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Sequential repetitive chemical reduction technique to study size-property relationships of graphene attached Ag nanoparticle

M. Salman Haider [a], Abimbola Comfort Badejo [b], Godlisten N. Shao [c, d], S.M. Imran [c], Nadir Abbas [c], Young Gyu Chai [b], Manwar Hussain [c, *], Hee Taik Kim [c, *]

[a] Department of Civil and Environmental Engineering, Hanyang University, 1271 Sa 3-dong, Sangnok-gu, Ansan-si, Gyeonggi-do 426-791, Republic of Korea
[b] Department of Molecular and Life Science, Hanyang University, 1271 Sa 3-dong, Sangnok-gu, Ansan-si, Gyeonggi-do 426-791, Republic of Korea
[c] Department of Chemical Engineering, Hanyang University, 1271 Sa 3-dong, Sangnok-gu, Ansan-si, Gyeonggi-do 426-791, Republic of Korea
[d] Department of Chemistry, Mkwawa College, University of Dar es Salaam, Iringa, United Republic of Tanzania

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ABSTRACT

The present study demonstrates a novel, systematic and application route synthesis approach to develop size-property relationship and control the growth of silver nanoparticles (AgNPs) embedded on reduced graphene oxide (rGO). A sequential repetitive chemical reduction technique to observe the growth of silver nanoparticles (AgNPs) attached to rGO, was performed on a single solution of graphene oxide (GO) and silver nitrate solution (7 runs, R1–R7) in order to manipulate the growth and size of the AgNPs. The physical–chemical properties of the samples were examined by RAMAN, XPS, XRD, SEM-EDAX, and HRTEM analyses. It was confirmed that AgNPs with diameter varying from 4 nm in first run (R1) to 50 nm in seventh run (R7) can be obtained using this technique. A major correlation between particle size and activities was also observed. Antibacterial activities of the samples were carried out to investigate the disinfection performance of the samples on the Gram negative bacteria (*Escherichia coli*). It was suggested that the sample obtained in the third run (R3) exhibited the highest antibacterial activity as compared to other samples, toward disinfection of bacteria due to its superior properties. This study provides a unique and novel application route to synthesize and control size of AgNPs embedded on graphene for various applications.

1. Introduction

There has been considerable evaluation and utilization of the unique properties of metal nanoparticles in biological applications [1–4], conductive polymers [5,6], sensors [7,8], electrochemical devices [9] and optoelectronic devices [10–12]. These properties mainly depend on the appropriate size of particular nanoparticles. Consequently, varying the size of nanoparticles can change the entire physicochemical properties of the materials [13]. For instance, size affects the surface area and distribution leading towards the formation of nanoparticles with significant properties as compared to the bulk material [13,14]. This has opened a new door to many other fields in terms of research and application. Therefore, the periodic change in the nanostructure of many materials as a function of size is the main topic of nowadays.

Meanwhile, the research on the coarsening techniques that controls the size of nanoparticles and its growth in solutions reflects tremendous scientific and technological importance. Recently, there has been a revived concern in the research of growth process of nanoparticles in solution. Qin et al. [15], reported the change in the color of gold nanoparticles with respect of their size. Sun et al. [16], explained the effect of size of metallic nanoparticles on various chemical and physical properties. In the process of providing a sequel of size-metallic nanoparticle applications correlation, Xu et al. [17] and Gogoi et al. [2], revealed that the AgNPs less than 10 nm are more toxic to bacteria such as *Escherichia coli*. Indeed, Elechiguerra et al. [18], explained that AgNPs ranging from 1 to 10 nm inhibit certain virus from binding to host cell by preferentially binding to the virus. Unfortunately, a comprehensive analysis of the available reports shows lack of or inconsistent information describing physicochemical properties of the nanoparticles in relation to their size variations [19]. Because of

* Corresponding authors.
E-mail addresses: manwarh@hanyang.ac.kr (M. Hussain), khtaik@yahoo.com, khtaik@hanyang.ac.kr (H.T. Kim).

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nonlinear relationship between the applications and size, extrapolation of the properties from bulk material to nanomaterials is quite difficult. Many researchers have been trying to make a bridge between the variation in size and application by computational approach [20,21] as performing experiments to obtain systematic data for size-application correlation involves complicated synthetic approaches and is costly [19]. Thus, there is great demand to provide facile synthetic approaches to fabricate metallic nanoparticles with desirable size for antibacterial applications.

In recent, various chemical and physical methods has been used for the synthesis of nanoparticles, such as chemical reduction [22], laser ablation [23], photocatalyst [10,24], molecular beam epitaxy [25], chemical vapor deposition [26] thermal decomposition inorganic solvents [27]. Nanoparticles synthesized by physical method require expensive equipment, high temperature, pressurized condition whereas chemical methods are comparatively facile and cost-effective. Moreover, chemical methods are the most convenient and reproducible routes for the fabrication of nanocrystals with controlled size and shape [28]. However, the main limitation of this process is over-reliance on the average size and size distribution [29]. Chemical reduction or solution synthesis approach is common to produce nanoparticles with a controlled particle size and distribution.

Metallic nanoparticles have interesting role in wide number of applications and insertion of these nanoparticles on graphene has been enormously studied [30]. Recently, numerous metals or metal oxide semiconducting nanoparticles such as Ag, Co, Pt and Au based materials have been incorporated into 2D graphene structures [31–34]. Particularly, Ag nanoparticles (AgNPs) have been extensively focused due to its unique applications on antibacterial effects [1,35–37]. Many efforts have been made to synthesize AgNPs decorated on graphene with controllable shape, size and distribution using chemical reduction method [38], radiolytic method [39] and polyol method [40]. However these reports overlook the co-relationship between synthetic methods, size and applications. Therefore carrying out a systematic investigation on this relationship would further stimulate the production of rGO–AgNPs with desirable properties for different applications. Among all the above methods, nanoparticles can easily be synthesized by chemical reduction method and so the mechanism of varying size of the nanoparticles can be studied using solution synthesis technique.

In this study a sequential repetitive chemical reduction technique was used to obtain AgNPs with different particles sizes embedded on graphene. In this novel technique same solutions of substrate and precursor was reduced repeatedly in order to increase the particle size of the nanomaterial. Using the sequential repetitive chemical reduction approach the particle size can be easily controlled depending on the desired applications. The antibacterial properties of the as-synthesized nanocomposites were tested using *E. coli* bacteria to reveal the practical reliability of the samples. Even though there is a few reports [3,29,37,41,42] have exquisitely investigated the antibacterial properties of the rGO–AgNPs but none have systematically shown the relationship between the size of nanoparticles and applications using the technique reported herein.

2. Material and methods

2.1. Materials

Graphite, Potassium permanganate powder (~325mesh, 97%) and $AgNO_3$ (ACS reagent 99+%) were purchased from Sigma–Aldrich. HCl (extra pure reagent, ~35.0%), H_2SO_4 and $NaBH_4$ were procured from Dae-Jung Chemical and Metal Co. Ltd, Korea.

2.2. Synthesis process

2.2.1. Preparation of graphene oxide

The graphene oxide (GO) samples were prepared by a modified Hummer's method [43] outlined in our previous study [44]. Briefly, the expandable graphite powder (2 g) was stirred in 98% H_2SO_4 (35 ml) for 2 h. $KMnO_4$ (6 g) was gradually added to this solution while keeping the temperature less than 20 °C.The mixture was then stirred at 35–40 °C for 30 min, and then at 65–80 °C for 45 min. The resulting solution was diluted by adding 46 ml of water and the mixture heated at 90 °C for 30 min. The reaction was terminated by addition of 150 ml of distilled water and 30% H_2O_2 solution (10 ml). The mixture was washed by repeated centrifugation with 5% HCl aqueous solution followed by deionized water until the pH of the solution becomes neutral. To obtain GO particles, 160 ml of water was added resulting precipitate and sonicated well to make a uniform suspension for graphene oxide, which was then confirmed by Raman and XPS analysis further explained in subsequent section. This reddish brown solution was used for the synthesis of Ag graphene nanocomposites.

2.2.2. Preparation of silver nanoparticles on GO

AgNPs were prepared by chemical reduction method in which a sequential repetitive chemical reduction of low concentration of $AgNO_3$ in GO suspension was performed in multiple runs i.e. R1, R2, R3, R4, R5, R6 and R7, consecutively. Initially, GO and $AgNO_3$ were simultaneously reduced with $NaBH_4$ and after every run the mixture was washed, centrifuged and used again in the next run. In R1 2.42×10^{-4} mol/L of $AgNO_3$ was mixed with GO suspension and stirred for 30 min. Then 5 ml of 0.25 M freshly prepared $NaBH_4$ solution was gently added to the solution under stirring at 1500 r.p.m. Reaction mixture was stirred for 12 h at room temperature to allow complete reduction of silver. The mixture was centrifuged, washed and a portion of the sample was dried for analysis while the rest of the sample was sonicated for use in the next run. The same synthetic routes were followed through decreasing the concentration of the $AgNO_3$ in order to yield R2 through R7 samples as is shown in Table 1.

2.3. Antibacterial activity of rGO–AgNPs

The antibacterial activity of the synthesized rGO–AgNPs was investigated through examining their disinfection performance of Gram negative bacterial strain (*E. coli*), acquired from the American type culture collection under the code ATCC 25922. The bacterial culture was maintained in Nutrient broth (NB) at 37 °C with continuous shaking at 200 rpm for 4 days. The bactericidal effect of the synthesized samples was carried out in a nutrient agar medium using zone of inhibition and concentration-contact testing methods.

Table 1
A summary of silver salt used and corresponding colony count of that sample in CFU/ml.

Name of samples	Amount of $AgNO_3$ (mg/L)	CFU/ml			
		0 min	5 min	10 min	20 min
R1	2.42×10^{-4}	4.9	4.2	3.8	2.4
R2	1.68×10^{-4}	4.8	3.8	2.6	1.3
R3	1.29×10^{-4}	4.7	2.3	0.1	0
R4	1.05×10^{-4}	4.9	2.7	0.5	0
R5	8.82×10^{-5}	4.7	3.0	1.1	0.1
R6	7.61×10^{-5}	4.8	3.6	2.5	1.4
R7	6.69×10^{-5}	5.0	4.5	3.7	2.8
Control	–	5.1	4.9	4.5	3.9