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# Study on the Process of Preparing Zinc and Silver-Loaded Sepiolite and Its Antimicrobial Ability

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The inorganic antibacterial agents (single metal ions), process for preparing double ion-type inorganic antibacterial agents was studied by using sepiolite as carrier and silver ions and zinc ions as antibacterial components. By determining the content of antibacterial ions loaded in sepiolite using atomic absorption spectroscopy, the effect of sodium phosphate in processing sepiolite and the loaded capacity of two ions in different processing methods were compared. The results showed that the antibacterial ion contents can be significantly improved with sodium phosphate pretreatment, followed by acid modification of sepiolite. When the initial concentration ratio of $Zn^{2+}$ and $Ag^+$ was 10:1 in sepiolite in this experiment, the content ratio of the two ions was more than 8:1. Thus, the content of $Zn^{2+}$ loaded in sepiolite was increased and that of $Ag^+$ decreased. The prepared sepiolite loaded with silver and zinc ions had good antibacterial property and resistance to discoloration. There was certain relationship between absorption of $Ag^+$ and $Zn^{2+}$ ions onto sepiolite and the contents of two kinds of antibacterial ions in sepiolite, but the correlation was weak.

**Key Words: Sepiolite carrier, Zinc and silver compound, Sodium phosphate, Antiseptic, Anticolour-change property.**

## INTRODUCTION

Inorganic antibacterial agent has good safety, high chemical stability, broad antibacterial spectrum and long-term effectiveness. It plays an important role in maintaining the environmental sanitation and preventing the occurence and spread of disease when used in fibers, plastics and all kinds of building materials products[1-3].

Silver ion antibacterial agent, which is the most widely used at home and abroad, has strong antibacterial capacity, but due to the silver ion activity, such antimicrobial agents can change colour easily, with antibacterial instability and limited scope of application[4,5]. In addition, silver as the noble metal has high price, which restricts its application as antimicrobial agents.

Zinc ion is stable and its price is low. Its anti-mold effect is good , but its antibacterial strength is lower than $Ag^+$. Furthermore, the absorption effect of $Zn^{2+}$ is better than $Ag^+$[6]. If $Ag^+$ and $Zn^{2+}$ can be used in combination, by reducing the content of $Ag^+$ and adding a large number of $Zn^{2+}$ to maintain the antibacterial strength, the cost can be reduce, while improving the broadness of antibacterial spectrum. This is an effective way to solve the existing problems of silver-type antibacterial agent.

Currently, most of the silver type antibacterial agents are based on artificially synthetic zeolite as carrier material, but the cost is high. Sepiolite is relatively low cost. Because of its special chain and layered structure, large surface area, good cation exchange absorption, it is highly suitable to be used as carrier material, Most of the studies treat sepiolite as the carrier material, but rarely as the carrier for metal antimicrobial ions and even less as the carrier for two antibacterial ions[7-9]. Based on the above analysis, this paper selected the sepiolite as carrier and used $Ag^+$ and $Zn^{2+}$ as the antibacterial components to prepare antibacterial agent. The effects of carrier modification, the concentration ratio of two ions and loading method on the loaded capacity of the two ions, antibacterial performance and discoloration of sepiolite were studied. Our experiment will provide scientific basis for the development of new-type antibacterial agent of low cost, high resistance to discoloration, good antibacterial performance and wide application.

## EXPERIMENTAL

The experiment used the sepiolite provided by Nanyang Yang Lei Sepiolite Co., Ltd; analytically pure hydrochloric acid, silver nitrate, zinc nitrate and sodium phosphate were provided by Chengdu Kelong Chemical Reagent Factory; peptone, beef extract, yeast extract and agar powder were provided by Beijing Aoboxing Biotech Company; the main equipments were Hitachi 18080 type atomic absorption spectrometer, Hitachi S-3000N type scanning electron microscope and Tensor 27 type infrared spectrometer of Germany Brooke Optical Company, *etc*.

**Modification of sepiolite as carrier material:** Immerse the sepiolite in distilled water and add a small amount of $H_2O_2$ and mix it well. Leave the mixture to stand for a while and then centrifuge the suspension. Dry the sepiolite and grind to fine powder. Mix the sepiolite powder with 15 % $Na_3PO_4$ solution at 1:20 ratio and stir the solution for 2 h. After filtering, washing, drying and grinding, sieve the sepiolite powder through a 150 μm stainless steel sieve. Add the sepiolite powder into the the 0.3 mol/L dilute hydrochloric acid solution at a 1:40 and mix it at 80 ºC for 4 h. Wash, dry and grind the sepiolite powder, before passing the sepiolite through a 150 μm stainless steel sieve.

**Preparation of zinc and silver-loaded sepiolite:** On the basis of early experiments on absorption of $Ag^+$ or $Zn^{2+}$ onto sepiolite, the concentration of $Zn^{2+}$ and $Ag^+$ was set as 0.1 and 0.01 mol/L, respectively. Absorption temperature was 60 ºC, pH = 5 and absorption time was 6 h. We mainly investigated the effect of the loading sequence of $Ag^+$ and $Zn^{2+}$ absorption onto sepiolite on the loaded contents of the two ions. There were three absorption mode.

**Absorption of $Ag^+$ after addition of $Zn^{2+}$:** Put 1 g of sepiolite into 20 mL of solution containing $Ag^+$ (0.01 mol/L concentration) for absorption for 6 h and then add the same volume of solution containing $Zn^{2+}$ (0.2 mol/L concentration) for absorption for 6 h.

**Absorption of $Zn^{2+}$ after addition of $Ag^+$:** Put 1g of sepiolite into 20 mL of solution containing $Zn^{2+}$ (0.1 mol/L concentration) for 6 h absorption and then add the same volume of $Ag^+$ (0.02 mol/L concentration) solution for 6 h absorption.

**Absorption of $Ag^+$ and $Zn^{2+}$:** Mix 20 mL of solution containing $Ag^+$ (0.02 mol/L concentration) with the same volume of $Zn^{2+}$ (0.2 mol/L concentration)solution and add 1 g of sepiolite for absorption for 6 h.

Atomic absorption spectroscopy was used to characterize the contents of $Ag^+$ and $Zn^{2+}$ in sepiolite. Before atomic absorption spectroscopy, the sample was digested by aqua regia.

**Antibacterial test of zinc and silver loaded sepiolite:** *Escherichia coli* and *Bacillus subtilis* are representative of gram-negative and gram-positive bacteria respectively. This experiment selected these two kinds of bacteria for antibacterial test. Septic distilled water was used to dilute the bacteria after activation in liquid culture medium at 30 ºC for 24 h, to prepare the bacterial liquid with concentration of 1.2-1.5 × $10^3$ cfu/mL. 0.1 g of sepiolite loaded with antibacterial metal ions and 10 mL of bacterial liquid were mixed and oscillated in sunlight at 30 ºC for 4 h. 0.5 mL of the bacterial liquid was coated on a solid medium. The medium plate was sealed and placed invertedly into the culture box. *Escherichia coli* and *Bacillus subtilis* samples were cultured in 37 and 25 ºC temperature for 72 h, respectively. The colonies were counted and the sterilization rate was calculated[10].

## RESULTS AND DISCUSSION

**Characteristics of acid modified sepiolite:** The purposes of using acid solution to process the sepiolite were: first, to dissolve the impurities in sepiolite and purify and clear the pores of sepiolite; second, to improve the microstructure of sepiolite as the carrier material, enlarge pore size and improve the loaded capacity of antibacterial ions.

In order to understand the modification effect of hydrochloric acid on sepiolite, we analyzed and observed the components, structure and morphology of sepiolite by infrared spectroscopy (IR) and scanning electron microscope.

It is seen from Fig. 1 in IR analysis that: in sepiolite ore spectrum (a) appears the strong calcite characteristic peak (at 1419 and 875 $cm^{-1}$), which indicated that sepiolite ore contained large amount of calcite impurities; the characteristic peak disappears in the modified sepiolite spectrum (b) after hydrochloric acid treatment. The infrared spectrum contained much less impurities[11]. This suggested that the treatment with hydrochloric acid on sepiolite effectively removed a large number of carbonate minerals without destroying the basic structure of sepiolite.

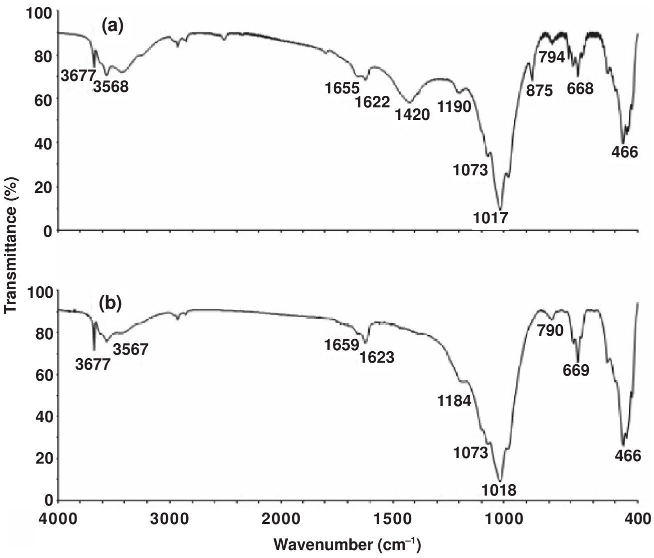


Fig. 1. Infrared spectrum of sepiolite (a) sepiolite ore; (b) hydrochloric acid modified sepiolite

Fig. 2 showed that the purity of the sepiolite sample had been improved by hot acid treatment. The modified sample was mainly composed of loosely interlaced fine sepiolite fibers and there was nearly no flake-like and granular impurities; the amount of sepiolite fiber bundles was also reduced.

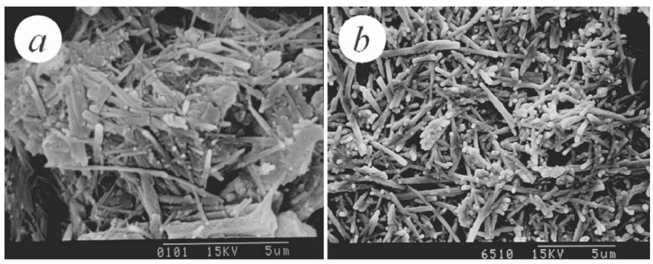


Fig. 2. Scanning electron microscope photographs contrast of sepiolite (a) sepiolite ore;(b) hydrochloric acid modified sepiolite

**Effect of sodium phosphate in processing sepiolite sample:** The effects of phosphates as dispersing agent are masking the electropositive edge of sepiolite, which is to increase its surface electronegativity[12] and complexing free $Ag^+$, which is beneficial to improve the absorption of metal antibacterial ions onto sepiolite and to avoid $Ag^+$ discoloration. Therefore, we observed the the actual effect and the role of sodium phosphate in processing sepiolite and how it took effect. The results are shown in Table-1. Analysis data showed: sepiolite modified by $Na_3PO_4$ could load more $Ag^+$ and $Zn^{2+}$; it is better to use $Na_3PO_4$ on sepiolite before acid modification. The reasons might be that more impurities were removed by $Na_3PO_4$, which increased the purity of sepiolite; during acid modification, the existence of $Na_3PO_4$ led to the better dispersibility of sepiolite in acid solution and hence better acid modification effect.

TABLE-1
PERFORMANCE OF SEPIOLITE DIFFERENTLY TREATED WITH $Na_3PO_4$

| Adding methods of $Na_3PO_4$ | $Ag^+$ content in Sepiolite (μg $g^{-1}$) | $Zn^{2+}$ content in Sepiolite (μg $g^{-1}$) |
|---|---|---|
| No $Na_3PO_4$ | 1813.2 | 3674.8 |
| Adding $Na_3PO_4$ before acid modified | 2195.44 | 5149.6 |
| Adding $Na_3PO_4$ after acid nodified | 1939.9 | – |

**Loading method of $Ag^+$ and $Zn^{2+}$:** Table-2 comprises the test results of sepiolite sample loaded with two kinds of antibacterial ions in three different loading modes. When the concentration ratio of $Zn^{2+}$ and $Ag^+$ was 10:1 (*i.e.*, the concentration of $Zn^{2+}$ and $Ag^+$ was, respectively 0.1 and 0.01 mol/L), the concentration ratio of $Zn^{2+}$ and $Ag^+$ in three samples was more than 8:1. This showed that controlling the initial concentration ratio of two kinds of ions in the solution can increase the amount of $Zn^{2+}$ loaded onto sepiolite and reduce the load capacity of $Ag^+$ in sepiolite. The loading sequence of $Ag^+$ and $Zn^{2+}$ affect the capacity of sepiolite in loading the two ions. But all samples showed higher load capacity of $Zn^{2+}$ than $Ag^+$, which was related to the differences of concentration, effective ionic radius and electrovalence between two ions. If $Ag^+$ was absorbed onto sepiolite after $Zn^{2+}$, the loaded contents of both $Zn^{2+}$ and $Ag^+$ were high,with stronger antibacterial effect. Considering the factors of convenience and time saving, loading of $Ag^+$ and $Zn^{2+}$ onto sepiolite at the same time is better.

TABLE-2
CONTENTS OF $Ag^+$ AND $Zn^{2+}$ IN SAMPLES OF DIFFERENT ABSORPTION MODES

| Order of metal ions adsorted by sepiolite | $Ag^+$ (μg $g^{-1}$) | $Zn^{2+}$ (μg $g^{-1}$) |
|---|---|---|
| $Ag^+$ before $Zn^{2+}$ | 496 | 4225 |
| $Zn^{2+}$ before $Ag^+$ | 407 | 4239 |
| $Ag^+$ and $Zn^{2+}$ at same time | 376 | 4052 |

**Antibacterial performance and resistance to discoloration of silver and zinc-loaded sepiolite:** Table-3 summarizes the antibacterial test results of zinc-loaded sepiolite, silver-loaded sepiolite, silver and zinc-loaded sepiolite. The table shows that the different types of antimicrobial agents had good antibacterial performance against two bacteria. The sterilization rate of zinc-loaded sepiolite was lower, while the silver and zinc-loaded sepiolite and silver-loaded sepiolite had similar antibacterial effect, but the $Ag^+$ content of the former was only 1/4 of the latter. The good antibacterial effect is associated with a significant number of $Zn^{2+}$. Because of the presence of $Ag^+$, though $Ag^+$ content was reduced, its antibacterial effect remained strong. Many studies show that the content of $Ag^+$ is not directly proportional to antibacterial strength. Excessive $Ag^+$ will accumulate on sepiolite surface, blocking the entry of ions and easily resulting in discoloration. In addition, the existence of $Zn^{2+}$ may be beneficial to improve absorption stability of $Ag^+$ and slow down the release of ions. Therefore, silver and zinc loaded sepiolite has strong antibacterial properties due to the synergy between antibacterial $Ag^+$ and $Zn^{2+}$ ions.

TABLE-3
ANTIBACTERIAL ACTIVITIES OF SEPIOLITE CARRYING ANTIMICROBIAL METAL IONS

| Antimicrobial type | Antimicrobial content (μg $g^{-1}$) | | Sterilization rate /% | |
|---|---|---|---|---|
| | $Zn^{2+}$ | $Ag^+$ | *Bacillus subtilis* | *Escherichia coli* |
| Zinc sepiolite | 5149.6 | 0 | 60 | 68 |
| Silver sepiolite | 0 | 1939.9 | 99 | 99 |
| Silver-zinc sepiolite | 4225 | 496 | 99 | 99 |

In order to understand the discoloration of silver and zinc-loaded sepiolite and silver-loaded sepiolite, two kinds of samples were exposed to ultraviolet irradiation for 48 h and then placed under unshaded environment for 1 month. The samples of the same weight were pressed into tablets. Zinc-loaded sepiolite sample was also made as reference for colour contrast. The results showed that zinc-loaded sepiolite had the lightest colour, basically retaining the original colour. With or without UV irradiation, the silver-loaded sepiolite sample turned dark gray after being placed under unshaded environment for 1 month. Silver and zinc-loaded sepiolite sample and zinc-loaded sepiolite sample underwent no significant colour change. Antibacterial test showed that the antibacterial properties declined by *ca.* 20 % for silver-loaded sepiolite after being placed under unshaded environment for 1 month; that for silver and zinc-loaded sepiolite was down by only *ca.* 4 %.

## Conclusion

The use of $Na_3PO_4$ and hydrochloric acid to modify sepiolite plays a crucial role in purifying, improving the absorption performance and enhancing the load of antibacterial metal ions onto sepiolite. Experimental results showed that to treat the sepiolite with $Na_3PO_4$ before acid modification has better effect that after acid modification.

The loading test of sepiolite samples showed that when the concentration ratio of $Zn^{2+}$ and $Ag^+$ reached 10:1, the content ratio of $Zn^{2+}$ and $Ag^+$ in sepiolite sample was greater than 8:1. The prepared sepiolite sample contained more $Zn^{2+}$ and less $Ag^+$. The loading sequence of $Zn^{2+}$ and $Ag^+$ had little effect on the loaded contents of the two ions.

This study showed that by loading two antibacterial ions, $Ag^+$ and $Zn^{2+}$ onto sepiolite can prepare antibacterial agent with good antibacterial property, high resistance to discoloration and low cost.

## ACKNOWLEDGEMENTS

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