Synthesis and Antimicrobial Activity of Nano-fumed Silica Derivative with N, N-dimethyl-n-hexadecylamine

Xia Xu, Shurong Li, Fayun Jia, Pu Liu

Laboratory Center of Medical Science, Zhengzhou University, Zhengzhou, Henan 450052, China

Abstract: Nano-fumed silica derivative with N, N-dimethyl-n-hexadecylamine was synthesized with γ-Chloropropyltrimethoxysilane as the roupling agent, and subsequently treated with N, N-dimethyl-n-hexadecylamine. The nano-fumed silica derivative was confirmed by Fourier-transform infrared spectroscopy (FTIR). The zeta potentials of nano-fumed silica and nano-fumed silica derivative were measured as a function of pH in suspensions and showed the isoelectric point of modified nano-fumed silica has increased in the direction of pH rising compared with nano-fumed silica. Antimicrobial properties of the nano-fumed silica derivative against selected microorganisms were tested by the quantitative suspension method. The results showed that the obtained polymer inhibited the growth of *E. coil*, *S. aureus* and *C. albicans*. It was found that the growth inhibiting effect of polymer varied with the time exposed to the microorganism. When the time exposed to the microorganism was 15 min, each of their inhibitory rates was 99.99%, 99.99% and 95.23%, respectively. [Life Science Journal. 2006;3(1):59-62] (ISSN: 1097-8135).

Keywords: nano-fumed silica; silane coupling agents; quaternary ammonium group; antimicrobial

1 Introduction

Quaternary ammonium compounds (QAC) belong to the group of compounds, which exhibit high antimicrobial activity. They are widely used in many of domains such as environmental disinfection, equipment surfaces and disinfection in hospitals. These compounds seem to be safer than chemically active disinfectants such as chlorine and glutaraldehyde. However, since the irritant and cytotoxic effects of these compounds on human cells/tissues such as keratinocytes, fibroblasts, cornea and respiratory mucosa have been shown previously (Augustin, 1995; Damour, 1992; Steinsvag, 1996; Tripathi, 1989), the improvement of QACs is necessary, not only for their antimicrobial activity but also for human cells' safety. To overcome these problems, anchoring the QAC to a polymer backbone by a covalent might be promising in developing materials which would have antimicrobial activity by themselves.

Nano-fumed silica (NFS) is utilized in industry, e.g. as fillers for elastomer reinforcement, additives in fluids, free-flow agents in powders, medicinal and industrial adsorbents, etc. And it is a kind of extremely important superfine inorganic material. There are many hydroxyl groups at the surface of nano-fumed silica that are allowed to react with silane coupling agents, and the resulting composites connected with many organic functional groups show expectable characteristics, such as biological activity (Demir, 2005), heat resistance (Fu, 2004), mechanical (Agnihotry, 2004) electrical properties (Paik, 2005), and other properties.

In our study, the nanometer antimicrobial material that contained organic antimicrobial agent was reported and the preparation of the nano-fumed silica derivative with quaternary ammonium group using the γ-Chloropropyltrimethoxysilane as silane coupling agent was investigated (It can avoid the small molecular antimicrobial losing). Furthermore, the antimicrobial activity was also studied.

2 Materials and Methods

2.1 Materials

Nano-fumed silica (mean particle size 40 nm) was purchased from Jibishi Chem. Lin (Guangzhou, China); N, N-dimethyl-n-hexadecylamine was kindly donated by the Feixiang Chem. Lin (Jiangsu, China). γ-chloropropyltrimethoxysilane was bought from Yingcheng Debang Chemical Industrial New Materials Co., Ltd. Toluene, acetonitrile are of reagent grades.

2.2 Tested microorganism

Tested microorganism included the Gram negative bacteria *Escherichia coli* (8099), Gram positive bacteria *Staphylococcus aureus* (ATCC 6538). Bacteria and fungi were maintained on Subourond agar slopes. LB medium was sterilized by autoclaving for 30 min at 121℃.

2.3 Reaction

The procedure was schematically shown in

Figure 1(Ⅰ). After a mixture of nano-fumed silica, the toluene and a little water was added to the three-necked with stirring for a period of time, and amount of γ-Chloropropyltrimethoxysilane was added and refluxed at 80℃ for 6 h. The resulting nano-fumed silica (MNFS-1) was washed with toluene to remove excess γ-Chloropropyltrimethoxysilane and then dried at 110℃ for 16 h in vacuo. The subsequent reaction with N, N-dimethyl-n-hexadecylamine is schematically shown in Figure 1(Ⅱ). A mixture of MNFS-1 and N,N-dimethyl-n-hexadecylamine and acetonitrile was refluxed with stirring at 80℃ for 8 h. The product containing the quaternary ammonium group (MNFS-2) was washed to remove excess N, N--dimethyl-n-tetradecylamine and dried in vacuo.

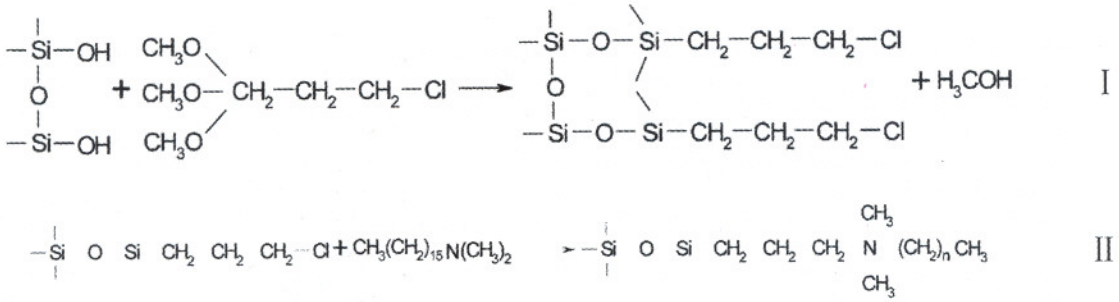

Figure 1. Scheme of reaction

2.4 Measurement

FT-IR spectra were recorded on a Fourier-transform infrared spectroscopy (MAGNA. 506 Nicolet USA). The mean size and the zeta potential of the NFS and MNFS-2 were measured with Zetarsizer Nano series (MALVERN).

2.5 Antimicrobial activity

In order to assess antimicrobial functions of MNFS-2, the suspension quantitative test was employed. The bacteria used were *E. coli* (8099), *S. aureus* (ATCC6538) and *Candida albicans* (ATCC 10231). They were characterized as the bacteria of Gram negative bacteria and Gram positive bacteria as well as the fungus, respectively. The control was the bacterial of the solution of N. S. The suspension quantitative test determining the antibacterial activity was as follows: 0.5 g MNFS −2 was added to 150 ml Erlenmeyer flask containing 5 ml 108 cfu/ml of *Escherichia coli* and 45 ml N. S. The resulted solution was shaken at 37℃ by a Burrell wrist action shaker for 5 min, 15 min and 30 min, respectively, and kept station for 30 min to obtain a mixture. 1 ml supernatant of the mixture was diluted gradiently, and then 1 ml dilution solution was added to agar plate and incubated at 37℃ for 24 h. After incubation, the colonies of bacteria were counted to indicate bactericidal activity.

3 Results and Discussion

3.1 FTIR Analysis 1

Figures 2(a), (b) and (c) showed the IR of NFS, MNFS-1 and MNFS-2, respectively. The absorption peaks of active hydroxyl group at the surface of NFS were observed at 3438 cm^{-1} and 1640 cm^{-1}. The absorption peaks of the Si-O-Si asymmetric stretching vibration, symmetric stretching vibration, and bending vibration were observed at 1110 cm^{-1} and 805 cm^{-1} and 475 cm^{-1}. As seen from Figure 2(b), the absorption peaks of the methyl and methylene stretching vibration were in the range of 2850 ~ 3000 cm^{-1}; The peak of the C-H symmetric deformation vibration was at 1460 cm^{-1}. The intensities of these absorption peaks increased with chain length of the alkyl group. As seen from Figure 2(c), the absorption peaks of the methyl and methylene stretching vibration were observed in the range of 2850 ~ 3000 cm^{-1}, and they were stronger than Figure 2(b) for the number of $-CH_2$ increasing.

3.2 Zeta potential analysis

0.05% (wt) of NFS and MNFS-2 were titrated with 0.25 mol/L HCl and 0.25 mol/L NaOH at 25℃, while zeta-potential changes with pH were tested, respectively. The results were as follows (Figure 3).

Figure 3 showed the zeta potential of NFS and MNFS-2. The isoelectric point of the NFS is usually known to exist at pH of 2 − 3. The isoelectric point of the NFS in this work has a pH value about 4.5 from Figure 3(a). The Si-OH at the surface of NFS (pH < 4.5) showed a positive charge for an added proton:

$$—Si—PH + H^* \rightarrow SiOH_2^+$$

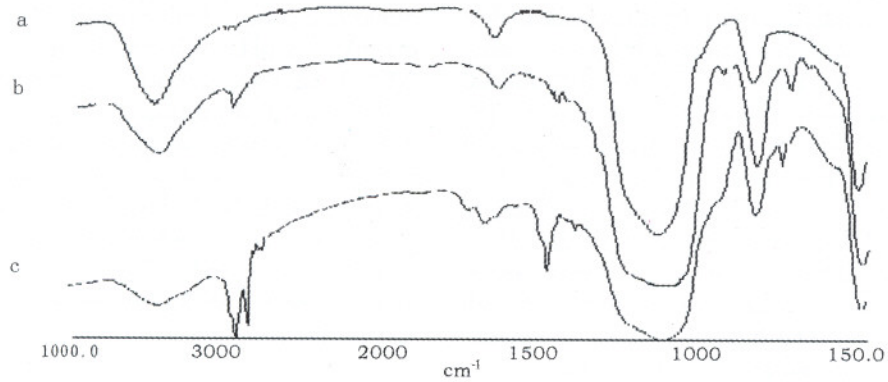

Figure 2. FTIR spectra of nano-fumed silica (a)NFS (b) MNFS-1 (c)MNFS-2

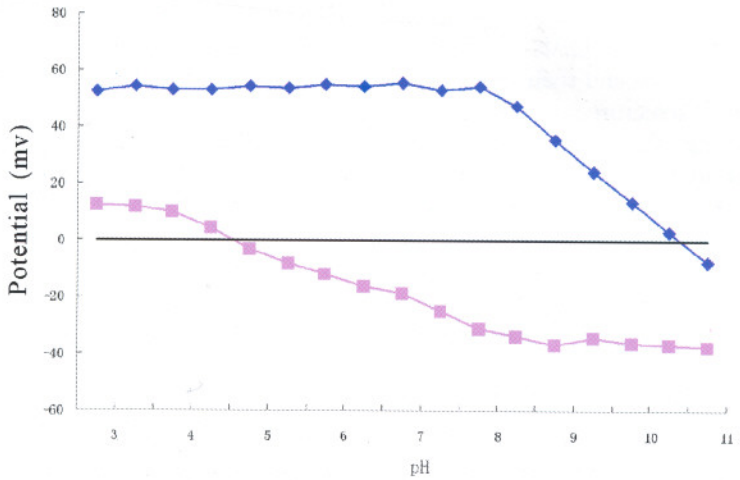

Figure 3. PH titration graph of NFS and MNFS: (a) NFS; (b) MNFS

The Si-OH at the surface of NFS ($pH > 4.5$) showed a negative charge for a liberated proton:

$$—Si—PH + H^* \rightarrow SiO^- + H^+$$

When pH of solution was more than 4.5, surface of NFS showed negative electric charge; as pH increases, absolute value of zeta potential increased.

In contrast, the zeta potential of MNFS-2 constantly showed a positive charge in the range of pH 3-10.5. The zeta potential rapidly decreased when $pH > 10.5$. Suhara (1995) measured the zeta potential of fine silica power modified with the quaternary ammonium group. In the pH range of 2-10, the zeta potential of fine silica power modified with the quaternary ammonium group showed the positive charge range was wider than that of the original fine silica powder and its value of the zeta potential ($pH < 10$) was almost constant. And the decrease in the zeta potentials of the quaternary ammonium group was considered to be due to the quaternary ammonium group being neutralized by the base in the range of $pH > 10$; the zeta potentials of fine silica powder may change the negative charge owing to adsorption of an anion or ionic bond. As described in previous papers; the decrease in the zeta potential of MNFS-2 was considered to be due to the N,N-dimethyl-n-hexadecylamine being neutralized by the base in the range of $pH > 10.5$. The mechanism of the change of the zeta potential is similar to the paper of Huhara.

3.3 Antimicrobial activity

Table 1. Bacteriostatic efficacy of MNFS-2 after exposure for different periods of time

Microorganism	Average bacterial count of control group($\times 10^6$)	Average bacteriostatic rate (%) after exposure for different period of time (min)		
		5	15	30
Escherichia coli	19.8	97.01	99.99	99.99
S. aureus	28.2	99.12	99.99	99.99
C. albicans	15.9	94.92	95.23	95.99

Note: The temperature was 19~22℃. The results are means of triplicate tests.

The antimicrobial activity of nano-fumed silica modified quaternary ammonium salts was investigated and shown in Table 1. The results obtained showed that as the exposure time increased, the antimicrobial activity increased. And it was found that bacteriostatic rates of inhibiting the growth of *E. coli* and *S. aureus* were higher than that of *C. albicans*. The polymer inhibited the growth of *E. coli* (8099), *Staphylococcus aureu* (ATCC6538) and *Candida albicans* increased with the exposed time. The inhibition became stronger as the sequence, *Candida albicans* < *Escherichia coli* < *Staphylococcus aureu*.

Quaternary ammonium salts possessing at least one alkyl substituted are able to kill microorganisms such as bacteria and fungi. So QAS belong to the membrane active compounds and their biological activity depends on their structure and physicochemical properties affecting the interaction with the phospholipid bilayer in the cytoplasmic membrane of bacteria and influencing cell metabolism.

The antimicrobial activity of the nano-fumed silica derivative with quaternary ammonium group is considered to be one of the important properties linked directly to the possible applications. Its mechanism was proposed for the antimicrobial activity exerted by antimicrobial polymeric derivative with quaternary ammonium salts. It is that the polycationic nature of nano-fumed silica derivative with quaternary ammonium group interferes with bacterial metabolism by electrostatic stacking at the cell surface of bacteria. This mechanism is evaluated in term of the value of the zeta potential of nano-fumed silica derivative with quaternary ammonium group. Because MNFS-2 shows positive charge in aqueous solution (pH=7), its antimicrobial activity is stronger.

4 Conclusions

1. Hydroxyl groups on surface of nano-fumed silica and tertiary amine can be connected with γ- chloropropyltrimethoxysilane, and powder antimicrobial material was prepared by quaternization.

2. From FTIR spectra, γ-chloropropyltrimethoxysilane bonding on the surface of nano-fumed silica by acting with hydroxyl groups on surface of nano-fumed silica can be seen. From alkyl radical peak on NFS modified with quaternary ammonium salts, we can see NFS carried alkyl groups.

3. After researches on change of isoelectric points of NFS and modified NFS, we can see that owing to isoelectric points of modified NFS increasing in the direction of pH enhancing by 4, ions of modified NFS in aqueous solution showed positive charge, and it can kill microbial.

4. By the quantitative suspension method, the bacteriostatic efficacy of powder antimicrobial material modified with N, N-dimethyl-n-hexadecylamine to *Escherichia coli* (8099), *Staphylococcus aureus* (ATCC6538) and *Candida albicans* (ATCC 10231) was 99.99%, 99.99% and 95.23%, respectively.

Correspondence to:
Xia Xu
Laboratory Center of Medical Science
Zhengzhou University
Zhengzhou, Henan 450052, China

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