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Investigations on Different Sol-gel Incorporation Methods of Green Synthesized AgNPs in Textiles for Antibacterial Activity

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Article

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ABSTRACT

Sol-gel is an excellent antibacterial agent carrier. Different researchers incorporated various antibacterial substances, including silver nitrate ($AgNO_3$), quarternary ammonium chloride (QAC), and titanium dioxide (TiO_2) in sol-gel. However, there is limited study on the influence of pH and acid hydrolysis time (ageing) to form sol-gel. Besides, few investigations have been made on the influence of fabric structure and silver nanoparticles (AgNPs) incorporation into fabrics by the sol-gel method. This study also compared the light and heavy fabrics in terms of sol-gel application and the advantages of sol-gel over other AgNPs incorporation methods. The sol-gel-AgNPs incorporated fabric samples were characterized by Ultra Violet Spectroscopy (UV-VIS), X-ray diffraction (XRD), Scanning Electron Microscope (SEM), and Energy Dispersion spectroscopy (EDS). For AgNPs synthesis in a green way, we used Calendula arvensis, a Mediterranean weed. This study found, that for sol-gel formation the minimum acid hydrolysis time was 5.30 hours, 6.30 hours, and 8.00 hours at 60 ^oC, 25 ^oC, and 3 ^oC, respectively. After ageing, a minuscule amount of alkaline was needed for gelation. Seven different methods for incorporating AgNPs through sol-gel have been illustrated. The lower molarity of $AgNO_3$, having a more significant portion in the sol-gel solution, had excellent antibacterial activity and wash fastness. Besides, the ex-situ method was better than the in-situ method. Among different types of cotton fabric, lightweight knit fabric showed much better antibacterial activity than heavier twill fabric.

KEYWORDS

sol-gel, green synthesis, silver nanoparticles, antibacterial textiles, cotton fabrics

INTRODUCTION

Sol-gel is used for making aerogel, the lightest material in the world, most rigid ceramics, protective coatings, biomedical, nano-powder, optomechanical products, medicines, antimicrobial, water repellent substances, etc. It also has a very promising scope in textiles. Sol-gel is a simple, effective, and one of the cutting edge technologies in functionalizing textile materials. It is believed to be an excellent host for different antibacterial agents. The most important benefit is that it needs only room temperature [1]. Mahltig et al. claimed that sol-gel is good at fixing silver and its compounds within a

modified silica matrix [2]. Other researchers used various methods to incorporate antimicrobial agents in sol-gel. Jeon et al. mixed $AgNO_3$ in Tetraethyl orthosilicate (TEOS), 2-ethoxy ethanol, and distilled water, waited 4 hours for ageing, and then stirred for extra 2 hours with $AgNO_3$ at room temperature [3]. Then they exposed it to a high temperature (200 ^oC - 600 ^oC) to get a yellow film. Singh et al. used TEOS, water, and ethanol at 1:4:3 proportions with HNO_3 as a catalyst (to maintain pH =2) [4]. They used a non-ionic surfactant (span-80) and paraffin for applying this silica sol. Desired materials (such as hydrophobic, antimicrobial, or other properties) can be added to the sol liquid [5]. Dai et al. prepared AgNPs having 1-5 nm size involving dodecanethiol by sol [6]. Tarimala et al. also used dodecanethiol for capping AgNPs incorporation [7]. In another investigation, Lin et al. immersed oxygen plasma-activated cotton into anhydrous ethanol, APP, TEOS, HPDMS mixture, and ammonia to control pH [8]. QAC-incorporated sol-gel showed 99% bacterial reduction for both gram-positive and gram-negative [9]. Similarly, Abramova et al. used TiO_2 and got similar antibacterial activity [10]. Zhang et al. used titanium (IV) butoxide polytetrafluorethylene to prepare sol and later incorporated AgNPs through a layer-by-layer technique to release silver ions to have antibacterial activity of the steel substrate [11]. They used N, N, N', N' – tetramethylethylenediamine to synthesize silver nanoparticles from silver nitrate. Rivero et al. incorporated AgNPs into textiles through the sol-gel method using borane dimethylamine complex (DMAB) to synthesize AgNPs from silver nitrate [12]. Silver nanoparticles (AgNPs) can be synthesized by physical and chemical methods. The physical methods are slow, costly, and imperfect whereas the chemical methods are fast, accurate, and cheap. To synthesize AgNPs in chemical methods, the silver ions (Ag^+) need to be reduced to silver atoms (Ag), The silver atoms tends to aggregate due to changes in free energy, hence the aggregation must be stopped after a certain level which is termed as capping or limiting growth. So it needs reducing and capping agents. Traditional reducing and capping agents are costly and sometimes toxic whereas the biological extracts (plants or microbes) can simultaneously act as both reducing and capping agents [13]. In this study, we used *Calendula arvensis,* a well-known medicinal plant in Mediterranean regions, to synthesize AgNPs.

Sol-gel has multiple applications to functionalize textiles. Abidi et al. modified cotton fabrics with titania nanosols for self-cleaning and UV protection [14]. Mahltig et al. produced water, oil, and soil-repellent antibacterial textiles with silica sols [2]. Brzeziński et al. showed hybrid SiO_2*/Al_2O_3 sols increase the abrasion resistance of cotton fabric by five times [15]. Additionally, Periyasamy et al. mentioned that sol-gel could be used in oil/water separation, heat storage, flame retardant, photochromic colour change, etc [16].

Sol-gel consists of subsequent hydrolysis and condensation processes [17]. The hydrolysis process takes time. Some researchers claimed this time should be 2-3 days [18]. Again, others mentioned it is 24 hours [19]. However, there is no study on the exact time of hydrolysis at different temperatures.

Moreover, in the textile application, we did not find any work on the effect of textile parameters on antibacterial properties. Besides, there is no study on the influence of different parameters such as loading methods, silver nitrate concentration, reducer amount, antibacterial activity, and wash fastness. There is also a lack of knowledge on comparing sol-gel to traditional AgNPs applications in textiles.

This study aims to investigate the required time for acid hydrolysis at three different temperatures. It compares seven different methods to incorporate AgNPs in textiles using sol-gel regarding the antibacterial properties of textiles. Then, the influence of different factors of silver, reducing agents (plant extract), and fabric parameters on antibacterial textiles are discussed. We also investigated how the sol-gel method is better than other methods for antibacterial activity regarding wash fastness.

EXPERIMENTAL

Materials and Methods

This study used analytical grade Tetra Ethyl Orthosilicate (TEOS), Silver Nitrate ($AgNO_3$), Ethanol, Muller Hinton agar, Nutrient broth, and Citric acid. All the chemicals were analytical grade and purchased from Merck, Germany. Two types of cotton fabrics were used: twill and knit. The fabrics were collected from local textile mills. Here the twill fabric was heavy (253 g/m^2), and the knit fabric was light (154 g/m^2). Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Klebsiella pneumoniae*) were used for the antibacterial test. The bacteria were obtained from the microbiology research unit of the university.

Fabric Pretreatment

Sol-gel needs slight alkalinity after acid hydrolysis. Hence, we pretreated the fabrics with NaOH so that the residual NaOH would suffice for the alkaline condition. For pretreatment, we used 4 g NaOH per 1 litre of water. First, the fabrics were immersed in NaOH solution and boiled at 90 °C for 1 hour. Then again, boiled for 1 hour with distilled water to reduce alkalinity. Finally, the samples were air-dried at 70 °C for 15 minutes. The induced alkalinity of fabrics acts as a catalyst for sol-gel formation.

Sol preparation

TEOS, ethanol, and dilute acid with a ratio of 5:21:1 were used for sol preparation. Hence, 20 ml TEOS was dissolved in 84 ml Ethanol and stirred. Then 4 ml of 0.01N HCl were added in stirring condition. The solution was aged for 24 hours for acid hydrolysis. Then the aged TEOS solution was used in all the sol-gel-AgNPs applications. As citric acid is a good cross-linker for cellulosic fabric, we tested citric acid instead of HCl and found the same results. So, the rest experiments were conducted with citric acid.

Plant extract preparation

The *C.arvensis* plant was collected from the university garden. First, the cut plants were dried in the shade for a few weeks. Then the plant's stalk and leaves were separated and cut into small pieces. Next, 2.5 g of dried *C.arvensis* plant stalk was placed in 150 ml distilled water and heated until the colour of the water changed. The plant extract solution was then filtered and stored at 3 ^{o}C for future use.

AgNPs preparation

First, the $AgNO_3$ solution was prepared with different molarity. In another beaker, the plant extract was heated at 60 °C. 100 ml of $AgNO_3$ solution was taken in a 500 ml Erlenmeyer flask and placed on a magnetic heater with a stirrer, and the temperature was constantly monitored. When the temperature rose to 60 °C four drops of heated plant extract were added in the flask with continuous stirring. The colour of the solution changed within a few minutes.

Sol-gel methods

Method -1 (In-situ- sol-gel)

In this method, 100 ml of 96% Ethanol was mixed with 23.5 ml TEOS in stirring condition. Then 4 ml of 2% citric acid (2gm citric acid in 100 ml water) was added to the solution. For acid hydrolysis, the solution was kept at room temperature (25 ^{o}C) overnight. Then this solution was poured on the scored fabrics. The scoured fabric samples were already alkaline; hence additional alkaline solution was not used in the TEOS solution. In this way, the sol-gel film formed on the fabrics. After drying at room temperature (25 °C), the fabrics were washed several times with tap water. No antibacterial agents were added here in the sol-gel.

Method-2 (Dip coating)

It was done in 2 steps:
Step-1 (AgNPs formation): 20 ml $AgNO_3$ (0.08 M) was heated to 60 °C, and 3 ml plant extract (also at 60 °C) was mixed. Within 3 minutes, the AgNPs were formed.
Step-2 (Sol-gel solution preparation): The fabrics were treated with 24 hours aged TEOS solution (TEOS + Citric Acid+ Ethanol). Then without any delay, the prepared AgNPs were applied to it (Figure 1). Then the samples were kept in the open air to dry. Finally, they were washed with tap water and air-dried. The fabric turned to violet colour.

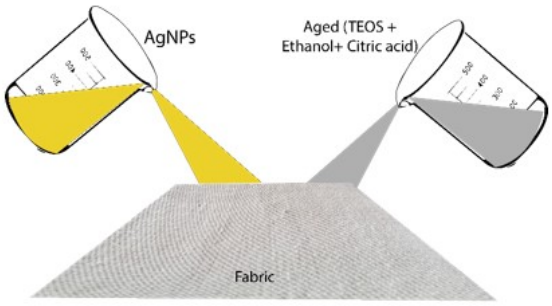

Figure 1. Process flow of method-2 (dip-coating)

Method-3 (In-situ AgNPs -0.08M)

TEOS-AgNO3 mixture: The TEOS mixture was prepared by mixing Tetraethyl orthosilicate (TEOS), Ethanol, and Citric Acid with the same ratio (5:21:1). 18 ml $AgNO_3$ (0.08 M) was mixed with 18 ml TEOS solution (1:1 ratio) and kept at room temperature (25 ^oC) overnight for acid hydrolysis. The plant extract solution was prepared by adding 13 drops of plant extract to 38 ml of water. Next, the scoured fabrics were immersed in the plant extract solution for 10 minutes. Then, the aged TEOS-AgNO3 (1:1) mixture was poured onto the fabric sample. The samples were kept at room temperature (25 ^oC) for simultaneous AgNPs synthesis and sol-gel formation. When the samples were dried up, they were washed with tap water. Then again, the samples were air-dried. In this method, the fabric also turned to a violet colour (such as in Method 2).

Method-4 (Spray)

The fabric was immersed in a mixture of 15 ml acid-hydrolyzed TEOS and 15 ml $AgNO_3$ (0.08 M). Then the samples were kept at 60 ^oC for 10 minutes. Next, on the treated fabrics, the plant extract (13 drops + 38 ml water) was sprayed on both sides of the fabrics. Later, the fabrics were kept at 60 ^oC. Finally, it was washed with tap water. This time the cloth turned to a brown colour.

Method-5 (in-situ-AgNPs-2mM)

Four drops of plant extract were added to 22 ml of water to prepare the plant extract solution. Next, the scoured fabrics were soaked in a plant extract solution. Next, the fabrics were immersed in a mixture of 50 ml of 0.002 M $AgNO_3$ and 50 ml acid-hydrolyzed TEOS solution (1:1). Next, the samples were dried in an air-drier. Finally, the samples were again washed and air-dried. The colour of the cloth remains white.

Method-6 (Immersion -1mM-2:1)

100 ml of 0.001 M $AgNO_3$ was synthesized by 3 drops of plant extract. Then 40 ml of these synthesized AgNPs was added to the 20 ml acid-hydrolyzed TEOS solution (AgNPs: TEOS = 2:1). Subsequently, the scoured fabric was immersed in this solution. After 3 minutes, the sol film with AgNPs formed. The colour of the fabric also remains white.

Method-7 (Immersion-1mM-1:1)

Same as Method-6 but the ratio of AgNPs: sol-gel was 1:1. The colour of the fabrics remained white.

Characterizations

Fourier-transform infrared spectroscopy (FTIR-6700, Jasco) was used to obtain the infrared spectrum of transmittance of the liquid plant extract. The spectral range was 449 to 4000 cm^{-1}. XRD (PANalytical, EMPYREAN XRD) was used to determine the physical properties of the synthesized AgNPs incorporated sol-gel samples by analyzing the crystal structure. The step size of 2theta was 0.0001 and ranged from 10 ° to 90 ° with Cu anode. The surface of the sol-gel coated fabrics was analyzed by SEM (FEI, model Quanta 650). At the same time, Ag and Si contents in the samples were confirmed by EDS.

Antibacterial Test

For the antibacterial test, *S. aureus* (a Gram-positive bacteria) and *K. pneumoniae* (a Gram-negative bacteria) were used, with a minor modification to the AATCC-100 test standards. In this procedure, cloth swatches measuring 4.8 cm x 4.8 cm square were employed. First, a 100 ml inoculum was cultured for 24 hours at 37 °C in nutritional broth. Muller Hinton nutritional broth was added to the mixture, bringing the bacterial concentration to 2 x 10^5 cells/ml. Next, 1 ml of this inoculum was pipetted onto each sample, and the vessel's cover was then secured to prevent evaporation. Each sample was then incubated once more for 24 hours at 37 °C. Following the incubation period, 100 ml of water was added to the vessel containing the fabric sample, and the samples were shaken vigorously for one minute. The necessary serial dilution was then carried out. Then, 0.5 µl of this diluted solution was applied to a plate of Muller Hinton Agar. Next, the plates were incubated for another 24 hours. On the agar plates, the number of live bacteria was counted. Finally, the following formula (1) has been used to get the antibacterial activity:

$$\text{Reduction rate \%} = (B-A)/B \times 100 \quad (1)$$

where B = bacterial colony for the untreated sample, A = bacterial colony for AgNPs treated sample.

Wash fastness

Samples were washed following AATCC 61-2010 (ISO 105-C06) standard. First, 120 ml liquor was made with 4 grams of AATCC standard reference detergent (without Optical Brightener). Next, the fabrics were dipped into it along with ten steel balls. Then the machine was started and run at 40 °C for 45 minutes. This method makes one wash cycle equivalent to five home laundry.

RESULTS AND DISCUSSION

This study focused on AgNPs synthesis and sol-gel formation. The AgNPs were synthesized using plant extract. The FTIR spectrum of the plant extract (*C.arvensis*) shows three major peaks at 3325.64 cm^{-1}, 1633.41 $cm^{-1,}$ and 1470 cm^{-1} (Figure 2). The peak at 3325.64 cm^{-1} corresponds to –OH stretching. On the other hand, the peak at 1633.41 cm^{-1} corresponds to the Alkene (>C=C<) group. Besides, the peak at 1470 cm^{-1} shows the presence of $–CH_3$ asymmetrical or symmetrical stretching. The presence of the groups showed that the *C.arvensis* plant extract has both reducing and capping abilities.

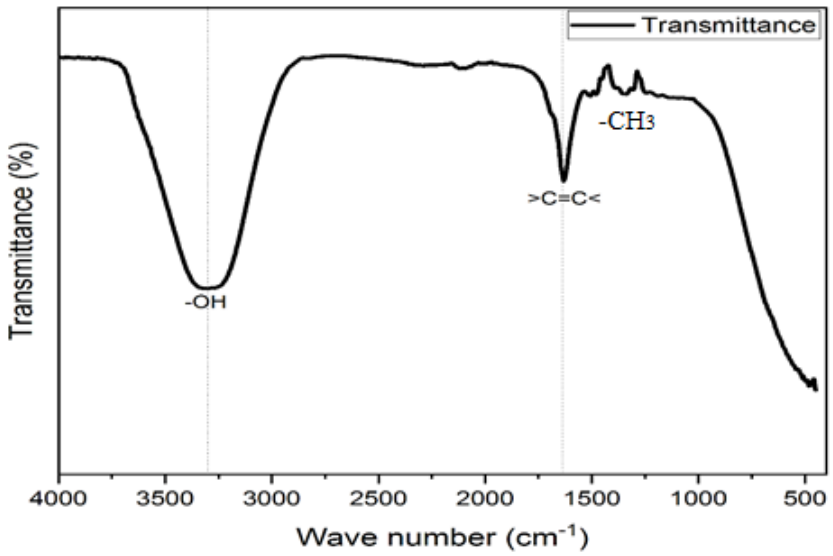

Figure 2. FTIR of C.arvensis

For confirmation of AgNPs synthesis, the UV-VIS spectrum was taken. The UV-VIS spectrum of $AgNO_3$ solution and AgNPs suspension are shown in Figure 3. It shows the highest wavelength of the UV-VIS peak is 421 nm. UV-VIS Surface Plasmon Resonance (SPR) of AgNPs ranges from 350 nm to 500 nm [20]. Hence, the obtained 421 nm SPR of the UV- VIS spectrum confirms the AgNPs synthesis.

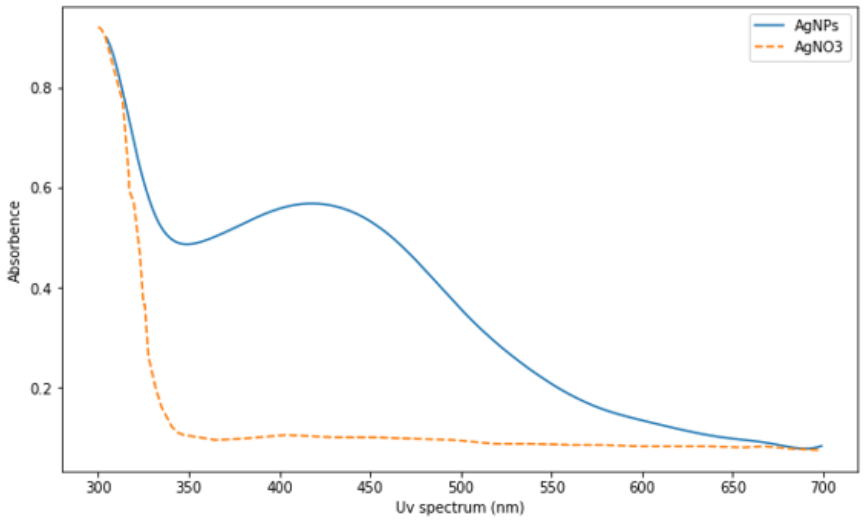

Figure 3. UV-vis of $AgNO_3$ and AgNPs

Aging

In sol-gel, aging is crucial. It is necessary to hydrolyze the TEOS and Ethanol solution using dilute acid. This reaction takes a very long time and is relatively slow. We investigated the duration of this acid hydrolysis time at various temperatures. The TEOS, Ethanol, and acid solution were kept at 3 °C, room temperature (25 °C), and 60 °C for this purpose. A few drops of NH_4OH were added to 5 ml of TEOS solution at every two-hour intervals to set the pH around 8.5 and mixed with a propeller stirrer to witness the development of colloidal gel. The results are shown in Table 1.

Table 1. Acid hydrolysis time and sol-gel formation

Sample	intervals	TEOS	Acid reaction temperature	Acid hydrolysis time	Result	Gelation Time
1		5 ml	3 °C	2 hours	Nothing	
2	Interval-1	5 ml	Room temp (25 °C)	2 hours	Nothing	
3		5 ml	60 °C	2 hours	Nothing	
4		5 ml	3 °C	4.30 hours	Nothing	
5	Interval-2	5 ml	Room temp (25 °C)	4.30 hours	Nothing	
6		5 ml	60 °C	4.30 hours	Colloidal gel	20 min
7		5 ml	3 °C	5.30 hours	Nothing	
8	Interval-3	5 ml	Room temp (25 °C)	5.30 hours	Nothing	
9		5 ml	60 °C	5.30 hours	Gel	13 min
10	Interval-4	5 ml	3 °C	6.30 hours	No gel	
11		5 ml	Room temp (25 °C)	6.30 hours	Gel	15 min
12	Interval-5	5 ml	3 °C	7.30 hours	Colloidal gel	20 min
13		5 ml	3 °C	8 hours	Gel	15 min

We may infer from Table 1 that increasing the temperature can accelerate sol-gel formation. Sol-gel formation takes longer in low temperatures. For instance, the acid reaction time at 60 °C should be around 5.30 hours. For 3 °C and room temperature (25 °C), this period should be more than 6.30 hours and 8.0 hours, respectively. However, factors like TEOS concentration, alkalinity, etc., may impact this acid reaction period. These findings are comparable to those of Jeon et al. [3], who mixed $AgNO_3$ at room temperature for 6 hours, whereas our finding shows it should be at least 6.30 hours. The environmental conditions of various regions might be the cause of the variance.

pH

The most crucial factor in sol-gel is probably pH. We experienced that unless a minuscule amount of alkaline (such as NaOH or NH_4OH) was added, sol-gel did not form even after several months. The alkaline addition dramatically sped up the procedure and finished quickly. It's interesting to note that since the acid and alkali react, there won't be any sol-gel if the alkali is added before completing the ageing (acid hydrolysis). Our results, however, differ from those of Venkateswara Rao and Bhagat [18], who reported that it would require about three days without alkaline.

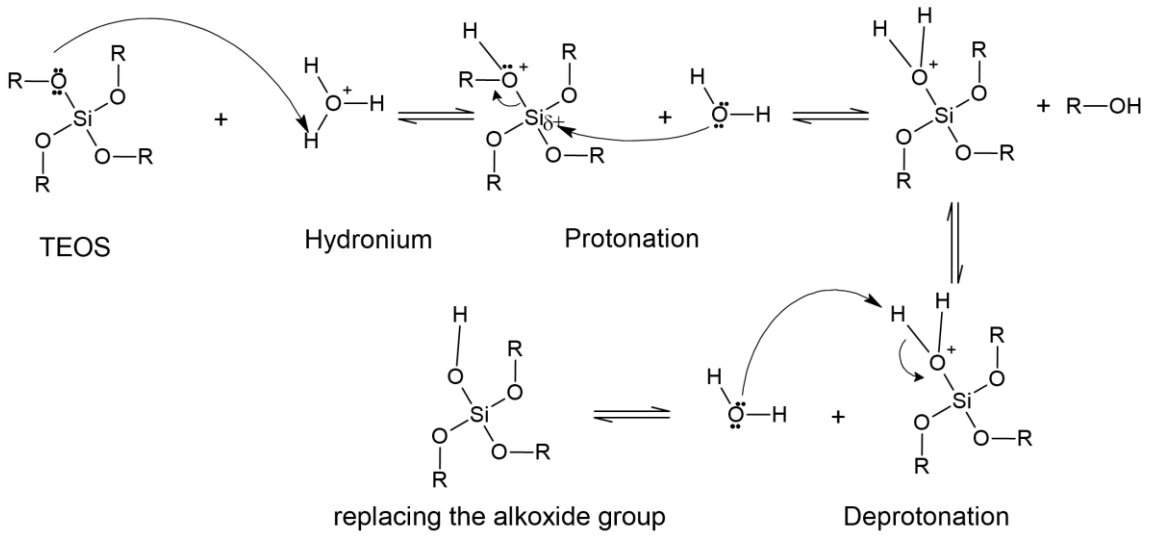

Figure 4. Acid hydrolysis of TEOS

Figure 4 depicts the acid hydrolysis mechanism of TEOS by acid. First, the added acid causes the hydronium ion (H_3O^+) that protonate the alkoxide groups (-OR). Next, the protonation releases the alcohol (R-OH) group as the -OH leaving group, which concurrently causes the silicon group to become partially positive and attract the water molecule. Following that, it deprotonates by leaving a silanol group (Si-OH). Finally, the hydroxyl group (-OH) completely replaces the alkoxide groups (-OR). Without an alkaline catalyst, these alkoxide groups won't attach since they are pretty stable. The moment an alkali is applied, the condensation process begins (as shown in Figure 5).

The –OH groups of alkaline solution (NH_4OH or NaOH) take the proton (H^+), leaving the nucleophilic O-. On the other hand, silicon (Si^+), an ideal electrophile, forms a partial positive ion as a result of oxygen's (O) pi-electron resonance (Figure 5). Therefore, silicon will connect with the nucleophilic (O^-). It results in the formation of a complicated and highly cross-linked polymer or gel. Typically pH ranges from 1 to 5 is required to promote a controllable gelation [17]. We found the pH of the aged TEOS was about 3, and if the pH of the solution was raised to 8.9 by adding alkali, then the gel formed within 8 minutes. However, the time may vary based on the TEOS concentration and other factors such as ageing time, temperature, etc.

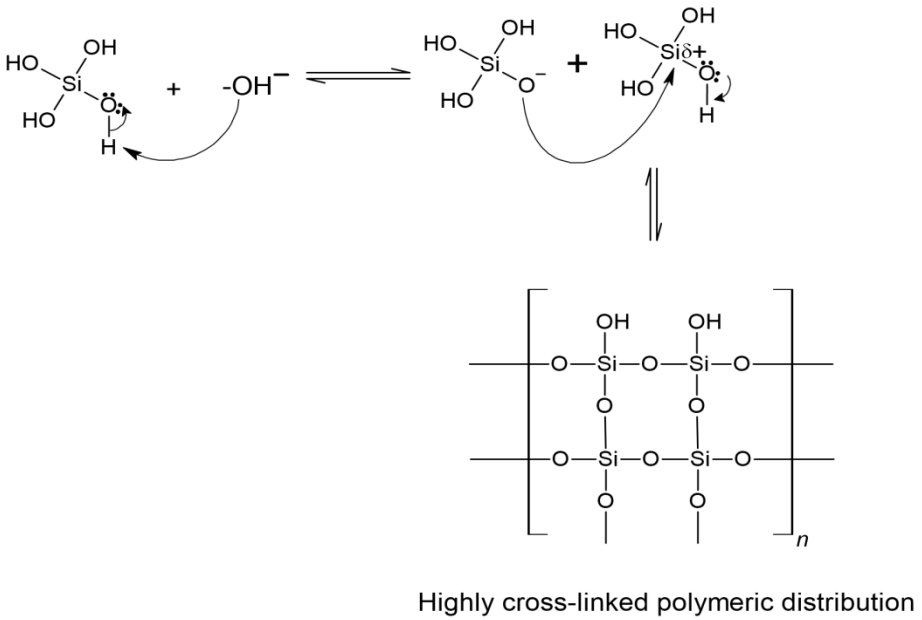

Figure 5. Condensation after acid hydrolysis

The visual appearance of different samples

The seven different methods were used to produce AgNPs and sol-gel. However, colour variation was noticed for the high molarity $AgNO_3$ (Figure 6). The high molarity $AgNO_3$ was synthesized by sufficient reducing agents (here, plant extract) and changed its colour completely. However, the lower molarity of $AgNO_3$ did not show any mentionable change in colour. Well-synthesized samples turned violet, while the insufficient plant extract for high molarity turned the sample brown colour. The nanparticle's colour depends on size and density [21]. When an adequate reducing agent (here plant extract) was given to the 0.08 M silver nitrate solution, numerous AgNPs were synthesized (Figure 6-a). The higher number of smaller-sized silver nanoparticles has a higher density of free electrons and a higher surface-to-volume ratio leads to stronger surface plasmon resonances and increased absorption at shorter wavelengths. Thus resulting in shorter wavelengths (i.e. higher energy) and a blue shift in nanoparticle colour. On the other hand, in the spray method, high molarity $AgNO_3$ (0.08 M) did not get a sufficient amount of plant extract so a few AgNPs were synthesized (for this reason the antibacterial activity of

this sample is only 89% as shown in Table 2). Low-density free electrons and a lower surface-to-volume ratio lead to weaker plasmon resonance and increased absorption at longer wavelengths which results in a red shift in nanoparticle color.

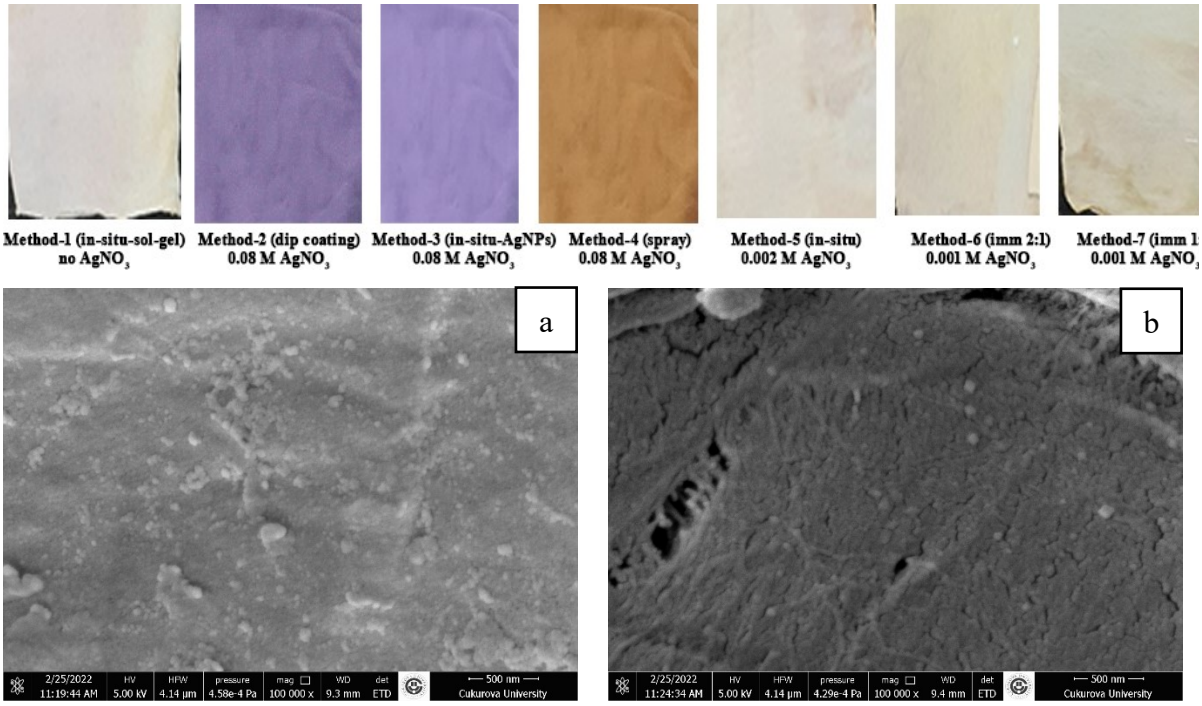

Figure 6. The visual appearance of different samples; a) SEM image of purple coloured sample (Method-2 dip coating 0.08 MAgNP3) high density with 36 nm average size; (b) SEM image of the brown coloured sample (Method-4 Spray 0.08 M $AgNO_3$) very few AgNPs with 44 nm average size synthesized

XRD

The XRD graphs compare the peaks of only sol-gel and AgNPs-sol combinations. Figure 7(a) compares 3 distinct ratios of Sol-gel to AgNPs. It demonstrates that the three graphs exhibit a variety of lattice structures and crystal systems. Figure 7(b) (only Sol) shows four intense peaks at 2-theta positions ranging from 20 to 80. The peaks match with ICSD: 170544 (Zeolite), which are 34.6, 39.04, 42.66, 45.18 having hkl values of (1,2,-1), (3,-1,-2), (0, 3,-2), (4,-2,0) and d-spacing 2.59 Aº, 2.3 Aº, 2.12 Aº,2 Aº, respectively. Here, the crystal system is Anorthic.

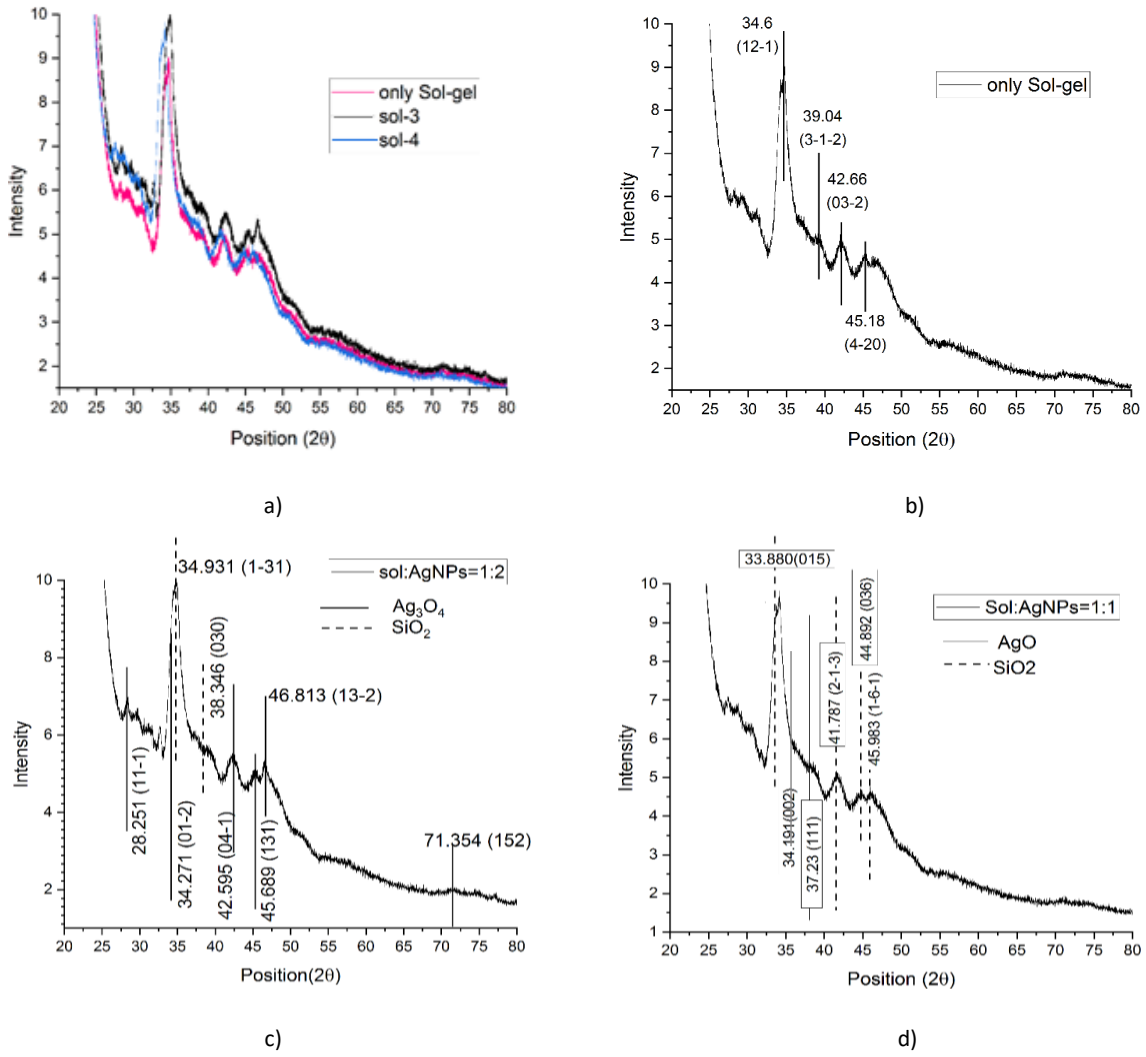

Figure 7. XRD of different sol-gel and AgNPs combinations: (a) XRD comparison of only sol-gel, sol: AgNPs= 1:2, and sol: AgNPs = 1:1; (b) XRD of only sol-gel; (c) XRD of sol: AgNPs = 1:2; (d) XRD of sol: AgNPs = 1:1

Figure 7(c) shows five peaks; three matched with Ag_3O_4 (ICSD: 59225) and 2 with Zeolite (ICSD: 170544). The Ag_3O_4 peaks are 28.251 (1, 1,-1), 34.271 (0, 1,-2), 42.595 (0, 4,-1), 45.689 (1, 3, 1), and 71.354 (1,5,2) where the digits inside the parenthesis indicate hkl values. The d-spacing of the peaks are 3.16 Aº, 2.61 Aº, 2.12 Aº, 1.98 Aº, 1.32 Aº, respectively. On the other hand, The Ag_3O_4 peaks have a Monoclinic crystal system whereas Zeolite peaks are 34.931 (1,-3,1), and 38.346 (0,3,0) have an Anorthic crystal system. Figure 7(d) shows six peaks; four peaks match with SiO_2 (ICSD: 171736), and the rest two match with Silver Oxide (ICSD: 605625). The 33.880 (0,1,5), 41.787 (2,-1,-3), 44.982 (0,3,6), and 45.983 (1,-6,-1) peaks match with SiO_2 having d-spacing 2.64 Aº, 2.15 Aº, 2.01 Aº, 1.97 Aº, respectively. The first three peaks have the Anorthic crystal system. The rest two peaks, 34.91 (0,0,2), and 37.23 (1,1,1) match with AgO and have 2.57 Aº and 2.41 Aº d-spacing, respectively. Here the crystal system is Monoclinic. Figures 7(c) and 7(d) show that the sol: AgNPs = 1:2 has more portion of Ag than that of sol: AgNPs = 1:1.

SEM images

Among the prepared samples with the mentioned 7 methods, Method 6 and Method 7 had the lowest silver concentration but Method 6 showed the highest antibacterial efficiency (as shown in Figure 11). We took SEM images of these two samples in various magnifications to investigate the difference in silver nanoparticle morphology produced by method 6 and method 7. The SEM images depict nanoparticle disposition in the sol film on the fibres. Figures of different magnifications show that the sol forms a film on the fibres, and the AgNPs are incorporated into the sol film (Figure 8 and Figure 9). Figure 8 shows that the prevalence of AgNPs is conspicuous here because of the high ratio of AgNPs (sol: AgNPs = 1:2). It is seen that the nanoparticles are distributed log-normally. The majority of AgNPs are between 60 and 80 nm in size. Nevertheless, the mean nanoparticle size is 100 nm.

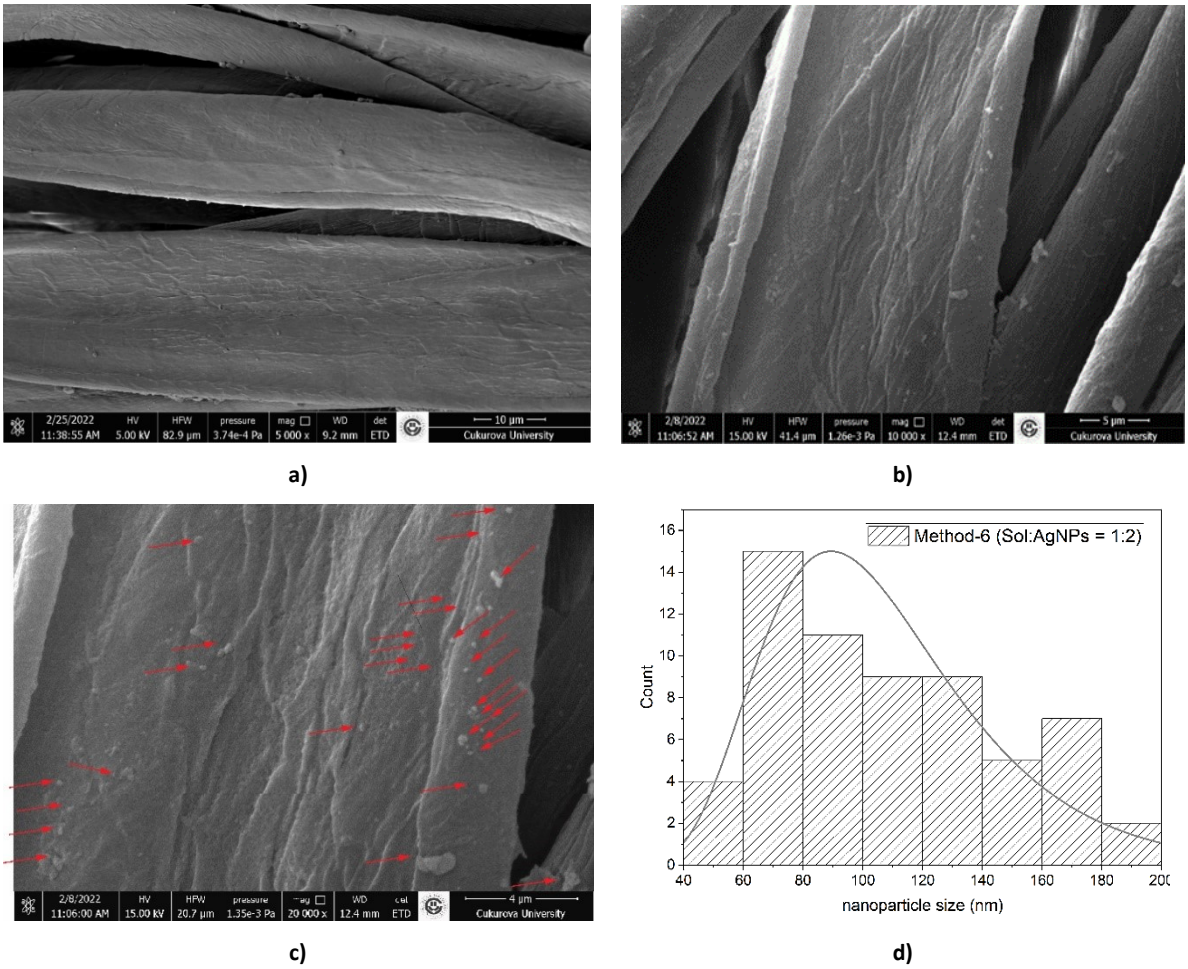

Figure 8. Method-6 (Sol: AgNPs = 1:2) at various magnifications: (a) Untreated fabrics; (b) Wrinkle sol-gel-film is visible; (c) AgNPs entrapped by the sol-gel film; (d) nanoparticle size distribution

Figure 9 depicts the AgNPs, synthesized by method-7 (sol: AgNPs 1:1). Here, the number of AgNPs is comparatively less than in the previous method (method-6). The solidified TEOS is present here in the

inter-fibre region because of the high ratio of TEOS. The AgNPs distribution is from 40nm to 80 nm. The mean nanoparticle size here is about 60 nm.

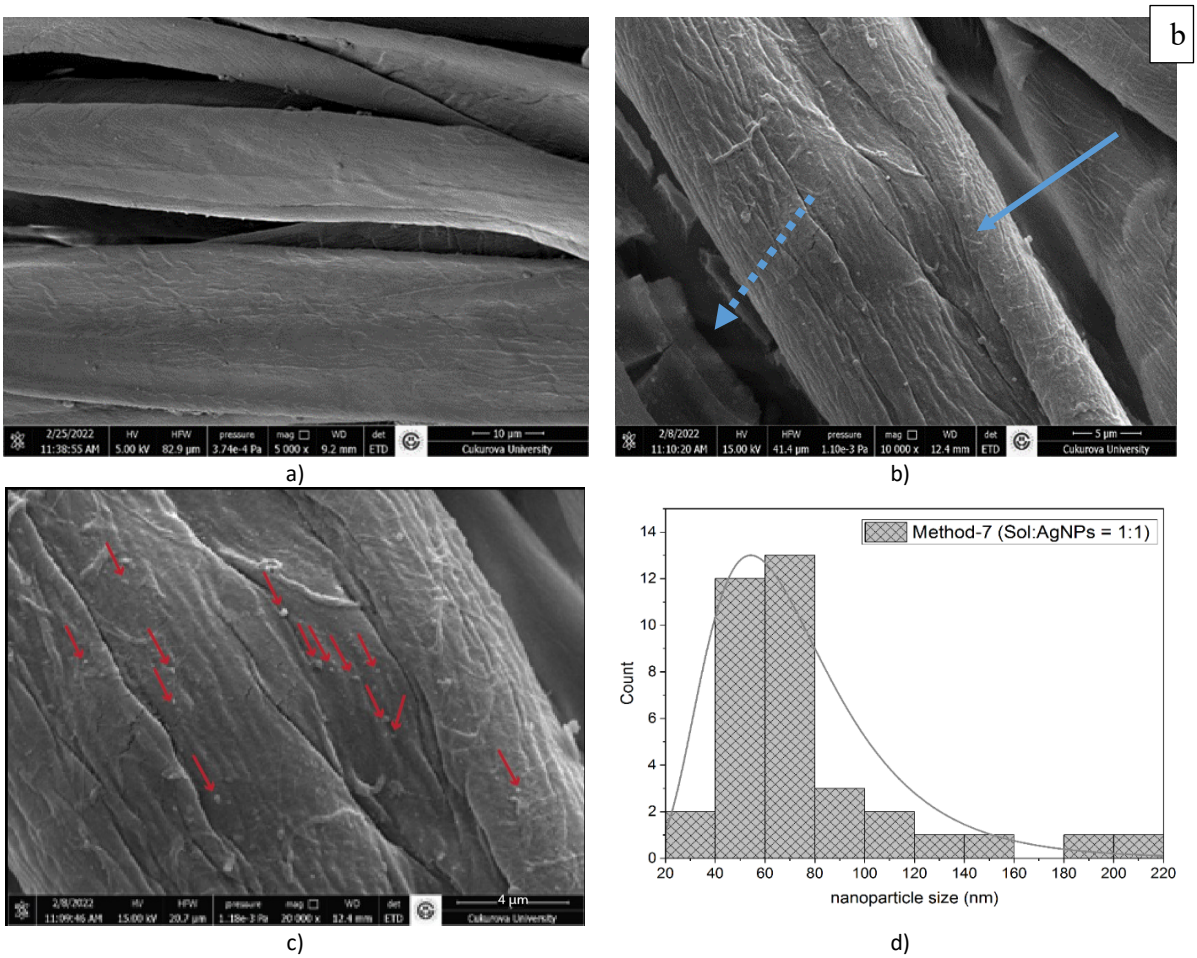

Figure 9. Method-7 (Sol: AgNPs = 1:1) AgNPs at various magnifications: (a) Untreated fabrics; (b) Formed sol-gel are visible here: the broken arrow indicates the dried sol and the normal arrow indicates the sol film; (c) AgNPs entrapped by the sol-gel film, due to the equal proportion of AgNPs and sol-gel chemicals the AgNPs are less abundant; (d) nanoparticle size distribution

Figures 8 (d) and 9 (d) make it abundantly evident that the average nanoparticle size is bigger in method 6 (100 nm), and the AgNPs size is smaller in method 7 (60 nm). It happened due to the aggregation of the abundance of AgNPs in alkaline conditions (pH 8.9).

EDS

The EDS (Energy Dispersive Spectroscopy) spectrum (Figure 10) has different peaks for Ag, C, O, and Si. The EDS profile shows Ag and Si peaks, confirming the AgNPs and Sol-gel film. Here, the only sol sample exhibits no Ag but Si. In contrast, there are several peaks of Ag in AgNPs: sol =1:1 sample. The C and O elements indicate the presence of Carbon and Oxygen available in cotton fabrics and plant extracts. Another strong peak is Au which occurred due to the gold plating of the sample.

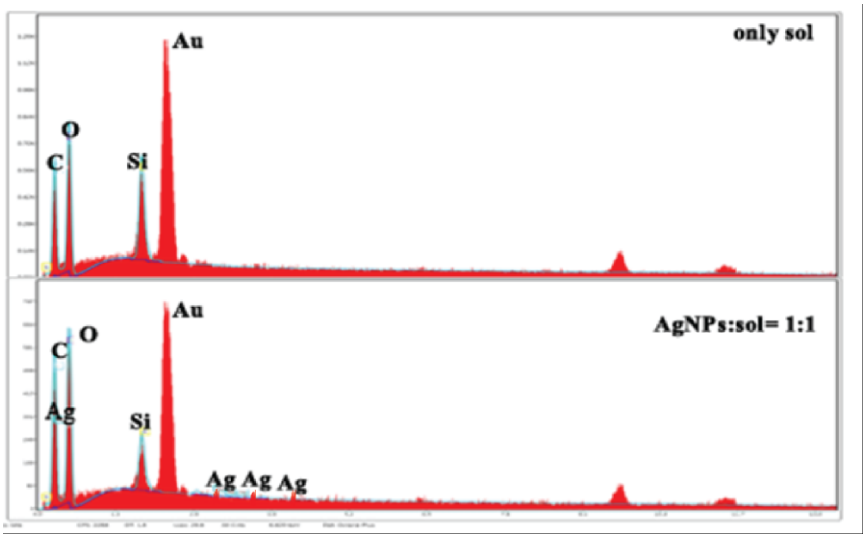

Figure 10. EDS of only sol and AgNPs incorporated sol

Antibacterial Test Results

The silver nanoparticle is an excellent antibacterial agent. It is effective against almost all types of bacteria, fungi, and viruses. However, the exact mechanism of the antibacterial activity of AgNPs is not known [22]. It is assumed, that the AgNPs inhibit the bacteria in 3 main stages: adhesion to the bacteria, penetrating them, and inducing cellular toxicity inside the microbial organelles [23]. When the incorporated silver nanoparticles (AgNPs) come in contact with the bacteria the AgNPs start to release silver ions (Ag+) [24]. The Ag+ ions start to damage the cell wall finally enter the cytoplasm and cause substantial damage to the organelles. In this way, the bacteria get annihilated.

According to this investigation, samples are approximately equally effective against both S.aureus and K. pneumonia. Hence, we are not mentioning them individually for convenience.

Different AgNPs loading methods on Antibacterial:

Among various methods, Method 6 performed best for both knit and twill fabrics (Figure 11). Here the initial antibacterial activity was 100% for both knit and woven fabrics, whereas the knit shows better wash fastness than the twill. Knit fabrics performed better for all methods except Method 3, where twill fabrics perform much better than knit fabrics (Figure 11).

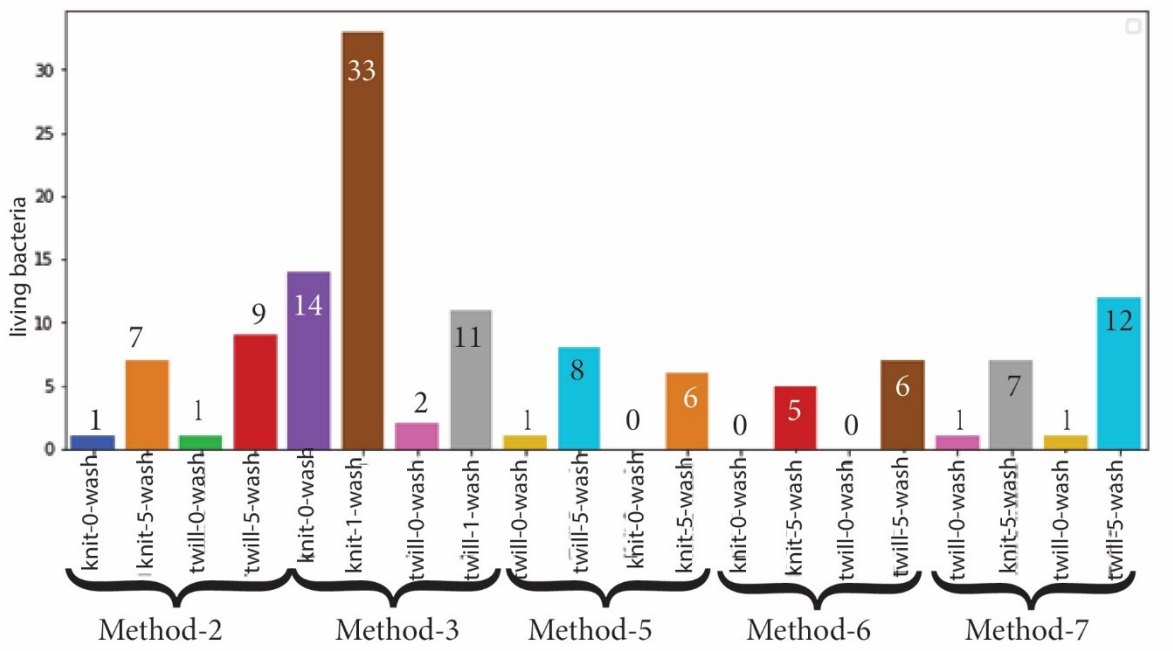

Figure 11. Various methods of antibacterial activity

Because the AgNPs were loaded by the *in-situ* method in Method 3. Here the molarity and volume of $AgNO_3$ were 0.08 M and 18 ml, respectively (Table 2). The heavier twill fabrics absorbed more $AgNO_3$ than knit fabrics. Henceforth, more AgNPs were synthesized inside the bulk twill fabrics. Similarly, the molarity and volume of $AgNO_3$ used in Method 2 are almost similar to Method 3, but the incorporation method was immersion here. Hence, the performance of both knit and twill was also much better than Method 3. The antibacterial efficiency of Method 2 and Method 3 was 99.87% and 98.14%, respectively (Table 2). On the contrary, lower $AgNO_3$ molarity samples (Method-5, 6, 7) were good for antibacterial activity. However, the spray method (Method-4) performed worst due to the insufficient plant extract sprayed on the fabrics.

Table 2. Antibacterial activity of sol-gel applied on different methods

index	samples	Fabrics	Method	AgNO3 conc. (M)	AgNO3 volume (ml)	living bacteria	Eff (%)	Eff (%) reduction due to wash
Method-2	Knit-0-wash	knit	immersion	0.08	20	1	99.84	
Method-2	Knit-5-washes	knit	immersion	0.08		7	98.84	1.00
Method-2	Twill-0-wash	twill	immersion	0.08		1	99.84	
Method-2	Twill-5-washes	twill	immersion	0.08		9	98.50	1.34
Method-3	Knit-0-wash	knit	In-situ	0.08	18	14	97.67	
Method-3	Knit-1-wash	knit	In-situ	0.08		33	94.50	3.25
Method-3	Twill-0-wash	twill	In-situ	0.08		2	99.67	
Method-3	Twill-1-wash	twill	In-situ	0.08		11	98.17	1.50

index	samples	Fabrics	Method	AgNO3 conc. (M)	AgNO3 volume (ml)	living bacteria	Eff (%)	Eff (%) reduction due to wash
Method-4	Knit-0-wash	knit	Spary	0.08	20	78	89.60	-
Method-5	Twill-0-wash	twill	In-situ	0.002		1	99.84	
Method-5	Twill-5-wash	twill	In-situ	0.002		8	98.67	1.17
Method-5	Knit-0-wash	knit	In-situ	0.002	100	0	100.00	
Method-5	Knit-5-wash	knit	In-situ	0.002		6	99.00	1.00
Method-6	Knit-0-wash	knit	immersion	0.001		0	99.84	
Method-6	Knit-5-washes	knit	immersion	0.001		5	99.17	0.67
Method-6	Twill-0-wash	twill	immersion	0.001	100	0	100.00	
Method-6	Twill-5-washes	twill	immersion	0.001		7	98.84	1.16
Method-7	Knit-0-wash	knit	immersion	0.001		1	99.84	
Method-7	Knit-5-washes	knit	immersion	0.001		7	98.84	1.00
Method-7	Twill-0-wash	twill	immersion	0.001	100	1	99.84	
Method-7	Twill-5-washes	twill	immersion	0.001		12	98.00	1.84

Advantages of sol-gel over conventional methods

We introduced AgNPs using the sol-gel approach and without the sol-gel method to examine the impact of the two methods on fabrics (Table 3). To keep the experiment free from biases, we kept similar parameters i.e. the fabric samples were twill the method was in-situ, keeping the plant extract, AgNO3 volume, etc., identical for both groups. It demonstrates that while the non-sol-gel methods had 8–10 live bacteria, the sol-gel samples had just one. Similarly, sol-gel samples performed twice as well as non-sol-gel samples regarding wash fastness (Table 3). Hence the sol-gel samples are much better than non-sol-gel samples regarding antibacterial activity and wash fastness.

Table 3. Efficacy of AgNPs incorporation through sol-gel over normal

sample	AgNO3 conc. (M)	Fabric	Method	AgNO3 volume (ml)	AgNPs: sol	Bacteria-before-wash	Bacterial after 5 washes
Sol-1	0.001	Twill	In-situ	100	1:1	1	10
Sol-2	0.001	Twill	In-situ	100	1:2	0	8
Sol-3	0.002	Twill	In-situ	100	1:1	1	9
No-sol	0.001	Twill	In-situ	100	1:0	10	21
No-sol	0.002	Twill	In-situ	100	1:0	8	15

Influence of different parameters on antibacterial activity

The correlated parameters on the antibacterial property have been identified using Pearson correlation and mutual information (shown in Supplementary 1). It demonstrates that increased Ag^{+}

content will result in high antibacterial activity. The remaining variables including $AgNO_3$ volume, plant extracts (PE), and $AgNO_3$ concentration, are also tangentially connected.

$AgNO_3$ volume on antibacterial activity

$AgNO_3$ volume is the most significant factor affecting antibacterial activity. We found that increased volume was beneficial for antibacterial characteristics, as shown in Figure 12(a). However, it relies on multiple variables, including plant extract and incorporation methods. AgNPs dispersed in a large volume have more antibacterial action. In addition, it will stop nanoparticle aggregation. Bamsaoud et al. and Ahmed, Ogulata, and Gülnaz reported similar results. They showed the antibacterial activity increased with the silver nitrate volume[24-25].

Concentration of $AgNO_3$

The quantity and size of the synthesized AgNPs rather than the concentration determine the antibacterial activity. For instance, Table 2 demonstrates that Methods-2 and Method-5 are identical, except for the $AgNO_3$ molarity (0.08 M and 0.002 M). The antibacterial activity (without washing) is the same in both situations. The bactericidal activity of Methods 2, 6, and 7 with concentrations of 0.08 M, 0.001 M, and 0.001 M was identical (only 1 survival bacteria). However, some researchers found antibacterial activity increased with the concentration [26] [27].

On the other hand, Leung, Wong, and Xie asserted [28] that the concentration of either the precursor or the reducing agent will increase the nanoparticle size. Following that our study shows, that synthesizing high-molarity $AgNO_3$ is challenging and requires a precise amount of plant extract as a reducing and stabilizing agent. If an excess amount of plant extract is administered, numerous AgNPs will form, but they will aggregate. On the contrary, insufficient plant extract will result in a few AgNPs, which cannot show antibacterial activity. Hence, the spray method (Method-4), having a minuscule amount of plant extract, showed poor antibacterial activity. Figure 12(b) summarizes the cumulative impact of $AgNO_3$ concentration (wash and without wash samples) on bacteria's survival ability. It demonstrates that there won't be any antibacterial properties without AgNPs. The effectiveness of $AgNO_3$ as an antibacterial agent decreases with increasing molarity. However, the quantity of plant extract has a significant impact.

Plant extract on antibacterial activity

The plant extract is also essential for antibacterial action. It is more than 60% linearly correlated with the AgNO3 molarity and $AgNO_3$ volume (shown in supplementary-1). Therefore, the plant extract should be according to the $AgNO_3$ molarity and volume. If plant extract is insufficient, there will be a

very low antibacterial activity (such as the spray method in Method 4). However, our experiments were not enough to establish any direct relation to antibacterial activity (Figure-12(c)).

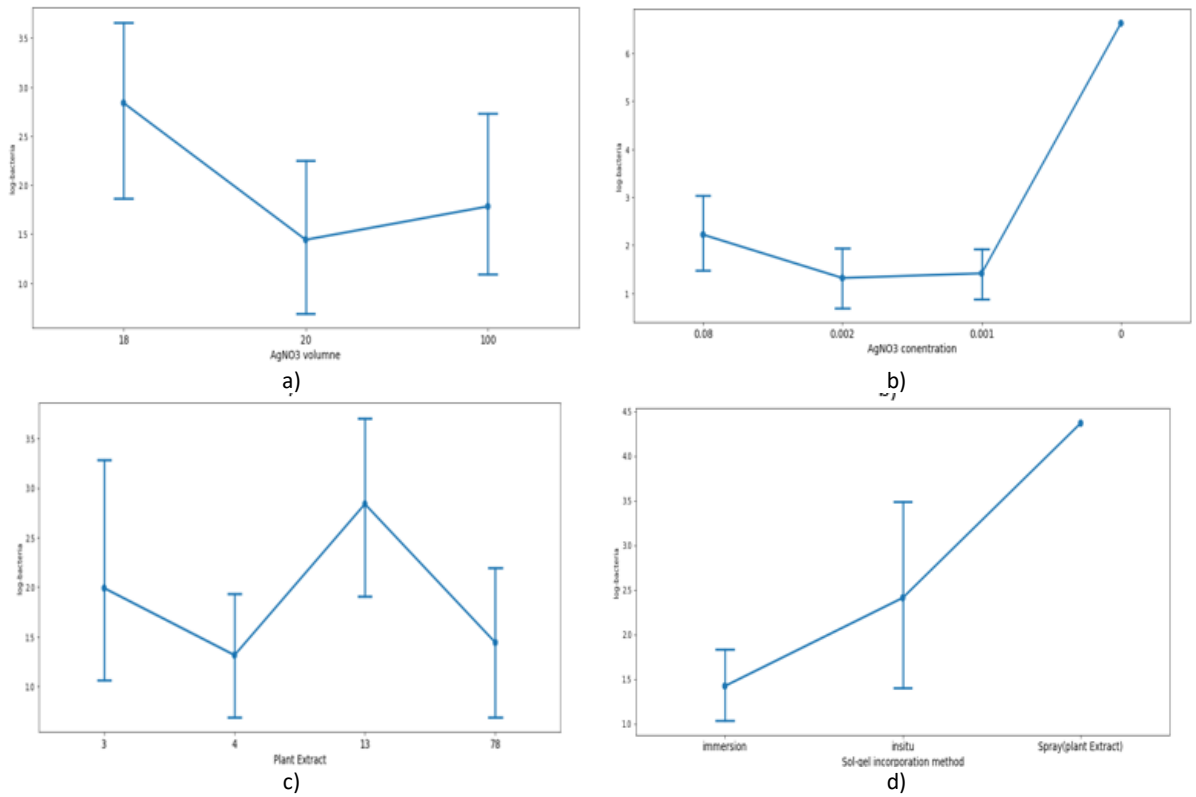

Figure 12. Various factors on antibacterial action: (a) AgNO3 volume on survival bacterial; (b) AgNO3 concentration on survival bacteria; (c) Plant extract on survival bacteria; (d) Sol-gel-AgNPs incorporation methods on survival bacteria

Incorporation methods on antibacterial property

Figure 12 (d) depicts the antibacterial properties of different incorporation methods. In this case, we applied three techniques: immersion, spray, and in-situ. It demonstrates that the spray was the worst since the plant extract was inadequate. The in-situ synthesis approach was also poor due to the influence of the fabric types, the alkaline nature of the sol, etc. On the other hand, an ex-situ method (immersion method) performed best among all methods. Because the immersion method synthesizes AgNPs outside the fabrics, the synthesis process is free from any external influence.

Fabric type on antibacterial activity

To determine the effect of fabric type on the antibacterial activity, we compared the antibacterial efficiency for knit and twill fabrics (Table 4), keeping all the parameters identical. The knit fabrics lost a little (only 0.67) antibacterial activity by washing, whereas the twill fabric's antibacterial activity loss was consistently around 1 to 1.84. Hence regarding wash fastness, knit fabrics functioned much better. The heavy-weight twill fabrics had a 3/1 interlacement which renders more inter-yarn friction during

washing. On the other hand, lightweight knit fabrics have lower inter-yarn friction due to high interlacement. Moreover, the yarn used in knit (yarn count 30 Ne) was much finer than twill (20 Ne), which also caused comparatively low interfiber friction. Hence, knit fabrics performed better than twill fabrics regarding antibacterial activity and wash fastness.

Table 4. Knit and twill fabrics on antibacterial activity and wash fastness

Samples	Methods	Antibacterial Efficiency		Eff% loss by 5 washes	
		Knit	Twill	Knit	Twill
Method-2	immersion	99.340	99.17	1.001603	1.342147
Method-6	immersion	99.505	99.34	0.671074	1.001603
Method-7	immersion	99.340	98.92	1.001603	1.842949

CONCLUSION

Sol-gel is a popular technique to functionalize textile materials for water repellency, antimicrobial, UV protection, etc. It can host various functionalizing agents. This article used sol-gel to host AgNPs for antibacterial activity and wash fastness of cotton fabrics. We investigated the importance of the acid hydrolysis time of TEOS solution (TEOS+ Ethanol+ acid) and concluded that at least 6.30 hours at room temperature (25 ^{o}C) and 5.30 hours at 60 ^{o}C are required. The alkaline condition (around pH 8.9) is crucial for gel formation from aged TEOS solution. The obtained sol-gel is very amicable to host the AgNPs. We analyzed seven different methods and found that the in-situ method with 0.001M $AgNO_3$ performed the best regarding antibacterial wash fastness. The sol-gel-hosted AgNPs are 8 to 10 times more efficient than no-sols. Regarding other parameters, high volume and low concentration of $AgNO_3$, lightweight fabric (low GSM), and immersion method are more congenial for antibacterial efficiency. However, there are several scopes to improve the study, such as using a high amount of plant extract, low molarity (lower than 1 mM), high volume of silver nitrate, and incorporation by padding method with various pressures.

Author Contributions

Conceptualization – Ahmed T, Ogulata RT; methodology – Ahmed T; formal analysis – Ahmed T; investigation – Ahmed T; resources – Gülnaz O and Ogulata RT ; writing-original draft preparation – Ahmed T; writing-review and editing – Ahmed T; visualization – Ahmed T; supervision – Ogulata RT and Gülnaz O. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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