Preparation and Properties of Essential Oil Microspheres
Antibacterial Alignate Films

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Abstract. In this paper, plant essential oil was used as antibacterial agent, dispersed into the sodium alginate solution by emulsifying effect, and then the essential oil microspheres antibacterial alignate films (EOA) were prepared. The particle size distribution of the oil microspheres was characterized by particle size analyzer (DLS) and scanning electron microscopy (SEM), respectively. The results showed that the oil microspheres dispersed evenly and the range of particle size was 0.5 ~ 2μm. The films showed good antibacterial property, and its antibacterial property increased with the increase of the essential oil content.

1. Introduction
The widespread utilization of synthetic materials has caused significant environmental impact. In recent year, increasing consciousness towards the environment has driven researchers to study the substitution of petro-based materials, leading to certain scientific research on environmentally friendly materials. Sodium alginate (SA) is a natural polysaccharide derived from β-D-mannuronic acid (M units) and α-L-guluronic acid (G units) linked by β-1,4-glycosidic bonds \cite{1}. And it has the advantages of non-toxicity, high water absorption, good biocompatibility, biodegradability, etc \cite{2-4}. Therefore, it is widely used in the medical and health industry \cite{5}. Making sodium alginate as films for packaging can effectively reduce pollution and protect the environment, but its antibacterial performance is insufficient. Therefore, it is of great significance to improve the application value of alignate films by giving it excellent antibacterial properties.

At present, there are two methods for preparing antimicrobial alignate films. One is a direct cross-linking method. For example, Wu Yan et al. crosslinked Cu\textsuperscript{2+} with sodium alginate to prepared antibacterial alignate fibers \cite{6}. The other was added antibacterial agents. Xihui Zhao et al. prepared nano silver/alignate fibers with good antibacterial properties \cite{7}. Zhu et al. bonded quaternary ammonium salt with calcium alginate and prepared antibacterial alignate fiber. The above studies mainly used inorganic and organic chemical antibacterial agents, while natural antibacterial agents, such as plant essential oils, are less application in alginate materials and need further investigation. Plant essential oil is a volatile and aromatic substance extracted from the plant. Among the plant essential oils that have been discovered, such as blue eucalyptus oil and peppermint oil have antibacterial and antiseptic properties and are used as antibacterial drugs. In this paper, the essential oil
is used as an antibacterial agent, the essential oil is embedded in the algae films, and the antibacterial properties of the films are preliminarily investigated.

Figure 1. Internal structure diagram of the essential oil microspheres alignate films

2. Experimental

2.1. Materials
Sodium Alginate was purchased from Jiejing Group (Shandong, China), Span 80, Anhydrous Calcium Chloride (Molecular weight 110.98), Glycerol, Anhydrous ethanol, Collodion glue were provided by Sinopharm Chemical Reagent Co., Ltd (Shanghai, China), Tea Tree Oil. All the reagents used were of reagent grade and used as received.

2.2. Preparation of essential oil microspheres antibacterial alignate films
The EOA were prepared as follows: 100ml of distilled water and 3g of alignate were mixed together under mechanical stilling (600rpm) for 2 hours. In the meantime, tea tree essential oil, span 80 and glycerol are mixed in a mass ratio of 3:2:1. Afterwards, the above-mentioned essential oil mixed solution was added dropwise to the sodium alginate solution. The final solutions were mixed using a homogenizer (15000rpm for 30min). According to the content (1%, 2%, 3%) of the essential oil, it is numbered as A\textsubscript{1}, A\textsubscript{2}, A\textsubscript{3}, and the pure alignate is numbered A\textsubscript{0}. Then pour the same mass of solution into watch-glasses of the same size and left to dry under ambient laboratory conditions.

2.3. Characterization

2.3.1. Particle size of microspheres in emulsion. After diluting the solution 50 times, 10-15 mL solution was loaded into the sample cell for particle size testing. The particle size test results show that all the samples had no sedimentation, adsorption, agglomeration. The test results are within the usable range. It can be seen from Figure 2 that the distribution of the particle size is in the range of 70 to 2000 nm and 1000-2000 nm. From A\textsubscript{1} to A\textsubscript{3}, the average particle size of essential oil emulsion microspheres decreased, and the content of microspheres increased. This may be due to an increase of emulsifiers.
2.3.2. *Scanning electron microscopy (SEM)*. The results indicated that pure alignate (Figure 3 A₀) exhibited no specific Morphology. In contrast, all the films showed that the mi have uniformly distributed in the range of 0.5-2μm. The density of microspheres from A₁ to A₃ gradually increased, and the average diameter also decreased. The SEM test results are consistent with the DLS test results.

2.3.3. *Antibacterial activity of EOA films*. In these experiments the activity of EOA films was tested against two different microorganisms, namely Escherichia coli, a model gram negative bacterium, and Staphylococcus aureus, a gram-positive, round-shaped bacterium. To assess the antibacterial behavior of EOA films, the overnight microorganism culture was diluted to a final concentration of 10⁶ cells/ml and 500μl of this solution was spread onto new LB medium agar plates. And then, the sample was glued to agar surface. The plates were then placed in the incubator at 37 °C for 18-24 h.

The average width of a zone of inhibition may be calculated using the following equation:

\[
W = \frac{(T - D)}{2}
\]

where:
- \(D\) = diameter of the test specimen in mm
- \(T\) = total diameter of test specimen and clear zone in mm
- \(W\) = width of clear zone of inhibition in mm

As can be seen from Figure 4, compared with the control sample, a significant inhibition zone was formed around the EOA. It can be seen from Table 1 that the EOA has very good antibacterial effect on both bacteria, and from A₀ to A₃, the width \(D\) of the inhibition zone of the films increases in turn, indicating the antibacterial activity of the films. The effect is closely related to the mass fraction of the essential oil in the films. The higher the mass fraction is, the better the antibacterial effect is.
Figure 4. Antibacterial performance test, Escherichia coli (left), Staphylococcus aureus (right)

Table 1. Antibacterial performance test

| samples | total diameter of test specimenand clear zone T (mm) | width of clear zone of inhibition W (mm) |
|---------|-----------------------------------------------------|----------------------------------------|
|         | Staphylococcus aureus    | Escherichia coli                        | Staphylococcus aureus    | Escherichia coli                        |
| A0      | 10.00                   | 10.00                                  | 0                        | 0                                      |
| A1      | 16.78                   | 16.37                                  | 3.39                     | 3.18                                   |
| A2      | 17.47                   | 17.70                                  | 3.73                     | 3.85                                   |
| A3      | 18.39                   | 19.85                                  | 4.19                     | 4.92                                   |

3. Conclusion
We successfully prepared essential oil microspheres antibacterial alignate films. The natural essential oil microsphere antibacterial alignate films were prepared. The essential oil microspheres can be uniformly dispersed in the films, with a particle size of 0.5 to 2μm. The prepared films have good antibacterial performance against Escherichia coli and Staphylococcus aureus, and its antibacterial performance increases with the increase of the essential oil content.

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