The Sonochemical Impregnation of Silver Particles into Fluff Pulp for Enhanced Antimicrobial Efficacies

Sunil Shafqat[1], Christy Munir[1] and Mudassar Abbas* [1,2]

[1]Department of Chemistry, Forman Christian College, Ferozepure Road 54600, Lahore, Pakistan.

[2]School of Textile and Design, University of Management and Technology, C-II Johar Town Lahore54770, Pakistan.

* Corresponding Author, mudassirabbas@yahoo.com , mudassarabbas@fccollege.edu.pk

Abstract

Herein, the preparation of silver particle containing microfibril cotton as filling material and fluid dispersant with intrinsic anti-microbial and anti-fungal properties is presented. Upon finding cherished results for such characteristics through different assays, it may be concluded that the protection against the bacterial and fungal infections can be improved and hence the contact time of such products with the body. Whereas, the overall appalling effects on health could also be reduced by application of such materials. The cotton fluff pulp was extracted from commercial diapers and without any further treatment, the loading of silver particles through chemical and sonochemical methods was established. The characterization of the material for silver-ion loadings was accomplished via using multiple instrumental techniques majorly UV-Visible spectrophotometer, thrmogravemetric analysis and elemental analysis whereas, for antimicrobial efficacies, disc diffusion method was incorporated for detailed antimicrobial action against *Escherichia coli* (*E. coli)* colonies. Finally, the optimal amounts of silver were designated to maximize the beneficial effects and it was observed that no less than 0.4 wt.% silver ion concentration when applied under sonification based reduction for fluff pulp, were effective for strong antimicrobial effects.

Keywords: Sonochemical method, Silver particles, Impregation, antimicrobial efficacies

Introduction

During the last two decades, silver nanoparticles have gained much attention due to exclusive antimicrobial applications introduced as the state of the art technology for controlling pathogens [1]. Since beginning, when this distinctive property has been revealed, it has always been an inquiry that what role of silver makes it special against microbes and so is preferred over other metals. Until lately, it has been proven that distributed silver-ions (Ag^+) over the material, are responsible for enhanced efficacy against drug resistant microbes [2]. Due to their small size silver nanoparticles are very effective and altruistic donor of silver ions. Acquiring the same characteristic, silver nanoparticles have been used in different materials where antimicrobial properties were desired [3,4,5]. Two worth-mentioning examples are incorporation of silver nanoparticles with bacterial cellulose for antimicrobial wound dressings [6] and impregnation into paper sheet for water treatment [7]. Apart from antibacterial effects, silver nanoparticles have also been found effective against different viruses [8,9]. For instance, human immunodeficiency virus (HIV) and genital herpes are becoming a big problem and silver nanoparticles based materials showed impressive inhibition of HIV-1 when applied on human cervical tissue [10]. Similarly, herpes simplex and hepatitis B viruses have also been inhibited by silver nanoparticles [11]. The presence of highly efficient particles giving outperforming results against microbes lead to designing of fabrics loaded with such material that later may be used for making cloths and garments containing self-cleaning functions within [12].

Several methods have been proposed for incorporation of silver nanoparticles into cellulose based materials. Majorly, it include; the borohydride reduction of silver ion in presence of bacterial cellulose [6], microwave assisted loading of nanoparticles into cellulose [13], greener route for synthesis and attachment to cotton using natural extract [10], and UV-irradiated cellulose acetate fiber with silver via electrospun with $AgNO_3$ [14]. In all of these examples, the reducing and stabilizing properties of cellulose were also utilized during nanoparticle incorporation [15]. However, the most attractive example for producing such materials at laboratory scale has been encountered by Fottesman et al. where sonochemical method for coating of paper with silver nanoparticles has been reported [16]. The sonochemical treatment is preferred over other methods for reduction due to its ease of availability and use, very low economic prices of the process and most importantly least side reactions and harmful wastages which generally are encountered when alternatives are applied. In the current study, similar method has been chosen as the test system for impregnation of silver particles into fluff pulp fiber for production of silver fluff pulp composite (SFPC). Fluff pulp fiber is the important part of absorbent core in diapers and feminine hygienic products. It was expected that such material when combined with silver ions to form composite material can help in reducing the major problems related to infections in women and children, generally caused by growth and sustainability of microorganisms [17]. The microorganisms like Candida albicans, Chlamydia trachomatis, Neisseria gonorrhoeae, Trichomonas vaginalis and Staphylococcus epidermidis are natural part of skin flora [18]. The change in bacterial microbiota due to antibiotic abuse can result in increase in fungal population and hence can cause infections [19].

Materials and methods

All chemicals were purchased from commercial resources or synthesized in laboratory if mentioned otherwise. Similarly, all the solvents were distilled according to standard procedures before use [20]. Silver nitrate ($AgNO_3$), aqueous ammonia (NH_4OH) and sodium borohydride ($NaBH_4$) were obtained from Merck chemicals, whereas ethanol was purchased from Scharlau chemicals. Fluff pulp was obtained from the regular zero size pampers (disposable baby diapers), a product of Uniliver and was used without any further treatment. 1 mg/ml or 0.01M Ag^+ solution was prepared by dissolving 1.70 g of $AgNO_3$ in 50 ml of deionized water and making the volume up to 1000 ml. $NaBH_4$ solution of 0.02M was prepared by dissolving 800 mg of it in 50 ml of deionized water and making the volume up to 1000 ml. 12.12 ml of the 33% of aqueous NH_3 solution was diluted to 100 ml to make 4% NH_3 solution.

Impregnation of silver particles using $NaBH_4$

50 g of the fluff pulp was weighed out. It was dipped in 100 ml of the $AgNO_3$ solution containing 10 ml of 4% ammonia solution for 30 min. Ammonia was added to minimize control the Ag^+ in the solution [21]. Afterwards, it was removed from the solution and excess amount of silver solution was allowed to drip off. The sample was washed with ethanol and placed in 100 ml of the 0.002 M $NaBH_4$ solution for 10 min [6]. The color of the fluff was observed to change from light yellow to the appearance of dark brownish black tint. The reaction followed between silver nitrate and sodium borohydride is given in scheme 1. [22].
Afterwards, the sample was removed from the solution and excess water was allowed to drip off. At final stage, it was washed with 1:2 ethanol and water mixtures and dried at 50 ^{0}C overnight in memmert oven.

Impregnation of Silver Particles via Sonochemical Method

50 g sample of fluff pulp was immersed in 100 ml of silver nitrate solution in a 250 ml beaker. The sample was irradiated in ultrasonic bath (FS-60 Fisher Scientific). Frequency of irradiation was 40 KHz at power of 130 watt. Irradiation was done for 3 hour, [H] and [OH] formed in the process reduced the Ag^+ [23] onto the fluff pulp fibers. The color of the fluff pulp was observed to change from white to light yellow and finally reddish brown tint appeared. The sample was removed from the solution. After removing the sample, it was washed with 1:2 ethanol water mixtures and dried at 50 ^{0}C overnight in memmert oven.

Characterization

The silver fluff pulp composite (SFPC) was characterized using different tools to observe silver nanoparticles on the surface of the fiber. UV-Visible (Double beam PC 8 scanning auto cell UVD-3200) was used to obtain UV-Visible spectra of the particles. A colloidal solution of silver fluff pulp was made in DMSO/ paraformaldehyde [24]. Excess of water was added and phase transfer into chloroform was performed to take UV-visible spectra. Thermogravimetric analysis (TGA SDTQ 600 TA) was performed to determine percentage silver loading in the sample [13]. Elemental analysis was carried out on pure fluff pulp sample obtained from the commercial diaper by using CHNS/O analyzer (Leco TruSpec® Series). Antibacterial property of the SFPC was qualitatively tested using modified Kirby-Bauer method on E.Coli. Sonochemically treated sample was then compared with SNFPC synthesized by sodium borohydride reduction in the presence of ammonia.

Antimicrobial Studies for SNFPC

Modified Kirby Bauer (diffusion method) was used of qualitatively testing the antibacterial activity of silver fluff pulp composite (SFPC) [25]. Mueller-Hinton agar (MHA) was used as a medium for growth of bacteria. Prepared agar plates were used for *E. coli* from healthy colonies on the plate was applied by cotton swab and it was used to prepared suspension in saline solution. Turbidity of the suspension was adjusted to 0.5 McFarland standards using Wickerham card [26]. This suspension was used to streak the agar plates, the agar surface was carefully streaked with the inoculum to cover the entire surface. Finally, the untreated fluff pulp, sonochemically treated silver fluff pulp and chemically treated silver fluff pulp samples were moisturized with previously autoclaved deionized water, the samples were then placed in the center of the plates with a forceps and pressed against the agar. The plates were incubated for 22 hours, after which the results were observed. An exemplary plate with incubated results for silver particles is shown in Fig 1.

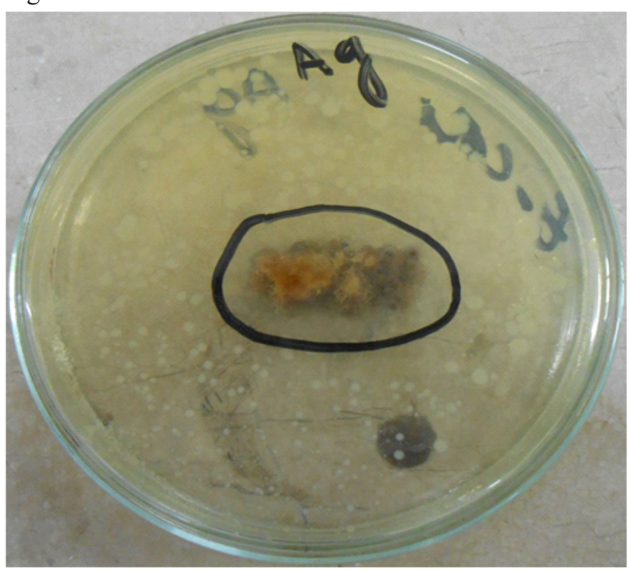

Fig 1: Silver particle loaded cotton incubated plate represnting resisted E.Coli bacterial growth after 22 hours.

Results and Discussion

The sonochemical method for the reduction of transition metal from respective salts has been reported multiple times in

Scheme 1.

$$AgNO_3 + NaBH_4 \longrightarrow Ag^o + B_2H_6 + H_2 + NaNO_3$$

literature [27,28]. The special phenomenon of cavitation occurs when ultrasonic compressional waves pass through the liquids. It is characterized by formation, growth and subsequent collapse of bubbles in the liquid. When the bubbles collapse, extreme conditions are generated. In sonification method, it is assumed that the variation in temperature and pressure of the liquid results in pyrolysis of water molecules [29]. Thereby, the Ag^+ ions from salt precursor are reduced in the solution by [H] produced by sonolysis of water. Hydroxyl groups containing oxygen in the cellulose/hemicellulose matrix can stabilize these particles through electrostatic interactions [30]. By employing the same principle, the aqueous solution containing fluff pulp and silver ion resource was chosen as the test system for the current investigation. To compare the results for incorporation of silver into the cellulose matrix borohydride reduction was chosen as the standard and the results were compared with the sonochemical incorporations. Elemental analysis for untreated fluff pulp was carried out for determination of different ratio of carbon, hydrogen, nitrogen, sulphur and oxygen CHNS/O and the results are given in Table 1. The empirical formula from the percentages given in Table 1 was found out to be $C_5H_9O_4$ which was in accordance to the data already found in literature [31].

Table 1: The elemental analysis of cellulose fiber from fluff pulp via CHNS/O analyzer

Element	Composition (%ge)
Carbon	45.065%
Hydrogen	6.765%
Oxygen	48.169%
Nitrogen	0.000%
Sulphur	0.000%

For the next experimental system, UV-visible absorption spectra were obtained for confirmation of silver particle containing solutions. Fig 2 shows comparison graph of sonochemically and chemically synthesized silver particles respectively. The absorption maxima were observed at 424 nm for sonochemically synthesized silver particles while a redshift was observed in case of chemically synthesized particles. The absorption maxima for chemically synthesized silver particles were observed at 428 nm. This can be accounted for a comparison between the reducing strength of sodium borohydride and sonochemical reduction. A large number of silver ions are reduced in first few seconds after coming in contact with borohydride. These small particles result in growth of larger particles by Ostwald ripping [32] as in the presence of excess of borohydride the equilibrium lie on the side of Ag^0. In case of sonification the equilibrium lies towards Ag^+ when the irradiation is discontinued. This can explain large population of small silver particles in case of sonochemical synthesis. As expected, the weak surface adsorption of silver ions in case of sonochemical impregnation leads to larger number of free particles in the fluff pulp solution available for phase transferring into the chloroform layer. Fluff pulp saturated with silver nitrate when reduced by borohydride must have resulted in strongly adsorbed or even chemically bonded silver particles. A whole new set of study is required to elaborate the detailed phenomena of these results that can be attributed as the topic for further investigation.

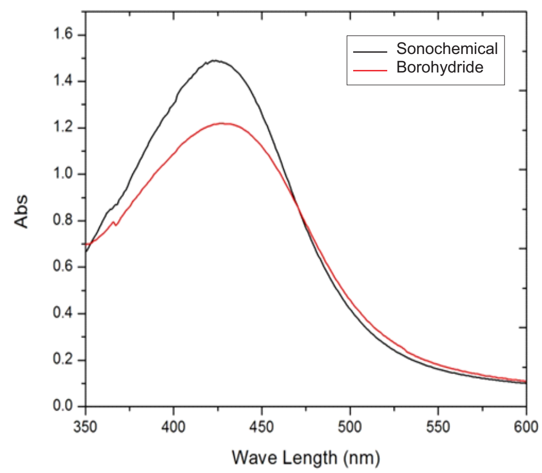

Fig 2: UV-visible spectra of sonochemically- and borohydride-reduced particles into fluff pulp.

Thermogravimetric analysis using (TGA SDTQ 600 TA) was carried out on the three samples to determine percentage silver loading by method of differences in values. The samples were heated from 25 ^{0}C to 800 ^{0}C and percentage weight loss with change in temperature was obtained for each sample. The percentage residual weight was obtained for untreated fluff pulp, sonochemically treated fluff pulp and chemically treated fluff pulp. The results are presented in Fig 2. The highest silver loading was observed in case of sodium borohydride sample. This observation is again consistent with the fact that all silver in solution absorbed by the 50 g of fluff pulp must have been reduced by an excess of sodium borohydride. As a comparison, in case of sonochemical treatment, where silver particles formed near the fiber liquid boundary were stabilized by adsorbing onto the fiber surface. This is evident from the absence of silver particles in the sonicated solution, where sharp changes in the color of fluff pulp were observed over the course of sonification. Similarly, more intense color change was observed in case of borohydride reduction. This physical change may be attributed to presence of some stray silver ions that were found on their way out of the silver fluff pulp composites (SFPC).

The silver fluff pulp composites (SFPC) after incubating for 22 hours with bacterial colonies were observed for growth in plates and the samples were compared with untreated ones. The samples of fluff pulp of all kinds treated with silver particles and untreated were observed for in detailed analysis over the surface and in depth core of the material. For sonochemically and chemically treated fluff pulp, no sharp growth of bacterial colonies was observed inside the sample and on the outer core whereas the bacterial growth could not be resisted under the untreated sample as compared to silver ion containing composites prepared by either method (*cf.* Fig1.). The inhibition zone around the samples was also observed which indicated qualitative antimicrobial activity of the composite.

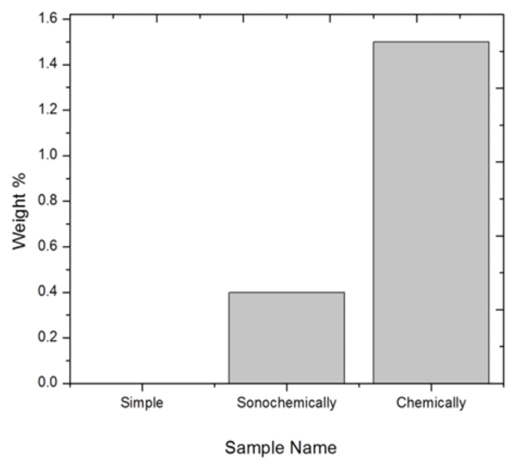

Fig 3: The percentage loadings of silver particles observed via TGA/DSC analysis

Conclusion

From the current study, it may be concluded that silver particles when introduced into fluff pulp (cellulose) obtained from commercially available baby diapers enhances the bacterial resistant properties of the material and hence may persist in low growth of bacterial colonies. The sonochemical method is preferred over chemical treatment, due to adverse loading of silver particle and strong variation in color of fluff pulp in the later case. In future, this material can be recommended for practical application at larger scale only after clearing the tests from dermatological laboratories for interaction of silver with skin.

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