Thiourea functionalized β-cyclodextrin as green reducing and stabilizing agent for silver nanocomposites with enhanced antimicrobial and antioxidant properties†

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Rapid development of microbial resistance against traditional antibiotics has generated a need for the synthesis of new more potent, less toxic, target specific, cost effective and biodegradable antimicrobial agents. Biopolymer based Ag-nanocomposites are a good alternative for this purpose. With the auxiliary privilege of being utilized as prospective stabilizer and emulsifier, a biopolymer reaffirms the edge over traditionally used chemicals in nano-chemistry. In view of the above context, the present investigation involves microwave assisted facile and green method for the synthesis of β-cyclodextrin silver nanocomposites (Ag-NPs). Herein, sulphur functionalized β-cyclodextrin itself acts as a green reducing and capping agent for silver ion. The constitution and framework of the synthesized thiorner and nanocomposite were characterized using FTIR, XRD and TEM techniques. These studies indicated the homogeneity of the silver nanocomposites with average size of ~4 nm via XRD results. Functionalized nanocomposites, with unique properties of –SH group and Ag-NP, were screened for broad spectrum antimicrobial and antioxidant activity. The antimicrobial results disclosed excellent antimicrobial performance with maximum inhibition zone of 38 mm and MIC value of 0.9 μg mL⁻¹ against E. coli. The sulphur functionalized β-cyclodextrin and their synthesized nanocomposites also exhibited eminent antioxidant properties even at 100 ppm.

1. Introduction

Infectious microorganisms are rapidly acquiring resistance to the traditional antibiotics. Their perseverance for antibiotic resistance has produced an interest for the synthesis of new potent and non-resistant antimicrobial agents. Along with resistance towards the microbial action, a good antimicrobial agent must retain precise shape and size that could help in targeting a specific disease site with excellent efficacy even in a small volume. This is where nanotechnology plays a very important role in the preparation of drugs or formulations, which are target specific, cost effective and less toxic to human cells. Silver based antimicrobial agents have received much attention due to strong antimicrobial properties against 650 types of disease-causing microorganisms and least toxicity to human cells. The high surface to volume ratio of silver nanoparticles (AgNPs) increases the association of silver ions inside the microorganisms, thereby exhibiting efficient antimicrobial action. Liberation of silver ions from a sol–gel coating has been reported to prevent the formation of a biofilm on indwelling surfaces over a period of 10 days.2

Although AgNPs exhibit remarkable antimicrobial properties, the sophisticated synthetic methods, instability and high production cost remain a major hindrance in their large scale production.3 Moreover the shape and size of silver nanoparticles are controlled via nature and concentration of reducing agents,4 nature of the capping agent5–8 and amount of metallic precursor used.9 The most common reducing agents employed for the AgNPs synthesis are sodium borohydride,10–14 citric acid,15 sodium citrate16 and ascorbic acid.17 Various stabilizing agents are also used during synthesis that attach onto the surface of nanoparticles to improve nanoparticle stability and prevent agglomeration.18 The use of above mentioned toxic reducing agents could ultimately produce health concerns. However such medical hazards could be prevented by synthesizing AgNPs in biocompatible and biodegradable chemicals, which themselves act as reducing and stabilizing agents.

In the last two decades gigantic effort has been made to develop such techniques, from which metal nanoparticles could be produced with environment friendly methods by utilizing
biopolymers like starch,19,20 alginate,21,22 chitosan23–26 and plant extracts.27–29 Biopolymers are undoubtedly the most appropriate alternatives and the most accomplished green choice for the present study as these are regenerative, cost effective and easily available. In addition, biopolymers could be restructured using simple chemical reactions to a large number of products for multidisciplinary applications. Among the various biopolymers, β-cyclodextrin (CD) is an excellent material of choice. Cyclodextrins (CDs) are cyclic oligosaccharides with six, seven, or eight glucopyranose units in α, β and γ forms, respectively. It possesses a hydrophobic core and hydrophilic surface that act as a surfactant to assist the good dispersion of nanoparticles. It could also form both channel and cage complexes to incorporate nanosize metal guests in its cyclic internal cavities. Suarez-Cerda et al.15 have reported β-CD as the best stabilizing agent amongst α-CD, β-CD and γ-CD for Ag-nanocomposites owing to its high activity due to the number of glucopyranose units in its structure. For effective coupling of various NPs like Au, Ag and Pt, the CD in thiol modification have been testified as stabilizing agents.30–36 Moreover –SH groups are well explored for their reductive activity in the synthesis of AgNPs.37–39

From the reported results, it could be observed that β-CD has been employed for the synthesis of nanoparticles of uniform size and shape with varying extent of success. However the majority of these methods suffer from one or more of the following imperfections, i.e., the need of additional or toxic reducing agent, uncontrollable shape and size control, and long reaction time. Inspired by these advances, we deduce that if we endow β-CD with a stronger stabilizing ability through the import of a few strong reductive moieties into the β-CD to formulate single ligand system, then the production of AgNPs with controllable size, shape and colloidal stability in aqueous solutions could be achieved under mild conditions and in a single synthetic process. Therefore, herein, we report the integration of –SH group with β-CD for remarkable reductive and stabilizing ability towards AgNPs via green chemistry concepts. Here, sulfur modified β-CD plays a dual role: it acts as both a reducing and a capping agent. This present investigation purposes a green, cost effective method for the synthesis of AgNPs stabilized with β-CD without the use of any other hazardous chemical. Moreover, we explore, herein, a new microwave synthesis to integrate the highest modification for sulphur derivatization and NPs synthesis. It is expected that this method would fabricate the functionalized NPs with superior antimicrobial and antioxidant properties due to synergistic effects of –SH and AgNPs for advanced industrial applications.

2. Experimental section

2.1. Materials and methods

Chloroacetyl chloride, silver nitrate, resublimed iodine, hydrochloric acid, sabouraud dextrose agar, nutrient agar, sodium chloride and sodium thiosulphate were purchased from LobaChemie India. β-CD, thiourea and DPPH (2,2-diphenyl-1-picylhydrazyl) was purchased from Hi-Media India. All other chemicals used were of analytical grade.

2.2. Synthesis of chloroacetyl and thiol functionalized β-CD

Chloroacetylation of β-CD was attained on pursuing a previous reported procedure with slight modification.40 Briefly, 1.0 g β-CD was completely dissolved in 12 mL of N,N-dimethylacetamide/lithium chloride (DMAc/LiCl) (5.0%) solvent system and charged into a three-necked flask kept in inert atmosphere. To this mixture, 1:1, 1:2 and 1:3 molar equivalents of chloroacetyl chloride (dissolved in 4.0 mL DMF) were added drop-wise with continuous stirring at ~5 °C. 2 M pyridine solution was also added as an acid acceptor to the reaction mixture. The resulting mixture was stirred for 1 h at room temperature and then irradiated for 2 min at 640 W with continuous stirring and intermittent irradiation at 30 s intervals. The obtained precipitates were washed thoroughly with water and methanol and dried at 50 °C. Resultant compounds are stated henceforth as β-CD-Cl-11, β-CD-Cl-12 and β-CD-Cl-13 for 1:1, 1:2 and 1:3 molar ratios, respectively. To attain –SH functionalization in β-CD, the above synthesized β-CD-Cl-11, β-CD-Cl-12 and β-CD-Cl-13 (1.0 g in 50 mL of deionized water) along with thiourea in 1:1, 1:2 and 1:3 molar ratio were irradiated, respectively, for 2 min at 640 W.41 The products obtained after hydrolysis were named as β-CD-SH-11, β-CD-SH-12 and β-CD-SH-13 for 1:1, 1:2 and 1:3 ratio, respectively.

2.3. Synthesis of surface functionalized silver nanocomposites

β-CD silver nanocomposites were synthesized following formerly reported method with slight modification.42 0.1 g of β-CD was dissolved in 50 mL of distilled water and was kept at 60–65 °C.43 1 mM solution of hot silver nitrate (10 mL) was slowly added to the two-necked flask furnished with dropping funnel, reflux condenser and magnetic stirrer under vigorous stirring. Upon the slow addition of silver nitrate to the thiolated β-CD solution the colour of the reaction mixture gradually changed from colorless to light brown and then finally to dark brown. The reaction mixture was stirred under the refluxing conditions for one hour to ensure the complete synthesis of β-CD based silver nanocomposites. All the synthesized thiomers and β-CD-Cl-13, the highest chloroacetyl modification, were also treated with silver nitrate solution to obtain β-CD based silver nanocomposites. The suspensions of different nanocomposites prepared were confirmed through their colour. The resulting colour suspensions were centrifuged and nanocomposites were collected and dried at 50 °C. The resulting compounds are referred to as β-CD-Cl-Ag-13 for chloroacetyl β-CD based silver nanocomposites and β-CD-SH-Ag-11, β-CD-SH-Ag-12 and β-CD-SH-Ag-13 for β-CD based thiomeric silver nanocomposites.

2.4. Antimicrobial activity

Synthesized β-CD thimer and their silver nanocomposites were screened for antimicrobial properties against broth culture of E. coli (MTCC 739), S. aureus (MTCC 737) and Candida albicans (MTCC 227) using the standard disc diffusion method. Different concentrations of synthesized thiomers and silver thimer nanocomposites were tested against standard strains. A total volume of 20 mL nutrient agar medium for antibacterial
studies and sabouraud dextrose agar (SDA) for antifungal studies was poured into petri-plates and allowed to solidify. 30 µL and 10 µL of synthesized compounds were taken from prepared different concentrations (30 to 180 µg) in three different wells, which were made using gel puncture technique, for antimicrobial and antifungal studies respectively. In case of antibacterial studies the plates were examined for possible clear zones after 24 h at 37 °C, while in antifungal studies the plates were inspected for possible clear zones after incubation at 25 ± 2 °C for 5 days. A distinct zone formed around the well is an indication of growth inhibition against the pathogenic species. The diameters of various zones were measured and the experiment was repeated in triplicates. To characterize the superiority of the synthesized products, the experiment was also performed with standard drugs ciprofloxacin and fluconazole.

Synthesized silver nanocomposites were screened against the strains of *S. aureus* (Gram-positive) and *E. coli* (Gram-negative) for antimicrobial activity by colony forming units (CFU). To conduct this experiment, three test-tube sets were employed per sample. The first test tube included 10 mL of nutrient broth and 1.0 mL of bacterial culture, which was marked as control test tube. In the second test tube, 500 µg mL⁻¹ of silver nanocomposites was added in addition to nutrient broth and bacterial culture. The third test tube was marked as reference (without bacterial strain) and comprised only nutrient broth and silver nanocomposites. For characterization, 3 mL suspension from each test tube was extracted and was diluted to 3–4 times to obtain a working suspension of approximately 10⁶–10⁷ CFU mL⁻¹. Further, the test tubes were placed in an incubator shaker at 37 °C for 24 h. The plates were investigated for colony forming units at 0 h and 24 h of incubation by plate count method. The results obtained were demonstrated as colony forming units per milliliter (CFU mL⁻¹) after multiplication by the dilution factor. The reduction percentage in bacterial colony growth was calculated by following equation.

\[
\% R = \frac{B - A}{B} \times 100
\]

where \( \% R \) is the reduction percentage of bacterial colonies, \( A \) is the number of bacterial colonies on the agar plate in test set, and \( B \) is the number of bacterial colonies on the agar plate for control.

MIC (minimum inhibitory concentration) of a particular compound is that concentration where no visible growth of bacteria is observed. The MICs of silver nanocomposites were assessed through 96-well microtiter plate utilizing broth micro-dilution assay with redox reagent resazurin. Concisely, bacterial suspensions were treated with different silver nanocomposites with a starting concentration of 30 µg. Serial dilution of each silver nanocomposite was implemented up to seven dilutions. The plates were sealed lightly (with ventilation) and were incubated at 37 °C for 24 h. Each plate exhibited seven dilutions of the compound, negative control with bacterial culture and antibiotic ciprofloxacin (1.0 µg mL⁻¹), a positive control having only bacterial culture without nanocomposites/antibiotic, and one for each synthesized nanocomposite. The MIC was determined by the turbidity of the culture media in wells. MIC values were distinguished as a colour shift from blue to red after 24 h at the lowest concentration of antimicrobial compound when resazurin dye was added. The experiment was repeated in triplicates.

### 2.5. Antioxidant studies

Antioxidant studies of synthesized compounds (chloroacetethylated β-CD, β-CD thiomers and their silver nanocomposites) were estimated using UV-Visible spectroscopy and cyclic voltammetry. Measure of antioxidant activity was examined through 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assays. Antioxidant activities were conducted by following a previously reported colorimetric method. Briefly, 2.0 mL of DPPH methanolic solution (0.1 mM) was incubated with 2.0 mL of test samples i.e. β-CD, β-CD-Cl-13, β-CD-SH-13, β-CD-SH-12, β-CD-SH-11, β-CD-Cl-Ag-13, β-CD-SH-Ag-11, β-CD-SH-Ag-12 and β-CD-SH-Ag-13 at three different concentrations (10, 50 and 100 ppm) in distilled water. The reaction mixture was transferred to an incubator maintained at 30 °C under dark conditions for 3 min. Following incubation, absorbance was estimated at 517 nm against a blank. Blank solution was composed of β-CD solution without the addition of DPPH. Another sample containing distilled water was also maintained and was marked as the control sample. Measure of antioxidant activity was determined by measuring the decrease in the absorbance of DPPH using the following equation.

\[
\text{DPPH scavenging effect (\%)} = \frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100
\]

where \( A_{\text{control}} \) is the absorbance of control solution containing DPPH and distilled water, and \( A_{\text{sample}} \) is the absorbance of DPPH solution containing samples.

Antioxidant studies were also performed using cyclic voltammetric analysis by 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assays. Cyclic voltammetric analysis reveals more precise estimation in scavenging assay over the traditionally used colorimetric spectrophotometric estimation of DPPH test. The observed voltammograms were assessed using a three electrode cell, where a stationary gold electrode was used as working electrode, platinum was used as a counter electrode and silver electrode served as a reference electrode. All the voltammograms were taken at 100 mV s⁻¹ and in 0.033 M KCl phosphate buffer (pH 7.4) as electrolyte in all voltammograms measurements. Scavenging activity was measured by using the following equation:

\[
\text{DPPH scavenging effect (\%)} = \frac{(I_0 - I)/I_0 \times 100\%}{I}
\]

where \( I \) is the peak current for sample and \( I_0 \) is the peak current for the DPPH at a particular concentration.

### 2.6. Characterization

The synthesized samples were subject to characterization by various techniques. IR spectra for the samples were obtained from Agilent technologies Cary 630 Spectrophotometer. XRD diffracgrams were recorded on Philips PAN analytical Xpert-PRO X-ray diffractometer and TEM was performed on TEM TECNAI G² 20 S-TWIN (FEI Netherlands). EDX analysis was performed on a Joel.
JSM 6100 instrument. UV-Visible absorption spectra were recorded using a double beam, UV-visible spectrophotometer (Systronics 2205) and photographs for colour change of solutions were captured using a Canon digital camera. Voltammetric study was carried out on a cyclic voltammetric system (BioLogic SP-200) and analyzed using EC Lab 10.37 software.

3. Results and discussion

Cyclodextrins are cyclic oligosaccharides with primary –OH of the anhydroglucose residues on the narrow side and the secondary –OH groups on the wider side. The chloroacetylation reaction was performed to achieve complete derivatization of –OH groups in reactive functionality. The percentage efficiency of the reaction was determined gravimetrically and was reported ~83%. The as-synthesized products were further functionalized in –SH group via thiourea reagent. A yield of 80–82% was registered gravimetrically for different molar equivalent reactions between chloroacetyl-CD and thiourea.

3.1. Characterization

In analytical determinations, the applied protocols were assessed for their efficiency via estimation of percentage of chloride and sulphur from the iodine titrations. The results reinforced the highest degree of substitution (DS) as 2.99 for β-CD-Cl-13 with maximum chloride content of 27.02%, while thiolation reaction supported maximum sulphur content of 25.25% in DS 2.95 (Table 1). EDX study was performed to confirm the titrimetric results of percentage modification for chloride and sulphur content in chloroacetylated and thiolated samples. The results indicated the chlorine atom content as 26.50% for β-CD-Cl-13 (Fig. S1a, ESI†) and sulphur atom content as 25.00% for β-CD-SH-13 (Fig. S1b, ESI†), and are in good agreement with titrimetric results (Table 1). The percentage of Ag was established as 2.11% in β-CD-SH-Ag-13 (Fig. S1c, ESI†). Therefore, the principal product formed in these reactions contains all the three –OH groups per anhydroglucose unit modified, i.e., they undergo –COCH$_2$Cl or –SH functionalization. The proposed structure is presented in Fig. 1 and the detailed results for chloride, sulphur and DS values are listed in Table 1.

The newly synthesized and well characterized β-CD-thiomers were explored for green synthesis of silver nanocomposite. During the reaction the –SH group of β-CD-thiomer reduces silver to dark yellow colour initially that changes to light brown and then to dark brown colour, confirming the formation of silver nanocomposites. The small size of the silver nanocomposites could be attributed to the stabilization of the structure via –SH groups on the periphery of the ring, which prevents agglomeration of particles.11,13 The proposed structure for β-CD based silver nanocomposite is shown in Fig. 2. Moreover the resultant CD-stabilized AgNPs exhibit sulphur functionalization on the surface with –SH molecular receptors (molecule with well-defined binding sites for interaction with complementary substrates for better applications). Such systems possess properties

| Table 1 Percentage of chloride, sulphur and DS values in functionalized β-CD |
|-----------------|-----|-----|------|
| Polymer         | % Yield | Cl [%] | S [%] | DS  |
| β-CD-Cl-11      | 80.52  | 16.39 |      | 1.16 |
| β-CD-Cl-12      | 83.01  | 22.51 |      | 2.00 |
| β-CD-Cl-13      | 83.39  | 27.02 |      | 2.99 |
| β-CD-SH-11      | 80.19  | 13.53 | 0.99  |
| β-CD-SH-12      | 80.45  | 18.69 | 1.64  |
| β-CD-SH-13      | 82.15  | 25.25 | 2.95  |

Fig. 1 Proposed structure for β-CD-thiomer.

Fig. 2 Proposed structure of β-CD thiomer stabilized silver nanocomposites.
in synergism of the two components, and the molecular recognition ability of the receptor exhibits synergistic effect of –SH and AgNPs for advanced industrial applications. In conclusion, the synthesized greener reagent is quite advantageous as it could be used as both reducing and stabilizing agent for synthesis of metal nanoparticles.

The as-synthesized thiomers and Ag-nanocomposites were characterized for their complete structure via UV, FTIR, TEM and XRD. UV-visible spectrum of β-CD stabilized silver nanocomposites exhibited an absorption band at 435 nm suggesting formation of a typical plasmon bond, which corresponds to the formation of AgNP (Fig. 3).49 The surface plasmon resonance (SPR) of NPs depends upon the size and shape of particles. For non-spherical NPs, the absorption spectrum consists of more than one SPR band. A single SPR band in the visible region accounts for the spherical shape of particles, which is also confirmed by the TEM results. The intensities of the peaks are also increasing from β-CD-Cl-Ag-13 to β-CD-SH-Ag-13 because of involvement of more silver in the complexation, where it forms bonds with electron rich species like sulphur of –SH group.

FTIR spectra of chloroacetyl β-CD and sulphur modified β-CD confirmed the integration of –COCH₂Cl and –SH group with β-CD backbone. In β-CD-thiomer spectrum a characteristic weak band appeared at 2694 cm⁻¹, which is ascribed to S–H group.45 Synthesized silver nanocomposites displayed FTIR spectra, which are similar to that exhibited by free β-CD-thiomer. The close inspection of these IR spectra reveals the following: weak S–H stretching peak at 2694 cm⁻¹ in the spectrum of free β-CD-SH-13, which disappears in the spectrum of dark precipitates collected for β-CD-SH-13-Ag (Fig. S2, ESI†). This is consistent with the anticipated conversion of S–H bonds in β-CD-SH to Ag–S bonds in silver nanocomposite.

The powder XRD analysis results for β-CD-SH-Ag-11 and β-CD-SH-Ag-13 indicated sharp diffraction pattern peaks at 2θ 36.77°, 45.45°, 66.17°, 75.45° and 83.94° (Fig. 4). The peaks observed are attributed to (111), (200), (220), (311) and (222) crystalline planes, respectively, of silver face centered cubic (FCC) lattice [JCPDS: 087-0719].50 The average size of the synthesized nanocomposites is calculated by broadening of peak at (111) reflection and by using Debye–Scherer equation.51 The results support an average size of 12 nm for β-CD-SH-Ag-11 and 4 nm for β-CD-SH-Ag-13.
The size of the nanoparticles was further confirmed through TEM analysis. Typical TEM images and histograms with the particle size distributions (obtained from individual measurements on at least 100 particles) are shown in Fig. 5. The TEM images of nanocomposites clearly show that silver nanoparticles are embedded in the β-CD cavity. The outer covering of β-CD, which acts as a matrix and Ag-NPs, are embedded like a reinforcement to create a perfect nanocomposite system. Moreover, the metal cores present good crystallinity with bright spots superimposed to the diffraction rings as evidenced by SAED (Selected Area Electron Diffraction) image (shown in inset of Fig. 5e). TEM analysis also revealed that the Ag-NPs stabilized via β-CD-SH are nearly spherical in shape (SEM image result in Fig. S3, ESI†). However, some of them are also observed as extensive aggregates particularly in β-CD-Cl-Ag-13 with an increase in both average size and polydispersity of the particles. Therefore the average diameter is calculated 32.5 nm with standard deviation of ±7.6 nm for β-CD-Cl-Ag-13. Thiomers based composites show an average diameter of 17.5 ± 4.2 nm for β-CD-SH-Ag-11 and 14.2 ± 3.1 nm for β-CD-SH-Ag-13. From the results obtained, it is clear that diameter of the nanoparticle is nearly structured with shape and size (limited polydispersity) in β-CD-SH-Ag-13 due to the high and uniform extent of modification via –SH group. However, it is very difficult to control the shape and size of nanoparticles when biological systems are used in the synthesis.52 In conclusion, the use of –SH integrated CD as stabilizing agent and water as a solvent medium aligns with the topic of increasing emphasis, i.e., the green chemical process. Moreover, water-soluble Ag nanoparticles are prepared using CD-thiomers in nearly controlled monodispersity (for β-CD-SH-Ag-13), and the surface is functionalized with –SH group for better interaction for enhanced technological potential.

3.2. Antifungal and antibacterial potential

The results obtained by agar well diffusion method have shown tremendous effectiveness of silver–thiol-nanocomposites on the clinical strains of E. coli and S. aureus. The results presented maximum zone sizes in the range of 29 to 38 mm for E. coli and for S. aureus the zone sizes were calculated in the range of 23 to 29 mm (Fig. S4, ESI†). The zones observed for the different synthesized compounds have been summarized in Fig. 6.

![Fig. 6 Zone of inhibition of various synthesized compounds in mm against (a) E. coli, (b) S. aureus and (c) Candida albicans.](Image)
The results revealed comparable antimicrobial activity with new silver nanocomposites when equated with standard antibiotic (ciprofloxacin at concentration 30 μg). This improved antimicrobial activity is due to the controlled nano-size and pseudospherical shape of nanoparticles. The smaller size accounts for greater surface area to bind on the receptor site of bacterial cell wall to alter its functional mechanisms for survival.53–55 The results reveal that zones observed for E. coli are better as compared to those of S. aureus. The native β-CD didn’t display any activity towards microorganisms, whereas chloroacetyl β-CD, β-CD-thiomers exhibited antimicrobial activity due to the presence of –Cl and –SH group. The synthesized silver nanocomposites also displayed antimicrobial activity against Candida albicans. The activity against Candida albicans showed maximum zone inhibition diameter between 21 to 27 mm (Fig. S5, ESI†) and are shown in Fig. 6c. The competence for the products was also equated with standard Fluconazole at 10 μg and results in a maximum supported zone of 32 mm.

This enhanced activity of silver nanocomposites could be attributed to the fact that in nanocomposites the admittance of silver particles to the biological system is high due to increase in bacterial cell membrane permeability.56 The appropriate surface functionalization is a necessary requisite for enhanced permeability and extraordinary application of nanoparticles. The surface functionalization via biocompatible biomolecules or polysaccharides creates an interface that is compatible and readily binds with the biological system.57 In particular, the presence of –SH group in silver-thiol composites could account for the enhancement of bioadhesive character of the suspension to control the passage of Ag nanoparticles through mucous membrane by the process of diffusion.58 The thiol group presence on the surface of Ag-NPs results in the functionalization of the surface of nanoparticles, which is attributed to their prolonged retention in the body and penetrating abilities for improved activity.59

The results obtained from the colony forming test were in accordance with the agar well diffusion method results. Chloroacetyl β-CD and thimer based silver nanocomposites showed excellent antimicrobial properties towards bacterial strains. As the thimer and chloroacetyl β-CD disclosed less activity towards clinical strains, further studies were performed only with β-CD-SH-Ag samples. The CFU mL⁻¹ was calculated for both the strains (E. coli and S. aureus). The observed CFU mL⁻¹ for the positive control of E. coli and S. aureus were calculated as 41.2 × 10⁶ and 42.5 × 10⁶ CFU mL⁻¹, respectively. A considerable decrease in CFU mL⁻¹ was observed when bacterial strains were treated with β-CD-SH-Ag samples. The maximum decrease was observed in β-CD-SH-Ag-13, which was 0.3 × 10⁶ and 0.6 × 10⁶ CFU mL⁻¹ in case of E. coli and S. aureus, respectively (Fig. 7a). These results revealed the reduction of CFU mL⁻¹ by 99.27% for E. coli and 98.58% for S. aureus in β-CD-SH-Ag-13 (Fig. 7b). The same trend was observed for all the synthesized silver nano-composites.

### 3.3. Determination of MIC

In the present study, we tested both strains (E. coli and S. aureus) against synthesized silver nanocomposites. In Table 2, values of MIC have been listed and it is clear from the results that β-CD-SH-Ag-13 displays lowest value for MIC. This is because of the highest bioavailability of silver ions due to the strong reducing effect of –SH group present in the nanocomposite. The lowest MIC value, 0.9 μg mL⁻¹, is reported against E. coli and 1.8 μg mL⁻¹ against S. aureus with β-CD-SH-Ag-13. The results are found to be preferential in comparison to previous studies, where MICs of silver nanocomposites have been calculated to be higher than 10 μg mL⁻¹ for both E. coli and S. aureus.60,61 The excellent antimicrobial activity of silver-thimer nanocomposites could be attributed to the better accumulation and dissolution of silver nanocomposites (via –SH group present on the surface of NPs) through the cell wall of the microorganisms.62 If a nanoparticle enters the bacterial cell, it brings in thousands of molecules or reactive agents against the resistant bacteria and enhances their bactericidal properties via altering the enzymatic action of protein.63,64

### 3.4. Antioxidant studies

Herein, a stable free radical, DPPH, has been used to reveal the antioxidant properties of synthesized compounds spectrophotometrically and electrochemically. In Fig. 8 the antioxidant...
activity of native β-CD, β-CD-thiomers and thiol–silver nanocomposites are shown. The studies were performed using three different concentrations, 10, 50 and 100 ppm, of various samples in distilled water and DPPH methanolic solution. High scavenging activity of 96% was calculated for ascorbic acid at 100 ppm. In these test sets, β-CD-SH-13 showed good scavenging activity of 89% while β-CD-SH-12 and β-CD-SH-11 revealed scavenging activities of 76% and 68% at 100 ppm, respectively. In addition, β-CD-Cl-13 exhibited a scavenging ability of 62% at 100 ppm. The β-CD silver nanocomposites also indicated antioxidant potential, but not up to the levels to which thiomers and chloroacetylated β-CD were evaluated. All these tests were also performed for β-CD, but no significant antioxidant activity was observed toward DPPH. As it is clear from UV-visible results that β-CD-SH-13 has shown the maximum scavenging activity against DPPH assay, hence it was further evaluated for its antioxidant activity by cyclic voltammetry using the same DPPH radical. The observed cyclic voltograms display two reversible peaks corresponding to oxidation and reduction reaction of DPPH radical attributed to two-electron change. A significant decrease in the DPPH peak current confirms the scavenging ability of the tested compound at various concentrations in the range of 10 to 50 ppm as shown in Fig. 9(a). The results show a maximum scavenging activity of ~50% for β-CD-SH-13 at 50 ppm through cyclic voltograms (Fig. 9b). The percent inhibition characterization is performed using the peak at 0.74 V in the plot. The results are preferential in comparison to UV-visible results.

4. Conclusions

In the proposed study, a green method has been reported for the synthesis of CD-thiol stabilized silver nanocomposites with unique properties of both the components, i.e., β-CD-SH and silver. No additional hazardous reducing agent was used to reduce the size of AgNPs as –SH functionalized β-CD itself acted
as reducing agent, stabilizer, emulsifier and capping agent. The prepared products were characterized using UV-visible, FTIR, TEM and EDS techniques to identify the structure and size/shape of the nanocomposites. The results supported the formation of nearly spherical-shaped nanocomposites that chiefly exhibited monodispersity in case of β-CD-SH-Ag-13 due to the high extent of –SH group, which contributes the strong reducing capability to the thiomer. The as-prepared compounds and nanocomposites displayed promising antimicrobial activity against E. coli, S. aureus and Candida albicans, which are resistant to common antimicrobial agents, even at very low concentrations. The nanocomposite β-CD-SH-Ag-13 exhibited excellent maximum zone size of 38 mm against E. coli. β-CD-SH-Ag-13 exhibited preferential results with lowest MIC value 0.9 μg mL⁻¹ against E. coli and 1.8 μg mL⁻¹ for S. aureus in comparison to the previous studies. The thiolated β-CDs and thiol based silver nanocomposites showed exceptional antioxidant properties at 100 ppm. The resultant nanocomposites provide an excellent alternative to be used as efficacious antimicrobial agents in the near future.

Conflicts of interest

There are no conflicts to declare.

References