Preparation and characterization of silver chloride nanoparticles as an antibacterial agent

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Abstract
Silver chloride nanoparticles were prepared by the precipitation reaction between silver nitrate and sodium chloride in an aqueous solution containing poly(vinyl alcohol) as a stabilizing agent. Different characteristics of the nanoparticles in suspension and in lyophilized powder such as size, morphology, chemical nature, interaction with stabilizing agent and photo-stability were investigated. Biological tests showed that the obtained silver chloride nanoparticles displayed antibacterial activities against Escherichia coli and Staphylococcus aureus.

Keywords: silver chloride nanoparticles, poly(vinyl alcohol), antibacterial activity
Classification number: 2.05

1. Introduction

Nanotechnology plays an ever more important role in diverse areas of science, including medicine and pharmacy. Among a wide range of applications, nanoparticles as a drug delivery system are believed to open the door to a new era for the whole pharmaceutical field, allowing drug molecules to be delivered temporally and spatially to specific targets. This potential is attributed to the special properties of nanoparticle in optics, electromagnetics and membrane permeability, which are not available in micro-scaled particles. Inorganic nanoparticles with unique physicochemical properties are being extensively investigated, and among them silver related compounds draw much interest [1]. They have been recently used in various fields, from photocatalysts to bactericides.

As concerns with antibiotic resistance increase, the interest in silver as a broad spectrum antibacterial agent has been revitalized. At present, silver is used in many cases for disinfection, such as in wound healing, medical implants and instrument sterilization [2, 3]. It is reported that the main factor that determines the antibacterial abilities of silver-related compounds is the silver ion [4]. Silver ions can inhibit bacterial multiplication by binding and denaturing bacterial DNA, which affects the ribosomal subunit protein and some enzymes essential for bacterial cell growth [5–7]. As a result, silver chloride (AgCl) as a sustainable resource of silver ions is a potential candidate for treating infections. Furthermore, AgCl in the form of nanoparticles could be more toxic to the bacteria than the bulk counterpart, because small size nanoparticles may pass through cell membranes, and the accumulation of intracellular nanoparticles can lead to cell malfunction [8]. Although having various applications, the preparation of AgCl nanoparticles with controlled size is limited to methods such as micro-emulsion technique, ultrasound irradiation, matrix-based technique or mixing silver nitrate (AgNO3) with hydrochloric acid in the presence of poly(vinyl pyrrolidone) [9–12]. Therefore, with the purpose of using AgCl as an antibacterial agent, it is necessary to develop a simple method for preparing AgCl nanoparticles.

In this study AgCl nanoparticles were prepared via a facile method from two precursors: AgNO3 and sodium chloride (NaCl). This nearly immediate reaction occurred in an aqueous environment containing stabilizing agent poly(vinyl alcohol) (PVA). Physico-chemical characteristics of the nanoparticles were evaluated with ultraviolet-visible (UV–vis) spectrophotometer, scanning electron microscopy (SEM), Fourier transform infrared (FTIR) spectroscopy and x-ray diffraction (XRD) spectroscopy. Furthermore, the antibacterial activities...
were tested on two susceptible strains *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*).

2. Materials and methods

2.1. Preparation of AgCl nanoparticles

AgNO₃ was purchased from Tianjin Yinlida Chemicals Co. Ltd, NaCl was purchased from Xilong Chemical Co. Ltd, NaNO₃ and PVA (partially hydrolyzed, viscosity from 5.2 to 6.2 cps) were purchased from Sekisui Chemical Co. The above chemical reagents were analytical grade and used without further purification.

AgCl nanoparticles were synthesized by the chemical reaction between silver ions from AgNO₃ and chloride ions from NaCl in the presence of stabilizing agent PVA according to the following procedure: 1.634 g of PVA and 0.122 g of AgNO₃ (0.72 mmol) were dissolved in 210 ml of distilled water. To this solution were added 14.4 ml of NaCl 0.1 M aqueous solution (1.44 mmol, 2 eq) at a steady rate over 30 min under vigorous stirring. The reaction mixture was stirred for further 2 h at room temperature.

Lyophilized powder was obtained from freshly prepared AgCl suspension using Alpha Christ 1-2 LD Plus freeze dryer (SciQuip).

2.2. Characterization

The size and zeta potential of the nanoparticles were investigated by dynamic light scattering and laser Doppler principles respectively using Zetasizer Nano ZS90 Malvern (Malvern Instruments) with a scattering angle of 90°. The morphology of the nanoparticles was investigated with a field emission scanning electron microscope (FESEM) S4800-NIHE (Hitachi), operated at an acceleration voltage of 5.0 kV. The XRD patterns were obtained by using x-ray diffractometer D-8 Advanced Bruker (Bruker) with Cu-Kα radiation at a scan rate of 0.03° per second in the 2θ range of 20° to 80°. The FTIR spectra were recorded between 4000 and 400 cm⁻¹, on an IR-Affinity 1s spectrometer (Shimadzu). UV–vis absorption spectra were measured by UV–vis spectrophotometer Cary UV-60 (Agilent Technologies) in the range of wavelength from 200 to 800 nm; all samples were diluted 10-fold in distilled water before measuring.

2.3. Biological evaluation

The antibacterial efficacy was evaluated by agar disc diffusion method against *S. aureus* (Gram positive bacterium, ATCC 1128) and *E. coli* (Gram negative bacterium, ATCC 25922), respectively, using benzathine penicillin (BZP, 20 IU/ml) and streptomycin (STM, 20 IU/ml) as positive controls. The final bacterial cell density was 1 × 10⁸ CFU/ml for both species. The AgCl nanoparticles were tested at different concentrations (460, 230, 115, 57.5 and 28.75 ppm) in comparison with silver sulfadiazine (Macsen Laboratories, 1000-62.5 ppm). Blank samples are an aqueous solution containing NaCl, NaNO₃ and PVA with the same concentrations as in the original AgCl nanosuspension and its 4 serial two-fold dilutions. Sterile discs (6–6.5 mm) were impregnated separately with different samples, and then placed over the surface of the agar medium. After incubation at 37 °C for 18–24 h, the zone of inhibition (D) was measured.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by dilution method, using distilled water as a negative control. AgCl nanoparticles (in the concentration range of 46-9.2 ppm) were inoculated with test strains (final cell density of 1 × 10⁶ and 1 × 10⁴ CFU/mL in agar culture medium and incubated at 37 °C for 18 h. The lowest concentration of AgCl nanoparticles showing growth inhibition (as seen visually) was considered as the MIC. The MBC was recorded as the lowest concentration of AgCl nanoparticles that showed no visible growth on agar medium.

3. Results and discussion

3.1. Size, zeta potential and morphology

The AgCl nanosuspension was simply prepared from AgNO₃ and NaCl in aqueous medium at room temperature, using stabilizing agent PVA. The average diameter of particles in suspension was about 80 nm. The suspension was polydisperse, as its polydispersity index (PDI) was about 0.15. The zeta potentials of the particles were around −10 mV. The SEM analysis confirmed the presence of nano-scaled particles and provided more information about their morphology. From figure 1, it is observed that the particles in suspension were cubic in shape. Meanwhile, the particles in lyophilized...
powder seemed to be spherical in shape, with the average diameter of about 90 nm and the PDI of about 0.25, which were slightly greater than those of nanocubes. These discrepancies are probably attributed to the PVA coating of AgCl nanoparticles in lyophilized powder.

Practically, it is difficult to control the synthesis of silver related nanoparticles. The changes in morphology and size of silver particles can be related to the initially formed nuclei of metallic silver, which is sensitive to the reaction conditions (concentrations, reduction agents, temperature, presence of additives) [12–14]. Also, many different kinds of AgCl nanoparticles have been reported in the literature. Besides spherical particles [15–18], cubic [11, 19] and wire shapes [11] were found, depending on the reaction conditions. The partial reduction process creating metallic silver probably contributed to these changes in the shape and size of AgCl nanoparticles [19]. In this study it can be seen that even after the end of the chemical reaction between AgNO₃ and NaCl, the shape of AgCl nanoparticles was still altered by the lyophilization process.

3.2. Chemical nature

The crystalline nature of obtained nanoparticles was investigated by XRD patterns. The diffraction sharp peaks observed in the 2θ range of 20° to 80° in XRD spectrum of centrifuged
powder were 27.91°; 32.32°; 46.27°; 54.85°; 57.49°; 67.42°; 74.53°; 76.84° (figure 2), and in lyophilized powder were 27.97°; 32.32°; 46.24°; 54.79°; 57.55°; 67.63°; 74.50°; 76.54°. They are respectively assigned to the (11̅1), (200), (220), (311), (222), (400), (331) and (420) planes of the face centered cubic structure of AgCl crystal.

3.3. Interaction with stabilizing agent

The possible interactions between PVA and AgCl were investigated by FTIR spectroscopy. In the FTIR spectra of lyophilized PVA and lyophilized AgCl nanoparticle (figure 3), the strong broad peak at 3319 cm⁻¹ was attributed to the presence of hydroxyl group (OH). The peak observed at 2940 cm⁻¹ was responsible for the CH₂ asymmetric (-CH₂-CH₂-) stretching. The peak at 1734 cm⁻¹ represented the carbonyl (C=O) stretching bond, while 1090 cm⁻¹ indicated the terminal polyvinyl group. The FTIR analysis showed no considerable chemical bonds between PVA and AgCl, which indicated that the main function of PVA was to facilitate the synthesis of AgCl nanoparticles and stabilize the suspension by hindering AgCl agglomeration [19].

3.4. Photostability

The AgCl nanoparticles are known to be photosensitive and to produce silver upon exposure to ambient light [20]. This was observed in the absorbance data of the suspensions with and without exposure to UV light at the wavelength of 254 nm in 5 h (figure 4).

During this period of time, the colour of the suspension changed from slightly opalescent to violet and finally, brown-yellow. The UV–vis spectrum of the 10-fold diluted suspension after UV irradiation showed an intense peak in visible region (about 420 nm), along with the strong absorption in the
wavelength below 300 nm, which was also observed in the spectrum of the original suspension. The visible light absorption might be the result of metallic silver layer formed by chemical reduction of AgCl during the UV irradiation [21]. There are two reasons supporting this assumption. Firstly, the direct and indirect band gaps of AgCl are 5.15 eV (240 nm) and 3.25 eV (380 nm) [22], respectively, which prevents AgCl from absorbing light with the wavelength above 380 nm. Secondly, it is reported that metallic silver deposited on AgCl particles exhibited the plasmonic absorption of visible light [11, 19, 23].

3.5. Biological evaluation

The antibacterial activities of metallic silver nanoparticles against E. coli and S. aureus were reported elsewhere [4, 24–26]. In this study, the antibiotic properties of AgCl nanoparticles were assessed against these two species using agar disc diffusion method (figure 5).

The zone of inhibition of BZP for S. aureus was 16.51 mm (s = 0.50) and that of STM for E. coli was 8.53 mm (s = 0.36).

From table 1, it can be seen that AgCl nanoparticles displayed antimicrobial activities against both Gram positive and Gram negative organisms comparable to those of silver sulfadiazine. The zone of inhibition (D) was found to increase in accordance with the increasing concentrations (C) of AgCl nanoparticles. E. coli was more sensitive to AgCl nanoparticles than S. aureus, which was shown by the larger zones of inhibition. Blank samples without AgCl exhibited no inhibitory effects on both species.

4. Conclusion

This study reported a simple method for the synthesis of AgCl nanoparticles from two precursors AgNO₃ and NaCl with the presence of PVA as a stabilizing agent. In suspension, the particles were cubic in shape, with an average size of about 80 nm. SEM studies revealed that there were changes in shape and size between particles in suspension and in lyophilized powder. The synthesized nanoparticles were AgCl crystalline structure, which was confirmed by XRD patterns. In the susceptibility test against S. aureus and E. coli, the AgCl nanoparticles showed bactericidal effects on both species. These results indicated that the AgCl nanoparticles could be a potential antibacterial agent.

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