CARBOXY-FUNCTIONALIZED POLYSACCHARIDE MEDIATED GREEN SYNTHESIS OF ANTIMICROBIAL SILVER NANOPARTICLES

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The present study deals with the green synthesis of silver nanoparticles (Ag NPs) using a chemically modified polysaccharide as a reducing and capping agent. Dextran succinate (Dex-Suc) was synthesized by succinylation of the dextran with succinic anhydride at 80 °C for 24 h under stirring and N_2. The Dex-Suc was used as a reducing and capping agent for the synthesis of Ag NPs in the presence of sunlight with high UV index. The Ag NPs were characterized using UV-Vis spectroscopy, SEM, and EDS techniques. The SEM data revealed the preparation of spherical Ag NPs having 50 nm size. The Ag NPs showed antimicrobial properties against different bacterial and fungal strains.

Keywords: antimicrobial, biopolymer, green chemistry, nanoparticles, polymer functionalization, polymeric materials

INTRODUCTION

The field of nanotechnology is developing quickly due to the attractive optical, catalytic and electrical properties of nanoparticles (NPs).[1] It has been established that the characteristics of NPs depend upon their shape and surface chemistry.[2] Ag NPs are synthesized (320 tons per year) mainly due to their microbicidal nature.[3] However, Ag NPs also found applications in bio-imaging, detecting, makeup materials, foodstuffs, interior paints, and tumor treatments.[4,5]

To fully take advantage of the benefits offered by Ag NPs in daily life, pharmaceuticals, and medicines; it is important to prepare Ag NPs with the help of green/non-toxic, biodegradable, and economical reagents.[6] So far, the preparation of Ag NPs has been reported through a number of methods, including photochemical and chemical reduction, laser ablation, electrochemical methods, electromagnetic radiation *etc*.[6-9] In this category, a few polysaccharide materials/reducing agents have been utilized effectively, such as hydroxypropylcellulose,[10] dextran,[11] cellulose,[12] chitosan,[13] arabinoxylans,[14] and rhamnogalacturonan[15] for the green synthesis of Ag NPs and to avoid the use of toxic reducing agents, like $NaBH_4$,[16] etc. Among polysaccharides, dextran is one of the most widely used biopolymers in pharmaceuticals due to its biodegradable and biocompatible nature.[17,18]

Keeping in view the benefits of natural polysaccharides for the green synthesis of Ag NPs, dextran will be chemically modified to get its carboxy functionalized derivative as dextran succinate (Dex-Suc). Dex-Suc will be then used as reducing, as well as a capping agent for the synthesis of Ag NPs with virtually no reagent. This versatile modified biomaterial (Dex-Suc) will be employed under diffused sunlight to synthesize Ag NPs. Hence, the present work reports on a green, easy and rapid synthesis of Ag NPs using Dex-Suc, which is an economical, non-toxic, and eco-friendly method. The aim is also to explore the antibacterial and antifungal activities of Ag NPs (Dex-Suc-Ag NPs) against strains like

E. coli (ATCC 25922), *B. subtilis* (ATCC 6633), *S. aureus* (ATCC 25923) and *A. niger*, respectively.

EXPERIMENTAL

Materials

Dextran (Mw 40000), solvents and other chemical reagents of analytical grade were procured from Sigma Aldrich, USA. The $AgNO_3$ was procured from Merck, Germany. Deionized water (DW) was used during this work.

Characterization techniques

The FTIR spectra were recorded on an IR-Prestige-21 (Shimadzu, Japan) after making the pellets of samples with KBr using a hydraulic press. The pellets prepared under pressure were dried for 10-15 min in a vacuum oven just before analysis. The UV-Vis spectra were performed on a UV-Vis spectrophotometer (UV-1700 Pharmspec, Shimadzu, Japan) in the range of 800–200 cm^{-1}. A scanning electron microscope (SEM, Quanta 250, FEI, USA) was used to characterize Ag NPs, whereas the images of the samples were recorded in transmission electron microscopic (TEM) mode of SEM (1530 Gemini LEO, Carl Zeiss). Energy-dispersive X-ray spectroscopy (EDS) was coupled to SEM and was employed to characterize Ag NPs regarding the elements available in samples.

Preparation of dextran solution

The pre-dried dextran (1.0 g, 110 °C, 5 h) was suspended in *N,N*-dimethylacetamide (DMAc, 30 mL) and heated at 80 °C for 30 min. To the homogenized dextran suspension, LiCl (1.0 g) was added and the mixture was stirred for another 30 min to get an optically clear solution of dextran.

Synthesis of dextran succinate

To the solution of pre-dissolved dextran (1.0 g, 6.17 mmol) in DMAc/LiCl, succinic anhydride (2.468 g, 24.67 mmol) was added in parts and the reaction proceeded at 80 °C for 24 h. The reaction mixture was precipitated in acetone (200 mL). Thick precipitates of the desired product – dextran succinate (Dex-Suc) – were obtained and washed thrice with acetone (100 mL) before drying in a vacuum oven set at 50 °C overnight.

Yield: 2.1 g, 79%; DS: 2.68 (calculated with acid-base titration after saponification); color: colorless; solubility: soluble in water, DMAc, and dimethyl sulfoxide (DMSO); insoluble in diethyl ether, hexane, and acetone.

FTIR (KBr): 3427 (OH), 1732 (CO_{Ester}), 1030 (COC_{AGU}) cm^{-1}

Dex-Suc mediated synthesis of Ag NPs

Freshly prepared Dex-Suc (1%, 2 mL) solution was taken in a tube and mixed with 2 mL of 25 mmol $AgNO_3$ solution. $AgNO_3$ (0.42 and 0.84 g) was dissolved in DW (100 mL) to prepare $AgNO_3$ solutions equivalent to 25 and 50 mmol, respectively. The reaction mixture remained unchanged in the dark, as no color change/reaction was observed. The reaction mixture was then exposed to sunlight (of high UV index) and samples were collected (3 mL) at different time intervals (0.5, 1, 1.5, 2.5, 5 and 10 min) from both samples (*i.e*., 25 and 50 mmol $AgNO_3$) and the change in color was monitored under a UV-Vis spectrophotometer.

Scanning electron microscopy (SEM)/Energy dispersive X-ray spectroscopy (EDS)

The Dex-Suc-Ag NPs solutions were centrifuged via ultracentrifugation and the residue was taken for SEM analysis recorded in TEM mode. The surface topology/morphology and elemental composition of Dex-Suc-Ag NPs were studied by blotting a drop onto a carbon coated copper grid using SEM-EDS operating at 10 kV. EDS was also recorded simultaneously.

Determination of DS of succinylation

The DS of succinyl moieties onto dextran was calculated using the acid-base titration method. The Dex-Suc (100 mg) was stirred in 0.02 mol/L $NaHCO_3$ solution (100 mL, 2 h, room temperature). After filtration, a known volume of the $NaHCO_3$ solution was titrated against 0.02 mol/L HCl. The methyl orange was used as an indicator of the reaction. From the volume of the acid (HCl) used, the DS of free COOH groups was drawn following Equations (1) and (2):

$$n_{Suc} = (V_{base} \times M_{base}) - (V_{acid} \times M_{acid}) \quad (1)$$

where n_{Suc} is the number of moles of free COOH moieties; V_{base} is the volume of $NaHCO_3$ titrated against V_{acid} which is the volume of HCl used; M_{base} is the molarity of $NaHCO_3$; M_{acid} is the molarity of HCl.

$$DS = [162.14 \times n_{Suc}]/[(m_{Dex\text{-}Suc} - 100) \times n_{Suc}] \quad (2)$$

where 162.14 (g/mol) is the molar mass of an anhydroglucose unit (AGU) of dextran; 100 (g/mol) is the net increase in the mass of an AGU for each substituted succinyl group; n_{Suc} is number of moles of free COOH groups; $m_{Dex\text{-}Suc}$ is the mass in g of Dex-Suc being analyzed.

Determination of yield of Dex-Suc

The following equation was used to calculate the theoretical yield of Dex-Suc:

$$Theoretical\ yield = m_{Dex\text{-}Suc} + [(m_{Dex\text{-}Suc}/M_{Dex}) \times DS \times M_{Suc}] \quad (3)$$

where $m_{Dex\text{-}Suc}$ (g) is the mass of Dex-Suc used; M_{Dex} is the molecular mass of the dextran (162.14 g/mol); DS is the degree of substitution of succinate moieties determined by acid-base titration after saponification; M_{suc} (g/mol) is the molecular mass of a succinate group.

Antimicrobial activities

Ag NPs were investigated for their fungicidal and bactericidal effects against some bacterial (*E. coli* ATCC 25922 (American-type culture collection), *B. subtilis* ATCC 6633, and *S. aureus* ATCC 25923,) and a fungal strain (*A. niger*) grown on Mueller-Hinton agar media (Thermo Fisher Scientific, Waltham, MA, USA) and Sabouraud dextrose agar (Hardy Diagnostics, Santa Maria, CA, USA), respectively. The protocol followed for these studies was reported earlier.[15,19] The negative control used consisted in deionized water. All experiments were repeated thrice to report average values.

RESULTS AND DISCUSSION

Synthesis of dextran succinate (Dex-Suc)

Dextran was chemically modified to its succinate derivative (Dex-Suc) using succinic anhydride. Succinic anhydride first reacts with free hydroxyl groups on dextran to form its ester derivative as Dex-Suc. The reaction proceeded under homogenous reaction conditions created by dissolving dextran into DMAc at 80 °C for 24 h. A high yield (2.1 g, 79%), with a high degree of succinyl substitution (DS 2.68), was obtained for the product. The Dex-Suc was soluble in water, DMAc and DMSO, whereas it remained insoluble in non-polar solvents, such as acetone, chloroform and hexane. The reaction scheme for the synthesis of Dex-Suc is depicted in Figure 1.

The structures of Dex and Dex-Suc were analyzed using FT-IR (KBr) spectroscopy. Figure 2 illustrates the conversion of Dex to Dex-Suc as a distinct peak of carbonyl present at 1732 cm^{-1} – this peak is typical for ester bond formation.[20]

Synthesis of Ag NPs

The Dex-Suc conjugate contains free carboxylic acid groups that can be exploited to reduce Ag^{+} to Ag^{0}. The zerovalent silver (Ag^{0}) got stabilized due to carboxylate anion termini of Dex-Suc as the reaction of $AgNO_3$ with Dex-Suc to form Ag NPs was performed in DW. Therefore, Dex-Suc not only effectively reduced the silver ions to synthesize Ag NPs, but also appeared as a stabilizing agent *in situ* to form $[Ag(Dex\text{-}Suc)]^{+}$. These Dex-Suc stabilized Ag NPs were therefore conveniently synthesized according to an economical and green protocol just under diffused sunlight. The mechanism of reduction and stabilization is depicted in Figure 3.

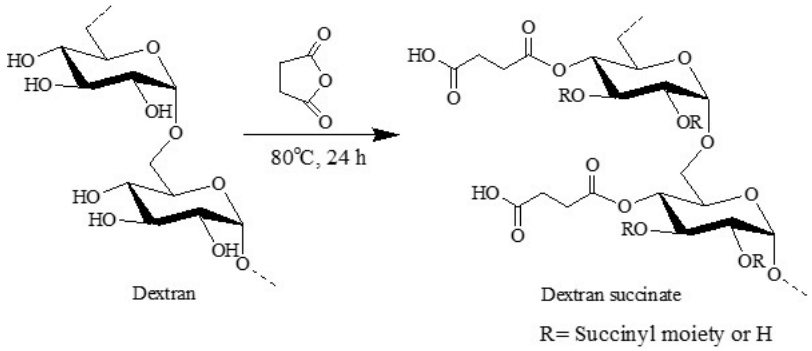

Figure 1: Succinylation of dextran using succinic anhydride

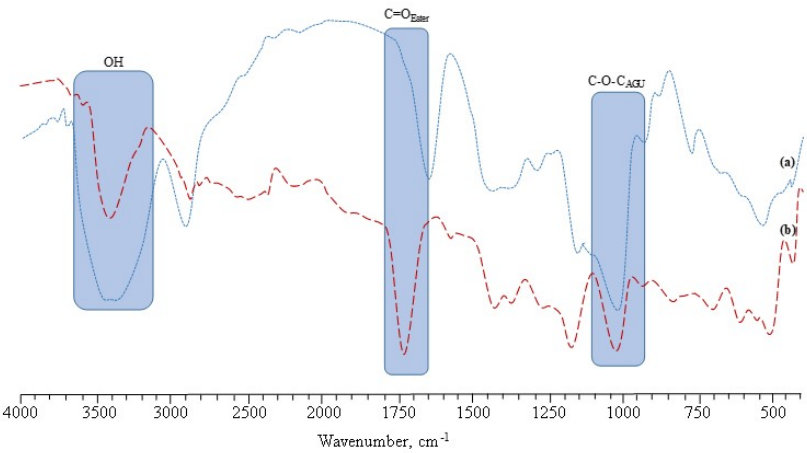

Figure 2: FT-IR spectra of: a) Dex and b) Dex-Suc

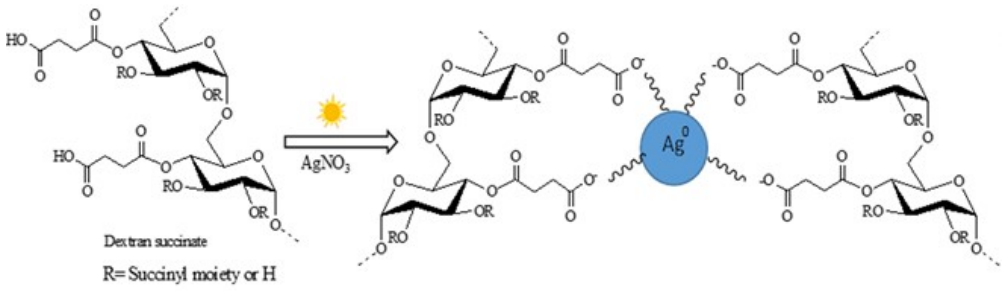

Figure 3: Diffused sunlight-assisted synthesis of Ag NPs capped by Dex-Suc conjugate

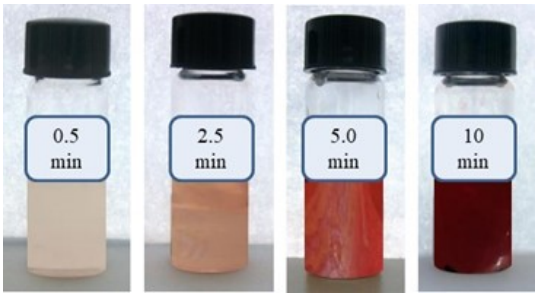

Figure 4: Pictures of Dex-Suc-Ag (Ag NPs) showing color change with reduction of Ag^{+} to Ag^{0} under diffused sunlight at different time intervals

The progress of the reaction was followed by noting the color of the reaction mixture at different time intervals, and color changes were recorded as an indication of the reduction of silver ions by Dex-Suc into the Ag NPs of different sizes.[11,15] The color change for 50 mmol sample (Fig. 4) indicates the red shift as color is being changed from colorless to light brown and reddish brown and dark brown, respectively. This is generally related to the growth in size of Ag NPs with passage of time on exposure to sunlight.[15] Similar red shift was observed in UV-Vis spectra of the synthesized Ag NPs with 25 mmol $AgNO_3$ solution.

Characterization of Ag NPs

UV-Vis spectrophotometry

The 25 and 50 mmol samples showed distinct absorption peaks in UV-Vis spectroscopic analyses. The peaks appearing at 402, 405, 412, 430, 441 and 446 nm for the samples corresponding to the time intervals of 0.5, 1, 1.5, 2.5, 5 and 10 min, respectively, for 25 mmol are shown in Figure 5 (a), whereas the peaks at 405, 408, 412, 425, 444 and 450 nm for the time intervals of 0.5, 1, 1.5, 2.5, 5 and 10 min, respectively, for 50 mmol are shown in Figure 5 (b). Moreover, the peaks appearing at 407, 410, 416, 430, 450, and 460 nm after 0.5, 1, 1.5, 2.5, 5, and 10 min are depicted in Figure 5 (c). UV-Vis results indicate a red shift as the time of nucleation is increasing up to 10 min.[14]

Scanning electron microscopy

After centrifugation of Dex-Suc-Ag (Ag NPs) samples (25 and 50 mmol), they were subjected to SEM and images were taken in TEM mode to observe the size range and morphology (Fig. 6). The SEM images in TEM mode revealed spherical geometry for Ag NPs, stabilized with Dex-Suc, showing almost uniform distribution. The optimal size of Ag NPs noted from the SEM images lies well under 50 nm. Almost similar size distribution (<50 nm) of the Ag NPs prepared from dextran or its modified form has been reported in the literature.[21,22]

Energy dispersive X-ray spectroscopy (EDS)

While recording the TEM images, the EDS spectra of the samples were also recorded (Fig. 7) to see the elemental profile of the sample. The EDS results indicated the presence of well-recognizable signals of Ag typical for the Ag NPs (Ag^{0}), along with the signals of carbon and oxygen coming from Dex-Suc (stabilizing agent). It is also a preliminary assessment of the capping of Ag NPs with Dex-Suc moieties.

Antimicrobial properties

The antimicrobial activity of the synthesized Ag NPs was evident through the determination of the zone of inhibition. The zones of inhibition of *E. coli*, *B. subtilis*, *S. aureus* and *A. niger* were measured as 25 and 21 mm, 15 and 13 mm, 17 and 13 mm, and 20 and 17 mm for Ag NPs prepared from 25 mmol and 50 mmol solutions of Ag NO_3, respectively (Fig. 8). It was observed that the zones of inhibition of the Ag NPs prepared from 25 mmol solution of Ag NO_3 were greater than those corresponding to the solution of 50 mmol. Moreover, the size of the Ag NPs prepared from the 25 mmol solution was lower than that of the Ag NPs prepared from the 50 mmol solution, as observed in the SEM images (Fig. 6). Therefore, due to the smaller size and increased surface area of the Ag NPs, the tendency of these Ag NPs to adhere to the surface of microorganisms and the penetration through the cell wall were increased.[23]

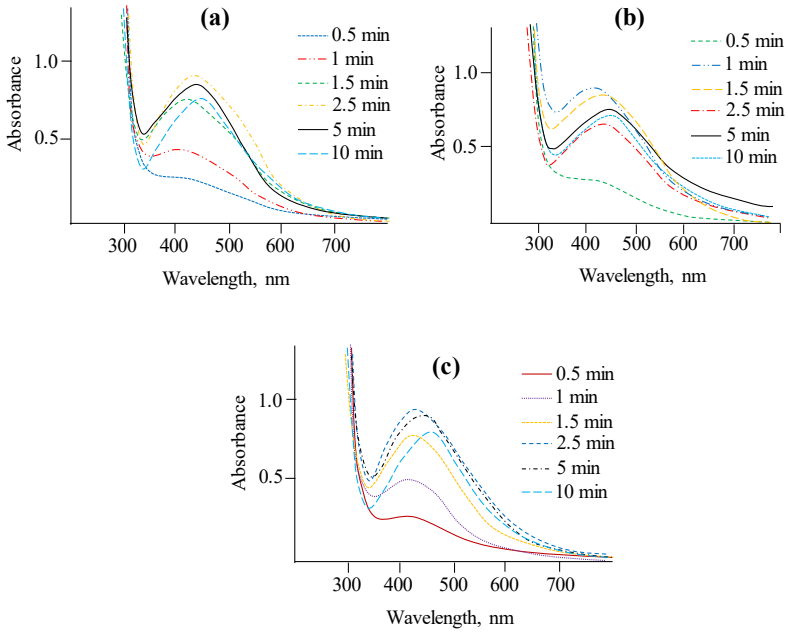

Figure 5: UV-Vis spectra of Dex-Suc-Ag: a) 25 mmol, b) 50 mmol, and c) 75 mmol solution

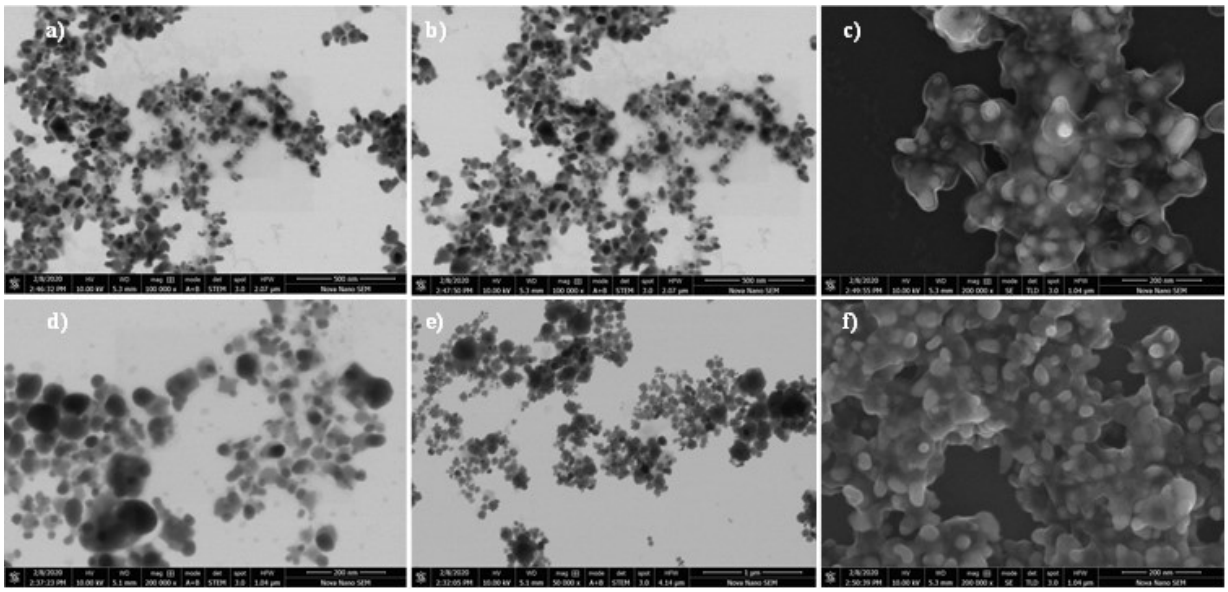

Figure 6: SEM images of Ag NPs: (a-c) 25 mmol $AgNO_3$ solution and (d-f) 50 mmol $AgNO_3$ solution

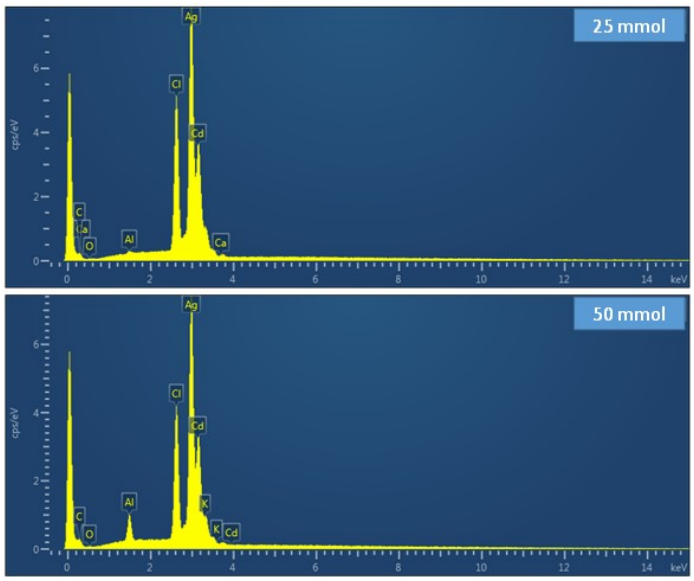

Figure 7: EDS results of Ag NPs samples using Dex-Suc as reducing and capping agent

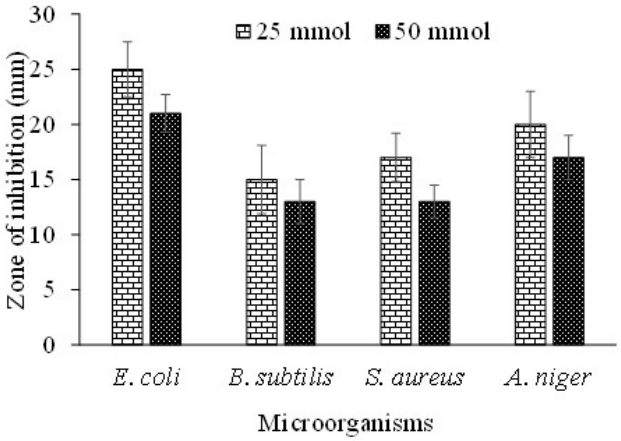

Figure 8: Antimicrobial activity of Ag NPs prepared from 25 mmol and 50 mmol $AgNO_3$ solutions against different bacterial and fungal strains

Consequently, the antimicrobial activity of the Ag NPs prepared from the 25 mmol solution was higher, compared to that of the Ag NPs prepared from the 50 mmol solution (Fig. 8).

CONCLUSION

An economical and green strategy for the synthesis of Ag NPs has been established herein. The developed method utilized a non-toxic, hydrophilic, biocompatible, biodegradable and chemically modified dextran (*i.e.*, dextran succinate) as a reducing as well as *in situ* stabilizing agent. As a result, small sized Ag NPs (<50 nm) were synthesized, as indicated by the TEM images and UV-Vis results. Such NPs can be potentially utilized for medical and biological applications, including wound healing, wound dressings, seed germination, as well as in materials for everyday use. Further studies (*i.e.*, electrochemical) are required to establish the capping mechanism of Dex-Suc onto the Ag NPs. Moreover, the synthesized Ag NPs proved a significant antimicrobial potential against common bacterial and fungal strains. As a future prospect, such Ag NPs may be investigated as a potential agent for coating different medical devices for sterilization.

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