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Zeolite X with potassium diformate as a sustained-release antibacterial agent

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Abstract. A reaction of 800 mesh stellerite and a concentration of 15% hydrochloric acid in a solid to liquid ratio of 1:3 was carried out for 2 h at 80°C. Most of its impurities were removed. It can be used as precursors for the preparation of high quality zeolite. Zeolite X which is octahedral crystal shape as cube, 96% of the degree of crystallinity has been synthesized hydrothermally from $n(SiO_2/Al_2O_3) = 3$, $n(Na_2O/SiO_2) = 1.14$ and $n(H_2O/Na_2O) = 37$ by controlling reaction temperature and time. The synthesis included zeolite X as carriers, chitosan as intermediates, a mass ratio of zeolite X:chitosan:potassium diformate = 3:1:2 in weight for 2 h at 40°C. X-ray photoelectron spectroscopy analysis showed hydrogen bond formation by the chitosan amino hydrogen, potassium diformate oxygen and zeolite X oxygen or hydrogen bond formation by free hydroxyl group of zeolite X and oxygen of chitosan's C–O, indicating effective grafting. Zeolite X sustained-release antibacterial agent was prepared with an inhibition rate of 78.16% by the antibacterial.

Keywords. Stellerite; zeolite X; potassium diformate; chitosan; sustained-release antibacterial agent.

1. Introduction

Potassium diformate is a new type of organic acid promoting agent and antibacterial agent [1]. Formic acid dissociates from H^+ and $HCOO^-$. Potassium diformate dissociates from K^+ and $HCOO^-$. H^+ is used to regulate the acidity and alkalinity of the gastrointestinal tract, to improve the activity of pepsin, so as to promote the digestion and absorption of protein and other nutrients, beneficial bacteria breeding [2], to inhibit the growth of *Escherichia coli* and can be used as an alternative to antibiotics. There is no damage to animals by adding potassium diformate below 6% (in weight) in the feed. Potassium diformate can inhibit the growth of *E. coli* and promote the conversion rate of feed and the internal balance after weaning of piglets. So it can be used instead of antibiotics [3,4]. But potassium diformate is decomposed and absorbed rapidly in the acidic environment of the animal stomach and does not reach the end of the intestines, so the pH value of the end of the intestines is not reduced effectively. We utilize the biocompatibility, biodegradability, non-toxicity and other properties of chitosan [5]. Chitosan can produce chemical reactions such as hydrolysis, carboxymethylation, oxidation, reduction, condensation and complexation. Chitosan is used as an ideal graft site for general inorganic amino groups [6,7]; chitosan and faujasite zeolite X with the function of the carrier activated to form the antibacterial agent with a sustained-release effect [8,9].

2. Experimental

2.1 *Preparation of sustained-release antibacterial agent*

An appropriate amount of 800 mesh zeolites (figure 1A-a) and 15% hydrochloric acid were mixed in a 1:3 solid–liquid ratio and stirred for 2 h at 90°C. The acidified zeolite used in the preparation of zeolite X was obtained by product washing and removal of chlorine ions (figure 1A-b). After acidification, the content of SiO_2 was 91.67%, the impurity Al_2O_3 was 0.33%, CaO was 0.11% and Na_2O was 0.23%.

The acidified zeolite, NaOH solution and $NaAlO_2$ solution were poured in the ratio of $n(SiO_2/Al_2O_3) = 3$, $n(Na_2O/SiO_2) = 1.14$, $n(H_2O/Na_2O) = 37$ for 7 h at 97°C. The zeolite X was prepared by washing and drying after the reaction (figure 1B-b). Figure 2A-a shows that the absorption peaks are 3500, 1000, 1643, 1390, 453–784 cm^{-1} and so on which are the characteristic peaks of the zeolite X [10]. Under certain conditions, that the mass ratio of zeolite X, chitosan and potassium diformate is 3:1:2.

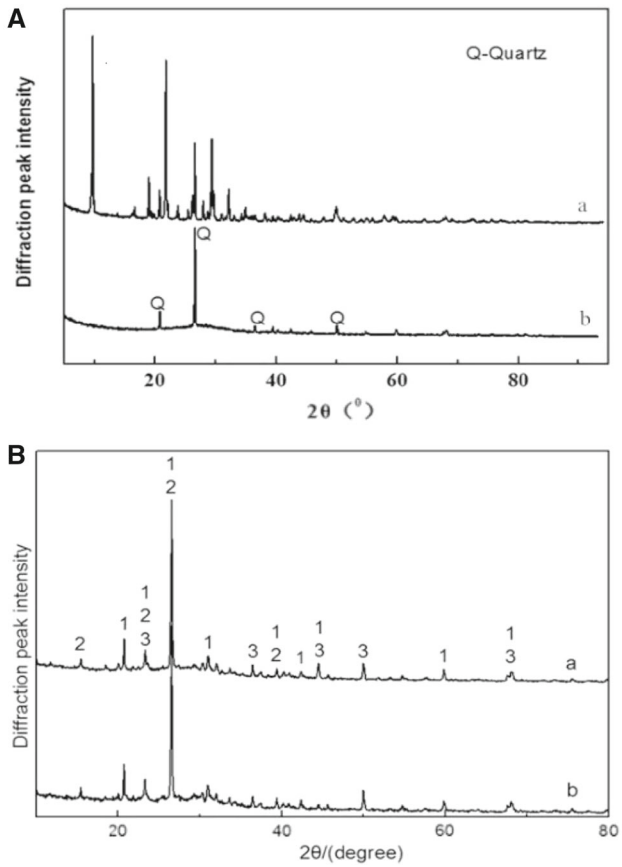

Figure 1. (**A**) XRD patterns of modified stilbite (a: ore, b: acidized zeolite). (**B**) XRD (a: the antibacterial, b: zeolite X).

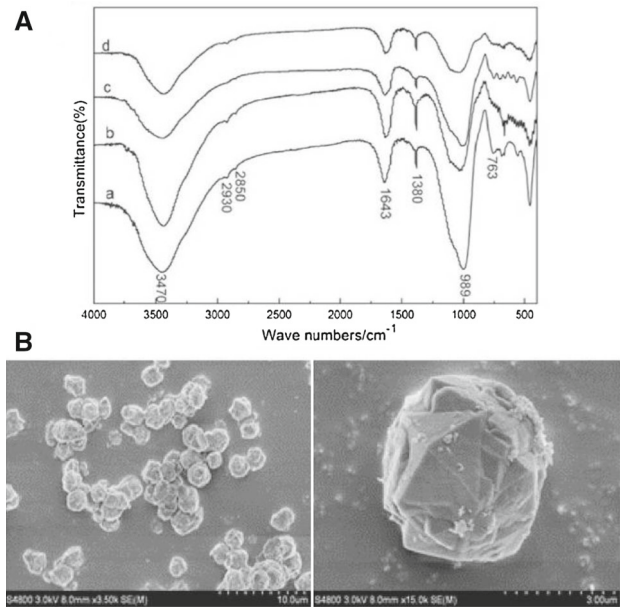

Figure 2. (**A**) FT-IR of the antibacterial agent with different material ratio. (**B**) scanning electron microscopy images of zeolite X, the antibacterial agent.

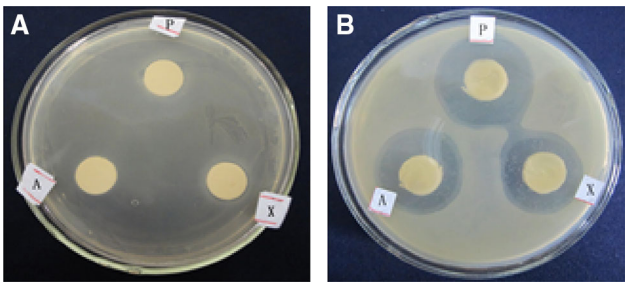

Figure 3. Comparison of the antibacterial effects on (**A**) zeolites and (**B**) antibacterial agents.

First, a certain amount of zeolite X was mixed with water and the pH value was adjusted to 6. Then a certain concentration of chitosan and potassium diformate were mixed to form a gelatinous mixture and poured into zeolite X and stir them. The zeolite X sustained-release antibacterial agent was prepared for 2 h at 40°C (figure 2A-b, c, d).

2.2 *Grafting characterization*

X-ray powder diffractometer was used for phase analysis (voltage 60 kV, electric current 55 mA, 2.2 kW, Cu target Kα radiation, $\lambda = 1.54060$ nm). The morphology was observed by scanning electron microscopy (figure 2B) (secondary electron resolution was 3.0 nm). A Fourier transform infrared spectrometer (Thermo Nexus 470FT-IR of Nicolet) was used to analyse the product structure by potassium bromide pressed-disc technique and the highest resolution was 0.5 cm^{-1}. Thermo Scientific ESCALab250Xi photoelectron spectrograph was used to analyse the composition of changes (the excitation source was monochromatic Al Kα X-ray, and the power was about 200 W. The analysis area was 500 μm, and the base vacuum in the analysis was 3×10^{-10} mbar). The antibacterial effect was analysed by Visible Spectrophotometers using the pressed-disc technique (figure 3). The specific site of hydrogen bond formation was speculated by X-ray photoelectron spectroscopy (XPS) test (figure 4A and B). The antibacterial rate was calculated by the coating method.

Figure 4A and B are the C1s XPS atlas of chitosan and the antibacterial agents. In figure 5, the b, c, d, respectively, represent the peaks of C–H, C–N, C–O and respectively there were 284.6, 285.4 and 286.3 eV. In figure 6, the b, c, d, e, respectively, represent the peaks of C–H, C–N, C–O, C=O and respectively there were 284.6, 285.2, 286.6 and 288.6 eV.

Comparing the peak changes of chitosan and the antibacterial agents [11,12], there was no change in the peak value of C–H, indicating that the hydroxyl group of the methyl group of chitosan did not form hydrogen bonds. The peak value of C–N decreased by 0.2 eV, indicating that hydrogen bonds were formed in the amino group of chitosan.

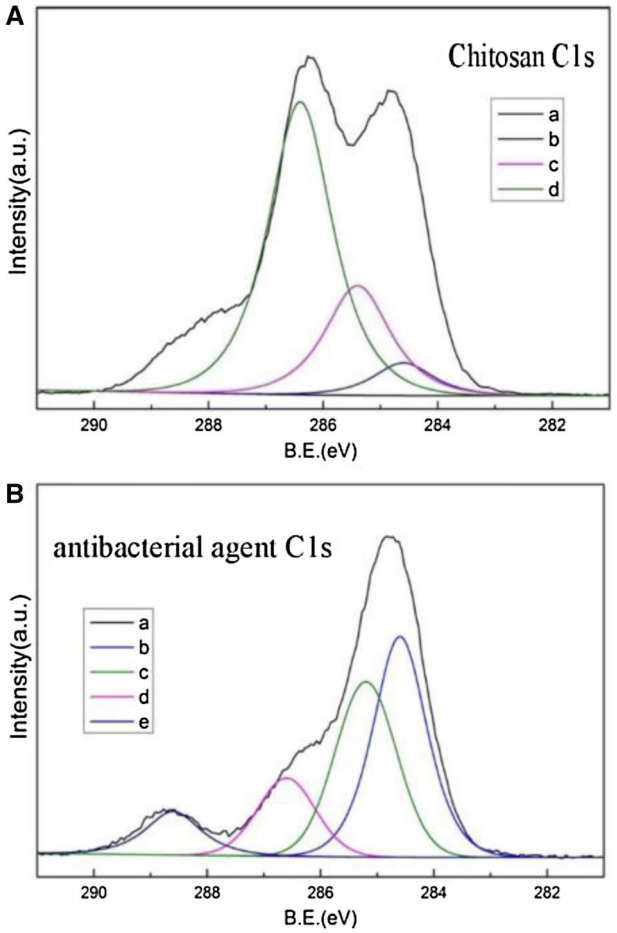

Figure 4. (**A**) XPS pattern of chitosan C1s. (**B**) XPS pattern of the antibacterial agent C1s.

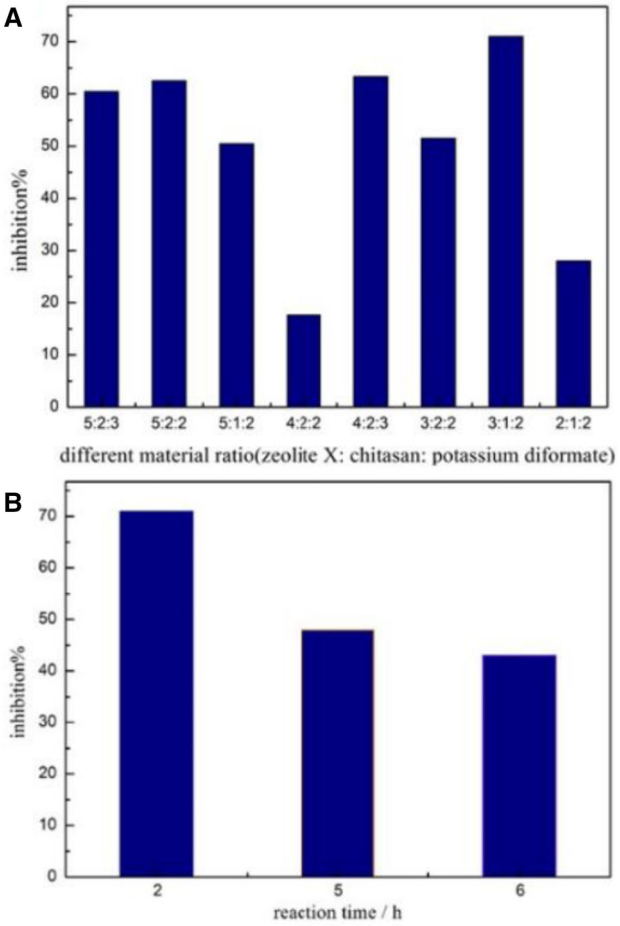

Figure 5. (**A**) Inhibition rate of *E. coli* by the antibacterial agent with material ratio (pH: 7.3×10^{-6}). (**B**) Inhibition rate of *E. coli* by the antibacterial agent with times (pH: 7.3×10^{-6}).

The formation of hydrogen bonds reduced group of electronegativity of N atoms. The spectral line moved to the low binding energy. The peak value of C–O increased by 0.3 eV. Since the hydroxyl hydrogen in potassium diformate has already formed intramolecular hydrogen bonds, the O atoms in chitosan form hydrogen bonds with the free hydroxyl groups of zeolite, which increases the electronegativity of oxygen atoms. The negative charge density around the C atom is lower than before the formation of hydrogen bond, leading to a 1S binding energy increase of C and a shift of the spectral line to a high binding energy. The peak value of C=O increased by 0.8 eV, indicating that the oxygen atom of C=O in potassium diformate generates hydrogen bonds in chitosan, resulting in a higher binding energy of C1s. It shows that hydrogen in the amino group of chitosan forms hydrogen bonds with oxygen in potassium diformate and oxygen in zeolite. The free hydroxyl groups in the molecular sieve form hydrogen bonds with the oxygen in the chitosan C–O to form an effective grafting process [13].

3. Analysis of bacteriostasis results

3.1 *Inhibition rate of the antibacterial agent to E. coli by material ratio*

After adding chitosan as an intermediate, the inhibition rate was improved obviously (figure 5A). When the ratio of zeolite:chitosan:potassium diformate = 3:1:2, the inhibition rate of the antibacterial agent was 71.01% and the pH value was weakly acidic. Different material ratios affect the hydrogen bonding between the three phases, and lead to a change in the inhibition rate [14].

3.2 *Inhibition rate of the antibacterial agent to E. coli by reaction time*

A ratio of 3:1:2 was fixed for the zeolite, chitosan and potassium diformate and the controlled reaction temperature was 40°C. With the increase in reaction time, the inhibition rate of antimicrobials on *E. coli* decreased (figure 5B). The graft

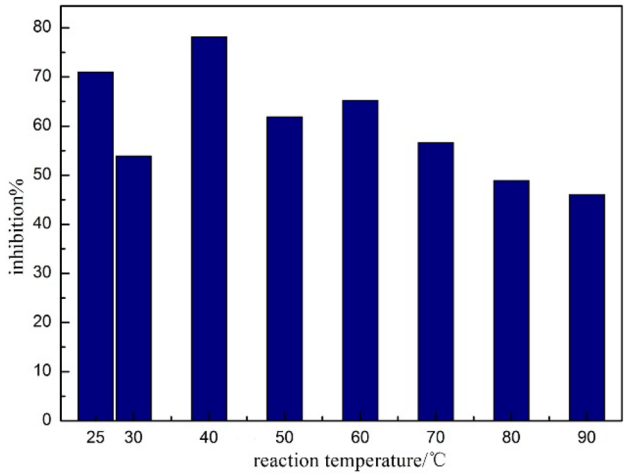

Figure 6. Inhibition rate of *E. coli* by antibacterial agent with temperature (pH: 7.3×10^{-6}).

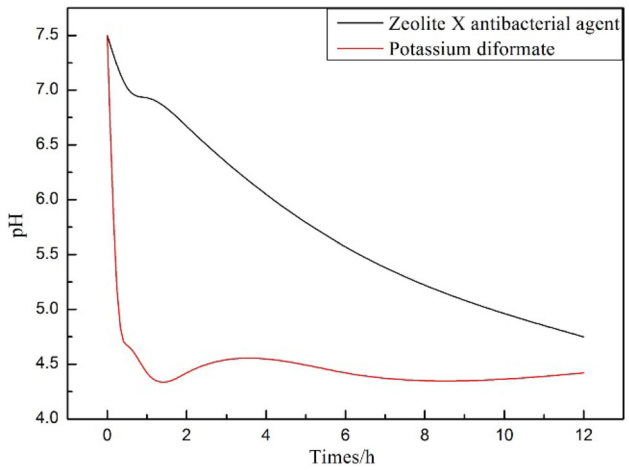

Figure 7. Different times on the pH value.

efficiency of zeolite X decreased with increased reaction time.

3.3 *Inhibition rate of the antibacterial agent to E. coli by reaction temperature*

A ratio of 3:1:2 was fixed for the zeolite, chitosan and potassium diformate and the controlled reaction time was 2 h. When the reaction temperature was 40°C, the highest bacteriostasis rate was up to 78.16% (figure 6). With the increase in temperature, the inhibition rate showed a downwards trend, the reason of this phenomenon probably because the temperature was too high to destroy the intermolecular hydrogen bond [15], which was not conducive to the effective grafting of the three phases.

The sustained-release antibacterial agents of zeolite X can rapidly ionize into zeolite X, HCOOH and HCOOK in the neutral and weakly acidic digestive systems. HCOOH can enter the bacteria through the cell wall to reduce the pH value of intracellular fluid, thereby decreasing various enzyme activities and the transportation capacity of nutrient supply systems in the bacteria, and then interfering with the protein synthesis. Inside the bacteria, HCOOK act on the cell walls of bacteria, causing proteins in the cell walls to be broken down, thus interfering with the synthesis of cell wall proteins [16,17].

3.4 *Acidity detection*

1 g of the zeolite X antibacterial agent was added to 100 ml buffer solution of pH = 7.50 and corresponding adsorption amount of potassium diformate was compared. The pH value of the buffer solution was measured at different times with a pH meter to simulate the acid–base environment in the intestinal system to detect the pH adjustment performance of the molecular sieve antibacterial agent.

Figure 7 shows the pH values of the zeolite X antibacterial agents at different times. The analysis shows that after adding zeolite X antibacterial agents, the pH value of the buffer solution slowly decreases by 2.75 units after 12 h, the corresponding adsorption amount of potassium diformate is directly added within 1 h, and the pH value of the buffer solution rapidly drops to the equilibrium point. This indicates that the zeolite X antibacterial agent has a certain sustained release effect, which can slowly reduce the pH value of the simulated intestinal buffer solution. Through sustained release, it is expected to increase the retention time in the intestinal tract of potassium diformate, which has improved the end-stage environment in the intestinal tract and reached the end of the intestinal tract. The pH of the segment changes from weakly alkaline to weakly acidic, which is suitable for the purpose of growth of beneficial bacteria.

4. Conclusion

1. The precursors of the zeolite can be effectively formed by acidizing the stellerite. Zeolite X shows an octahedral crystal shape as a cube, 96% of the degree of crystallinity has been synthesized from $n(SiO_2/Al_2O_3) = 3$, $n(Na_2O/SiO_2) = 1.14$, $n(H_2O/Na_2O) = 37$ for 3 h at 97°C [18].
2. The antibacterial agent with sustained release has been synthesized hydrothermally from the ratio of zeolite X:chitosan:potassium diformate = 3:1:2 in weight for 2 h at 40°C. It is effective to improve the grafting efficiency with the suitable material ratio and it is not effective to improve the antibacterial efficiency by adding reaction times. The best inhibition rate of the *E. coli* was 78.16% at 40°C.

Acknowledgements

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