Research Article

Enzyme-free colorimetric sensing of glucose using L-cysteine functionalized silver nanoparticles

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Abstract

The study demonstrates an efficient, simple and on-site viable approach for sensitive determination of blood glucose using colorimetric sensing approach. The devised colorimetric sensor works based on cysteine functionalized silver nanoparticles. The as-synthesized Ag NPs were elaborately characterized using advanced analytical techniques such as ultra-violet visible spectroscopy, Fourier transform infrared spectroscopy, transmission electron microscopy, atomic force microscopy and X-ray diffraction. The detection of glucose was carried under ambient air conditions where change in the optical characteristics of Ag NPs and subsequent interaction between glucose and functional moiety (i.e. surface bind cysteine) of Ag NPs was considered as the signal response. This interaction led to direct detection of glucose in the concentration range between 0.01 to 0.17 µM with limit of detection up to 1×10^{-4} µM. It is worthwhile mentioning that success of the assay lies in application of the developed colorimetric sensor in real blood glucose measurements, which also proved its capability for field based analysis. In addition its simple design, low cost, and more reliability signifies the usefulness of colorimetric sensor and it can be extended monitor other biologically important molecules.

Keywords Glucose · Enzyme free · Colorimetric probes · Functionalized Ag NPs

1 Introduction

As diabetes mellitus have become pressing health problem around the globe when dealing with glucose metabolism disorders. This chronic disease, associated with insulin deficiency/resistance may lead to heart or kidney failure, high blood pressure, nerve damage and blindness due to continuous variation in blood glucose [1]. Thus, research on portable devices and chemical assays which can allow fast, sensitive and reliable monitoring of blood glucose level is an area of scientific research gaining significant interest of the people. Conventional approaches which include high-performance liquid chromatography (HPLC) [2], fluorescence spectrometry and chemiluminescence methods [2], despite being sensitive, fail to offer advantages like fast analysis, affordability, portability and ease of analysis. In contrast, colorimetric assay based on the use of nano-sized noble metal nanoparticles such as gold or silver have proven efficient, cheap and capable for on-site monitoring or screening of biologically important molecules. Among various metals available, silver and gold are most extensively used for the development of colorimetric probes based on their excellent surface plasmon resonance (SPR) characteristics. However, the use of such noble metals for probing biologically important molecules for example glucose is normally associated

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with the application of enzyme (glucose oxidase, GOx) for the detection [1, 2]. In this connection many reports have already been published likewise Zhang et al. [3] reported the use of Ag NPs for detection of glucose based on the change in SPR response as a consequence of H_2O_2 produced from the enzymatic reaction between glucose and glucose oxidase previously immobilized on surface of Ag NPs. In a similar scenario, use of gold nanoparticle (Au NPs) coupled with Ag NPs have also been proposed for the detection of glucose [4]. Literature reveals that recently efforts have been carried out for developing fluorescence probe based on GOx loaded nanocomposites of carbon nanodots supported over Ag NPs for gulucose detection. Nanocomplex based on Ag NPs and graphene quantum dots (AgNPs/GQDs) loaded with glucose oxidase have also been used for the development of colorimetric probe for glucose detection [5]. In addition to these, use of bimetallic composite particles and metal oxides has also been reported for the enzyme based optical probes [6, 7].

Despite the extreme selectivity offered by enzymatic assays, the use of such probes for practical application is restricted by the limitations offered by the enzyme. The temperature depended stability, high cost and immobilization complexities of the enzyme marks these assays as unfavorable candidates for diverse application. Recently, enzyme-free approaches have gained substantial attention due to their improved sensitivity and selectivity. An optical probe based on nano-mimicking capability of gold and iron oxide magnetic nanoparticles composite has been proposed by Shin et al. [8]. Despite the success of nano-mimicking assays, efforts for finding newer, simpler and cost-efficient protocols for reliable pre-screening of clinically important biomolecules are still under way. In this context, colorimetric sensor which rely on the interaction between the probe (nanoparticle) and target analyte are gaining reasonable consideration [9]. These assays are not only simple in mechanistic consideration but also involve minimum chemicals and complexity which make them suitable to be used practically in low-resource situations [10]. A number of such assays have already been developed for various heavy metal ions and other toxic compounds such as pesticides and drugs [9, 10]. In the present study we have functionalized Ag NPs with L-cysteine amino acid and carried out direct detection of glucose (i.e. enzyme free) on their surface via colorimetric sensing approach. The assay rely on the competitive interaction between functionalizing agent (L-cysteine) and glucose molecules leading to particle aggregation and subsequent change in SPR response. The developed assay provides a simplest route towards reliable detection of glucose without the need of any enzyme or any additional chemicals. The practical characteristic of the assay has been validated by considering it for glucose detection from human serum samples.

2 Materials and methods

2.1 Chemicals

All chemicals were analytical grade and used without further purification. Pure Milli-Q water as the preparatory medium. Silver nitrate ($AgNO_3$–97%), was obtained from E. Merck. L-cysteine ($C_3H_7NO_2S$), sodium hydroxide (NaOH–98%) and hydrochloric acid (HCl–37%) were purchased from Sigma–Aldrich. The stock solutions of metal salts were prepared in pure Milli-Q water and all experiments were performed at ambient temperature (25 ± 2 °C).

2.2 Instrumentation

UV–Visible spectroscopy (Lambda 35 of PerkinElmer) was considered for monitoring the variation in SPR signal of Ag NPs within the spectral range of 300–800 nm. Confirmation of surface functionalization was achieved using fourier transform infrared (FTIR) spectroscopy (Nicolet 5700 of Thermo) with ATIR mode. Further, characterizations of functionalized Ag NPs were obtained using transmission electron microscopy (TEM) (Jeol JEM 1200 EX MKI), atomic force microscopy (AFM) and X-ray diffraction (XRD) (model D-8 of Bruker). The change in color was noted by capturing photographs of the colloidal Ag NPs solutions before and after interaction with glucose using 18 mega pixel digital camera.

2.3 Synthesis and functionalization of silver nanoparticles (Ag NPs)

The Ag NPs were synthesized and functionalized using one-pot wet-chemical reduction approach. In a typical experiment, a 10 ml $AgNO_3$ (1 M) was homogenized with 1 ml of L-cysteine (1 mM) under constant stirring condition. The clear solution was then introduced into 60 ml of $NaBH_4$ (1 M) until a stable bright yellow colloidal solution was obtained with SPR wavelength of 395 nm. The pH of formed colloidal solution was maintained at 6.5 in accordance to pk_a value of L-cysteine to obtain effective functionalization and smaller size. The use of L-cysteine was considered based on its capability to act both as efficient surface protecting agent and mild reducing agent. This allowed preserving homogeneity of synthesized Ag NPs with generation of an inert atmosphere, which controlled the use of protective gas and hence tedious strategies to avoid oxidation.

2.4 Colorimetric sensing of glucose using cyst-Ag NPs

The colorimetric sensing of glucose using cyst-Ag NPs was carried out at room temperature in aqueous solution. For a representative experiment, a certain range of glucose concentration (0.05 mM to 1.5 mM) was introduced within a fixed aliquot of cyst-Ag NPs. The mixtures were allowed to stabilize for few minutes followed by SPR measurement using spectrophotometer. The absorbance was measured at SPR wavelength of 395 nm, in reference to a blank sample (without glucose). The change in absorbance (ΔA) was taken as the analytical signal for the corresponding concentration, while the change in color from bright yellow to colorless was considered the visual response of the devised sensor. The color change was recorded using an 18 mega pixel digital camera after stabilizing the colloidal mixture for 5 min.

3 Results and discussion

3.1 Characterization of cyst-Ag NPs

Optical characteristics of nano-sized noble metal particles are a function of geometry and size; thus the optical response of such particles is highly sensitive to change in size of the particles [9]. Owing to the surface plasmon modes of noble metallic NPs which belong to the optical region of electromagnetic spectrum [11–13], one can easily use optical spectroscopy as an efficient tool to observe different molecules based on change in the intra-particle distance of noble metals. In this case, the most stable and small sized cyst-Ag NPs (with blue shift in spectrum) were obtained after careful optimization of reaction parameters like concentrations of the precursor salt and functionalizing agent, pH of colloidal solution (not shown). Figure 1 shows the UV–Vis absorbance of a represented sample of the as prepared Ag NPs with SPR wavelength noted at 395 nm.

The morphological characteristics were evaluated using TEM analysis. Figure 2 shows a typical image for as-synthesized cyst-Ag NPs. The formed Ag NPs were evident of high surface homogeneity and dispersion indicating the success of surface functionalization with L-cysteine molecules. The average size of cyst-Ag NPs were estimated to be in range of 10–35 nm ± 1.4 nm. The EDX spectra provided as the inset of Fig. 2, further confirms the surface purity of the as-synthesized Ag NPs.

The AFM images for the same Ag NPs are presented in Fig. 3. The findings are apparent of narrow and homogenous distribution which is less likely in case of biological methods where use of plant extracts are considered as reducing/capping agents [14]. The topographical map shows significant variation in topography along the surface. This reflects the smaller cross-section of the synthesized nanoparticles with larger number of active sites available at the surface for reaction participation.

The XRD pattern shown in Fig. 4 consists of peak indexed to (111), (200), (220) and (311) planes of face centered cubic structure (FCC) as referenced against ICCDD (file no: 89-3722). The absence of peaks other than metallic phase silver confirms the compositional purity of the as-synthesized cyst-Ag NPs.

Confirmation for the functionalization was obtained using FTIR analysis. Figure 5 shows the FTIR spectra of both standard L-cysteine and Cyst-Ag NPs. The characteristics band for L-cysteine includes bands at 1630 and

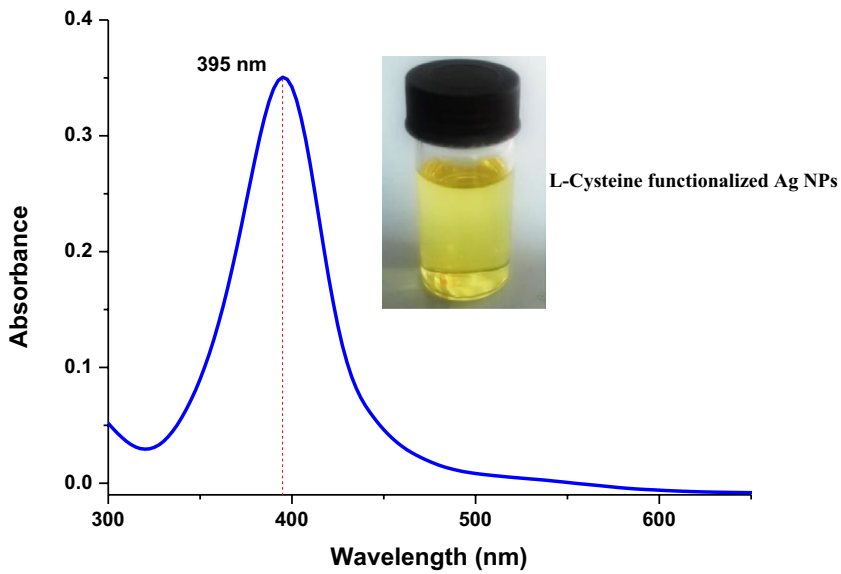


Fig. 1 SPR wavelength for cyst-Ag NPs with inset figure showing the corresponding bright yellow colloidal sol

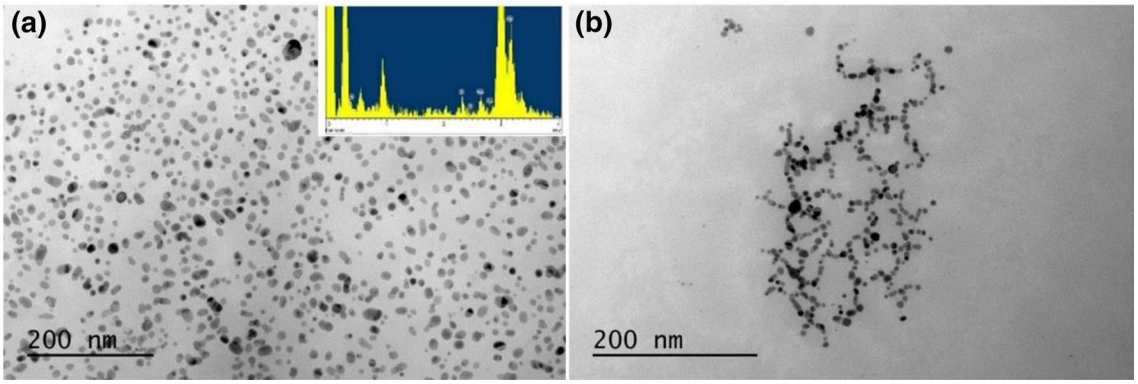


Fig. 2 TEM analysis of **a** Cyst-Ag NPs with EDX spectra as inset figure and **b** Cyst-Ag NPs in the absence and presence of glucose (0.01 µM) respectively

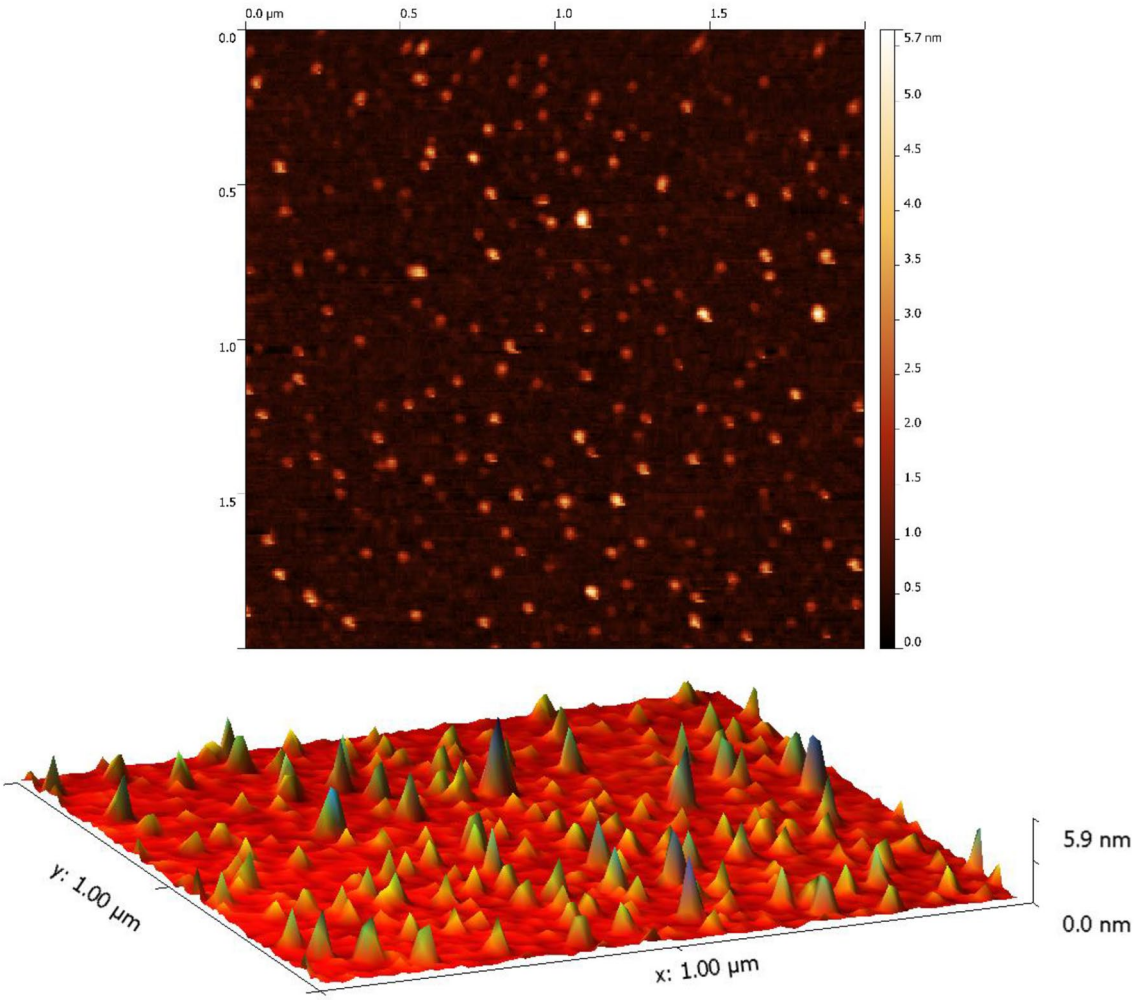


Fig. 3 AFM Height profile with topographical plot indicating the uniform distribution of as-synthesized cyst-Ag NPs

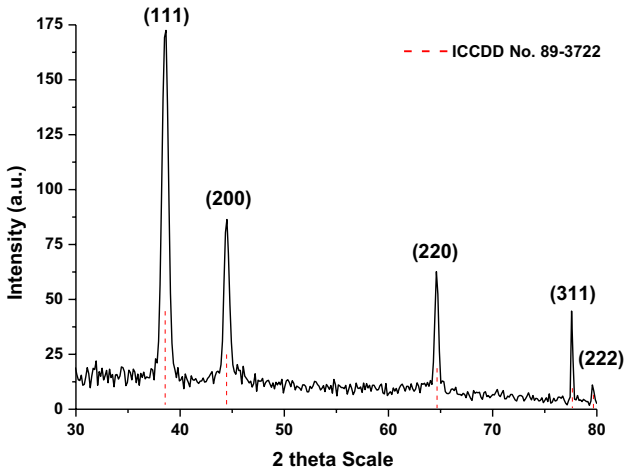


Fig. 4 XRD pattern recorded for Cyst-Ag NPs with major peaks indexed to cubic unit cell of FCC crystal structure for standard silver

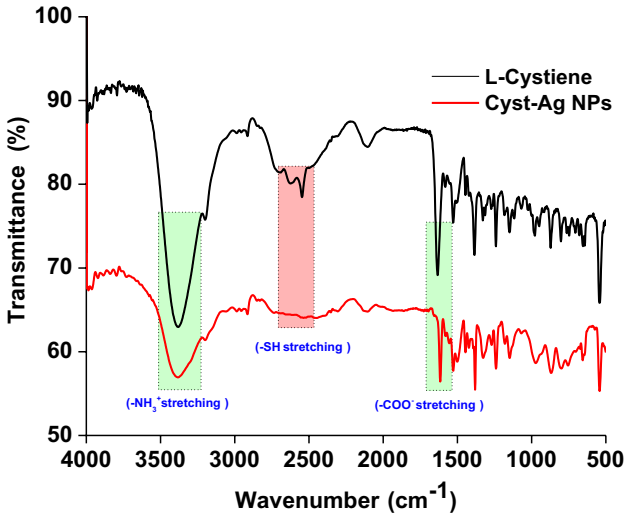


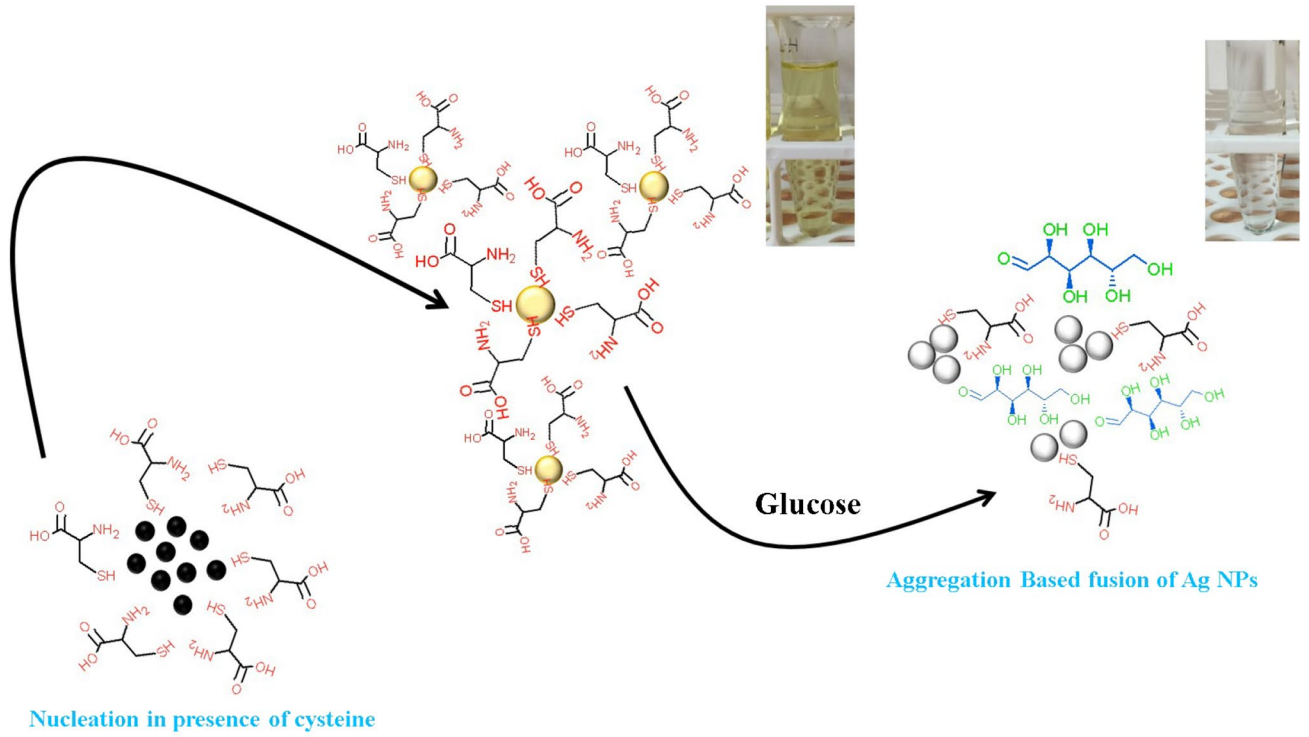
Fig. 5 FTIR profile for standard cysteine and cyst-Ag NPs

1390 cm^{-1} for asymmetric and symmetric stretching of (COO^{-}) and 1530 cm^{-1} for –NH bending vibration.

A broad band near 3000 for $-NH_3^+$ stretching and a band near 2500 cm^{-1} for –SH [15–17]. In case of cyst-Ag NPs, the band for –SH was not observed. This confirms the interaction of Ag with cysteine molecules via thiol group. This interaction was further strengthened by interaction of carbonyl from cysteine over Ag NPs surface, as indicated by shift in the vibrational frequency of COO^{-} from 1630 to 1620 cm^{-1}. A slight shift in the overall frequencies was also observed which can be explained on the bases of dipole change as a result of surface functionalization of Ag NPs with cysteine molecules [18].

3.2 Enzyme free colorimetric sensing of glucose

Due to the bright yellow color of colloidal solution of Ag NPs it exhibit a sharp SPR band near 400 nm in UV–Vis spectral window. Therefore a typical narrow SPR peak at 395 nm was evidenced by size homogeneous and crystalline Cyst-Ag NPs as shown in Fig. 1. This noted optical characteristic can be attributed to the effective capping/functionalizing agent of Ag NPs with cysteine molecules [19, 20]. The detection mechanism of glucose can be explained on the basis of inter-particle fusion caused by favorable intermolecular forces as a consequence of addition of glucose. This generalized mechanism is illustrated in Scheme 1. It is well-known that SPR of Ag NPs is highly sensitive towards change in the refractive index near the vicinity of metal nanoparticles. In this case when glucose is introduced into colloidal sol of Cyst-Ag NPs, it tends to contribute to the existing inter-ionic forces that prevail between the cysteine functionalized Ag NPs. The glucose based on its hydroxyl functionality can easily facilitates the amine-carbonyl interactions subsequently allowing nanoparticles to fuse together. Unlike the absorption-based colorimetric sensing where change in inter-particle distance is responsible for the shift in SPR wavelength [21], the said process results in complete aggregation of NPs leading to decline in the SPR intensity. The proposed mechanism is further supported by the TEM analysis of cyst-Ag NPs before and after the introduction of glucose molecules. Figure 3a shows that in the absence of glucose, the cyst-Ag NPs tends to be spherical and well-dispersed. In contrast, the TEM image Fig. 3b for cyst-Ag NPs with glucose shows evidence of particle fusion and formation of aggregates with size greater than 100 nm. Quantitative response measurements were carried out by monitoring the changes in absorbance (ΔA) for cyst-Ag NPs with glucose in concentration range of 0.01–0.17 µM (Fig. 6). The linear regression analysis was proof of good linearity with LOD ($3\times\partial$/slope) and LOQ ($10\times\partial$/slope) estimated to be 1.2×10^{-4} and 0.03 µM respectively. Table 1 compares the analytical characteristics of the described sensor with other competitor. It is clear that, the discussed sensor offer greater analytical leverage with additional advantage of working without enzyme and cost-friendly. The selectivity of the assay was evaluated by monitoring the change in SPR response against common co-existing interfering species such as ascorbic acid, sucrose and metal ions like Cu^{2+}, Zn^{2+} and K^{+} ions with concentration ten folds higher than glucose (0.01 µM). The spectral profile shows negligible variation in the SPR intensity of Ag NPs in the presence of interferents (Fig. 7). This negligible variation in the SPR characteristics of Ag NPs, is proof of the high tolerance offered by of cyst-Ag NPs against these interferents.



Scheme 1 A generalize illustration representing formation and aggregation of Ag NPs in the presence of cysteine and glucose respectively

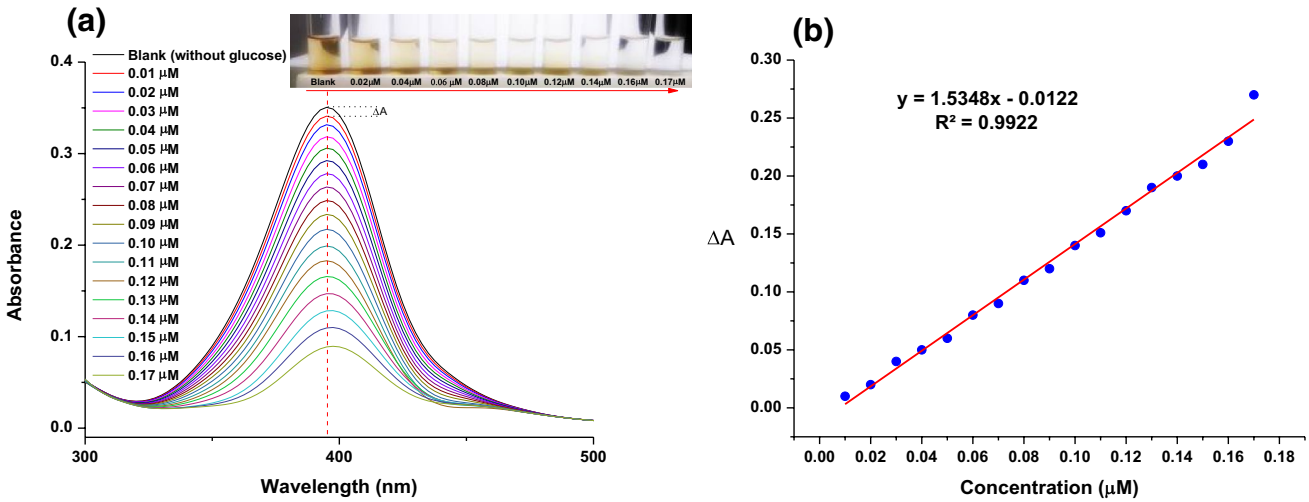


Fig. 6 **a** Variation in the noted SPR of Cyst-Ag NPs against successive addition of glucose solution with inset figure representing the corresponding decline in the colour intensity and **b** Calibration plot for the devised colorimetric sensor with linear fit analysis for glucose in concentration range of 0.01–0.17 µM

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Table 1 Analytical comparison of designed sensor with various other competitors

S. no	Type of nanoparticles	Concentrations (μM)	LOD (μM)	References
1.	Ag Nanoprism	0.2–100	0.2	[21]
2.	Ag NPs	868.6	–	[22]
3.	Ag NPs	215.50–3448	99.13	[23]
4.	Au–Ag NPs	5–70	3	[24]
5.	Ag NPs/GQDs	500–8000	30	[5]
6.	Isotropic silver nanoparticles (iAg NPs)	0.2–32	0.09	[25]
7.	GQDs/Ag NPs	0.5–400	0.17	[26]
8.	Ch-Ag NPs	500–200	0.1	[27]
9.	C Dots/Ag NPs	2–4000	1.39	[28]
10.	L-cyst-Ag NPs	0.01–0.17	1×10^{-4}	This work

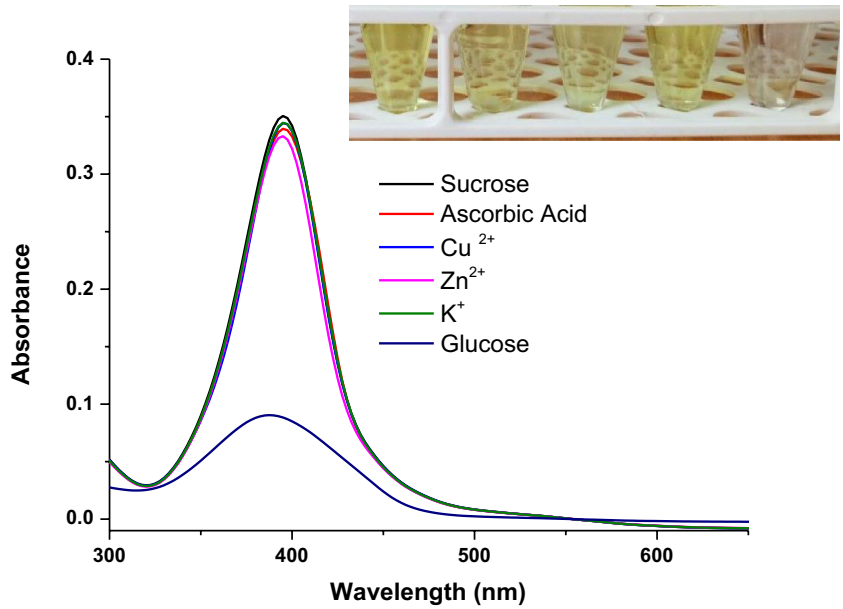

Fig. 7 The spectra profile representing the selectivity for cyst-Ag NPs against common interferents with inset figure depicting the corresponding color variation

Table 2 Blood glucose determination from real blood serum sample using devised colorimetric probe in reference to measured values obtained using commercial glucometer

Sample	Blood glucose (Glucometer) mmol/L	Blood glucose (Colorimetric sensor) mmol/L[a]	Relative standard deviation (RSD %)
1	4.5	4.3	4.0
2	4.8	4.4	9.0
3	5.2	5.0	4.0
4	5.6	5.4	3.7

[a]The measured concentration is the mean value for three consecutive measurements

3.3 Testing glucose from human serum samples

To validate the practical applicability of the developed colorimetric assay, the cyst-Ag NPs were subjected to human serum for glucose analysis taken from lab volunteers with informed consents. Serum was obtained after centrifugation of whole blood followed by two fold dilution for easiness of analysis. The serum samples were then tested for the concentration of glucose according to the procedure mentioned in Sect. 3.2. The concentration measured using Ag based colorimetric assay was cross-checked with the results obtained with commercial glucometer. Table 2 shows the obtained data with RSD value for each samples < 10% indicating the reliability of the assay for practical applications.

4 Conclusion

In conclusion, the study demonstrates the use of L-cysteine functionalized Ag NPs as colorimetric probe for enzyme free detection of blood glucose. The detection system relied on direct interaction between the

glucose molecules and functional groups of surface bound cysteine molecules. The assay demonstrated excellent working linearity for enzyme free glucose detection in range of 0.01–5.4 µM. The assay provides a non-enzymatic approach for the detection of glucose avoiding use of toxic chromophores or unstable enzymes like glucose oxidase or HRP. The successful application of the developed sensor in real blood glucose proves its feasibility for practical application and its applicability as detection strips for enzyme free glucose screening method. Moreover, the preparation of this sensor is very simple, fast, inexpensive and extendable to monitor other biologically important molecules.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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