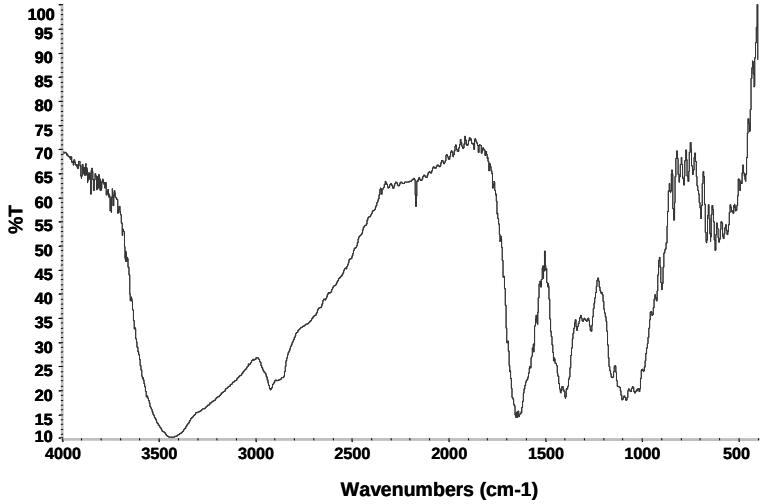

Figure 3: Infrared spectra of chitosan

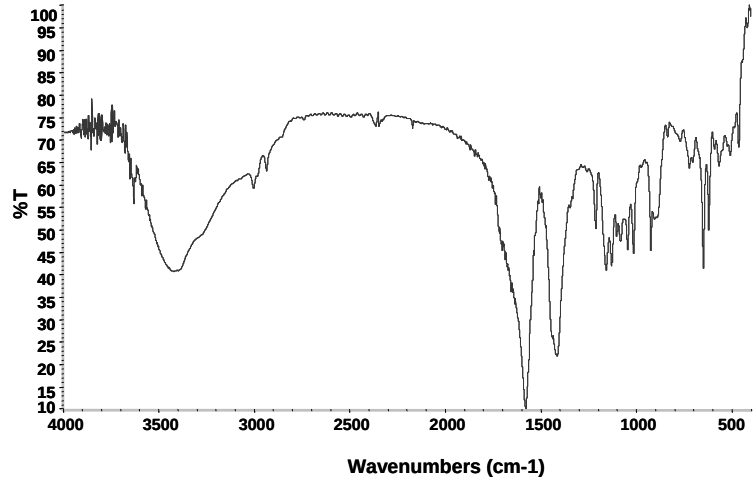

Figure 4: Infrared spectra of chitosan nano-particles

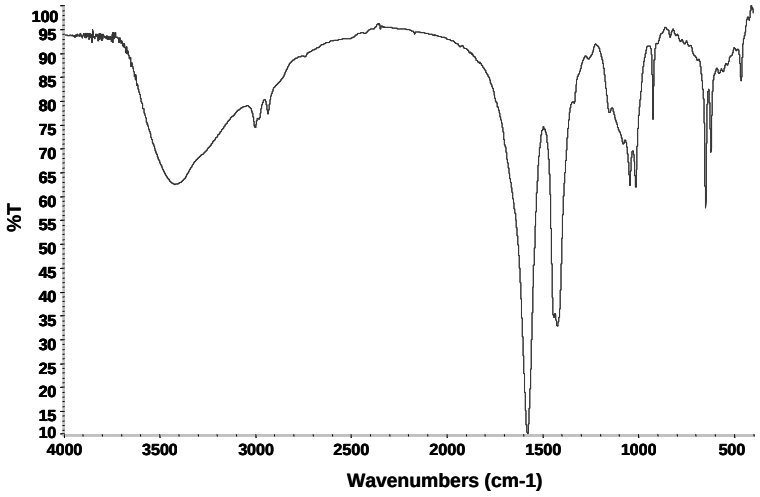

Figure 5: Infrared spectra of chitosan nano-particles with adsorbed nuclease P1

Sorption Kinetics for Nuclease P1

a) Influence of Nuclease P1 Concentration

The effect of different initial concentration of nuclease P1 solution on the sorption kinetics is illustrated in Figure 6. The higher the initial concentration of nuclease P1, the greater time it takes to reach equilibrium and the lower the sorption rate that is obtained. Nuclease P1 was adsorbed fast, more than 60 % in 10 min. When the initial concentration of nuclease P1 was 1.0 mg/mL, the sorption rate reached 80.0 % at 10 min. And the equilibrium time was 24.5 min. When the initial concentration of nuclease P1 was increased to 5.0 mg/mL, the sorption rate was 79.0 % at 10 min. The sorption reached equilibrium after 30 min. And the sorption rate increased to 97.5 %. Chitosan nano-particles could adsorb nuclease P1 quickly, and exhibited a high sorption capacity.

b) Influence of Nano-Particles Concentration

When the number of nano-particles was increased the amount of nuclease P1 adsorbed by chitosan nano-particles also increased. Figure 7 shows the results obtained for chitosan nano-particles in contact with nuclease P1 solution.

c) Influence of Temperature

Figure 8 shows the sorption kinetics obtained at different temperatures with chitosan nano-particles. The residual nuclease P1 concentration slightly increased when temperature was increased from 25 to 45°C. The sorption rate began to decrease at 45°C. This phenomenon might be explained if chitosan nano-particles were assembled at high temperatures. Previous studies have shown little differences for usual temperatures (in the range 5–55°C) for copper, zinc or mercury sorption (McKay and Blair et al., 1989).

d) Influence of Mean Size

Figure 9 shows a higher sorption capacity when the mean size of chitosan nano-particles decreases. Chitosan nano-particles with mean size 60 nm had a high sorption capacity when they were contacted with nuclease P1 solution. Increasing the size of the nano-particles, increased the time required to reach equilibrium. The contact surface of little size nano-particles could explain the differences for nuclease P1 sorption among various mean sizes of nano-particles. The sorption performance of chitosan can be affected significantly by the particle size and the conditioning of the absorbent due to the diffusion restrictions caused by the low porosity and crystallinity of the raw chitosan (Guibal and Jansson et al., 1995).

e) Influence of Agitation Speed

Figure 10 shows that the sorption rate of nuclease P1 was increased significantly with the increase of the agitation speed in a short contact time, while the sorption rate showed little difference at equilibrium. Therefore, nuclease P1 sorption rate was independent of the agitation speed. Similar results for cadmium sorption on chitosan were obtained by Dzul et al., (Dzul and Saucedo et al., 2001).

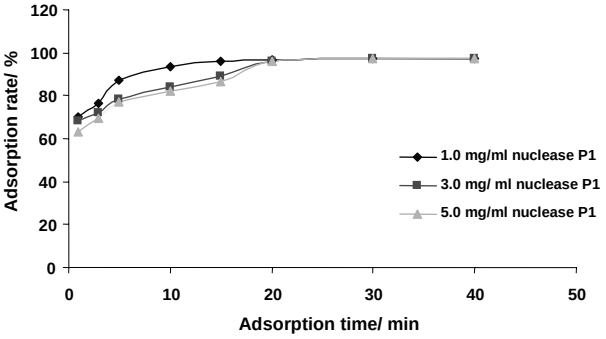

Figure 6: Effect of nuclease P1 concentration on adsorption

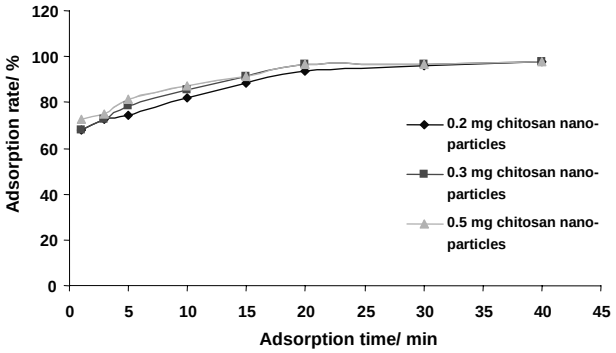

Figure 7: Effect of nano-particles concentration on adsorption

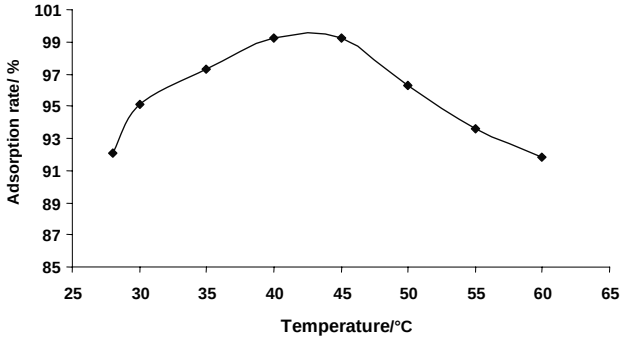

Figure 8: Effect of temperature on adsorption

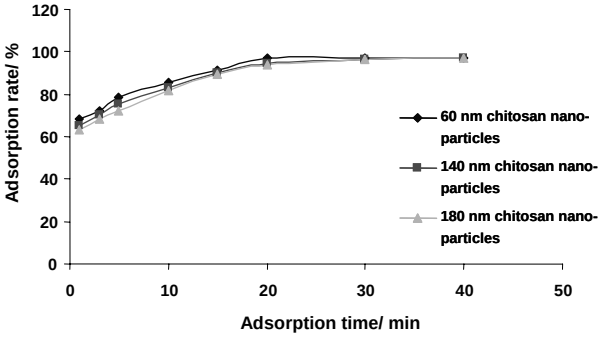

Figure 9: Effect of size of nano-particles on adsorption

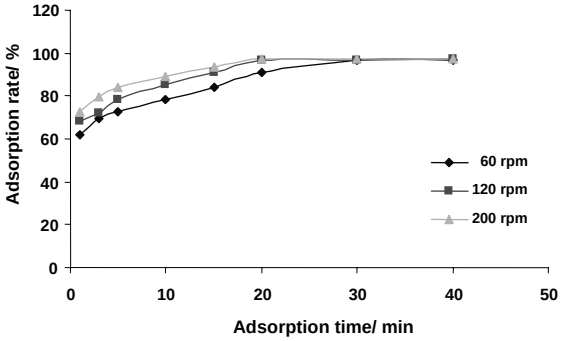

Figure 10: Effect of agitation speed on adsorption

Influence of pH Value on Sorption

Figure 11 shows the effect of pH on the sorption of nuclease P1 by chitosan nano-particles. It was found that the sorption efficiency increased when the pH of the solution was increased from 6.0 to 7.3. At low pH values of the solution, the amine and tri-poly-phosphoric groups were protonated to varied degrees, reducing the number of binding sites available for nuclease P1 uptake so that the extent of nuclease P1 uptake was low at high concentrations of protons. Moreover, the protonation of amine and phosphoric groups induced an electrostatic repulsion of nuclease P1 cations. When the pH of the solution was higher than 7.3, chitosan nano-particles began to resemble. So the sorption rate began to decrease. Similar results were shown using chitosan gel beads to study the influence of pH on metal sorption (Dzul and Saucedo et al., 2001).

Sorption Isotherms

Figure 12 shows the experimental equilibrium isotherms for sorption of nuclease P1 on chitosan nano-particles. Nuclease P1 was absorbed very quickly within 24 min. After 24 min, the adsorption reached equilibrium.

The first-order rate expression of Lagergren is

given as:

$$\log(q_e - q) = \log(q_e) - k_1 t / 2.3$$

where q_e and q are the amounts of nuclease P1 adsorbed on chitosan nano-particles at equilibrium and at time t , respectively, and k_1 is the rate constant of first-order adsorption. The slope and intercepts of the plots of $\log(q_e - q)$ Vs t were used to determine the first-order rate constant k_1. The adsorption of nuclease P1 was fit to first-order of Lagergren, k_1 was calculated as 22.98 h^{-1}.

The most important model of monolayer adsorption came from the work of Langmuir (Langmuir, 1918). Their sorption behaviors could be described with the Langmuir adsorption equation (Bayramoglu and Denizli et al., 2002) as:

$$\frac{C_e}{Q} = \frac{C_e}{Q_{max}} + \frac{1}{Q_{max}} b$$

where Ce is the equilibrium concentration of nuclease P1 (mg/L), Q the amount of nuclease P1 adsorbed per unit weight of chitosan nano-particles at equilibrium (mg/mg), Q_{max} the maximum sorption at monolayer coverage (mg/mg) and b is the Langmuir sorption equilibrium constant (mL/mg) and it is a measure of the energy of sorption.

A linearized plot of C_e/Q versus C_e (Figure 13) gave Q_{max} and b, the results obtained were: Q_{max} = 2.56 mg/mg and b = 0.0016 (mL/mg). The equation is C_e/Q= 0.3913C_e +0.0038. The plots demonstrated that the Langmuir equation provided a reasonable description of the experimental data.

The other well-known isotherm used to describe adsorption behavior was the Freundlich isotherm (Figure 14). The isotherm was another form of the Langmuir approach for adsorption on a heterogeneous surface. The amount of adsorbed material was the sum of adsorption on all sites. The Freundlich isotherm described reversible adsorption and was not restricted to the formation of the monolayer. This empirical equation took the form:

$$C = K_F (Q_e)^{1/n}$$

where K_F and n are the Freundlich constants characteristics of the system, K_F and n are indicators of the adsorption capacity and adsorption intensity, respectively. The slope and the intercept of the linear Freundlich equation are equal to 1/n and Log K_F, respectively. In this experiment, n was 0.10, the adsorption intensity to nuclease P1 was weak.

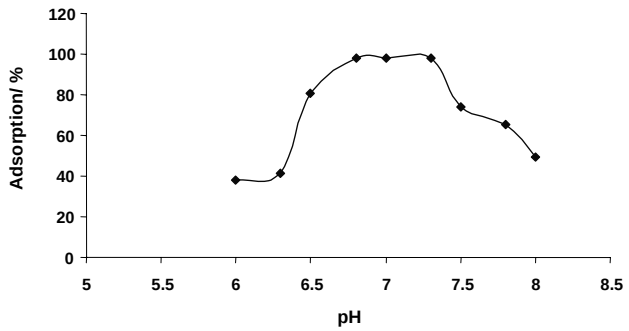

Figure 11: Effect of pH on adsorption

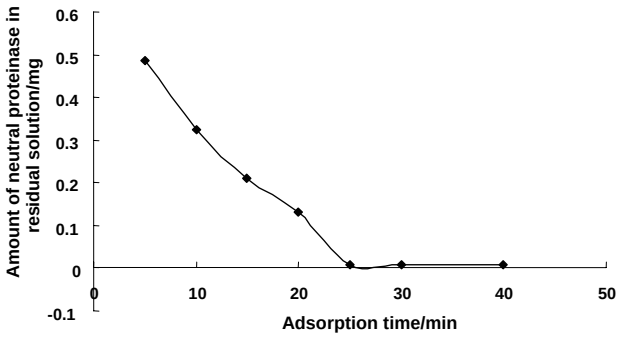

Figure 12: Adsorption isotherm Adsorption temperature: 30°C; Agitation speed: 120 rpm; Bulk concentration: 3.0 mg/ml

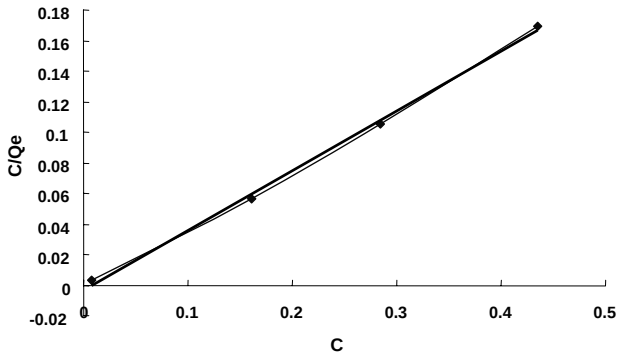

Figure 13: Langmuir adsortion isotherm

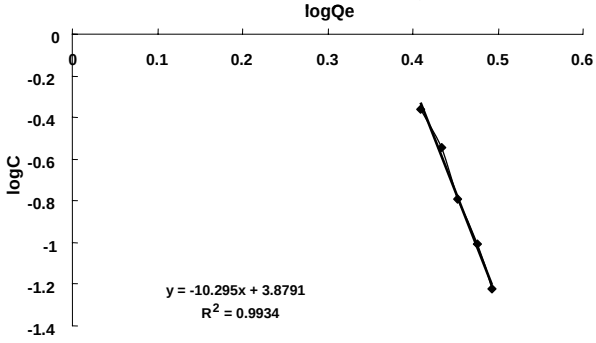

Figure 14: Freundlich adsorption isotherm

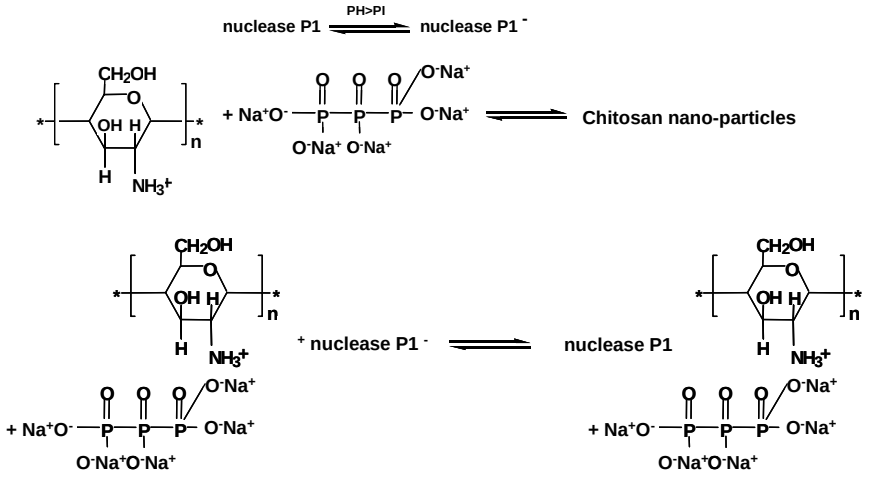

Figure 15: Hypothesis of adsorption mechanism

Hypothesis of Adsorption Mechanism

Nuclease P1 was a cation when the initial solution pH was 7.0, chitosan nano-particles were anions (Figure 15).

The n is less than 0.5 in the Freundlich equation. We can observe it was very easy to adsorb nuclease P1 with chitosan nano-particles. The interactivity strength between NH_2 group, hydroxyl group and nuclease P1 was hydrogen bond force.

The hydrogen in the carboxyl group was connected with electronegative oxygen. Electron pair was attractive to oxygen, so the hydrogen atom became cation and proton. When electronegative nuclease P1 was close to chitosan nano-particles, the hydrogen bond might be formed (Figure 15). It was the main force between hydroxyl group, NH_2 group and nuclease P1.

CONCLUSION

Chitosan nano-particles were prepared by ionic gelation of chitosan and tri-polyphosphate. The results of the experiments showed that chitosan nano-particles could adsorb nuclease P1 from aqueous solution effectively. The experimental data of the sorption equilibrium from nuclease P1 solution correlated well with the Langmuir isotherm equation. The high sorption capacity of chitosan nano-particles for nuclease P1 indicated a promising adsorbent. FT-IR spectra revealed the functional groups of chitosan nano-particles and the interaction with nuclease P1, the amine and hydroxyl group of nano-particles provided sorption sites for nuclease P1.

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