

RESEARCH ARTICLE

One pot synthesis of PMMA/silver nanoparticles and its antibacterial properties

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Abstract

Ag nanoparticles were prepared via one-pot reduction method using aniline and NaBH₄ as two different reducing agents whereby the effect of different factors such as the order of mixing the reactants, presence of a stabilizer and time on stability, size and concentration of the silver nanoparticles were studied using UV-Vis. The resulted colloidal Ag was then incorporated into poly (methyl methacrylate), PMMA nanoparticles through miniemulsion. The obtained products were characterized with UV-Vis DR and TEM to investigate the presence of Ag in the samples and study its morphology. The occurrence of the polymerization was analyzed with FTIR and ¹HNMR and the antibacterial activity of the samples was tested via Kirby-Bauer method against Gram-negative bacteria, E. coli. Results confirmed the successful synthesis of PMMA/Ag nanoparticles and measurements of the clear zone of inhibition from antimicrobial tests showed that by increasing the concentration of the Ag nanoparticles in the samples, the antibacterial activity was increased.

Keywords: Silver nanoparticles, polymer nanoparticles, one-pot synthesis, miniemulsion, antibacterial

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INTRODUCTION

As the reports on the number of antibiotic resistant bacteria strains are increasing rapidly, the search to find alternative antimicrobial agents has been pursued more earnestly. These resistant bacteria pose a serious threat to the health care system that is battling against the spread of terminal infectious diseases [1]. To prevent such diseases in the near future where the common antibiotics may not effective, alternative agents which limit bacterial resistant are crucial. Among the recently developed antibacterial agents, silver (Ag) based compounds and Ag nanoparticles have been receiving a lot of attentions and thus have been widely studied [2, 3].

Silver, Ag nanoparticles have been incorporated into variety of polymers to be used as biocides for different applications. Polymers impregnated with Ag have shown efficient antimicrobial properties and sustained release of Ag [4]. The synthesis of Ag-coated poly(ethylene terephthalate) fabric via deposition of Ag onto polymeric substrate was studied which effectively prevented the attachment of microorganisms onto silver functionalized surface of the fabric [5]. In another study, Ag ions was reduced and methyl methacrylate was polymerized simultaneously by γ -ray irradiations to produce poly(methyl methacrylate)/silver (PMMA/Ag) nanocomposite which was effectively biocidal against E. coli [6].

In general, a variety of techniques have been applied to produce polymer/Ag nanomaterials however, among them methods that are easy, straight forward and environmentally friendly are more in demand. Miniemulsion polymerization among several heterophase processes that allow the formation of nanoparticles in water is a very versatile method for production of a wide range of polymers in confined geometries and the fact that it is performed in water rather than in organic solvents makes it more environmentally friendly [7-9]. Different polymer nanoparticles have been synthesized and functionalized via miniemusion process for different applications. For instance, in an attempt to replace lubricating powders on the latex gloves with an alternative material in order to reduce the contamination and/or allergic problems, poly (methyl methacrylate) (PMMA) were prepared via miniemulsion method [10]. The antibacterial properties was also introduced to latex gloves and to reduce the coating steps of the particles onto natural rubber, miniemulsion technique was applied to synthesis PMMA-chitosan nanoparticles. Thus, a direct deposition of polymer particles onto rubber films can be carried out, however no antibacterial activity could be detected against Escherichia coli and Staphylococcus [11].

Antibacterial nanoparticles that can be potentially used as a coating powder for latex gloves was also prepared. PMMA/Ag nanomaterials are specially used in medical and dental devices due to their antibacterial activity. Different methods have been reported for the synthesis of Ag nanoparticles embedded in PMMA such as spincoating to prepare PMMA/Ag films [12], radical-mediated dispersion polymerization to produce PMMA/Ag nanofibers [3], γ-ray irradiation to synthesize PMMA/Ag nanocomposites [6], and suspension polymerization to fabricate PMMA/Ag microspheres [13]. Nevertheless, there were not many studies reported the convenience and environmentally friendly process with simultaneous polymerization and incorporation of Ag into polymer in order to synthesis PMMA/Ag nanoparticles. In this work, we reported a onepot synthesis of PMMA/Ag nanoparticles via miniemulsion method and investigated the antibacterial properties of the product.

EXPERIMENTAL

Preparation of silver, Ag nanoparticles

Aniline as a reducing agent

For the preparation of silver, Ag nanoparticles, AgNO3 solution (0.01 mol dm⁻³) and Cetrimonium Bromide, CTAB (0.01 mol dm⁻³) were used, respectively, as a metal salt precursor and a stabilizing agent. Aniline solution (0.01 mol dm⁻³) was used as a reducing agent. The transparent colorless reaction mixture containing AgNO3 + CTAB was converted to the characteristic pale-yellow color after the addition of a required solution of aniline. The appearance of color indicated the formation of Ag nanoparticles. To study the effect of different order of mixing the chemicals, stirring and time on production, stability and size of the nanoparticles, several experiments were conducted using same concentrations as stated above. Once aniline was mixed with CTAB, then AgNO3 solution was added while stirring. In the second experiment, AgNO3 solution was mixed with CTAB and then aniline was added with stirring. These two settings were repeated but without any stirring. All samples were characterized via UV-Vis once after 1.5 h and then after 24 h.

Sodium Borohydride as a reducing agent

A 10-mL volume of 1.0 mM AgNO₃ was added dropwise (about 1 drop per second) to 30 mL of 2.0 mM sodium borohydride, NaBH₄ and sodium dodecyl sulfate, SDS solution that had been chilled in an ice bath. The reaction mixture was stirred vigorously on a magnetic stir plate. The solution turned light yellow after the addition of 2 mL of AgNO₃ and a brighter yellow when the rest of the AgNO₃ solution was added. NaBH₄ and SDS solution was stirred for half an hour in the ice bath before the addition of AgNO₃. The nanoparticles were synthesized at the condition of SDS/AgNO₃ weight ratio of 10. The reaction was repeated using different initial concentration of silver nitrate (0.0005 M, 0.0002 M and 0.0001 M). To prepare all the solutions deionized water was used to minimize the effects of other ions present in water later in the production of PMMA/Ag nanoparticles.

Preparation of PMMA/Ag nanoparticles

PMMA and Ag nanocomposites were prepared via miniemulsion polymerization technique. The desired amount of sodium laureth sulfate, SLS (0.144 g) was dissolved in 20 g of prepared Ag nanoparticles solution with the concentration of 0.001 M with stirring. In a separate container, the dodecyl mercaptan, DDM (0.6 g) was dissolved in 10 g of MMA. The monomer and aqueous Ag solution were then mixed together and the mixture was sonicated for 10 min. The reactor was sealed and purged with nitrogen for 5 minutes and then transferred to an oil bath and kept at 65°C. The potassium persulfate initiator (0.156 g) was then dissolved in 5 g of water and injected into the reactor with a syringe. The reaction mixture was left over night at 60°C. The white product then was filtered and washed repeatedly and left to dry in 60°C oven over night. Synthesis of pure PMMA was conducted in the same way but instead of the Ag solution, 20 mL of deionized water was used.

Antimicrobial test

The antibacterial effect of PMMA doped with AgNPs was carried out using Escherichia coli. E. coli was cultivated in sterilized LB broth and then incubated overnight at 37°C with a shaking incubator. The bacterial suspension then was used in Kirby-Bauer test. An equal amount of solid state PMMA and PMMA/Ag with different concentrations of silver were pelletized by a hydraulic press. The pellets then were placed on the E. coli growth LB agar plates and incubated overnight at 37°C. The zone of inhibition was then measured.

RESULTS AND DISCUSSION

Colloidal Ag synthesis

Silver, Ag nanoparticles were synthesized via reduction method with the use of aniline and NaBH₄ as different reducing agents and

characterized by UV-Vis Spectroscopy. It was found that the high concentration of AgNO₃ along with stabilizer and reducing agent in the process resulted in aggregation of the nanoparticles [14, 15]. Thus, only low concentration of the chemicals required in the synthesis which led to the production of Ag nanoparticles.

In the first series of synthesis of Ag nanoparticles, aniline was used as a reducing agent and CTAB was used as a stabilizer. It is well known that Ag nanoparticles exhibit yellowish brown color in aqueous solution due to excitation of surface plasmon resonance band in the UV-visible region [15]. Different orders of mixing the chemicals with each other were examined and their absorption in the UV-Vis region was recorded after 1.5 hour and also after 24 h. In the first order, the solution of aniline was mixed with CTAB and then Ag solution was added with stirring. Next set of experiment was involved with the same order of mixing the chemicals without stirring. The second order of mixing was conducted while Ag solution was mixed with CTAB and then aniline was added with stirring. The same experiment was repeated without stirring. The effect of different conditions on the production of Ag nanoparticles studied using UV-Vis Spectroscopy is shown in Fig. 1.



Fig. 1 UV-Vis spectra of Ag nanoparticles in different conditions: a) aniline, CTAB solution then Ag solution, b) aniline, CTAB solution then Ag solution 24 h, c) aniline, CTAB solution then Ag solution without stirring, d) Ag, CTAB solution then aniline solution, e) Ag, CTAB solution then aniline solution then aniline without stirring.

As the aniline solution was mixed with aqueous solution of the Ag nitrate and CTAB, a pale-yellow color started to appear after few minutes indicating the formation of silver nanoparticles [16]. The same observation was true when Ag solution was added to the mixture of aniline and CTAB. The absorption spectra of Ag solution consist a single sharp surface plasmon resonance band at 400 nm [17]. A broad surface plasmon resonance band seen in sample (c) is due to aggregation and/or adsorption of aniline onto the surface of Agnanocrystals [15]. It appears that when Ag was added to the solution of aniline and CTAB without stirring, the probability of Ag aggregation is higher comparing to the solution of the same sample (a) with stirring. The spectrum of samples that were taken the next day shows a sharper peak comparing to the those taken after 1.5 hour, showing the production of higher amounts of Ag nanoparticles. This indicated that reduction by aniline is a slow process. Meanwhile, samples in which aniline was added to the solution of Ag and CTAB show sharper and narrower peaks comparing to the other samples. This may be due to the fact that in first set of samples where aniline was added to a solution of Ag and CTAB, Ag ions were dispersed in between CTAB molecules and were not concentrated in one spot. After the addition of aniline, the reduction process occured slowly while Ag ions had less chance of getting aggregated. Comparing sample (d) and (f) shows that stirring in this set of experiments

increases the chances of aggregation since the spectrum of the sample with stirring showed a broader peak with a lower intensity.

As reduction of Ag ions by aniline is a slow process, a stronger reducing agent was used. In this study, the production of Ag nanoparticles by NaBH₄ has been investigated using different concentrations of the chemicals. In the formation of Ag nanoparticles by the chemical reduction method, the order of reactant addition by dropping Ag nitrate solution into NaBH₄ solution with stabilizer, is important to obtain stable Ag nanoparticles. The reverse order of reactant addition causes the immediate precipitation of Ag nanoparticles [18]. Fig. 2 shows the UV-vis spectra of colloidal Ag nanoparticles prepared with different initial AgNO₃ concentrations (0.0001 M, 0.0002 M, 0.0005M and 0.001M). The nanoparticles were synthesized at the conditions of NaBH₄/AgNO₃ molar ratio of 2 and SDS/AgNO₃ weight ratio of 10.



Fig. 2 UV-Vis spectra of Ag nanoparticles with different concentrations using NaBH_4

The color of the solutions depended on the concentration of added AgNO₃ solutions. By increasing the initial AgNO₃ concentration, the color of solution changed from yellow to brown. The absorption peak at around 400 nm in Fig. 2 is attributed to the surface plasmon excitation of Ag nanospheres, indicating the formation of Ag nanoparticles. At low concentrations of AgNO₃ (0.0001 M and 0.0002 M), weak absorption maximum of surface plasmon peaks were observed at 396 nm, showing that Ag nanoparticles were produced at a relatively low concentration. With increasing the AgNO₃ concentration, the intensity of the maximum plasmon peak increased, indicating that higher concentrations of Ag nanoparticles were formed [19].

The UV-Vis spectra of two different prepared colloidal Ag nanoparticles in terms of reducing agents and the mode of mixing the chemicals after 2 hours is shown in Fig. 3. All the samples were prepared with the same initial concentration of AgNO₃ (0.0005M).



Fig. 3 UV-Vis spectra of colloidal Ag nanoparticles, a) Ag reduced by aniline and CTAB solution, b) Ag and CTAB solution reduced by aniline, and c) Ag reduced by $NaBH_4$

It is observed that sample c which was prepared using NaBH₄ as a reducing agent and SDS as a stabilizer has a very intense and narrow peak at around 397 nm. This indicated a higher concentration of

unishaped silver nanoparticles. On the other hand, sample a and b which were prepared using aniline as a reducing agent and CTAB as a stabilizer show weak broad absorption peaks at 392 and 396 nm respectively. Aniline is a weak reducing agent, therefore a lower amount of Ag ions were reduced to Ag nanoparticles. The broadness of these two peaks can be due to different shapes and sizes of Ag nanoparticles formed using aniline [15].

The size and the shape of the Ag nanoparticles synthesized using NaBH4 as the reducing agent were further investigated by transmission electron microscope (TEM). The images revealed (Fig. 4) most of the nanocrystals had spherical shapes and the size distribution was quite uniform (5-15 nm). This particle size range is consistent with the obtained maximum absorption of the Ag nanoparticles at around 396 nm in UV-Vis spectra. The maximum absorption peak of the larger particles tends to shift to longer wavelengths [20].



Fig. 4 TEM images of Ag nanoparticles synthesized using NaBH₄.

10 nm

PMMA/Ag synthesis

In general, the miniemulsion is composed of submicron monomer droplets stabilized with a surfactant and against coalescence and with a costabilzer to minimize Ostwald ripening (Fig. 5) [21]. These droplets are not equal in size but have a droplet size distribution. Depending on the amount of surfactant used in the formulation and the homogenization procedure, micelles may be present [22].



Fig. 5 Schematic representation of miniemulsion polymerization using water soluable surfactant (Phase I is water and phase II is oil).

Miniemulsion as a one-pot synthesis method is efficient since several synthetic transformations and bond-forming steps is carried out in a single vessel and therefore, it minimizes chemical waste, saves time and simplifies preactical aspects [23]. Methyl methacrylate in this work was polymerized via miniemulsion with the use of sodium lauryl sulfate as a water soluble surfactant. Fig. 6 shows the structural representation of the reaction.



Fig. 6 Methy methacrylate polymerization.

FTIR spectroscopy of synthesized polymers was conducted using KBr plates. Fig. 7 shows the FTIR spectra of PMMA and PMMA/Ag which confirms the polymerization process. As it is shown in the spectrum of the monomer (methyl methacrylate), the peak at 1638.31 cm-1 is assigned to C=C stretching. The absence of this peak in PMMA samples shows that the polymerization was successfully conducted [24].



Fig. 7 FTIR spectra of methyl methacrylate and polymethyl methacrylate.

From the spectra, a sharp intense peak appeared at around 1720 cm⁻¹ is due to the presence of ester carbonyl group stretching vibration, C=O stretching. The peak at around 1157 cm⁻¹ is attributed to the C-O-C stretching vibrations. The band at around 2955 cm⁻¹ can be assigned to the C-H bond stretching vibrations of the -CH3 and -CH₂- groups. The intensity of the peaks at 2955 cm⁻¹ region in PMMA is higher due to the higher content of -CH2 and -CH3 vibration [24]. It is appeared that the presence of Ag in the samples did not have much of an effect on the position of the characteristic bands of PMMA. This can be due to a very low concentration of the Ag in the samples or it can be explained by the interactions between Ag nanoparticles and the polymer. The interaction between Ag nanoparticles and PMMA probably occurs through a weak physical force rather than a strong chemical bonding. This observation may also indicated that most of the PMMA molecules are either physically linked to the nanoparticles or are present without any contact with the nanoparticles [6].

The samples were further investigated in terms of occurrence of the polymerization by ¹HNMR spectroscopy. In the ¹HNMR spectra (400 MHz, CDCl3) of PMMA/Ag with SDS (a), PMMA/Ag (b) and PMMA (c) as shown in Fig. 8, there are three peaks centered at around 0.9 ppm, which correspond to the protons of the methyl groups. The peaks at around 1.3 and 3.6 ppm are attributed to methylene and protons of methyl group attached to ester functional group [26]. This data confirmed the occurrence of the polymerization in all three samples since the peak corresponding to vinylic hydrogens at around 5-7.5 ppm which can be observed in MMA spectra has been disappeared.



Fig. 8 ¹HNMR spectra of a) MMA, b) PMMA/Ag SDS, c) PMMA/Ag and d) PMMA.

The presence of Ag nanoparticles in the samples was confirmed by UV-Vis DR spectrum of the PMMA/Ag nanocomposites (Fig. 9). The PMMA/Ag synthesis was done using different Ag nanoparticles concentrations. Samples were synthesized using stabilized Ag nanoparticles with sodium lauryl sulfate that were then used in the polymerization process. However, in one sample no stabilizer was used during the Ag nanoparticles synthesis.



Fig. 9 UV-Vis DR spectra of PMMA/Ag in different concentrations with SDS and without SDS.

The absorption bands observed at approximately 400-415 nm in different samples correspond to the characteristic peak of metallic silver [27]. Studying these peaks shows that samples with higher concentration of Ag have higher intensities.

The morphology of PMMA and PMMA/Ag nanoparticles were studied by FESEM and TEM. Fig. 10 shows the FESEM images of PMMA and PMMA/Ag nanoparticles. The diameter of the spherical particles of PMMA was between 50 to 100 nm. The low tendency of aggregation in the PMMA nanoparticles as shown in FESEM image suggested that the miniemulsion system was stable by using monomer as oil-phase during the polymerization process.



Fig. 10 FESEM images of PMMA nanoparticles at a) 2,500 X, b) 10,000X and PMMA/Ag nanoparticles at c) 25,000 X d) 10,000.

The morphologies of PMMA/Ag nanoparticles shown in the FESEM image indicated non-agglomerated and some agglomerated particles which were dispersed throughout the nanocomposite. However, the fraction of non-agglomerated particles was smaller. The presence of these agglomerated particles in the sample may be due to the corporation of metallic Ag with polymer particles in PMMA/Ag composites.

TEM images of pure PMMA and PMMA/Ag are shown in Fig. 11. These images revealed that no crystalline structures were observed in pure PMMA as this an amorphous polymer. However, when Ag was incorporated with the polymer, spherical crystalline structures could be observed in the polymer matrix. To confirm these crystalline structures were Ag nanoparticles, selected area electron diffraction (SAED) pattern of Ag in the sample was captured (Fig. 11(c)). The result of d-spacing calculations on the software (2.36 Å, 2.03 Å, 1.43 Å) complied with d-spacing value of silver nanoparticles and its respective crystal planes of (111), (200) and (220) which agrees with crystalline and face centered cubic structure of Ag nanoparticles [28].



Fig. 11 TEM images of pure PMMA (a) and PMMA/Ag (b) and SAED pattern of Ag c)



Fig. 12 photograph image of the zone of inhibition of a) PMMA/Ag 0.0001 M, b) PMMA/Ag 0.0005 M, c) PMMA/Ag 0.00002 M, d) PMMA/Ag 0.00001 M and e) pure PMMA after 24h of incubation.

Antibacterial studies

Polymer/Ag nanoparticles were evaluated for antibacterial properties using Kirby-Bauer test. Gram-negative bacteria, E. coli was chosen for this test [3]. Pure polymer nanoparticles and samples containing Ag were made into pellets and placed on a lawn of bacteria in an agar plate. The antibacterial properties of the samples were then measured by the clear zone of inhibition around the pellets after 24 h incubation (Fig. 12). The diameter of the zone of inhibition for PMMA/Ag nanoparticles with the highest concentration of Ag nanoparticles (0.001 M) was ca. 26 mm where the size of the pellets

was ca. 13mm (Table 1). It was observed that the antibacterial activity of the samples reduced when lower concentrations of Ag were present in samples. Comparing the results of the polymer/Ag samples with the result of Tetracycline (30 μ g) antibiotic disk used as a standard in this test with the clear zone of inhibition of 53 mm, it can be observed that PMMA/Ag nanoparticles show relatively low antibacterial activity. This might be due to the low concentration of Ag in the synthesized samples or entrapment of Ag nanoparticles in polymer matrix which makes it difficult for them to be released.

 $\ensuremath{\text{Table 1}}$ Diameter of the zone of inhibition for PMMA and PMMA/Ag nanoparticles.

Polymer sample	Zone of inhibition (d)
Pure PMMA	0 mm
PMMA/Ag 0.00001 M	16 mm
PMMA/Ag 0.00002 M	19 mm
PMMA/Ag 0.0005 M	21 mm
PMMA/Ag 0.0001 M	26 mm

CONCLUSION

In this work, one-pot synthesis of PMMA/Ag nanoparticles and its antibacterial activity was reported. The silver, Ag nanoparticles were prepared via reduction method and then were introduced to the monomer mixture. The polymerization occured in the presence of Ag nanoparticles and the resulted nanoparticles were characterized by various methods: UV-Vis which conformed the surface plasmon excitation of Ag nanospheres both in colloidal silver and the final product, ¹HNMR and FTIR which proved successful polymerization. The TEM and FESEM showed the morphology of the product and further proved the presence of Ag nanoparticles in the samples. The antibacterial activity of prepared nanoparticles was tested on gramnegative bacterial strains. The result of the antibacterial tests indicated that increasing the concentration of Ag nanoparticles in the samples can significantly enhance the elimination of bacteria.

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