Synthesis and characterization of silver-loaded mesoporous alumina antibacterial agents by hydrothermal and adsorption methods

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MS received 21 July 2017; accepted 5 January 2018; published online 3 December 2018

Abstract. In view of the compatibility between carriers of antimicrobial agents and ceramic glaze, mesoporous alumina was employed as the carrier of silver ions and silver-loaded mesoporous alumina was synthesized by hydrothermal and adsorption methods. The liquid medium microdilution method and the spread plate methods were studied to evaluate antimicrobial efficiency of silver-loaded mesoporous alumina. N₂ adsorption/desorption, HRTEM images and low-angle X-ray diffraction experiment prove the existence of fine mesoporous structure of mesoporous alumina and silver-loaded mesoporous alumina. The chelation of ammonia can improve the adsorption capacity of silver nitrate on mesoporous alumina and adsorption capacity increases by 18 times in the presence of ammonia. The adsorption behaviour of silver nitrate can be described by a multilayer adsorption isotherm of BET and the adsorption principle of silver nitrate on mesoporous alumina is multilayer adsorption in the presence of ammonia. The surface area, pore volume and pore-size distribution of silver-loaded mesoporous alumina are obviously smaller than those of mesoporous alumina due to the blockage of channel by silver-ammonia complex. The antibacterial test results indicate that silver-loaded mesoporous alumina have favourable antibiotic property and MIC $\leq 240 \mu\text{g ml}^{-1}$ against *Escherichia coli*, *Staphylococcus aureus* and *Salmonella*. The diameter of inhibition zone of silver-loaded mesoporous alumina is 26 mm against *Escherichia coli*, 24 mm against *Staphylococcus aureus* and 22 mm against *Salmonella enterica*.

Keywords. Mesoporous; alumina; adsorption; BET; silver; antimicrobial.

1. Introduction

In our daily life, sterilization is the most effective way for avoiding transmission of disease and food poisoning triggered by bacteria. The methods of sterilization can be categorized as thermal and cold sterilizations. The former sterilizes the microorganisms by steam, boiled water, hot blast, extreme infrared and microwave. The later mainly utilizes antimicrobial agent [1–4]. Antimicrobial agent includes inorganic, organic and natural types. In addition to poor usability, security and stability of organic and natural antimicrobial agents, the development of resistance is the main problems of organic and natural antimicrobial agents. However, inorganic antimicrobial agent overcomes many disadvantages of organic and natural antimicrobial agents, as it is excellent not only in security, stability and long effectiveness, but also in non-resistance and heat resistance. Now, the synthesis of inorganic antimicrobial agents and research of antimicrobial properties have become a prime issue in the field of materials [5–7].

The main effective component in inorganic antimicrobial agent is metal ions such as silver, copper and zinc, which were adsorbed and immobilized on the surface and channel of

porosints such as zeolite, phosphate, porous silica and activated carbon by physical and chemical adsorptions [8–12]. Then, these inorganic antimicrobial agents are added to the plastics and silicate products to prepare diverse antibacterial materials [13–16]. In addition, metal ions such as silver, copper and zinc must be loaded onto carriers, thereby resulting in good safety and no toxic or side effects during use.

In numerous inorganic antibacterial agent studies, inorganic antibacterial agents-loaded silver has become one of the most widely studied antibacterial agents at present. Previous carrier study of silver has focussed on phosphate-based silica-metal nanocomposites and metal oxides, and much work was done to study those carriers of silver [17–24]. Despite the great progress has achieved in this field, a problem of adaptability remains between the ceramic glaze and those carriers of silver, for the addition of those carriers of silver to ceramic glaze will change the property of the ceramic glaze and decreases surface smoothness of the glaze. It is well-known that alumina is the main component of ceramic glaze, in addition, mesoporous alumina to ceramic glaze will not change the property of the ceramic glaze, and mesoporous alumina with a high specific surface area favours the improvement of silver sorption

amounts on mesoporous alumina. According to the present literatures, study on the preparation and characterization of silver-loaded mesoporous alumina is seldom reported.

In previous studies, we have synthesized mesoporous alumina by hydrothermal process in the presence of non-ionic surfactant PEG [25,26].

In this paper, silver nitrate was chosen to evaluate the adsorption properties of mesoporous alumina, then, the silver-loaded mesoporous alumina was prepared by static adsorption experiments. The structure and morphology of silver-loaded mesoporous alumina was systematically characterized by X-ray diffraction (XRD), high resolution transmission electron microscopy (HRTEM), and N₂ adsorption-desorption. The possible adsorption mechanism of silver nitrate on mesoporous alumina was discussed according to adsorption isotherm in the presence of ammonia. Gram-negative (*Escherichia coli* and *Salmonella enterica*) and Gram-positive (*Staphylococcus aureus*) bacteria strains were chosen to evaluate the antimicrobial activity of silver-loaded mesoporous alumina.

2. Materials and methods

2.1 Material

All reagents for the synthesis of silver-loaded mesoporous alumina are analytic grade, aluminium nitrate (Al(NO₃)₃ · 9H₂O) (Aladdin Industrial Corporation, Shanghai, China; 99.99% purity), silver nitrate (AgNO₃) (Aladdin Industrial Corporation, Shanghai, China; 99.99% purity), polyethylene glycol 600 (PEG 600) (KeBang Chemical Products Co. Ltd., Tianjin, China; 99.99% purity), ethanol (CH₃CH₂OH) (KeBang Chemical Products Co. Ltd., Tianjin, China; 99.99% purity), carbamide (CO(NH₂)₂) (KeBang Chemical Products Co. Ltd., Tianjin, China; 99.99% purity). All reagents without additional purification and deionized (DI) water were used in all the processes.

The reference quality control strains *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923) and *Salmonella enterica* (ATCC 13076) were originally acquired from the American Type Culture Collection (Rockville, MD).

2.2 Synthesis of silver-loaded mesoporous alumina antibacterial agents

Firstly, mesoporous alumina was synthesized during the process described in our earlier publication [25,26]. Then, silver-loaded mesoporous alumina was prepared by chemisorption method. In a typical synthesis, 0.1500 g of silver nitrate was dissolved in 50 ml deionized water in a Erlenmeyer flask, and ammonia was added until the solution is clear. Then, 0.3000 g of mesoporous alumina was added. The Erlenmeyer flask was placed in an electronic temperature-controlled shaker (Taihua HZQ-F160, Taichang, China) and shaken at 150 r min⁻¹ after supersonic dispersion. The

concentration of residual silver nitrate was determined by Volhard method after adsorption at 25°C for 12 h. The experiment was performed in duplicate and the averaged value was recorded.

2.3 Sample characterization

Phase composition of silver-loaded mesoporous alumina was studied by XRD (Bruker D8 Focus, in 10–70° 2θ range with a step of 0.02° and a residence time of 0.5 s), diffraction patterns were analysed using international centre of diffraction data (ICDD, PDF4) in EVA (Bruker) software. The microstructure of silver-loaded mesoporous alumina was assessed by HRTEM (JEM-2100F, JEOL, Japan). Quantachrome Nova Station A (Quantachrome, NOVA 2200e) was used to obtain N₂ adsorption-desorption isotherms at 77.3 K. The sample was out gassed at 300°C for 6 h before nitrogen adsorption. The pore-size distribution was calculated based on the BJH (Barret-Joyner-Halenda) method. Specific surface areas were determined by the BET (Brunauer-Emmett-Teller) method (Monosorb Analyzer MS-13, Quanta Chrome, USA) using liquid N₂ as adsorbate.

2.4 Evolution of antibacterial activity

Silver-loaded mesoporous alumina was evaluated for their antibacterial activity against Gram-negative (*Escherichia coli* and *Salmonella enterica*) and Gram-positive (*Staphylococcus aureus*) bacteria. The quantitative assay of the antimicrobial activity against bacteria was performed using the liquid medium microdilution method in a Petri plate to establish the minimal inhibitory concentration (MIC). For this purpose, two-fold serial dilutions of silver-loaded mesoporous alumina ranging between 0 and 270 µg ml⁻¹ was performed in a 25 ml volume of LB nutrient agar, and each was seeded with 25 µl of microbial inoculum (10⁷ CFU ml⁻¹). The Petri plate was incubated for 24 h at 37°C. The MIC value was considered as the lowest concentration of the tested silver-loaded mesoporous alumina that inhibited the visible growth of the microbial overnight culture.

The bactericidal activity of silver-loaded mesoporous alumina was further analysed using the spread plate method to determine the diameter of inhibition zones in millimetre. Briefly, 1.00 g of silver-loaded mesoporous alumina was compressed into a wafer with standardized diameter 10.00 mm. LB nutrient agar was appropriately sterilized and then deposited on the surface of a Petri plate. The inoculation was performed using a sterile glass rod. The bacteria were inoculated on the whole substrate surface on which a silver-loaded mesoporous alumina wafer was then placed. The plate was incubated at a temperature of 37°C for 24 h. Subsequently, photographic documentation of the Petri plates was performed, the measurement of inhibition growth zone diameter was performed using vernier calipers.

3. Results and discussion

3.1 XRD of nanostructures

Silver-loaded mesoporous alumina was characterized by XRD (figure 1).

As figure 1 illustrates the silver-loaded mesoporous alumina and mesoporous alumina are of the same structure. According to ICDD, powder diffraction file (PDF) standard card, both samples match a typical XRD of η - Al_2O_3 . Result of XRD indicates that silver nitrate, which was adsorbed on surface of mesoporous alumina has not converted to other silver compounds, such as silver oxide. Silver nitrate was adsorbed in the form of silver-ammonia complex by mesoporous alumina in the presence of ammonia. The particles of mesoporous alumina were negatively charged under alkaline conditions, i.e., the higher the value of pH, the more negative charge on the surface of mesoporous alumina. So, the adsorption efficiency of silver-ammonia complex increased with pH value.

3.2 Effect of ammonia on adsorption

To determine the adsorption mechanism of mesoporous alumina for silver nitrate, the effect of ammonia on the adsorption of silver nitrate on mesoporous alumina was studied. Firstly, 0.1500 g of silver nitrate was dissolved in 50 ml deionized water in Erlenmeyer flask, then, ammonia was added until the solution is clear. Then, 0.3000 g of mesoporous alumina was added. The Erlenmeyer flask was placed in an electronic temperature-controlled shaker and shaken at 150 r min^{-1} after

Figure 1. XRD patterns of mesoporous alumina and silver-loaded mesoporous alumina.

supersonic dispersion. The concentration of residual silver nitrate was determined by Volhard method after adsorption at 25°C for 12 h.

For comparison of the impact of ammonia on adsorption performance of mesoporous alumina, the above-mentioned experiments were repeated under the same condition without ammonia. All the experiments were performed for six times and the results of experiments were statistically analysed. The results are shown in table 1.

Table 1 shows that adsorption capacity of silver nitrate on mesoporous alumina increased by 18 times in the presence of ammonia. Firstly, the point of zero charge (pHzpc) of the mesoporous alumina was close to 7–9, so the particles of mesoporous alumina were negatively charged and the amount of negative charge increased correspondingly with pH in the presence of ammonia. Conversely, silver-ammonia complex was positively charged under alkaline condition. So, silver-ammonia complex was adsorbed on mesoporous alumina by their opposite charge, and the augment of solution alkalinity improved the adsorption capacity of silver-ammonia complex on mesoporous alumina. Secondly, the surface of mesoporous alumina is rich in oxygen atom due to the surface effect and quantum size effect of mesoporous alumina nanoparticles. The surface oxygen atoms incline to form stable chemical bonds with hydrogen atoms of silver-ammonia complex. The adsorption mechanism of silver-ammonia complex on mesoporous alumina is depicted in figure 2. So, the chelation of ammonia improved the adsorption capacity of silver-ammonia complex on mesoporous alumina.

3.3 Isotherm and mechanism of adsorption

The influence of various initial quantities of mesoporous alumina on adsorption performance in the presence of ammonia was investigated (figure 3). The concentration of

Figure 2. The adsorption mechanisms of silver-ammonia complex on mesoporous alumina.

Table 1. Effect of ammonia on absorption efficiency.

$\text{NH}_3 \cdot \text{H}_2\text{O}$		$Q_e \text{ (mg g}^{-1}\text{)}$					$\overline{Q_e} \text{ (mg g}^{-1}\text{)}$	RSD (%)
5.00 ml	79.16	79.18	79.23	79.25	79.30	79.35	79.24	0.091
0.00 ml	4.49	4.46	4.46	4.43	4.37	4.31	4.42	1.5

Figure 3. (a) Freundlich and (b) BET adsorption isotherms of silver nitrate.

Figure 4. TEM micrographs and low-angle XRD analysis of mesoporous alumina and silver-loaded mesoporous alumina. (a) Mesoporous alumina; (b) silver-loaded mesoporous alumina; and (c) low-angle XRD of mesoporous alumina and silver-loaded mesoporous alumina.

silver nitrate was kept constant ($3.0 \times 10^5 \text{ mg l}^{-1}$), while the quantity of mesoporous alumina varied between 2 and 10 g l^{-1} . The Freundlich ($\lg q = \lg k + 1/n \cdot \lg c$), Langmuir ($c/q = 1/q_e b + c/q_e$) and BET isotherm ($c/q \times (c_s - c) = (1/a \times B) + (B - 1/a \times B) \times c/c_s$) equations were used for the equilibrium modelling of adsorption systems.

Here, q is the amount of silver nitrate adsorbed at equilibrium concentration (mg g^{-1}); c the equilibrium concentration of silver nitrate in solution (mg l^{-1}); q_e the maximum adsorption capacity (mg g^{-1}); c_s the solubility of silver nitrate ($2.22 \times 10^5 \text{ mg l}^{-1}$); and n , k , a , b and B are the constants.

Figure 5. (a) Nitrogen adsorption–desorption isotherm and (b) BJH pore-size distribution of mesoporous alumina and silver-loaded mesoporous alumina.

Table 2. Mesoporous parameters of mesoporous alumina and silver-loaded mesoporous alumina.

Samples	S_{BET} ($\text{m}^2 \text{g}^{-1}$)	V_t (cc g^{-1})	D_p (nm)
Al ₂ O ₃	189.899	0.329	8.31
Ag-Al ₂ O ₃	133.631	0.226	6.64

The results show that adsorption isotherm of silver nitrate on mesoporous alumina accorded with Freundlich equation ($\lg q = 4.36 \lg c - 12.7$, $R^2 > 0.99$), but apparently did not accorded with Langmuir monolayer adsorption equation ($c/q = 603 - 0.312c$, $R^2 > 0.99$), because the slope of $1/q_e$ is negative and the q_e cannot be negative. But BET multilayer adsorption isotherm equation ($c/q \times (c_s - c) = 2.79 + 0.455 \times c/c_s$, $R^2 > 0.99$) can better describe the adsorption behaviour of silver nitrate on mesoporous alumina (figure 3). The analysis of adsorption isotherm equation shows that the adsorption principle of silver nitrate on mesoporous alumina is multilayer adsorption in the presence of ammonia.

3.4 Analysis of HRTEM images and low-angle XRD

The structure of silver-loaded mesoporous alumina can be observed from HRTEM images (figure 4). As shown in figure 4, all the samples exhibit a uniform rod-like morphology with length from 0.2 to 1 μm and well-defined mesoporous structure according to the HRTEM images. The surfaces of silver-loaded mesoporous alumina were more slippery than that of mesoporous alumina because the surfaces were covered by silver-ammonia complex. Low-angle XRD of mesoporous alumina and silver-loaded mesoporous alumina (figure 4c) show the direct evidences for a long range order of pore structures.

3.5 N₂ adsorption–desorption isotherm

The N₂ adsorption–desorption isotherm of mesoporous alumina and silver-loaded mesoporous alumina belongs to Langmuir type IV and the hysteresis loop belonged to type H2 (figure 5). The adsorption isotherm is relatively far from desorption isotherm at high pressure, proving the mesoporous structure of the samples. Type H2 hysteresis loop usually results from a complex, interconnected pore network. H2 hysteresis observed for the sample indicates that relatively uniform pores are present in the samples. The broad hysteresis loop centred at higher P/P_0 indicated the larger mesopores [27]. The verdict of N₂ adsorption–desorption isotherm is in accordance with results of HRTEM images and low-angle XRD experiment. All the results proved the existence of fine mesoporous structure of mesoporous alumina and silver-loaded mesoporous alumina.

From the BET result, the surface area of silver-loaded mesoporous alumina is found to be $133.631 \text{ m}^2 \text{g}^{-1}$ and that of mesoporous alumina is $189.899 \text{ m}^2 \text{g}^{-1}$ (table 2). The surface area, pore volumes and pore size distribution of silver-loaded mesoporous alumina are obviously smaller than those of mesoporous alumina due to the blockage of channel by silver-ammonia complex.

3.6 Antibacterial activity of silver-loaded mesoporous alumina

Escherichia coli, *Salmonella enterica* and *Staphylococcus aureus* are the most commonly used organisms associated with nosocomial infections and postsurgical wound infections. The results of qualitative antibacterial tests reveal that silver-loaded mesoporous alumina has an important inhibitory activity on Gram-negative and Gram-positive bacteria (table 3). The concentration of silver-loaded mesoporous alumina has a prominent influence on the

Table 3. Antibacterial activity of silver-loaded mesoporous alumina.

Antimicrobial concentration ($\mu\text{g ml}^{-1}$)	0	180	210	240	270
<i>Escherichia coli</i> (cfu ml^{-1})	1.25×10^7	0.38×10^7	9.60×10^5	1.36×10^3	80
<i>Golden staphylococcus</i> (cfu ml^{-1})	1.23×10^6	0.42×10^6	3.20×10^4	1.06×10^2	0
<i>Salmonella enterica</i> (cfu ml^{-1})	1.58×10^6	0.23×10^6	4.72×10^4	1.33×10^2	0

Figure 6. Silver-loaded mesoporous alumina for (a) *Escherichia coli*, (b) *Golden staphylococcus* and (c) *Salmonella enterica* 24 h antibacterial test results.

bacteriostatic efficacy against *Escherichia coli*, *Salmonella enterica* and *Staphylococcus aureus*, and three bacteria were inhibited for silver-loaded mesoporous alumina concentration above $240 \mu\text{g ml}^{-1}$.

Inhibition zone value was obtained from silver-loaded mesoporous alumina against Gram-negative (*Escherichia coli* and *Salmonella enterica*) and Gram-positive (*Staphylococcus aureus*) bacteria (figure 6). Result of the inhibition zones shows that silver-loaded mesoporous alumina have high and similar antibacterial activity against Gram-positive and Gram-negative bacteria. The diameter of inhibition zone of silver-loaded mesoporous alumina is 26 mm against *Escherichia coli*, 24 mm against *Staphylococcus aureus* and 22 mm against *Salmonella enterica*. The mesoporous structure of silver-loaded mesoporous alumina was in favour of the release of silver ions into the agar plate, so silver ions can easily reach the nuclear content of bacteria by destroying the membranes of bacteria.

4. Conclusion

Silver-loaded mesoporous alumina was synthesized by hydrothermal and adsorption methods. N_2 adsorption/desorption, HRTEM images and low-angle XRD experiment prove the existence of fine mesoporous structure of mesoporous alumina and silver-loaded mesoporous alumina. Silver nitrate is adsorbed in the form of silver-ammonia complex by mesoporous alumina in the presence of ammonia. The adsorption capacity of silver nitrate on mesoporous alumina increases by 18 times and the adsorption principle

of silver nitrate on mesoporous alumina is multilayer adsorption in the presence of ammonia. The surface area, pore volume and pore-size distribution of silver-loaded mesoporous alumina are obviously smaller than those of mesoporous alumina due to the blockage of channel by silver-ammonia complex. The antibacterial test results indicate that silver-loaded mesoporous alumina have favourable antibiotic property and $\text{MIC} \leq 240 \mu\text{g ml}^{-1}$ against *Escherichia coli*, *Staphylococcus aureus* and *Salmonella*. The diameter of inhibition zone of silver-loaded mesoporous alumina was 26 mm against *Escherichia coli*, 24 mm against *Staphylococcus aureus* and 22 mm against *Salmonella enterica*.

Acknowledgements

We are greatly indebted to the National Natural Science Foundation of China for the financial support (Project no. 51279073).

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