Preparation of Sustained Release Mixture Aerogel Antibacterial Agent

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Abstract. In this study, Hybrid aerogels of chitosan (CS), carboxymethyl cellulose (CMC) and Zeolite P molecular sieve (zeolite P) are successfully prepared by using Calcium chloride as the Chelator. pH-controlled drug delivery with CS/CMC/Ca²⁺/Zeolite P as the carrier is investigated using antibacterial agents potassium diformate (KDF), as the model drug. CS and CMC have pH sensitivity. The Zeolite Phas a large specific surface area which contributes to the enhancement of drug-loading amount. Thus, the synthesized microparticles were characterized by FTIR and XRD, and study the surface morphology of particles by SEM. The swelling and in vitro antibacterial activity of the prepared composites were studied. The particle systems developed are promising for adjusting the pH of the intestine and modulate gut microbiota in the intestinal tract with the purpose of improving piglets health.

1. Introduction
In the process of livestock breeding, Escherichia coli is prone to cause diarrhea in piglets, causing a large number of deaths and bringing great losses to farmers [1,2]. In order to reduce the intestinal disease of weaned piglets, it is common to add therapeutic or sub-therapeutic levels of antibiotics to the feed. However, long-term frequent use of antibiotics may induce problems such as the production of resistant strains of drugs and drug residues, posing a potential threat to human health and the ecological environment.

In 2001, potassium diformate was approved by the European Union as a growth promoter for antibiotic substitutes. Potassium diformate [3] is easier to use than pure formic acid [4,5], and can promote the growth of weaned and fattening pigs [6], and has a good bactericidal effect on Escherichia coli and Salmonella [7-9]. The synthesis and application research of domestic potassium diformate has also achieved rapid development. 85% potassium diformate enters the duodenum through the stomach of the pig in intact form. The recovery rate of formate in the duodenum was 83%, 38% in the anterior
segment of the jejunum, and 17% in the middle segment of the jejunum [10]. It can be seen that potassium diformate mainly plays a role in the anterior segment of the small intestine.

Chitosan (CS) [11] and carboxymethyl cellulose (CMC) [12] are pH-sensitive drug carriers. The pH sensitivity of CS is attributed to pH-induced protonation/deprotonation of the amino group (NH$_2$) on the CS chain. The change in pH can result in a transition between CMC (COOH) and the base form (COO$^-$), resulting in a non-obvious swelling rate of CMC. However, due to the poor mechanical strength of CS and CMC, drug burst release in controlled delivery systems based on CS or CMC cannot be completely ignored.

The incorporation of inorganic rigid materials improves mechanical strength and stability. Among the commonly used inorganic materials, molecular sieves have received great attention in recent years due to their excellent biocompatibility, low toxicity, outstanding thermal stability and mechanical properties, and have been applied in sewage treatment, biomedicine and medicine, and Animal husbandry production [13-15]. Due to its extremely large specific surface area and strong adsorption capacity, molecular sieves greatly promote the increase of drug loading and can be used for drug combination and sustained release effect. Molecular sieve-based drug delivery systems have received increasing attention in recent years.

Using CaCl$_2$ as a chelator, CS, CMC and Zeolite P were prepared by electrostatic self-assembly to prepare a hybrid aerogel CS/CMC/Ca$^{2+}$/Zeolite P, which was used as a carrier for drug delivery, and potassium diformate (KDF) was used as a model drug. Thereby preparing composite microspheres. The delivery of CS/CMC/Ca$^{2+}$/Zeolite P/KDF is pH sensitive, allowing potassium diformate to function in the posterior segment of the small intestine. Provide reference data for the application of new green slow release antibacterial agents through in vitro E. coli antibacterial testing.

2. Materials and methods

2.1. Materials

Zeolite; chitosan(CS), degree of deacetylation > 90.0%; carboxymethylcellulose (CMC), viscosity: 800-1200 mPa·s; potassium diformate, purity > 80%.

2.2. Preparation of Zeolite Ps

Natural stilbite and 15% diluted hydrochloric acid (solid-liquid ratio = 1:3) were put in a three-necked flask, they were heated at 90 °C for 2 h. After dried, the product was reacted with NaOH at a constant temperature of 80 °C for 15 min. The supernatant mixed with NaOH and NaAlO$_2$ underwent hydrothermal process at a certain temperature for 7 h. The hydrothermal product was centrifuged and dried to obtain Zeolite P [16].

2.3. Preparation of CS/CMC/Ca$^{2+}$/Zeolite P/KDF Composite Microspheres

Add appropriate molecular sieves to 3% CMC solution, heat and stir to make it evenly dispersed, and then add appropriate amount of KDF and stir evenly to obtain solution A. CaCl$_2$ and CS were dissolved in a 2% acetic acid solution to prepare a mixed acetic acid solution of 3% CaCl$_2$ and 2% CS to obtain a solution B. Slowly add solution A to solution B using a 5 ml syringe and stir for half an hour. Washed with deionized water to obtain CS/CMC/Ca$^{2+}$/Zeolite P/KDF composite microspheres. The preparation process diagram is shown in Figure 1.

2.4. Swelling test

The composite microspheres dried to constant weight were weighed 0.05 g, and the PBS buffer solution was set at pH 1.2, 5.5, and 7.4, respectively. The weighed composite microspheres were placed in PBS buffer solution at different pH, stirred slowly, and the surface water was weighed off with filter paper every 30 min. The equilibrium swelling capacity (Q) of the composite microsphere was calculated using the following equation:

$$Q = \frac{M_t - M_0}{M_0} \times 100\%$$
where Mt and Mo are the weights of the swollen and the dry samples, respectively.

2.5. Antibacterial test
The composite microspheres were separately added to the sterilized medium at 6, 12, 24, 48, 96 mg/mL and ultrasonic for 15 min. The diluted bacterial suspension (E. coli) was pipette into the test solution without the antibacterial microspheres (0 mg/mL) as a positive control. Place in a constant temperature incubator at 37 °C and incubate for 20 h. Pipette 200 μL of the culture medium to the plate (two replicates for each sample), measure the absorbance (OD630 value) at a wavelength of 630 nm and calculate the inhibition rate.

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\text{Bacteriostatic rate} = \frac{\text{OD}_0 - \text{OD}_2}{\text{OD}_0} \times 100
\]

3. Result and Discussion
Figure 2 shows the FT-IR spectrum of Zeolite P. The main vibrational characteristic peaks of Zeolite P in the vibration range of 4000-400 cm\(^{-1}\) were 3459 cm\(^{-1}\), 1641 cm\(^{-1}\), 1008 cm\(^{-1}\), 746 cm\(^{-1}\), 682 cm\(^{-1}\), 607 cm\(^{-1}\) and 443 cm\(^{-1}\). The absorption peak at 3459 cm\(^{-1}\) was the vibrational peak of the hydroxyl group. The weak peak at 1641 cm\(^{-1}\) was the vibrational absorption peak of the hydroxyl group adsorbing water. The characteristic absorption peak at 1008 cm\(^{-1}\) was mainly represented by the antisymmetric stretching vibration peak of Si-O-Si in the silicon tetrahedron. The absorption peak at 746 cm\(^{-1}\) was caused by the symmetric stretching vibration of Si-O-Si. The absorption peak at 607 cm\(^{-1}\) was the D4R vibration absorption peak of Zeolite P, and the absorption characteristic peak at 443 cm\(^{-1}\) was the bending vibration absorption peak of Si-O bond in silicon tetrahedron.

Figure 3 is a scanning diagram of a Zeolite P. It can be seen that the Zeolite P is spherical in shape and has a regular shape with a particle size of about 1.2 μm.
The FT-TR spectra characterizing CS/CMC, CS, CMC are shown in Figure 4. For the CMC curve, the antisymmetric and symmetric tensile vibrations of COO\(^-\) are characterized by 1425 and 1629 cm\(^{-1}\), respectively. In the infrared curve of CS, the absorption peak at 3434 cm\(^{-1}\) was assigned to the N-H stretching vibration absorption peak, and the absorption peak at 1637 cm\(^{-1}\) was assigned to the N-H bending vibration absorption peak. In the infrared curve of CMC, the absorption peak at 3434 cm\(^{-1}\) was assigned to the N-H stretching vibration absorption peak, and the absorption peak at 1637 cm\(^{-1}\) was assigned to the N-H bending vibration absorption peak. In the CS/CMC spectrum, the peak value of 2000-3400 cm\(^{-1}\) was broadened, and the N-H bending vibration absorption peak shifts to 1617 cm\(^{-1}\), indicating that the amino group in CS was protonated to form NH\(^3+\). The C=O peak in the carboxyl group coincides with the N-H bending vibration peak, and the C-O stretching vibration absorption peak shifts to 1419 cm\(^{-1}\), indicating that COO\(^-\) still exists. Therefore, NH\(^3+\)-COO\(^-\) ion bond may exist in CS/CMC to form polyelectrolyte complex. In addition, there was a certain shift in the -OH peak of CS/CMC in the spectrum, which may be due to intramolecular and intermolecular interactions with CS and CMC.

![Image of Zeolite P.](image_url)

The FT-IR of CS, CMC, CS/CMC.

The curve a in Fig. 5 were two characteristic diffraction peaks of CS at $2\theta = 10^°$ and $20^°$. Strong diffraction peaks of CMC at $20^°$ and $45^°$ in curve b. The characteristic diffraction peaks in the composite microspheres become wider, and the characteristic peaks of CMC almost disappear, indicating that they are not simply mixed. There was a strong interaction between the two components of CS and CMC in the composite microspheres, which changes some crystalline forms of the original components. It may be due to the electrostatic interaction of -COO\(^-\) of CMC and -NH\(^3+\) of CS, which indicates that the two form a relatively uniform structure.
Figure 5. XRD patterns of CS, CMC, CS/CMC.

Figure 6. Images of CS/CMC/Ca\(^{2+}\)/Zeolite P/KDF wet spheres and freeze-dried microspheres.

Figure 6 is a photograph of wet and freeze-dried microspheres of composite microspheres. From the photo of the wet spheres, the composite microsphere is relatively round and regular, without cracking. The composite microspheres will shrink when they were freeze-dried, and there will be more small pores on the surface. These pores will become the channel for the release and release of potassium diformate.

Figure 7. Swelling rate of composite microspheres at different pH.
Swelling performance is considered to be one of the most important properties that determine the release rate of a drug from a biocomposite hydrogel microsphere. It can be seen from Figure 7 that as time passes, the swelling ratio increases, the expansion speed gradually decreases, and reaches a maximum after 330 min. The shape of the microspheres in an acidic environment remains the same, but as the pH increases, the swelling rate doubles. After the pH rises, the external CMC chain was induced, and the ionizable carboxyl group is deprotonated. After the water-swelled outer shell was destroyed, the phosphate ion replaces Ca^{2+}. The electrostatic repulsion between these charged groups was enhanced, resulting in an increase in osmotic pressure, and more water molecules penetrate into the carrier. The prepared composite hydrogel microspheres can be applied to a sustainable drug delivery system under different pH conditions. Figure 8 is a comparison of the maximum water absorption of composite microspheres at different pH. We have known from Figure 8 that the composite microspheres have the largest water absorption at a pH of 7.4, and the maximum value was 2.2242 g.

![Figure 8. Maximum water absorption of composite microspheres at different pH.](image1)

Figure 9 shows that the inhibition rate of the composite microspheres increases with the concentration of the sample, and the inhibition rate also increases. The composite microspheres have a good antibacterial effect on Escherichia coli, and the inhibition rate was up to 81.78%.

![Figure 9. Antibacterial rate of Escherichia coli at different concentrations.](image2)
4. Conclusions
The Zeolite P is successfully prepared by hydrothermal synthesis, and the molecular sieve has good morphology. Zeolite P is used as skeleton support for carboxymethyl cellulose while increasing the drug loading of potassium diformate. The prepared composite microspheres have good sphericity. There is a strong interaction between the two components of chitosan and sodium carboxymethylcellulose, which can form a relatively uniform structure. The microspheres have better swelling behavior under the condition of pH 7.4 and have the largest water absorption. Under acidic conditions, the microspheres have poor water absorption. The vitro antibacterial test showed that the prepared composite microspheres have a significant inhibitory effect on the growth of E. coli, and the maximum inhibition rate was 81.78%.

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