Research Article

Green synthesized silver NPs: fluorescence sensor for Cl^- ions in aqueous solution in biological pH and cell viability study

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Abstract

New green synthesized silver nanoparticles (NPs) (Ag-CS) were prepared by a single step photochemical reaction at room temperature from sugarcane extract and its morphology, structure and spectral properties were characterized by transmission electron microscopy, energy dispersive x-ray analysis, x-ray diffraction, UV-vis and Fourier transform infrared spectroscopy and spectrofluorometry. The NPs were found to be selective and turn-on sensitive fluorescence probe for chloride ions (Cl^-). Cell viability of Ag-CS NPs has also been studied.

Graphic abstract

Keywords Silver nanoparticles · Sugarcane extract · Turn-on sensor · Chloride sensor · Cell viability

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1 Introduction

Nanobiotechnology indicates the link of nanotechnology and biotechnology, which is a promising field devoted to the generation, improvement, and efficacy of nanoscale structures for advanced biotechnology [1]. The nanomaterials are synthesised by using physical and chemical methods. However, most of these techniques are energy and resource intensive. The usual methods, involving capping agents, hazardous solvents, insensitive chemicals, and other additives, cannot be used in biomedical and clinical fields. Therefore development of green protocol for nanoparticle preparation that spurns the use of solvents and stoichiometric reagents, removes byproducts, and uses accessible renewable resources is necessary. A number of efforts have been made toward biosynthesis of metal nanoparticles (NPs) using bacteria [2], fungi [3], actinomycetes [4], yeast [5], and viruses [6]. In addition to the aforementioned techniques of generation, a new scheme that uses parts of whole plants for synthesis of NPs, known as phytosynthesis, is under constant development and is an advantageous and economical approach. Phytosynthesis of noble metal NPs (Ag, Pd, Pt, and Au) has attracted much attention in the past decade [7–9] because of its utility in catalysis, biomedical sensors, drug delivery, anticancer agents etc. In comparison with methods involving use of microorganisms, phytosynthesis is a fast and productive approach which can be scaled up for bulk production of NPs very easily [10]. The phytosynthetic approach for the preparation of NPs is reportedly more fruitful as the resulting NPs have consistency in size and shape and are well dispersed. So, there is considerable prospect for new protocols to use plant extracts for synthesis of new nanomaterials with added advantages over customary chemical and physical techniques. *Saccharum officinarum* (sugar cane) is the crop that belongs to *poaceae* family. They have solid jointed fibrous stalks which are rich in sugar, mainly accumulated at internodes. The sugar cane juice is a mixture of reducing (glucose) as well as non-reducing sugars (sucrose), organic acids such as oxalic acid, citric acid, maleic acid, succinic acid, and D-gluconic acid, and inorganic ions such as calcium, sodium, chloride etc. [11–13]. This is the reason why we opted for sugar cane juice as starting material as it is full of reducing agents (glucose) and well-known capping agents (organic acids) for preparation of metallic nanoparticles.

Determination of chloride ion concentration is very important in certain areas, such as clinical diagnosis [14, 15], food, industrial applications [16], water quality and salinity [17], environmental monitoring [18, 19] and some others. Such wide applications imply an urgent

requirement for increased selectivity and sensitivity to detect chloride ions. Several methods including chromatography [20, 21], light scattering [22], turbidimetry [23], ion selective electrodes [24–27], near-infrared spectrometry [28], spectroscopy [29], flow-based methods coupled with different detectors [30–32], visual sensing [33], laser-induced breakdown spectroscopy [34] have been used for determination of chloride ions. However, these methods are costly, time consuming and require bulk sample volumes as well as huge amount of organic solvent with obscure separation and extraction processes. So it is very much important to develop a simple, less time consuming, and sensitive method for determination of chloride ions. In recent past, several fluorescent chloride indicators based on calix [4] arene derivatives [35], fluorescent proteins [36], collisional quenching of quinolinium and acridinium compounds [37–41], fluorescence-lifetime-based organic fluorophores etc. [42], have also been developed. But fluorescence-based sensors have evinced much interest owing to their ease of handling and operation, inexpensiveness, selectivity, and high sensitivity. A few fluorescence based nanosensors have been reported till date. Recently, green synthesized Ag nanoparticles based fluorescence sensors for various metals are being reported in the literature [43–46].

In this current report we describe an easy way to synthesize Ag NPs using cane sugar extract with the influence of solar energy and show its application towards chloride ion sensing with high selectivity and sensitivity using fluorescence spectroscopy. The synthesized nanoparticles also show cytotoxic effect towards HeLa cell line.

2 Experimental

2.1 Materials and methods

Silver nitrate (AgNO_3) and HEPES buffer were purchased from Sigma Aldrich Ltd. All other chemicals were purchased from Merck, India. All chemicals and solvents purchased were reagent grade materials and were used as received without further purification. Milli-Q (Millipore, Billerica, MA, USA) and nanopure (double distilled water, filtered through 200 μm filter) water was used in all experiments. Stock solutions of different ions (0.01 M) and the HEPES buffer solution were prepared freshly in Milli-Q water. The sugar cane juice used for the synthesis of silver NPs was prepared following the method described below.

2.2 Preparation of sugar cane juice

Saccharum officinarum (sugar cane) stems were purchased from local market and were crushed to extract the juice using a two roller crusher machine. The extract was collected and filtered through a normal filter paper, filtered again through Whatmann 41 filter paper and the filtrate was collected in stoppered glass bottles.

2.3 Photochemical synthesis of silver NPs

20 mL of sugar cane juice filtrate was added to 1.7 mg of silver nitrate taken in a beaker and mixed well. 2 mL of this solution was then taken in different centrifuge tubes. These mixtures were then kept into sunlight for 2 min. The pale yellow colour of the solution turned brown red which indicates the formation of silver (Ag-CS) NPs. This brown-red solution was stored in a sealed centrifuged tube under cold condition which was later duly characterized by UV-vis spectroscopy, TEM, HRTEM, EDX and powder XRD analysis.

2.4 Characterization of Ag-CS NPs

UV-vis spectra of the sample were taken on a Shimadzu UV 1800 (200–900 nm) (1 cm quartz cell) spectrophotometer. High resolution transmission electron microscopy (HRTEM) images of Ag-CS NPs were obtained on a JEOL JEM-2100 transmission electron microscope (Hitachi, Japan) to study the size and morphology of NPs. Elemental analysis of the silver NPs was performed using EDX on the TEM. X-ray diffraction (XRD) pattern of Ag-CS NPs was measured at UGC-DAE Consortium for Scientific Research, Indore, using Bruker D8 Advance X-ray diffractometer, with CuK α radiation ($\lambda = 1.5406 \text{ \AA}$), 40 kV–40 mA, $2\theta/\theta$ scanning mode.

2.5 Fourier transform infrared spectroscopy (FTIR) analysis

FTIR measurement of sugar cane embedded silver NPs was carried out using the Perkin-Elmer L120-000A spectrometer using KBr pellet in the range of 450–4000 cm^{-1} to study the presence of various functional groups in biomolecules responsible for the bio-reduction of Ag⁺ and capping/stabilization of silver NPs.

2.6 General procedure for detection of chloride ions

For detection of chloride ion, the stock solution was prepared by adding 0.5 mL of Ag-CS ($5 \times 10^{-4} \text{ M}$) in 1.5 mL HEPES buffer of pH 7.2 at 25 °C. For fluorescence titrations, fluorescence measurement of the Ag-CS nano was done with varying concentration of Cl[−] ions keeping the

total volume 2 mL. Fluorescence measurements were performed using 5 nm $\times$ 5 nm slit width. All the fluorescence spectra were taken after 10 min of mixing to get the optimized spectra using a Perkin-Elmer LS55 Spectrofluorimeter equipped with FLWINLAB software with a rectangular quartz cuvette of path length 1 cm. All the fluorescence measurements were repeated for three times for more reliable result.

2.7 Cytotoxicity analysis

2.7.1 Cell culture

Human cervical cancer cell line (HeLa) was procured from National Centre for Cell Science, Pune, India. HeLa cells were cultured in DMEM supplemented with 10% fetal bovine serum and antibiotic solution (100U/mL penicillin G and 100 $\mu\text{g/mL}$ streptomycin sulfate) at 37 °C with a constant supply of 5% CO₂.

2.7.2 Cell viability assay

The percentage of viable cells after treatment with silver NPs (AgNPs) was estimated by MTT assay. Cells (1×10^5 cells/mL) were incubated in 96-well microplates for 24 h. The cells were treated with different concentrations of AgNPs (0.1, 1, 10, 50 and 100 $\mu\text{g/mL}$) for 24 h. Cells which were not exposed to any of the NPs were kept as untreated control. After the end of the exposure time, the 3-(4,5-dimethylazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) reagent was then added to each well and incubated for the next 4 h. The intracellular formazan crystals formed were solubilized with dimethyl sulfoxide (DMSO) and the absorbance of the solution was measured at 570 nm by using a microplate reader (Thermo-Scientific, Multiskan ELISA, USA). The percentage of cell survival was calculated as: (mean experimental absorbance/mean control absorbance) $\times$ 100%. Samples were placed in triplicate for each set of experiment. Each data are represented as mean $\pm$ standard deviation (SD). The statistical significance of differences between the experimental points was analyzed by ANOVA test and differences were considered significant when $p < 0.05$.

2.7.3 Morphological changes

Biocompatibility of the silver NPs was carried out by cell staining study. To evaluate further whether the NPs are discerningly cytotoxic to cancer cells, the cells were assessed for some other protocols as mentioned below.

Fig. 1 (Left) TEM image of Ag-CS NPs (right) particle size distribution from TEM image

2.7.4 Microscopic observation of DAPI and PI staining

Briefly, the HeLa cells were treated with 10 $\mu\text{g/mL}$ of AgNPs for 24 h at 37 $^{\circ}\text{C}$. After completion of treatment, the cells were washed with HEPES buffered saline (HBS), fixed and incubated for 10 min with DAPI and PI stain and observed under fluorescence microscope (Motic, BA410) and captured with MoticCam Pro 285B.

2.7.5 Microscopic analysis of mitochondrial membrane potential by Rhodamine123

To investigate the effect of NPs in any modification on the potentiality of the mitochondrial membrane of the cell Rh123, staining was performed. Briefly, the HeLa cells were treated with 10 $\mu\text{g/mL}$ of AgNPs for 24 h at 37 $^{\circ}\text{C}$. Treated cells were then washed with PBS and incubated with Rh123 for 30 min and photographs were captured under a fluorescence microscope.

Fig. 2 HR-TEM image of Ag-CS NPs

3 Results and discussion

3.1 Characterization of Ag-CS NPs

Transmission Electron Microscopic image of Sugar cane capped silver NPs (Ag-CS) is shown in the Fig. 1. It shows mono-dispersed spherical Ag-CS nanoparticles of diameter 11–21 nm (left) and particle size histogram plot on the right by counting 50 NPs from TEM image. For better understanding of the surface morphology of Ag-CS NPs, some other TEM images at different resolutions were taken, and are added to the Supplementary Information (Fig. S1). The high resolution TEM (HRTEM) image in Fig. 2

Fig. 3 EDX and elemental analysis of Ag-CS NPs

(a magnified version of the same figure is shown as Fig. S3, Supporting Information) showed clearly two distinct lattice fringes of 0.203 nm and 0.229 nm which correspond to the interplanar separation of the (200) plane and (111) planes of FCC cubic silver lattice respectively. The energy dispersive X-ray analysis (EDX) (Fig. 3) shows strong signal at 3 keV which confirms the formation of silver NPs [47]. Elemental analysis of the silver NPs was performed using EDX on the TEM.

Results indicate that silver (20.21%) was present as a major constituent element along with a trace amount of oxygen (1.71%), which may have originated from the bio-molecules that are bound to the surface of AgNPs, indicating the reduction of silver ions to elemental silver. Other peaks in the EDX correspond to Cu and carbon, which is an artifact of the carbon-coated Cu-grid on which the sample was placed. The absence of other peaks confirms complete reduction of silver compounds to silver NPs [48].

The X-ray diffraction (XRD) pattern of the Ag-CS NPs was recorded and is shown in Fig. 4. The obtained data is in good agreement with the standard powder diffraction data on JCPDS (silver file No. 04-0783). All the information/data obtained from XRD analysis are presented in Table 1. Four peaks appeared at 2θ values of 37.42, 45.35, 64.88, and 76.85 degrees in the experimental diffractogram, corresponding to (111), (200), (220) and (311) planes of FCC cubic silver respectively [49]. The XRD study has thus confirmed that the resultant particles in the prepared sample are silver NPs having FCC cubic crystal structure. The interplanar spacing ($d_{\text{calculated}}$) values are 2.402, 2.0002, 1.436 and 1.239 Å for (111), (200), (220) and (311) planes respectively and matched with standard values for metallic silver [47]. The average crystalline size is calculated using Debye–Scherrer formula,

Fig. 4 Powder X-ray diffraction plot of Ag-CS NPs

Table 1 Data analysis of powder XRD of Ag-CS nanoparticles

Sl. no.	1	2	3	4
2θ	37.42	45.35	64.88	76.85
$\cos\theta$	0.9471	0.9228	0.8439	0.7834
$\sin\theta$	0.3207	0.3851	0.5364	0.6215
FWHM degree	0.6402	0.592	0.622	0.736
β radian	0.01117	0.01033	0.01086	0.01284
Crystalline size 'D' nm	13.6	15.1	15.8	14.63
Inter-planar spacing 'd' Å ⁰	2.4019	2.0002	1.4360	1.2395
hkl	111	200	220	311
$h^2 + k^2 + l^2$	3	4	8	11
Lattice const. 'a' from d Å	4.1602	4.0004	4.0616	4.1109
Cell volume Å ³	71.99	64.02	66.92	68.31

$$D = k\lambda / \beta \cos\theta$$

where D is the average crystalline size of the NPs, k is geometric factor (0.94), λ is the wavelength of X-ray radiation source and β is the angular FWHM (full-width at half maximum) of the XRD peak at the diffraction angle θ [50]. The calculated average crystalline size of the AgNPs is ~ 14.78 nm. This matches well with the result from the TEM images.

3.2 Spectral characteristics

3.2.1 Selectivity study

The fluorescence response of Ag-CS NPs towards the different anions (Cl^- , SCN^- , SO_3^{2-} , F^- , $\text{S}_2\text{O}_7^{2-}$, H_2PO_4^- , HPO_4^{2-} , $\text{S}_2\text{O}_3^{2-}$, Br^- , SO_4^{2-} , AsO_4^{3-} , PO_4^{3-} , NO_2^- , OAc^- , I^- , $\text{C}_2\text{O}_4^{2-}$,

Fig. 5 Fluorescence change of Ag-CS NPs in presence of different anions

NO_3^- , HCO_3^- , CO_3^{2-}) are shown in Fig. 5. The fluorescence effects of various ions on Ag-CS NPs were investigated under excitation at 290 nm and emission was recorded at 422 nm. It reveals that Ag-CS NPs have excellent selectivity towards Cl^- ions in comparison to other anions. Initially, 500 μl of 5×10^{-4} M Ag-CS was added to 1.5 mL of 20 mM HEPES buffer, adjusted with 0.2 M NaOH to pH 7.2, and mixed well for taking blank emission spectrum of Ag-CS NPs. Then same volume of different buffered ion solution of same concentration were added to maintain the ratio of Ag-CS to various ions at 1:20 and selectivity was checked for each ion after 10 min of incubation time. Figure 6 shows that due to the presence of Cl^- ions, fluorescence intensity at 422 nm greatly increases in comparison to that from other ions. Therefore the Ag-CS NPs are highly selective towards Cl^- ions.

3.2.2 Fluorescence detection of Cl^- ions: binding constant, LOD and interference study

Fluorescence titrations were carried out upon incubation of CS capped Ag nanoparticles (100 μM) with aqueous sodium chloride (NaCl) solution (0–3 equiv.) for 10 min at room temperature. In this report chloride ions greatly enhance the fluorescence of Ag-CS NPs in HEPES buffer at pH 7.2 as shown in Fig. 7. As displayed in Fig. 7, a significant increase in the fluorescence emission at 422 nm was observed upon addition of 3 equivalent of NaCl solution and significant maximum fluorescence enhancement factor of ~ 3.0 fold were observed. These titration results

Fig. 6 Fluorescence change of Ag-CS NPs in presence of different anions in HEPES buffer at pH 7.2 at room temperature ($\lambda_{\text{ex}} = 290$ nm) where, 1) blank Ag-CS NPs, 2) Cl^- , 3) SCN^- , 4) SO_3^{2-} , 5) F^- , 6) $\text{S}_2\text{O}_7^{2-}$, 7) H_2PO_4^- , 8) HPO_4^{2-} , 9) $\text{S}_2\text{O}_3^{2-}$, 10) Br^- , 11) SO_4^{2-} , 12) AsO_4^{3-} , 13) PO_4^{3-} , 14) NO_2^- , 15) OAc^- , 16) I^- , 17) $\text{C}_2\text{O}_4^{2-}$, 18) NO_3^- , 19) HCO_3^- , 20) CO_3^{2-} ions

Fig. 7 Fluorescence titration of Ag-CS NPs with NaCl soln in 20 mM HEPES buffer at 27 °C

clearly indicate that chloride ions can effectively interact with CS capped Ag NPs as reflected by fluorescence enhancement.

Based on the fluorescence enhancement at 422 nm, the detection limit was calculated from the calibration curve focusing on the lower concentration region of aqueous sodium chloride solution (Fig. 8). From the slope of the curve (S) and the standard deviation of seven replicate measurements of the zero level (σ_{zero}), the detection limit was estimated using the Eq. 3 σ/S [51, 52]. This study indicates that the detection limit of Ag-CS NPs for chloride ion was found to be 1.08×10^{-5} M. The limit of quantification (LOQ) was also calculated and it was found to be

Fig. 8 Calibration curve for the micromolar range for calculating the LOD of Cl^- by Ag-CS NPs in 20 mM HEPES buffer at 27 °C

Fig. 9 Fluorescence titration of Ag-CS NPs with water from tap 1, tap 2 and standard solution in 20 mM HEPES buffer at 27 °C

3.59×10^{-5} M. Linearity range of the Ag-CS nanoparticles with chloride ion was calculated and it was found that linearity range was maintained up to the concentration of 59.69 μ M of chloride ion (Figure S4). Using the emission titration data the association constant value was calculated from the Benesi-Hilderband plot and it was found to be 3.538×10^3 M^{-1} (Figure S5). To confirm further the practical applicability of CS capped Ag NPs as a Cl^- selective fluorescence sensor, we carried out competition experiments (Figure S2). There is no noticeable change in emission spectra on addition of the selected competitive ions to Ag-CS NPs and chloride ion solution (Figure S6). These results demonstrate that other surveyed ions are non-interfering in nature and it can be said that this proposed Ag-CS nanoparticles can be practically and efficiently applicable for chloride ion detection.

3.2.3 Determination of Cl^- ions concentration: real sample analysis

For the sake of real sample analysis and to have a better application of the synthesized Ag-CS NPs, we analyzed the concentration of the chloride ion of water from two taps of Kalyani University and a standard solution. To have an answer over the question of the reliability of the determination ability of the synthesized nanoparticles towards the chloride ions, we compared the data from the fluorescence titration with the data obtained from ion chromatography for the same solutions i.e. water samples from tap-1, tap-2 and standard solutions. Tap water was taken from the Kalyani University campus, filtered through filter paper and used for the experiment without any further treatment. All the experiment

Fig. 10 Calibration curve for the linearity range with the data for tap1, tap2 and standard solution

Table 2 Estimation of Cl^- ions in water by fluorescence test

Sample	Cl^- (ppm)
Tap-1	1.983
Tap-2	2.022
Standard solution	1.465

Table 3 Comparison of two different analytical techniques

Sample	Cl^- estimated (ppm) (Present method)	Cl^- estimated (ppm) [53] (Ion chromatographic method)
Tap-1	1.983	2.015
Tap-2	2.022	2.046
Standard solution	1.465	1.476

was repeated three times and the satisfactory results obtained by the proposed sensor were summarized in Table 3. The titration spectra for tap-1, tap-2 and standard solution are shown in Fig. 9 and the corresponding fluorescence emission intensity at 422 nm were plotted in the calibration curve of linearity range (Fig. 10). From the spectra, the concentration of the chloride ions were calculated and the values are tabulated in Table 2. Ion chromatography was also used to test the values and the results are in a good agreement. Hence it can be said that the synthesized Ag NPs can be used in real sample analysis with good confidence. The comparative statement of results from the two different analytical techniques is tabulated in Table 3 below. The ion chromatogram of tap-1 is given in Figure S7.

3.2.4 Effect of reaction time on the formation of AgNPs by UV-vis study

When silver salt (AgNO_3) is added to aqueous cane sugar extract, it did not result any colour change. But under exposure to sunlight, the colour of the solution changed from pale yellow to yellowish brown and finally to dark brown. The corresponding UV-vis spectra of the mixture of AgNO_3 and cane sugar juice are shown in Fig. 11 with increase of the sunlight exposure time. The colour change of the solution is due to the occurrence of silver NPs produced by reduction of the silver salt. The reduction of silver salt to silver NPs is due to the presence of reducing agents present in CS juice. The reducing agents that are responsible for the reduction of silver salt to AgNPs are glucose and fructose, which are present in cane sugar extract. In aqueous solution, the brown colour of AgNPs is due to excitation of surface plasmon vibrations [54]. It is noticed that the complete colour change took place after about 2 min under the exposure to sunlight. No further colour change of the reaction mixture was observed under constant exposure to sunlight. Most surprisingly, the colour change from light yellow to dark brown did not take place for the reaction mixture when it was kept under darkness for as long as 30 h. This observation clearly suggest that sunlight is very much essential for successful preparation of Ag NPs for providing the crucial activation energy. Colour change in the solution clearly indicates that the silver salt present in the reaction mixture had been reduced entirely. The creation of Ag NPs was inspected and verified from the corresponding absorption spectra. A peak/hump in the UV-vis spectrum was obtained due to the strong surface plasmon resonance (SPR), i.e. due to resonant absorption of photons by Ag NPs. The observed absorption band is

Fig. 11 Time dependent UV-vis plot of Ag^0 nano-particles generation under sunlight exposure

size dependent, as SPR band is known to depend on size of the NPs and refractive index of the solution [55]. Figure 11 shows the absorption spectra of AgNPs obtained from the reaction of cane sugar extract and AgNO_3 recorded in the range of 200–900 nm at different reaction time. At darkness for long time or under no sunlight exposure, there was no absorption band centered at 460 nm. But in sunlight, with increase in time, the absorption maximum centered at 460 nm gradually developed and the absorbance didn't increase in scalable level till after 2 min of exposure time.

Here the absorption band is at higher energy similar to that obtained by Shankar et al. [54]. This indicates that the prepared AgNPs are smaller and has uniform size distribution. The observed increment in the absorbance with the passage of time indicates increase in concentration of Ag NPs. Change in colour was observed initially after 30 s of exposure to sunlight after the addition of the salt solution to the cane sugar extract. After 2 min, the colour of the solution became virtually steady, demonstrating that no silver salt was present for further reaction. The results are in good agreement to that of reported by Shankar et al. [54]. No shift in the SPR band intensity as well as no shift of the peak wavelength was observed with the passage of time after 2 min. As per Mie theory [56], absorption spectra of spherical AgNPs show single SPR band, and with increasing anisotropy, the number of peaks also increases. In the current study, SPR band suggests that the synthesized NPs are spherical in shape as confirmed by TEM study in supplement.

3.2.5 Synthesis of silver NPs and sensing mechanism of chloride ion from FTIR Study

FTIR analysis was carried out to check the role of sugar cane juice as capping agent as well as a reducing agent for the successful generation of Ag NPs. FTIR spectra of CS, Ag-CS and Ag-CS + Cl^- is shown in Fig. 10. The FTIR of sugar cane juice shows characteristic bands at 3422 and 1654 cm^{-1} , which may be due to the stretching of O–H and C=O bonds of hydroxyl and carbonyl groups either from sucrose, glucose or fructose of cane sugar juice. The FTIR spectrum of sugar cane juice capped Ag NPs show the peaks at 3384 and 1638 cm^{-1} , which may be attributed due to the stretching frequency of hydroxyl and carbonyl groups. The O–H stretching frequency significantly shifted by 38 cm^{-1} to a lower value, which may be due to the interaction of hydroxyl groups with the NPs. The stretching frequency due to carbonyl group also shifted to lower value, which also indicates the change of aldehyde group to carboxylic acid group during the generation of silver NPs. The FTIR data provides good support for the claim that reducing sugars from cane sugar play dual role as reducing agent and

also capping agent. To have a better understanding how the Ag NPs interact with chloride anions, a solution of CS coated Ag NPs with chloride ions was condensed gradually to obtain a powder. This powder was filtered out and given for FTIR analysis. This is shown at the bottom of Fig. 10. It is clear that the stretching band due to the

hydroxyl group was shifted from 3384 cm^{-1} in Ag NPs to 3372 cm^{-1} in chloride treated NPs. The downward shift of 12 cm^{-1} can be attributed due to the hydrogen bonding of hydroxyl groups to the Cl^- anion. Hydrogen bonding is important to explain sensing as well as bonding mechanism for the anions by NPs (Fig. 12).

Fig. 12 FTIR spectra of cane sugar (CS), Ag-CS NPs and Ag-CS NPs with chloride ion

Fig. 13 TEM images of Ag-CS NPs treated with chloride ions at different resolution

3.2.6 Interaction of Ag–CS NPs and chloride ion from the perspective of TEM, EDX and powder XRD studies

To have in-depth understanding about the interaction of the Ag–CS NPs with chloride ions, we have carried out TEM and powder XRD studies of the Ag-NPs treated with Cl^- ions. From the TEM studies (Fig. 13) it is clear that upon addition of the Cl^- ions to the Ag NPs, there is an aggregation of the NPs which may be attributed to the effective interaction of Cl^- ions with the hydrogen atoms of the hydroxyl groups of the capping sugar moieties. The generation of fluorescence upon addition of Cl^- ions may be due to the same fact of aggregation. As there is hydrogen bonding between the Cl^- ions with the hydroxyl groups of the capping agent, there is effective aggregation induced cluster formation which is indeed the probable cause of fluorescence enhancement. Similar aggregation induced fluorescence enhancement (and generation) is evident in the cases of metal mediated aggregation induced fluorescence generation of silver nanoparticles [43–46]. For better understanding of the aggregation phenomena some other TEM images of Ag–CS NPs (after treating with chloride ions) are added to the Supplementary Information (Figure S2). The surface elemental analysis was done by EDX analysis (Fig. 14) and from the analysis it's clear that a new peak appears due to additional presence of chloride ions. Powder XRD spectrum of Ag-NPs, treated with chloride ions is shown in Fig. 15. The powder XRD data clearly reveals that there is a slight shift of the peaks of the Ag NPs along with generation of new peak, from which it can be concluded that there is an interaction between the Ag NPs and Cl^- ions.

Fig. 14 EDX and elemental analysis of Ag–CS NPs treated with chloride ions

Fig. 15 Powder X-ray diffraction plot of Ag–CS NPs treated with chloride ions

3.3 Cell Imaging and cytotoxicity study

3.3.1 Cell viability assay

The viability assay gives information about cell death, survivability and metabolic activities. The MTT assay reveals that when cancer cells (HeLa) were treated with AgNPs at different doses, the cells appear to show a dose-dependent viability pattern as NPs concentration significantly affected their impact on the viable cells. There were only 27% viable cells found at a much higher concentration (100 $\mu\text{g}/\text{mL}$). The lowest death rate was obtained at 0.1 $\mu\text{g}/\text{mL}$ and about 74% cells were found viable at this NPs concentration as shown in Figure S8. The HeLa cells

exposed to Ag NPs had shown alteration in cell shape and morphology. The half-maximal inhibiting concentration value (IC_{50}) of AgNPs was found to be 10 $\mu\text{g/mL}$ and this concentration was used to carry out to explore the further cell-based experiments.

3.3.2 Morphological changes

To elucidate the extent and cell death modality caused by AgNPs on HeLa cells, several parameters such as the number of living cells (unstained cells), the number of cells undergoing necrosis and/or apoptosis were measured by fluorescence microscopy. Cytotoxicity of AgNPs was examined by determining their effects on the cell membrane integrity and permeability by DAPI and/or PI stains, and on the changes in the mitochondrial membrane potential activity by Rhodamine 123 dye. Significant morphological changes compared with untreated cells were observed under a microscope. Nuclear condensation was notable in HeLa cells when treated with NPs. The fluorescence

microscope images reveals that cells treated with Ag NPs have more dead cells with significant changes in the membrane integrity, because the DAPI and PI are much taken up by cells. Figure 16 clearly shows the increase of DAPI/PI stained cells in Ag-NP treated cells as compared to untreated control cells which are not exposed to Ag NPs.

To evaluate the integrity of membrane potential and to find the explanation behind loss of viability of the cells, membrane depolarization assay was performed with help of Rhodamine123 dye. Rhodamine fluorescence is used to measure the membrane polarization in living cells within mitochondria. Our data reveals distortion of membrane integrity as seen clearly in Figure S9. Cells treated with Ag NPs changed the potential of the mitochondrial membrane as evident by the decrease in fluorescence intensity which clarifies that NPs bring change in the potential of the cellular membrane. Untreated cells on the other hand show good fluorescence intensity, which indicates that the integrity of mitochondrial membrane was intact and cells are in good viable condition.

Fig. 16 Fluorescence images of control and NP treated HeLa cells stained with DAPI and PI dye

4 Conclusions

The present work introduces a robust, low-cost synthetic platform to prepare silver NPs stabilized by sugar cane extract, using sunlight. The Ag NPs act as fluorescence turn-on sensors for chloride ions which are indeed robust in nature for the application of real sample analysis. Most importantly, the nanoparticles function as fluorescent probe in aqueous solution under biological pH. Chloride is an essential electrolyte that maintains homeostasis within the body, where abnormal chloride levels in biological fluids may indicate various hazardous physiological effects. In this connection, the present study may provide a cheap and green strategy to prepare sensors with improved chloride sensitivity. Particularly, extending chloride sensing to biological pH may lead to critical developments in organic bio sensing, enabling intracellular studies of abnormal transmembrane chloride regulation. At the same time, Ag-CS NPs have also cytotoxic activity, checked in HeLa cell. There are several reports on cytotoxic effect Ag NPs with various shapes and sizes. Methods outlined in this article will be helpful to find out in-depth toxicity data which will enable the development of regulations on the safe use of Ag NPs in extensive applications of cancer therapy.

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Data availability Data is available on request to the corresponding author.

Compliance with ethical standards

Conflict of interest There are no conflicts of interest to declare.

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