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Facile synthesis of silver nano particles with highly efficient anti-microbial property

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Abstract

A simple surfactant assisted aqueous solution approach, based on the conventional Tollen's reaction has been applied for the facile syntheses of silver nano-assembly. Nano particle morphologies strongly depend on the temperature adopted during the synthesis. Two-dimensional (2 D) nano-disks and three-dimensional (3D) nano-globules with "Cauliflower" like morphology were observed. The unique and distinctive feature of the synthesized silver nano particle in solution is its very high anti-microbial activity. This is evident in very low (4 μg/ml) inhibitory concentration (MIC) value for *Escherichia coli*, *Vibrio cholerae*, *Shigella flexneri*, *Salmonella typhimurium* and three varieties of *Staphylococcus aureus*. This low MIC value is comparable to that of Penicillin and in the cases of *E. coli* and *S. aureus* (ML 422), the MIC value is as low as 2 μg/ml which beats even Penicillin.

Keywords: Tollen's reaction; Silver nano; 2D and 3D nano aggregates; Anti-microbial activity

1. Introduction

Research in the area of synthesis methodology of nano materials is mainly oriented in controlling their shape, size and composition. Each of these parameters is an important factor in determining the properties of materials that lead to various technological applications [1,2]. Among the large varieties of these nano materials, the metal nano particles both in the pure and composite form [3–6] have been extensively investigated because of their unique optical as well as electronic properties [7,8]. The noble metals, especially silver and gold, have attracted great attention due to their innumerable applications in various branches of science namely catalysis [9], photonics [10,11], photography [12], chemical sensing [13], surface enhanced Raman scattering (SERS) [14] and most importantly in the medicinal field as anti-microbial agents [15–21]. A number of synthetic methods for achieving controlled production of Ag and Au nano particles have been proposed [22–27], but the quest is still on for more easier and commercially viable methods for production of these important nano materials. The surfactant capping method is being used for the last few years due to its relative synthetic simplicity. Sodium dodecyl sulphate (SDS) [28], cetyl trimethyl ammonium bromide (CTAB) [31,32], sodium bis-(2-ethylhexyl) sulfosuccinate (NaAOT) [29] and poly-(oxyethylene) isooctylphenyl ether (Triton X-100) [30] are few of the commonly used surfactants. But a majority of these surfactants assisted syntheses of nano sized silver and gold, use the seed-mediated growth strategy [31,32]. Recently, Chu et al. [33] have demonstrated a facile and faster method of preparation of Au nano-plates through thermal reduction of $HAuCl_4$ using CTAB without using any seed particles. In this contribution, we report an equally facile and faster (just 5–15 min for the completion of the reaction) thermal reduction process for the production of Ag nano

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particles using a simple modification of the well-known Tollen's reduction in the presence of SDS. Utilization of silver nano particles as anti-microbial agent is well established though the underlying mechanism behind the activity is still ambiguous. In this paper the most important aspect of the synthesized Ag nano particles is its high efficacy as anti-microbial agent against a range of harmful microbes [*Escherichia coli* (ATCC 10536), *Vibrio cholerae* (7), *Shigella flexneri* (80903), *Salmonella typhimurium* (NCTC 74) and three varieties of *Staphylococcus aureus* (ATCC 29737, ML 276 and ML 422)] with a very low MIC value (2 μg/ml) in aqueous solution. To the best of our knowledge such a low value of MIC showed by silver nano particles is unprecedented.

2. Experimental

2.1. Reagents

All the reagents were of analytical grade. $AgNO_3$ (Aldrich) was used as received. Sodium dodecyl sulphate (SDS), *m*-hydroxy benzaldehyde, ammonia were purchased from Merck and also used as received. De-ionized water was used throughout the reaction.

2.2. Instruments

The prepared samples were characterized by FESEM and EDAX analyzer (JEOL JSM-6700 F), X-ray Powder Diffractometer (Bruker D-8 Advance) and the UV–Vis spectra of the as prepared colloidal solutions was recorded in a Perkin–Elmer UV Lambda 25 spectrophotometer.

2.3. Synthesis of Ag nano particles and study of anti-microbial activity by Agar dilution technique

Silver nitrate solution {0.02 (M)} was prepared by dissolving the required amount of $AgNO_3$ in (1:1) ammonia. Similarly, the aldehyde solution and the surfactant solution having equal concentration to that of the silver nitrate solution were prepared in dehydrated ethanol and water, respectively. Then 1 ml silver nitrate solution was taken in a well-cleaned dry beaker; 1 ml SDS solution was added to it and was mixed well for a few minutes (5 min for each set) by continuous stirring.1 ml aldehyde solution was finally added to this mixture. A light yellow color appeared at room temperature (30 °C). The solution was heated on a water-bath and the temperature was noted. When the temperature of the solution reached 80 °C, the light yellow color of the solution started turning into deep yellow. Then the color gradually changed through yellowish brown (82 °C) to reddish brown (84 °C) and finally turned into brownish black (86 °C) (Fig. 1). On further increasing the temperature of the solution, no perceptible change in color was observed. It is interesting to note that the range of reduction temperature is nearly identical to that reported by Chu et al. [33] for the case of Au. The gradual change of color in this temperature range indicated the formation of Ag nano particles of different dimensions [34]. So we have continued our study by preparing five different set solutions at the above-mentioned temperatures (i.e. at 30, 80, 82, 84 and 86 °C).

The anti-microbial study was carried out with the Ag nano particles (i.e. prepared at 86 °C) by standard agar dilution technique [36]. In short: (i) different amounts of the Ag nano particles were dispersed in phosphate buffer to get colloidal suspension; (ii) agar powder was dissolved in ultra pure water under mild heating; (iii) the molten agar solution was mixed with the prepared suspension in culture plates; (iv) the plates were cooled to turn the mixture into hydrogel; (v) diluted bacterial suspensions were transferred to the wells of an inoculum-replicating apparatus which in turn was used to transfer the inocula to the series of culture plates including a control plate without silver nano particles; and (vi) the inoculum spots were allowed to dry at room temperature and then the plates were incubated at 35 °C in air for 18 h. Finally, growth was observed and MIC values were determined in each case.

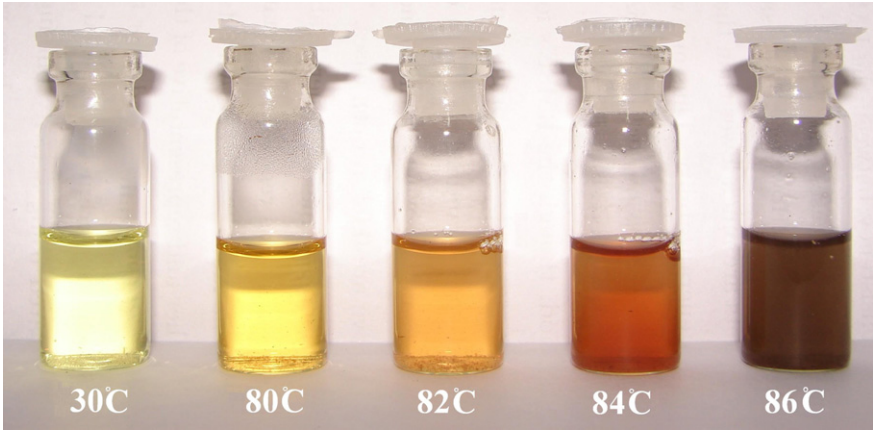

Fig. 1. The temperature-dependent color change during the progression of the reaction.