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Research Article

Siloxane-Silver Nanofluid as Potential Self-Assembling Disinfectant: A Preliminary Study on the Role of Functional Alkoxysilanes

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Abstract: Antimicrobial drug resistance among bacterial and fungal communities have been created a huge challenge for clinicians in treating infections. Conventional antibiotics get non-functional with time due to the rapid adaptation of microorganisms to the environment. Therefore, exploring alternative antimicrobial drugs/nanomaterials to treat such infections is highly needed. Therefore, an alternative to conventional antibiotics, functional alkoxysilane capped Ag-NPs synthesized from 3-aminopropyltrimethoxysilane mediated conversion of silver cations in the presence of three different organic reducing agents, i.e., cyclohexanone, 3-glycidoxypropyltrimethoxysilane and formaldehyde. The antimicrobial potential of synthesized silver nanoparticles was tested against *Acinetobacter bauminnii*, *Candida albicans* (mostly causes nosocomial infections) and sporangiospores of Mucorales (*Rhizopus arrhizus*), which showed a promising result. In addition to low MIC values, these Ag-NPs have shown variable killing dynamics as a function of reducing agents. Further, these functionalized silver nanoparticles were mixed with siloxane polymer to prepare three different siloxane-silver nanofluids. Siloxane-silver nanofluid can be self-assembled when diluted in a desirable volatile solvent on any inanimate surfaces such as medical catheters, surgical clothes and surgical bandages. Finally, the sprays were converted into thin films on sterile plastic strips and examined for their antibacterial activity against drug-resistant bacteria *A baumannii*. The antibacterial activity of nanofluid thin film has been found as a function of organic reducing reagents that control the morphology of the self-assembled film.

***Keywords*:** silver nanoparticles, nanofluid spray, nosocomial infections, siloxane, Mucorales

1. Introduction

In ancient times the most spectacular effect of nanoscale material (nanoparticles, NPs) was seen as a colour pigment in the glass and luster industry [1]. Metal nanoparticles (MNPs) have been used to color glasses. Lycurgus cup, kept in a British museum, is one famous example of nanoparticles used in coloring glasses. With the consistency and advanced nanotechnology research, the silver nanoparticle (Ag-NPs) has become one of the most diversified researched nanomaterials [2]. The attention received by metal nanoparticles from the scientific community can be ascribed to the large surface-to-volume ratio, physicochemical characteristics and surface electronic properties [3]. Therefore, the use of metal nanoparticles for applications like molecular recognition, biomedicine, energy transfer, sensors, and catalysts are

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well established [4]. Ag-NPs have received tremendous attention because of many important features: (i) They exhibit unique size-dependent physicochemical properties. (ii) These properties can be tuned by controlling the size, shape and surrounding environment (solvent and ligand). (iii) These Ag-NPs have potent antimicrobial and antifungal activity with non-cytotoxic, and (iv) stable Ag-NPs of desirable shape and functionality can be synthesized easily.

Considerable efforts have been devoted over the last two decades to synthesizing metal nanoparticles, especially silver and gold, focusing on control over their size, shape, dispersibility and functionality. The approaches for preparing metal nanoparticles include "top-down" and "bottom-up" approaches. In 1951, J. Turkevisch developed a method referred to as the 'Turkevisch method', which remains the most popular method for synthesizing NPs due to the ease of synthesis and cheap and non-toxic nature of the stabilizer. In this method, sodium citrate is used both as a stabilizer and reducer. It initially acts as a reducer to Ag^{+} producing Ag^{0} and then as a stabilizer due to the electrostatic repulsion between the negatively charged oxygen on citrate. The NPs thus produced are spherical with a size of around 20 nm [5]. Subsequently, Brust Method was given by Brust and Schiffrin to produce NPs in organic solvents that normally form a separate layer with an aqueous solvent. This method for synthesizing thiol-protected NPs was a breakthrough in NPs synthesis. It is a two-phase synthesis strategy in which thiol-gold interactions protect gold nanoparticles from agglomeration using thiol ligands. Synthesis protocol involves the transfer of metal cation from aqueous to toluene media by using surfactant tetraethylammonium bromide and reduced by sodium borohydride ($NaBH_4$) in the presence of octane thiol. A change in color from yellow to deep brown occurs in organic media with the addition of sodium borohydride, $NaBH_4$ [6]. The first report on the use of amine for NPs synthesis was reported [7]. These workers produced hydrophobic NPs using dodecyl amine or oleyl amine in place of dodecane thiol of the Brust-Schiffrin method. Further, the phase transfer technique has been used to generate organically soluble monodispersed NPs using amines like Lauryl amine and octadecyl amine [8], Oleyl amine [9], aromatic amines [10], amino acids [11], diamines [12], tetraethylammonium [13], porphyrins [14] and hyperbranched polyethyleneimines [15-16] have been used as reducing/capping agents in the synthesis of NPs, while a direct one-pot synthesis of amine-stabilised AuNPs using 3-(trimethoxysilylpropyl)-diethylenetriamine has been reported [17].

The conventional routes of nanoparticle synthesis described above have been used exhaustively by the scientific community for their application in different fields. However, such techniques for Ag-NPs synthesis have the following limitations: (i) Generally; the Ag-NPs are produced as aqueous suspension [11]. The use of such NPs in organic solvent causes agglomeration. Similarly, the Ag-NPs compatible with organic solvent (Brust-Schiffrin method) are incompatible in an aqueous system. (ii) The NPs fabricated through conventional routes are generally produced as a dilute solution. Therefore, the initial concentration of NPs precursors (i.e., Ag^{+}) is very low. (iii) The NPs made through conventional routes are not very stable to changes in pH and Salt concentration which limits their use for several applications. The NPs made through the Turkevisch method agglomerate by adding a single drop of salt. Therefore, the NPs should be able to adapt to different conditions (change of solvent, change of pH and change of salt concentration) without changing their size or shape. (iv) Attempt to convert the homogenous suspension of NPs to a heterogeneous matrix by adsorbing over some solid support (TiO_2, Al_2O_3) causes an increase in the size of the NPs, i.e., undergo agglomeration. To meet these challenges, sol-gel science and technology, which are the most widely accepted methods of producing nanomaterials, seem to be more reasonable.

Accordingly, the use of several functionalized alkoxysilanes in an optimum ratio of hydrophilic character [trimethoxy silane and 3-APTMS] and hydrophobic character [3-glycidoxypropyltrimethoxysilane (3-GPTMS), 2-(3,4-Epoxycyclohexyl)ethyltrimethoxysilane] have been demonstrated for yielding organically modified Ag-NPs and siloxane thin films for multiple applications [18-26]. Functional alkoxysilanes have been used for casting thin films (through sol-gel processing) that can be used for encapsulating enzymes (glucose oxidase), redox material (ferrocene and ferricyanide) and noble metal ions (palladium, ruthenium) and for its use as biosensor [18-20]. The property of the thin film depends on the concentration and composition of the alkoxysilanes used, justified by the functional reactivity of the moiety linked to alkoxysilane-alkoxysilanes while fabricating nanostructured thin films of organically modified silicates through the reduction of palladium cations [27]. Some of the functional alkoxysilanes like 3-GPTMS and trimethoxy silane have the potential to act as reducing agents for Palladium chloride ($PdCl_2$) that allowed the introduction of Pd together -Pd-Si- linkage within a nanostructured network of organically modified silicates [23]. Accordingly, such findings were directed to examine functional alkoxysilane's role in synthesizing noble metal nanoparticles. Indeed, real-time synthesis of all noble metal nanoparticles and their trimetallic analogues have been made analogues [YouTube

links: https://youtu.be/ASayCJ0WV_M, https://youtu.be/Zl-QT574j8Q] [21-25]. These findings demonstrated that functional alkoxysilanes assisted the controlled and rapid synthesis of noble metal nanoparticles and their multi-metallic analogues. However, using functional alkoxysilanes to yield Ag-NP or other noble metal nanoparticles is most effective. The Ag-NP formulation can be converted into thin film over the surface for biomedical applications like a surgical catheter or normal catheter-like, Ag-NP nanofluid allowing assembling of Ag-NP over any desired substrate just by spraying it, embedding the Ag-NP over the nylon cloth or other cotton cloth [https://youtu.be/ViQ9ivQ8msg].

Further, one study have reported that siloxane-polyindole-gold nanoparticles could be easily made from 3-APTMS mediated formation of siloxane polymer in acetone that undergoes nanofluid formation with polyindole-gold nanoparticles sol made again in acetone [26-27]. Indole undergoes polymerisation into polyindole in the presence of gold cations allowing the reduction of gold cations into gold nanoparticles in acetone, thus forming polyindole-gold nanoparticles sol. These sols assemble to form siloxane-polyindole-gold nanoparticles nanofluid that allowed the assembling of thin film as reported earlier [26-27]. It is of great interest if such nanofluid could be made involving siloxane and silver nanoparticles as a spray for various applications as a possible disinfectant thin film. The findings on these lines are reported in this communication.

Acinetobacter baumannii is the predominant cause of nosocomial infections in intensive care units (ICU) admitted patients, a potent biofilm former in hospital settings, and surgical items such as urinary catheters. The established biofilm is challenging for clinicians to treat with conventional antibiotics due to emerging antibiotic resistance. Over the past few decades, the use of nanotechnology approaches has achieved momentum to tackle the challenge of antimicrobial resistance; for example, silver nanoparticles (Ag-NPs) applied as an alternative to conventional antibiotics to treat infections caused by multidrug-resistant (MDR) microorganisms due to their broad-spectrum and potent antimicrobial properties [28-32]. Chemically, the silver cation is considered a Lewis acid, which reacts with a Lewis base, including phosphorous and sulphur-containing biomolecules that constitute the cell membrane, proteins and DNA bases [33-36]. Due to the net negative charge of the microbial cell surface, the Ag-NPs initially accumulate on the cell wall and cell membrane and exert their antibacterial action such as morphological changes, i.e. shrinkage of the cytoplasm, membrane detachment, formation of numerous electron-dense pits, and final membrane disruption [36]. In this study, the antibacterial and antifungal activity of alkoxysilane functionalized Ag-NPs, synthesized using 3-GPTMS, cyclohexanone and formaldehyde, were investigated. However, only the preliminary stage of antibacterial activity assessment of siloxane-silver nanofluid has been discussed.

2. Materials and methods

2.1 *Materials*

All of the reagents in this study were utilized as received; Silver nitrate, 3-APTMS, 3-GPTMS, 1-vinyl-2-pyrrolidone, Ethylene glycol di-acetate, acetone and other solvents were purchased from Sigma Aldrich and Merck (Bengaluru, India). In addition, bacterial culture media were purchased from Hi-Media (Mumbai, India). All other chemicals utilized in this study were of analytical grade.

2.2 *Synthesis and characterization of siloxane-silver nanofluid*

The 3-APTMS functionalized silver nanoparticles were synthesized as reported previously [23]. In brief, 120 µl of ethylene glycol di-acetate was taken in a vial with a methanolic solution of 1-vinyl 2-pyrrolidone (540 mM), followed by adding a methanolic solution of $AgNO_3$ (10 mM). Then 3-APTMS (0.5-5 M) was added to the solution, followed by cyclohexanone/3-glycidoxypropyltrimethoxysilane/formaldehyde (20 µl) to yield AgNP-1, 2 and 3, respectively. All reagents were mixed in a sequence, maintained a total reaction mixture volume at 1 ml with methanol, and carefully vortexed on cyclomixer for 30 s. Next, the reaction mixture was placed in a microwave oven to synthesize NPs. An average of 5-8 cycles of 15 s each pulse was given for synthesis. The appearance of deep yellow color indicates the formation of Ag-NP, as shown in the video link https://youtu.be/ASayCJ0WV_M .

The siloxane-sol was made as described earlier [26-27]. The experiments were performed using 12 mM 3-Aminopropyltrimethoxysilane (3-APTMS) and 9 M acetone, followed by the mixing on vertex cyclomixer. The mixture was allowed to stand at 30 °C for 6-12 h, forming a yellow-colored siloxane polymer sol. Both siloxane

polymer sol and silver nanoparticles solution were diluted in methanol and mixed in a 1:1 ratio under stirring to yield homogeneous colloidal suspension and kept in a glass vial for 1-2 h resulting in the formation of siloxane-silver nanofluid and can be used as a spray on surgical catheter over both out and inner portion of the catheter. A similar film can be made over surgical cotton bands and on any desired surface.

The absorption spectra of nanoparticles were recorded using a U-2900 spectrophotometer (Hitachi, Tokyo, Japan) by diluting the synthesized silver nanoparticles in methanol. Transmission electron microscopy (TEM) images were recorded using a Tecnai G2 20 TWIN (FEI, Hillsboro, Oregon™), operated at 200 kV by drop casting of synthesized silver nanoparticles on a 300-mesh sized carbon-coated grid. Light microscopy of silver nanoparticle-treated C. albicans cells was performed with an Olympus binocular compound light microscope (Tokyo, Japan).

2.3 *Antimicrobial assessment and MIC determination of synthesized silver nanoparticles and siloxane-silver nanofluid*

The minimum inhibitory concentration (MIC) values of AgNP-1, 2 and 3 were determined against freshly harvested *R. arrhizus* sporangiospores, *A. bauminnii* and *C. albicans* cells using the two-fold serial dilution method in a flat bottom sterile 96-well microtiter plate as performed previously [37-38]. First, an active suspension of 50 µg/mL Ag-NPs was prepared in water for AgNP-1 and DMSO for AgNP-2 and 3; 20 µL of each suspension was distributed in the wells using the double dilution approach; the final concentration was between 0.10 and 50 µg/mL. Subsequently, 100 µL of the bacterial (10^8 cells/ml) and fungal (10^3 sporangiospores/cells/ml each) suspension was added to each well. Amphotericin B, polymyxin B and sterile distilled water were used as a positive and negative control, respectively. Next, the microtiter plate was incubated in a static position at 37 and 28 °C in separate incubators for 24 h; visual demonstration of turbidity (i.e., a visually clear well) was recorded as the MIC. After that, a 10 µL aliquot from each well was sub-cultured on the Sabouraud dextrose agar (SDA) and Muller Hinton agar (MHA) plates for 24 h; growth was subsequently observed. The MIC value was determined as the concentration of Ag-NPs at which the growth was inhibited or slowed compared to the standard control.

To develop the antibacterial silver nanofluid thin film, the synthesized silver nanoparticles (AgNP-1/AgNP-2/AgNP-3) were diluted in acetone and mixed with siloxane suspension in an equal volume to form a thin film for self-assembling. The antibacterial activity of siloxane, thin film immobilized silver on sterile plastic stripes made from polyester was used. The plastic stripes were sterilized in 70% v/v ethanol under aseptic conditions. Silver nanoparticle-siloxane films using AgNP-1, AgNP-2, and AgNP-3 were prepared manually on sterile stripes and dried under the aseptic condition at 50 °C for 2 h. A log phase cells of *A. baumannii* (10^6 cells/ml) were taken and spread on MHA plates under aseptic conditions, followed by fixing siloxane thin film-coated strips on MHA plates aseptically and incubated for 24 h at 37 °C.

2.4 *Light and scanning electron microscopic examination*

The 24 h treated C. albicans cells were centrifuged, washed with phosphate buffer saline (PBS) twice and stained with Gram's stains, followed by visualizing on a binocular compound light microscope (Olympus, Tokyo, Japan). However, ultrastructural changes in AgNP-1, 2 and 3 treated *A. bauminnii* cells and *R. sporangiospores* were investigated using SEM. Initially, *A. bauminnii* cells and sporangiospores suspensions were treated with AgNP-1, 2 and 3 for 24 h at 28 °C and 37 °C, respectively, followed by centrifugation at 3500 rpm for 6 minutes. The condensed cells were fixed with 2.5% glutaraldehyde and postfixed with 1% aqueous OsO_4, followed by the washing with 0.1 M (pH 7.0) phosphate buffers. Subsequently, the samples were dehydrated in an ascending concentration of ethanol in 30, 50, 70, 80, 90 and 100% for 15 minutes and dried in a vacuum oven. Finally, the cells were placed on the glass coverslip, coated with gold, and observed using an EVO-Scanning Electron Microscope MA15/18 (Carl Zeiss AG, Germany)

3. Results and discussion

It is necessary to first understand the need for two functional alkoxysilanes during the synthesis of Ag-NPs and to investigate the difference in nanoparticle synthesis as described earlier for NPs synthesis [39]. Zhu et al. reported the

synthesis of AuNPs utilizing the active role of amines as a capping and reducing agent containing trimethoxy silane moiety [40]. They used 3-(trimethoxysilylpropyl) diethylenetriamine (TMSP diene) for AuNPs synthesis and found that the ratio of TMSP/Au^{3+} controls the morphology of resulting nanoparticles. The time required for nanoparticle synthesis varied from 2 h to 23 h depending on the ratio of TMSP/cation. The unique structure of the TMSP diene-gold complex has shown a key point to the rapid auto-reduction. They also observed very slow conversion to nanoparticles when TMSP is replaced with 3-APTMS since prolonged auto reduction tenure enables the hydrolysis and polycondensation of silanol residue. These findings revealed that the reducing and stabilizing ability of 3-APTMS, however, predict the essential requirement of additional reducing agents for real-time and controlled nanoparticle synthesis.

Also, the mechanistic role of 3-APTMS and GPTMS mediated synthesis of Ag-NPs is precisely evaluated to have deeper insight. 3-APTMS and GPTMS are hydrophilic and hydrophobic, respectively. It is found that GPTMS on mixing with water generates two separate layers, whereas a single homogeneous layer is observed in the methanolic medium, as shown in Figure 1. In this process, methanol acts as a reactant converting hydrophobic GPTMS to a hydrophilic methanolic solution. The addition of 3-APTMS to GPTMS and vice versa drastically affects the interaction dynamics, which has been one of the findings of the present investigation.

The results based on the above work revealed the followings; (1) the addition of GPTMS to 3-APTMS, present in the reaction system enables faster interaction between glymo- and amino-residue as compared to that of 3-APTMS to GPTMS, justifying the optimum requirement of necessary APTMS molecules per GPTMS molecule, (2) addition of 3-APTMS to GPTMS treated Ag^+ allowed the slow interaction as justified from relatively small variations in absorbance at 280 nm as compared to that of the same when GPTMS is added to 3-APTMS treated silver ion, again justifying the role of an available number of 3-APTMS molecules per GPTMS at the site of glymo and amino-residue interaction and doesn't enable the synthesis of Ag-NPs, (3) when GPTMS is added to 3-APTMS in the presence of $AgNO_3$, the new absorbance peak at 280 nm and 420 nm is more facilitated than that of the same without $AgNO_3$, validating that Ag^+ is catalyzing the reaction between glymo- and amino-residues and enable the synthesis of Ag-NPs [41]. Therefore, the findings discussed above provided valuable. information related to the synthesis of Ag-NPs and confirmed that only 3-APTMS capped Ag^+ undergo GPTMS mediated reduction of Ag-NPs [41].

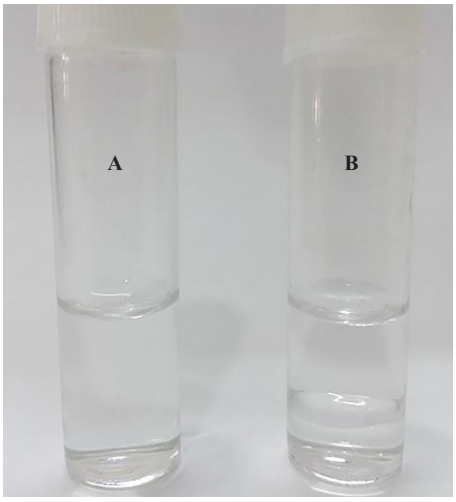


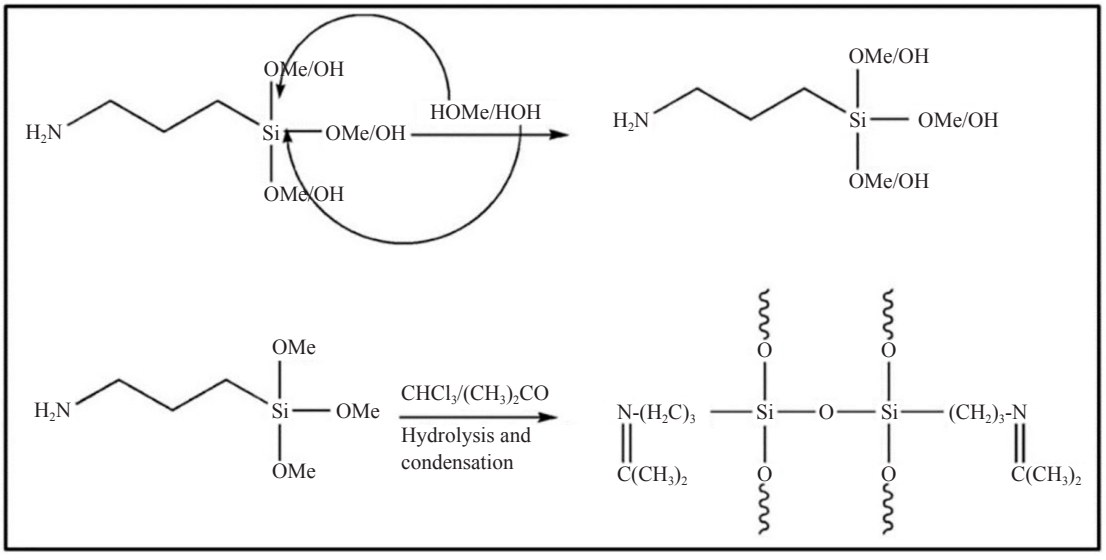
Figure 1. Image of methanolic and aqueous suspension of 3-GPTMS

Methanolic suspension of -GPTMS (A) The reactivity of functionalities linked to tri-alkoxysilane has been precisely investigated in synthesizing monometallic, bimetallic and trimetallic noble metal nanoparticles [23-24], especially organic amine and glycidoxy-group linked alkyl-trialkoxysilane. And the organic aldehydes (formaldehyde/ acetaldehyde/dimethyl ketone.) has also been explored as reducing agent along with 3-APTMS during synthesis of Ag-NPs. The chemistry of imine formation during the synthesis of AgNP-3 revealed the following observations: (a)

imines are typically formed by the condensation of primary amines and aldehydes, (b) imines are formed readily with formaldehyde, and (c) Imines (made from 3-APTMS and formaldehyde) has shown catalytic activity for the reduction of noble metal cations.

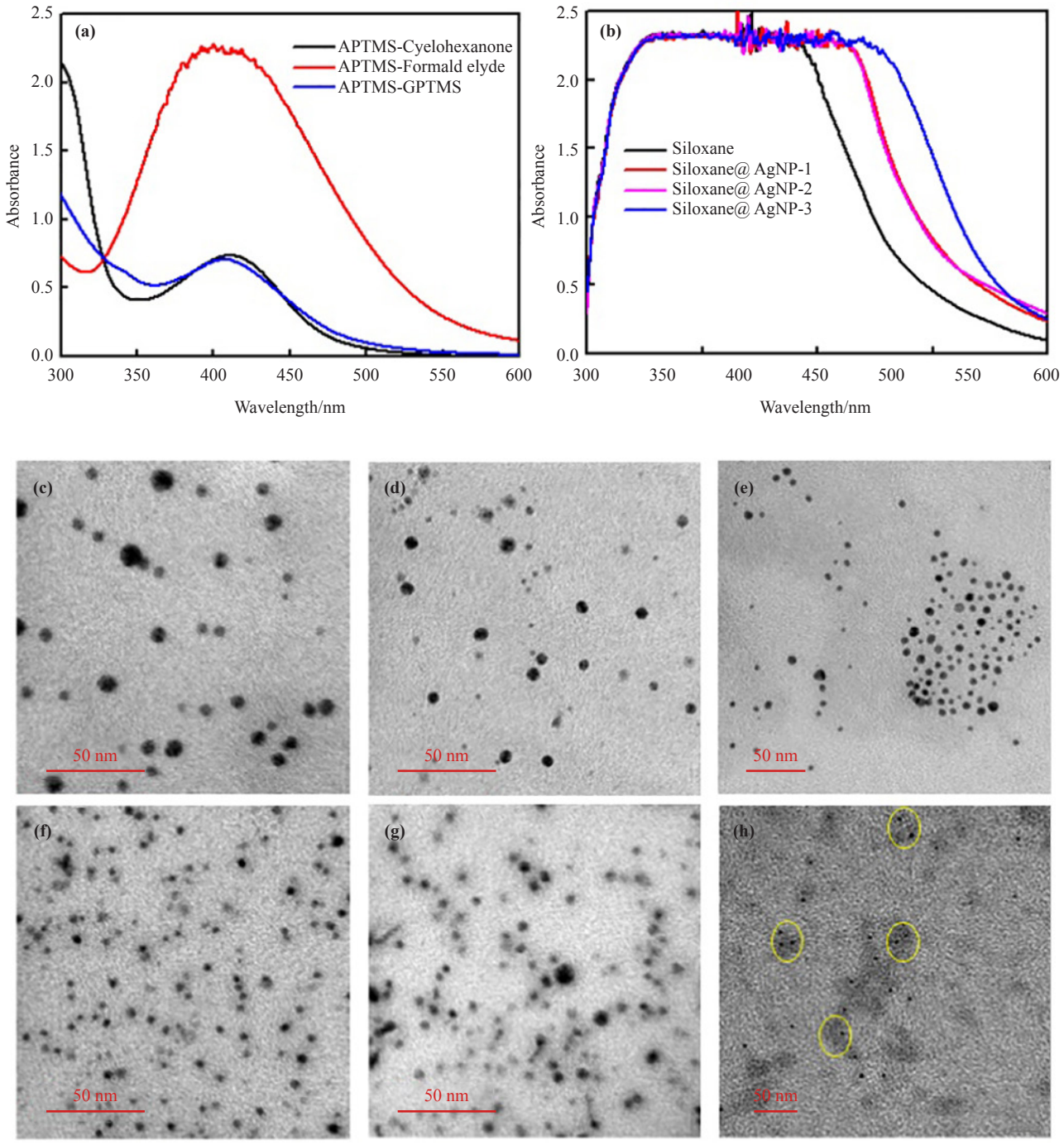
Since silver nanoparticles have shown potent activity against drug-resistant bacteria and viruses, we focused on using organic amine to synthesize silver nanoparticles where the presence of cationic charges was explored in the selective interaction of microbial cells with silver nanoparticles [37, 42-45]. In order to improve the stability and homogeneity of silver nanoparticles, 1% v/v of the total content of 1-vinyl-2-pyrrolidone and ethylene glycol diacetate was used during 3-APTMS mediated formation of silver nanoparticles. These additives significantly improved the stability, dispersibility and morphology of the silver nanoparticles (AgNP-1/AgNP-2/AgNP-3), involving the role of cyclohexanone for AgNP-1, 3-GPTMS for AgNP-2 and formaldehyde for AgNP-3 as shown in Figure 2a, 2c, 2d, and 2e. The synthesized silver nanoparticles had an λmax between 404-415 nm with a size range of ~6-12 nm. The visual photograph of Ag-NP 1-3 is shown in Figure 2(i).

Siloxanes are an important class of polymers used extensively for various applications, preferably involving alkyl silane with the active participation of -Si-H linkages [26-27, 46]. However, the high reactivity of -Si-H restricted the proper exploration of siloxane and directed the making of such polymer using highly stable functional organo-tri-alkoxysilane that has been used in making monometallic/bimetallic and trimetallic noble metal nanoparticles. The current findings demonstrate the use of 3-aminopropyltrimethoxysilane in siloxane formation that subsequently allows the formation of nanofluids for self-assembling. In addition, 3-APTMS also enables the rapid formation of noble metal nanoparticles and their multi-metallic analogues in the presence of a small organic reducing agent [47]. The choice of such a reducing agent is very important as it impacts the physical properties of NPs, like dispersibility in solvents, pH and salt tolerance, etc. Also, the imine linkage between 3-APTMS and a few carbonyl moieties impacts the catalytic activity as it acts as a catalyst. Recently, acetone-induced polymerization of 3-APTMS in chloroform has been observed [46]. Using ^{1}H, ^{13}C and ^{29}Si NMR techniques, they have proved that acetone reacts with the silane amino group to form an imine [$(CH_3)_2C = N(CH_2)_3Si\ (OCH_3)_3$], IPTMS or N-isopropylidene-3-aminopropyltrimethoxysilane. The water released during the imine formation hydrolyses the methoxy silane, thereby inducing the formation of siloxane and Si-O-Si bridges [46]. The mixing with acetone in aprotic solvents forms an imine [$(CH_3)_2C = N(CH_2)_3Si\ (OCH_3)_3$], IPTMS or N-isopropylidene-3-aminopropyltrimethoxysilane [47]. The water molecules released during the imine formation hydrolyze the methoxy silane moieties of 3-APTMS, inducing the formation of siloxane and Si-O-Si bridges upon condensation [46]. The hybrid materials made of siloxane polymer and Ag-NPs are obtained in the case of aprotic organic solvents like chloroform and acetone (acetone is used both as a solvent and mild reducing agent) as shown in scheme 1. Conversely, only discrete and spherical Ag-NPs are obtained using protic solvents like water and methanol.



Scheme 1. Formation of siloxane

Accordingly, it is justified that 3-APTMS in several non-aqueous solvents enables the formation of siloxane and simultaneously enables the formation of silver nanoparticles as silver nanofluid having property for casting thin film via spraying the nanofluid encapsulating the noble metal nanoparticles and directed the present work on siloxane-silver nanofluid for antimicrobial applications. Furthermore, the choice of ethylene glycol di-acetate provided a valuable addition to silver nanoparticle synthesis in the presence of a methanolic solution of 1-vinyl 2-pyrrolidone on precise reduction of silver cations into silver nanoparticles under microwave exposure [25].



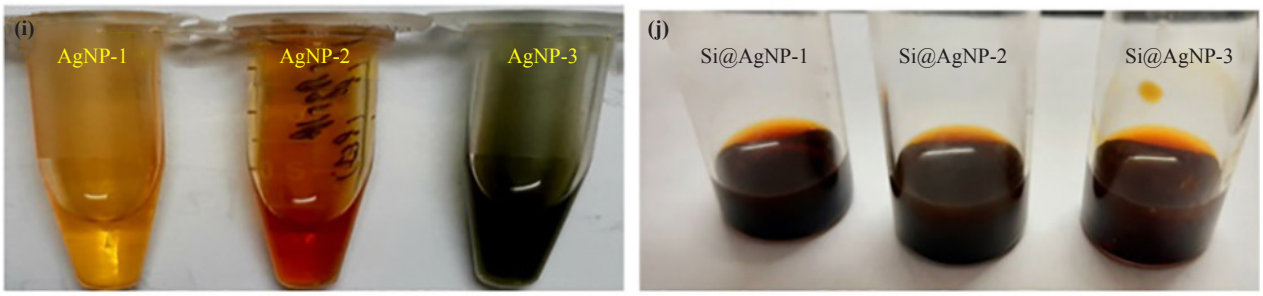


Figure 2. UV-VIS spectra of silver nanoparticles, siloxane polymer sol and corresponding visual photograph and TEM images of AGP-1, AgNP-2 and AgNP-3. (a) representing UV-VIS spectrum of synthesized AgNP-1, 2 and 3; (b) UV-VIS spectrum of Siloxane and siloxane dispersed silver nanoparticles; (c, d and e) representing the TEM images of AgNP-1, 2 and 3 respectively; (f, g and h) representing TEM images of siloxane dispersed AgNP-1, 2 and 3 respectively; (i and j) showing visual photograph of synthesized silver nanoparticles and siloxane dispersed silver nanoparticles.

The UV-VIS spectra of siloxane polymer are shown in Figure 2; b, i and j, along with Ag-NP-1-3. The siloxane polymer sol was yellow colored with λmax between 360-450 nm. Once made, siloxane sol undergoes self-assembled thin film formation over a variety of solid-substrate that could be explored in evaluating many properties of siloxane film for practical applications. The siloxane sol, as shown in Figure 2, when mixed under stirring with silver nanoparticles derived from 3-APTMS, enabled the formation of silver nanofluid. The nanofluid can be diluted in methanol in the desired ratio, as shown in Figure 2 (j). The TEM analysis has been performed for siloxane dispersed Ag-NPs and is observed that the size of silver nanoparticles was reduced compared to the non-dispersed. The result is shown in Figure 3, describing the impact of siloxane mixing on the size of Ag-NPs. It is observed that siloxane polymer induces the reduction process that makes Ag-NPs more favorable for biomedical applications.

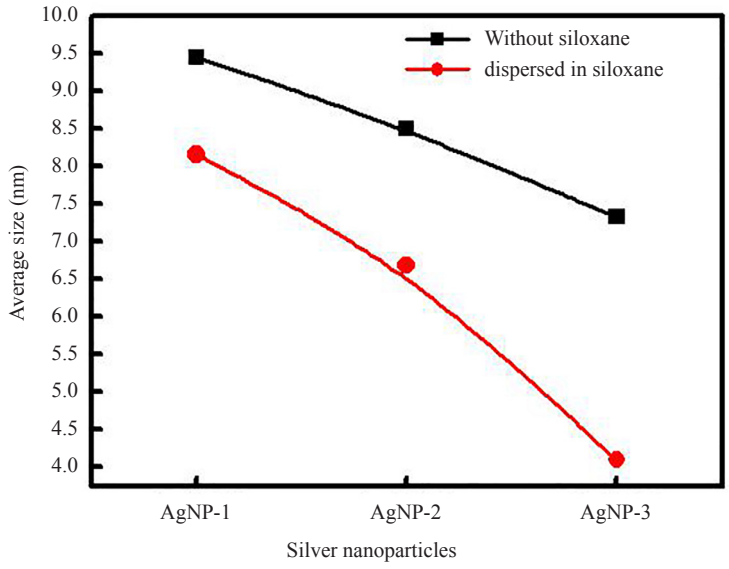


Figure 3. The silver nanoparticle average size comparison of siloxane non-dispersed and dispersed AgNP-1, 2 and 3.

The MIC of synthesized silver nanoparticles (AgNP-1, 2, and 3) determined against *A. bauminnii*, *C. Albicans* and sporangiospores of *R. arrhizus* were 7.3, 6.5 and 5.6 µg/ml for AgNP-1; 26.7, 23.1 and 24.0 µg/ml for AgNP-2 and 27.8, 21.3 and 22.4 µg/ml for AgNP-3 respectively, as shown in Figure 4. The ultrastructural changes induced by silver nanoparticle treatment on *A. bauminnii* cells and *R. arrhizus* sporangiospores were examined as shown in Figure 5 and 7. However, the structural changes in *C. albicans* were examined using light microscopy, as shown in Figure 6. The result

has shown that all three nanoparticles drastically affected the cellular structure of tested microorganisms. AgNP-1 has induced less cell damage than AgNP-2 and 3 against all tested microorganisms; however, the MIC value was too low, justifying the biocidal dynamics based on reducing agents.

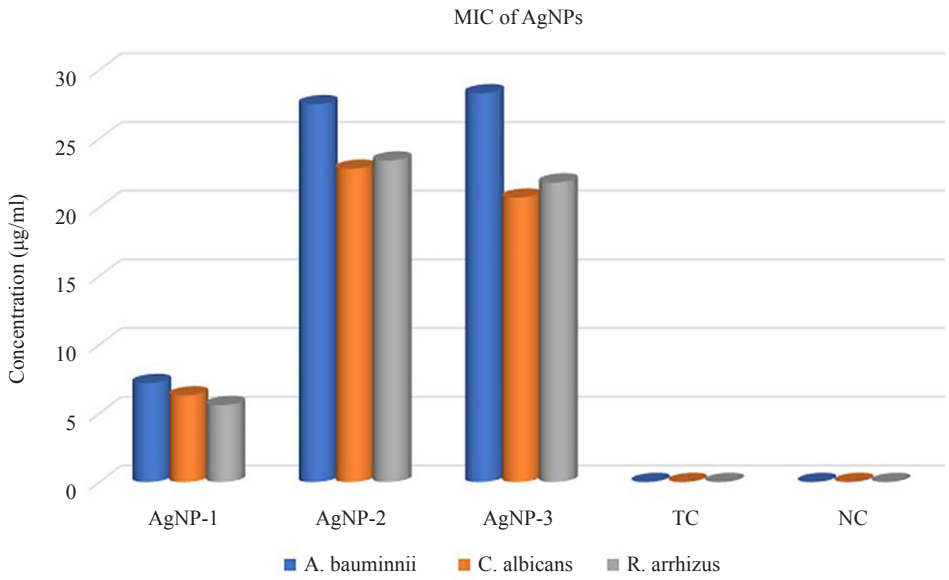


Figure 4. Comparative MIC values of AgNP-1,2 and 3 against *A. bauminnii, C. albicans* and *R. arrhizus* sporangiospores

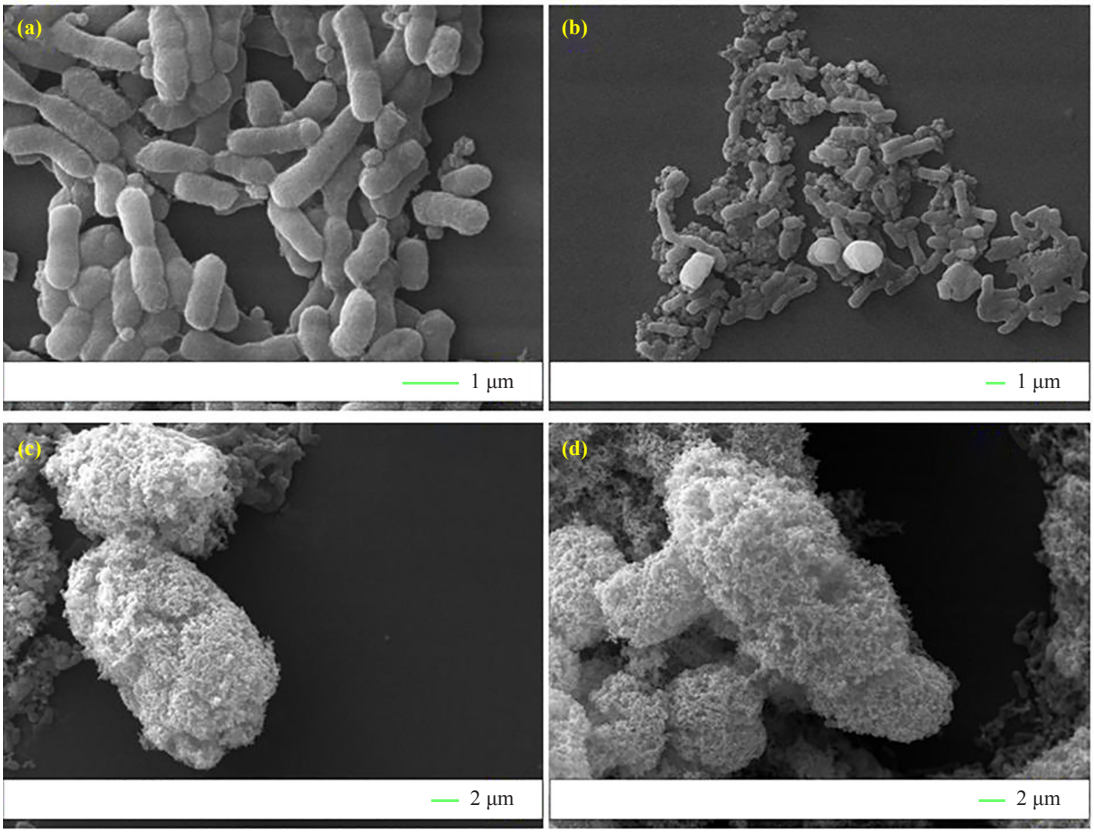


Figure 5. SEM images of *A. bauminnii* cells treated with AgNP-1 (b); AgNP-2 (c); AgNP-3 (d) and control cells (a).

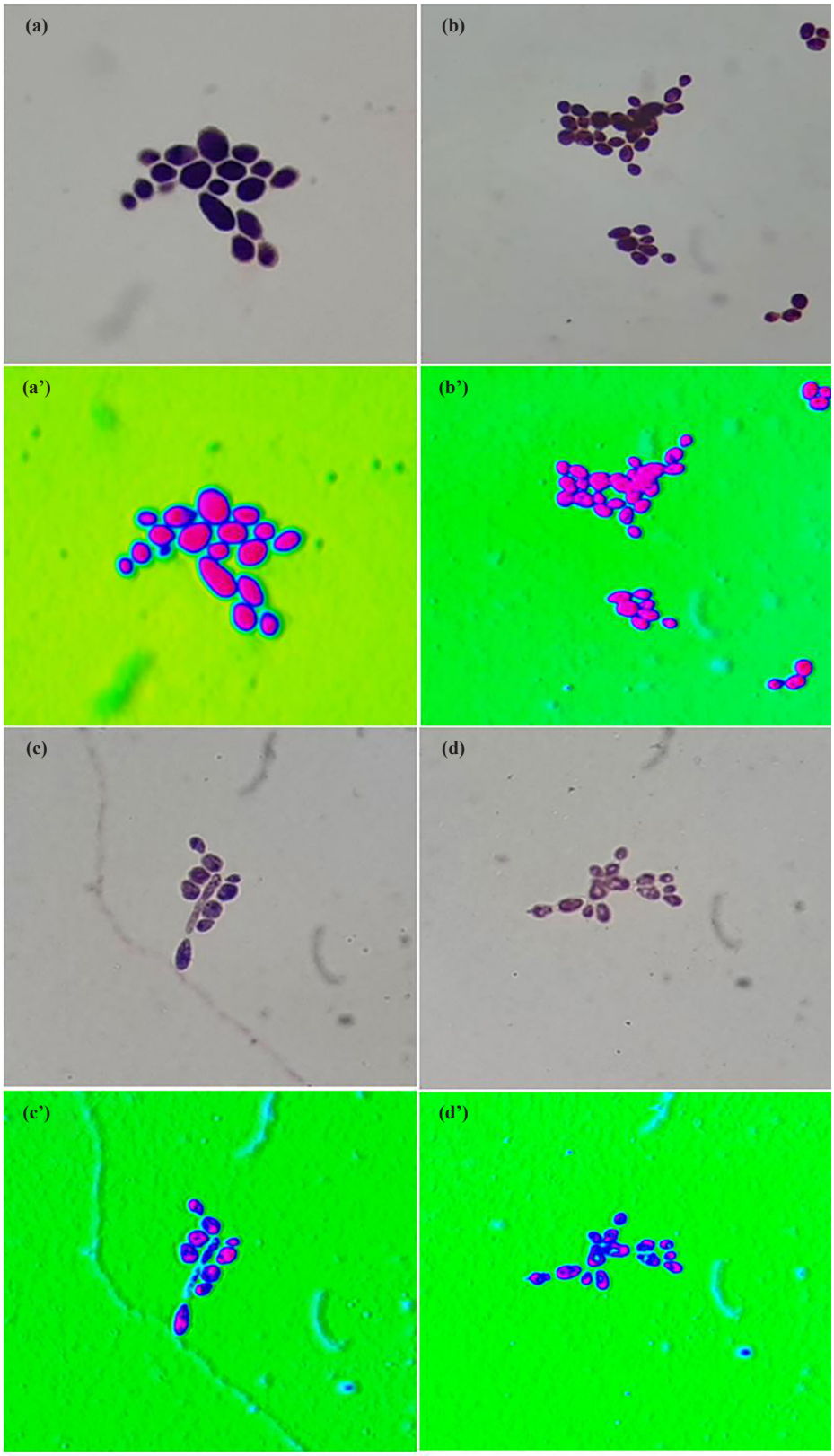


Figure 6. Light microscopy images of *C. albicans* cells with their respective 3D images; treated with AgNP-1 (b, b'); AgNP-2 (c, c'); AgNP-3 (d, d') and control cells (a, a').

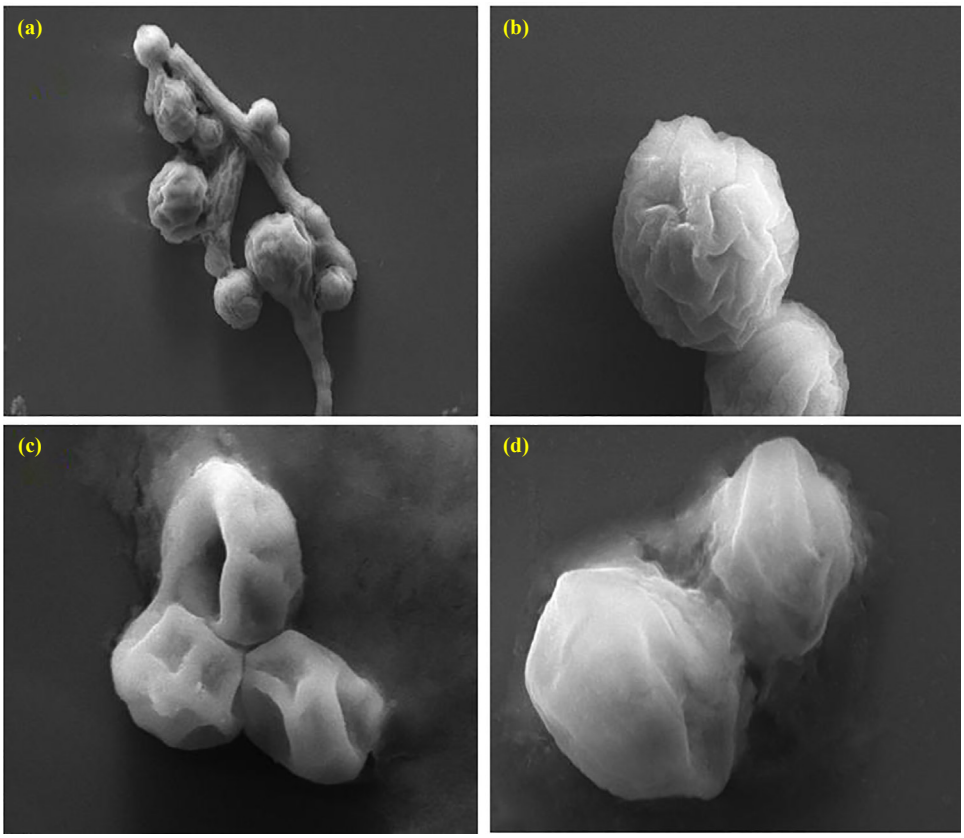


Figure 7. SEM images of *R. arrhizus* sporangiospores treated with AgNP-1 (b); AgNP-2 (c); AgNP-3 (d) and control sporangiospores (a).

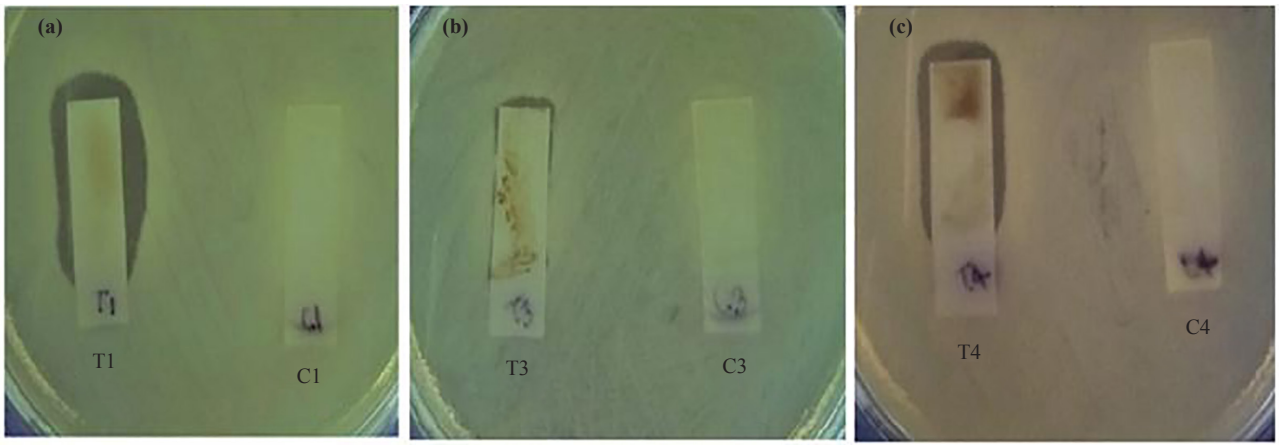


Figure 8. Antibacterial assessment plates of siloxane-Ag-NP film (T) casted on polyester sheet of 0.25 mm diameter and pasted over sterile agar plat with *A. baumannii* and with only siloxane polymer film (C) containing all ingredient used in silver nanoparticles formation except silver cations: with siloxane-Ag-NP film containing AgNP-1: T1, C1 (A), AgNP-2: T3, C3 (B), and AgNP-3: T4, C4 (C).

The Siloxane-silver nanofluid formed a thin film on sterile solid plastic strips with 0.25 mm thickness and has shown potential disinfectant activity against drug-resistant bacteria, and viruses as a function of organic reducing agent participated during the synthesis of AgNP-1, AgNP-2, AgNP-3. *A. baumannii,* a multidrug-resistant bacterium, has been examined for antibacterial activity using silver nanofluid thin films made on sterile polyester sheets. First, the polyester sheets were sterilized, followed by making self-assembled thin films with Ag-NP and placed in agar plate (T1) along

with subsequent loading of control (C1) containing all the components of siloxane without silver cations, and the film made with siloxane-silver nanofluids as shown in Figure 8a, 8b and 8c for AgNP-1, AgNP-2 and AgNP-3 respectively. As shown in Figure 8, the results demonstrated that (i) the control film made with a siloxane containing all other components except silver nanoparticles is insensitive to bacterial strain, (ii) *A. baumannii* interactions with siloxane-silver nanoparticles film suggested selective binding of siloxane silver nanoparticles present in the film as a function of organic reducing agents, e.g., cyclohexanone for AgNP-1, 3-GPTMS for AgNP-2 and formaldehyde for AgNP-3. It must be noted that many silver nanoparticles, when present in a thin film, lose their antibacterial activity; however, the self-assembled thin film of siloxane-silver nanoparticles showed variable antibacterial activity against multidrug-resistant bacteria *A. baumannii*. resistant bacteria *A. baumannii*.

It is noted that all controlled films C1, C3 and C4 of siloxane do not show antibacterial activity, whereas Ag-NP, Ag-NP2 Ag-NP-3 immobilized films showed the following observations: (a) The Ag-NP film made with cyclohexanone displayed excellent antibacterial activity, (b) The Ag-NP film made with 3-GPTMS showed poor antibacterial activity as compared to that for AgNP-1 and AgNP-3 and (c) The AgNP-3 film also showed good antibacterial activity to a somewhat lesser extent as compared to that of the AgNP-1 film. Such observation predicts the release of Ag-NPs from a self-assembled thin film. Since the presence of 3-glycidoxypropyltrimethoxysilane (3-GPTMS) converts the siloxane film relatively less porous with tight encapsulation of Ag-NP-2 due to their sol-gel processing of tri-alkoxysilane during film formation and constitute relatively less porous film of siloxane as compared to that of AgNP-1 and AgNP-3 made using cyclohexanone and formaldehyde respectively. These findings predict that innovation in the antibacterial thin film can be precisely controlled by the nature of reagents explored in nanoparticle formation when needed. A prototype of siloxane-silver nanofluid coated endotracheal tube and disinfectant nanofluid for the biomedical application is shown in Figure 9. The siloxane-silver nanofluid thin film prepared by dipping the catheter in a beaker filled with diluted nanofluid. The concentration/ratio of silver nanoparticles in nanofluid can be optimized by diluting in methanol, acetone or other volatile solvents.

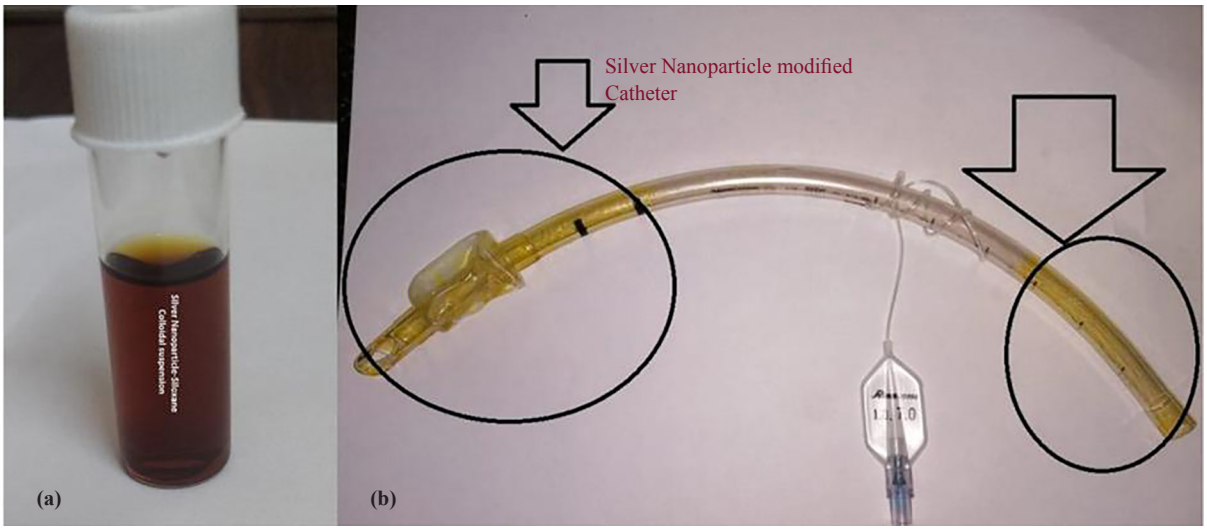


Figure 9. (a) Visual image of siloxane-silver nanoparticles spray, (b) image shown modification in catheter surface made by the silver nanofluid spray.

4. Conclusions

This study developed and discussed the synthesis and antimicrobial activity of functional alkoxysilane capped silver nanoparticles and siloxane-silver nanofluid. Three silver nanoparticles had variable particle sizes, i.e., AgNP-1, AgNP-2 and AgNP-3, were synthesized as a function of organic reducing agents (cyclohexanone/3-glycidoxypropyltrimethoxysilane/formaldehyde) along with 3-aminopropyltrimethoxysinae as capping agent. All three sized Ag-NPs has shown potent antimicrobial activity and the MIC values of each silver nanoparticles were dependent

on the microbial species and nature of reducing agent used. Additionally, 3-APTMS enabled the formation of siloxane polymer in the presence of acetone and allowed the formation of silver nanofluid as a potential antimicrobial spray for making a self-assembled thin film of Ag-NP over any desired solid support. The thin films of Ag-NPs on solid synthetic strips have shown potential antibacterial activity against drug-resistant bacteria as a function of reducing agents, i.e., cyclohexanone/3-glycidoxypropyltrimethoxysilane/formaldehyde that allows controlling the porosity of the nanofluid film. The findings justified the noble report of antibacterial activity of nanofluid thin film as a disinfectant for biomedical applications.

Authors contribution

Study conceptualization, A.K.T., M.K.G. and P.C.P.; methodology, A.K.T. and M.K.G.; investigation, A.K.T. and M.K.G.; resources, A.K.T., M.K.G., and P.C.P.; data curation, A.K.T. and M.K.G.; writing-original draft preparation, A.K.T., G. P. and P.C.P.; writing-review and editing, G.P., P.C.P.; visualization, P.C.P.; supervision, P.C.P., All authors have read and agreed to the published version of the manuscript.

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Data availability statement

The datasets generated during and/or analysed during the current study are included in this article.

Conflict of interest

The authors declare no conflict of interest.

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