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Heavy-metal ion sensors using chitosan-capped gold nanoparticles

A. Sugunan[a], C. Thanachayanont[b], J. Dutta[a,*], J.G. Hilborn[c]

[a]*Microelectronics, School of Advanced Technologies, Asian Institute of Technology, P.O. Box 4, Klong Luang, Pathumthani 12120, Thailand*
[b]*National Metal and Materials Technology Center, 114 Thailand Science Park, Phaholyothin Road, Klong 1, Klong Luang, Pathumthani, 12120, Thailand*
[c]*Polymer Chemistry, Materials Chemistry, Uppsala University, P.O. Box 538, SE-751 21 Uppsala, Sweden*

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Abstract

We report a novel strategy for using gold nanoparticles capped with chitosan for sensing ions of heavy metals. Acidic anions (glutamate ions in our case) are expected to cap the nanoparticle surfaces similar to conventional methods of stabilization of gold nanoparticles by citrate ions. The polycationic nature of chitosan enables attachment of the polymer to the negatively charged gold nanoparticle surfaces through electrostatic interactions. Use of chitosan serves dual purpose of providing sufficient steric hindrance ensuring stability of the colloid and also to functionalize the nanoparticles for use as sensors. The well-documented chelating properties of chitosan and the sensitivity of the optical properties of gold nanoparticles to agglomeration have been employed to detect low concentrations of heavy metals ions (Zn^{2+} and Cu^{2+}) in water. A comparison of the optical absorption spectra of the colloidal suspension before and after exposure to metal ions is a good indicator of the concentration of the heavy metal ions.

Keywords: Capping agent; Gold nanoparticles; Chitosan; Heavy metals; Sensors; Steric hindrance

1. Introduction

The chemical inertness and resistance to surface oxidation make gold an important material for use in nano-scale technologies and devices [1]. This property is crucial when particle size approaches the nano-scale and the dominance of surface atoms results in an enhanced chemical reactivity [2]. Though the resistance to surface oxidation is maintained in nanoparticles of gold, the chemical inertness of bulk gold is lost, and a heightened catalytic behavior is observed [3,4]

Uniform spherical nanoparticles of gold are used as seeds for synthesizing nano-wires of gold [5,6], which have potential applications in future electronic devices [7,8]. Other materials that share similar corrosion resistance as gold are silver and platinum. However, silver is considered too reactive and platinum is significantly more expensive than gold [8]. The wide variation of optical properties of gold nanoparticles with particle size, particle–particle distance, and the dielectric properties of the surrounding media due to the phenomenon called surface plasmon resonance [9–11] enables construction of simple but sensitive colorimetric sensors for various analytes [7]. Most modern applications of surface plasmon resonance (SPR) are in sensing bio-molecules [1,12,13]. Such sensors require ‘metal clusters’ i.e. high uniformity in size and shape (usually spherical) of the nanoparticles. A recent report of fluorescence from strictly size controlled Au clusters, with the emission wavelength tuned by controlling the number of Au atoms in the cluster [14], demonstrates another potential application offered by controlling the size and shape of nano-sized gold particles.

Synthesis of aqueous suspensions of gold nanoparticles involves reduction of a gold salt solution ($AuCl_3$ or $HAuCl_4$) by tri-sodium citrate to form gold crystals, which grow by Ostwald’s ripening to form nanoparticles of gold [15,16]. The excess citrate ions in the medium are adsorbed on the nanoparticles until there is sufficient electrostatic repulsion to keep them from agglomerating. This method was developed by Turkevitch [15] and later refined by Frens [16] who varied the ratio of gold salt to citrate concentration in the medium to achieve size controlled synthesis of nanoparticles. Though this method

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* Corresponding author. Tel.: +2 524 5680; fax: +2 524 5697.
E-mail address: joy@ait.ac.th (J. Dutta).

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is still one of the most commonly used strategy for synthesizing aqueous suspensions of gold nanoparticles, the resultant nanoparticles are monodisperse for sizes less than about 30 nm [17]. Other methods of obtaining gold nanoparticles, including the two-phase Brust-Schiffrin method usually involves organic solvents [15]. Gold nanoparticles synthesized by using organic solvents are reported to offer excellent control and uniformity of size and can be dried and re-dispersed easily, since the protective capping agent (usually a thiol) is chemically bound by a coordinate bond to the surface of gold nanoparticles, unlike the physical adsorption of excess ions as in the case of the Turkevitch process [15]. However, the organic solvents used in these techniques render them unsuitable for solution-based sensors for detection of bio-molecules like proteins, and saccharides.

Applications of gold nanoparticles as sensors are usually based on detecting the shifts in SPR peak, due to either change in the dielectric constant around the nanoparticles as a result of adsorption of analyte molecules, or due to analyte-induced agglomeration of the nanoparticles [18]. Both these effects rely on the selectivity provided by the functionalized capping agents (in the form of ions or specific polymers), highlighting the significance of the chemical methods of synthesis and stabilization of these nanoparticles for use as solution based sensors.

In this regard, we have synthesized aqueous suspension of gold nanoparticles by employing salts of acidic amino acids as the reducing agent [19,20]. Adsorption of excess glutamate ions on the gold nanoparticles induces electrostatic repulsion among the nanoparticles in effect keeping them from agglomerating. The synthesized nanoparticles were capped with chitosan, which is well known as a heavy metal-chelating agent [21]. Chitosan has free amines in some of its repeat units, which gets protonated in dilute acidic media. These protonated amines form the multiple bonding sites that are useful in chelating heavy metals like Cu^{2+} and Zn^{2+} [22]. Though chelation of heavy metal ions by chitosan has been widely studied, relatively less attention has been given for the development of simple colorimetric sensors to detect the presence of heavy metal ion contaminants in water. Since gold nanoparticles are an ideal candidate for the construction of colorimetric sensors, the electrostatic attachment of chitosan over gold nanoparticles has been studied in order to demonstrate a simple colorimetric sensor for indicating the concentration of heavy metals ions (Cu^{2+} and Zn^{2+}) in a solution.

2. Experimental

Synthesis of gold nanoparticles was based on the well-documented Turkevitch process with the exception that tri-sodium citrate has been replaced with monosodium glutamate that has been described elsewhere [20]. In brief; 100 ml of 0.1 mM of $HAuCl_4$ (Sigma-Aldrich) is heated until boiling. Upon boiling, 0.75 mM (final concentration) of monosodium glutamate (Merck) is added to it. After a few minutes, the color changes from almost colorless solution of diluted gold salt to the typical deep red color of gold nanoparticles. At this point, it is rapidly quenched to room temperature. While quenching, 37% chitosan (Aldrich, medium molecular weight) solution in dilute

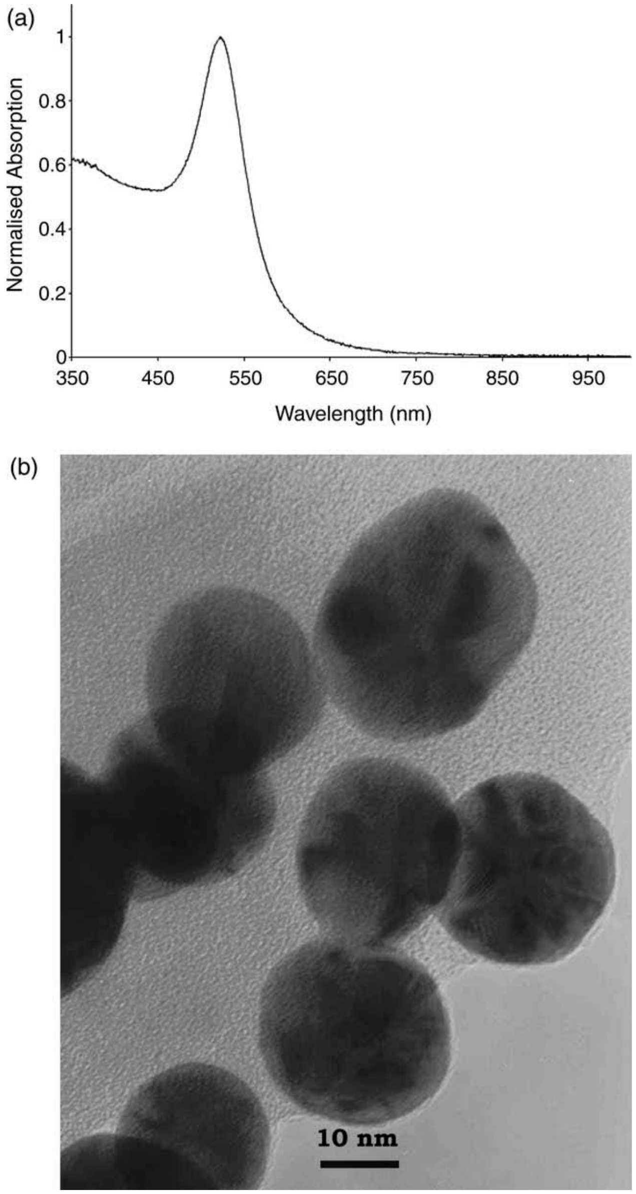

Fig. 1. (a) Optical absorption spectrum of typical colloidal suspension containing spherical 20 nm gold nanoparticles synthesized with monosodium glutamate as the reducing and capping agent. The absorption maximum is at 519 nm. These nanoparticles are solely stabilized by electrostatic repulsion due to the adsorbed glutamate ions. (b) TEM micrographs of the obtained glutamate stabilized gold nanoparticles. Particles are ∼20 nm in diameter, and are fairly spherical. The uniformity in shape and size is comparable to the nanoparticles obtained by tri-sodium citrate reduction. By varying the gold salt to glutamate ratio, nanoparticles with ∼10 nm diameters have also been achieved.

(1%) HCl (Merck) is added to it such that the final concentration of chitosan is 3.3% in the solution. The obtained gold nanoparticles were analyzed with a TEM (JEOL-JEN2010).

In order to demonstrate the detection of heavy metals, stock solutions of 0.01 M Copper(II)sulfate (Univar) and 0.2 M of zinc acetate (Univar) were prepared. The synthesized gold colloidal solution was exposed to various concentrations of Zn^{2+} and Cu^{2+} ions. Optical measurements were carried out using a UV–Vis spectrophotometer (ELICO-SL-164) and range of wavelengths for the experiments was from 350 to 1000 nm since most optical-absorption effects of gold nanoparticles are visible within this range.

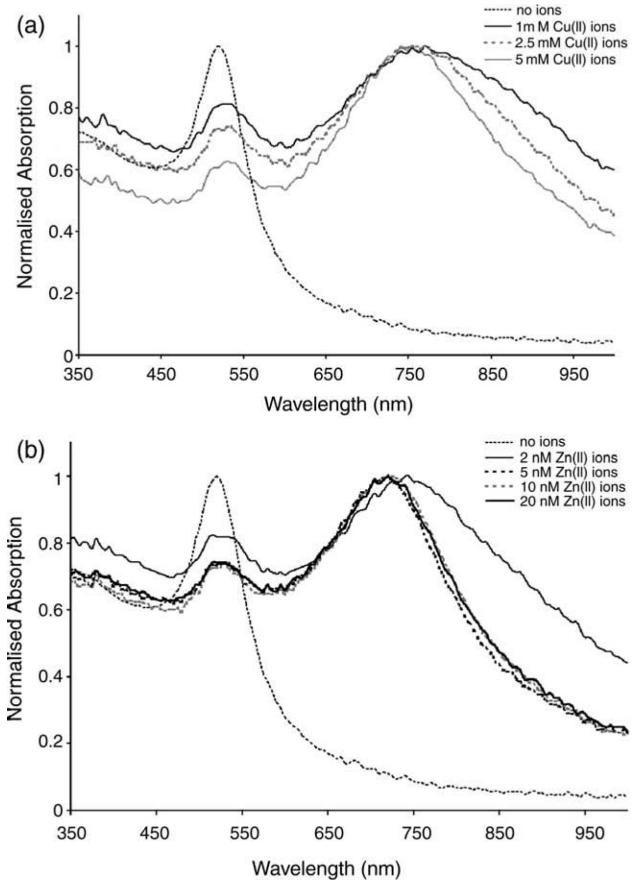

Fig. 2. (a) Series of optical absorption spectra obtained by exposing gold colloid to varying concentrations of Cu(II) ions without prior surface treatment with chitosan. The broad and red-shifted peak is a distinct sign of agglomeration of gold nanoparticles. Though the signal caused by agglomeration is apparent even with 1 mM of Cu(II) ions, there is no significant difference between the signals obtained for various concentrations, ranging from 1 to 5 mM. This agglomeration is probably caused due to the disturbance of the ionic equilibrium, resulting in loss of the protective glutamate capping from the gold nanoparticle surfaces. (b) The optical absorption spectrum obtained by exposing gold colloid to varying concentrations of Zn(II) ions is very similar to that obtained for Cu(II) ions. Though signs of agglomeration are apparent, spectral changes are minimum making it difficult to distinguish between the different concentrations of ions.

3. Results and discussions

Optical absorption spectrum of gold nanoparticles is a good indicator of their size and shape [9]. UV–Vis absorption spectrum of the as-synthesized nanoparticles show a sharp peak centered at 519 nm (Fig. 1a), corresponding to particles ranging from 10 to 20 nm in diameter [16]. TEM analysis confirms that the nanoparticles are on an average ∼20 nm in diameter (Fig. 1b). These nanoparticles are stabilized by physical adsorption of excess anions (glutamate ions) in the solution medium. Consequently, such colloidal suspensions are very sensitive to change in the concentration of ions in the solution. Addition of salts that change the ionic balance will result in loss of adsorbed anions from the nanoparticles, or exchange of the capping ions, resulting in agglomeration of the nanoparticles in both the cases. Surface-treatment of such nanoparticles becomes

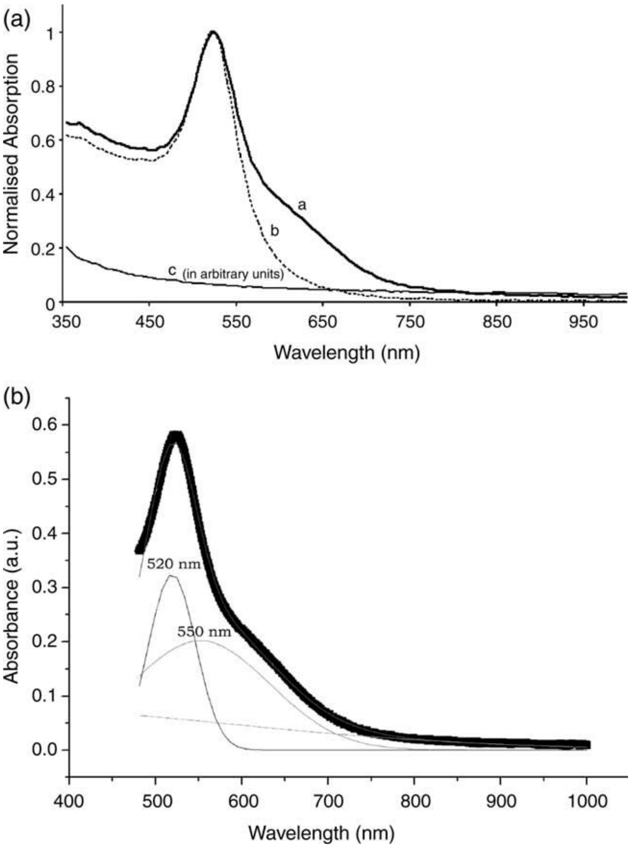

Fig. 3. (a) Absorption spectrum of gold nanoparticles after surface treatment with chitosan (plot a). A noticeable deviation in shape, when compared to spectrum of untreated colloids (plot b), in the range 600–750 nm can be observed. The absorption spectrum of chitosan solution in the absence of gold nanoparticles is essentially flat in this range of wavelengths (plot c). Possibly the change in the surrounding dielectric constant due to chitosan attachment over the nanoparticle surface causes this deviation in the spectral features. (b) De-convolution of the absorption spectrum shows that it neatly fits into three Gaussian peaks. The narrow peak at 520 nm is the original plasmon absorption peak, while the broader red-shifted peak centered at 550 nm is probably due to the ensemble of chitosan capped nanoparticles. The third peak is the absorption spectrum of unattached chitosan.

necessary for applications such as quantitative sensor, to predict the concentration of the analyte. This is because any concentration of the analyte will result in agglomeration, and it is impossible to obtain a unique optical signal for varying amounts of analyte. As an example, the ‘as made’ nanoparticles were exposed to varying amounts of Zn(II) and Cu(II) ions. The absorption spectra of the nanoparticles after exposure to varying amounts of analyte ions are almost identical (Fig. 2a and b). It is difficult to estimate the concentration of the analyte through optical characterization due to the lack of a unique signal for different concentrations. Hence it becomes important to cap the nanoparticles with a material that is not sensitive to the minor changes in electrolyte composition.

Chitosan, derived from chitin by partial deacetylation, is a polymer with poly-cationic properties in dilute acidic solutions, enabling electrostatic attraction towards the negatively charged gold nanoparticle surfaces. Addition of aliquots of chitosan into the gold colloid shows a distinguishable change in the transmitted color and its optical absorption spectrum show a slight departure from that of ‘as-made’ gold colloid (Fig. 3a). De-convolution of the absorption spectrum shows that the change is caused by the presence of an additional broad peak centered at 550 nm (Fig. 3b). Since it is highly improbable that the shape of the nanoparticles change so quickly under ambient conditions, the presence of the additional peak implies that either the particles have agglomerated to some extent, or chitosan has effectively wrapped around the nanoparticles and consequently changing the effective dielectric constant of its surrounding. TEM analysis confirms that no change in the shape of the nanoparticles had occurred and no indication of the particle agglomeration was found (Fig. 4a and b), confirming that the spectral features were caused due to chitosan capping of the gold nanoparticles.

Since chitosan is known as an effective chelating agent for heavy metal ions [21,22] it was expected that the chitosan capped gold nanoparticles will undergo agglomeration upon exposure to cations like Zn^{2+} and Cu^{2+}. As the chitosan capped nanoparticles are no longer stabilized by physically adsorbed charges, upon exposure to varying concentration of these analytes, a concentration-dependant

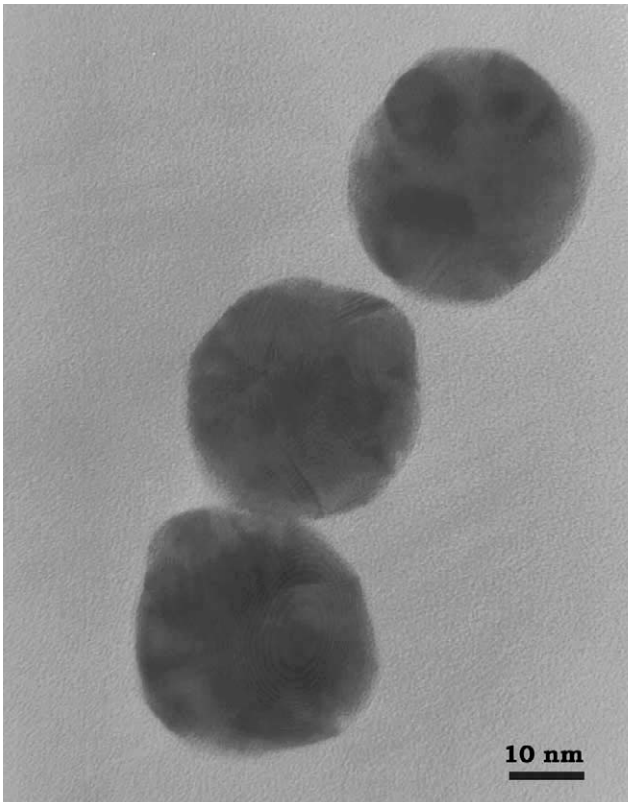

Fig. 4. TEM micrograph of the nanoparticles after surface treatment with chitosan. There is no noticeable change in the shape, and no signs of agglomeration of the nanoparticles hence it can be concluded that the deviation in the absorption spectrum of chitosan treated nanoparticles is due to attachment of chitosan on the surfaces.

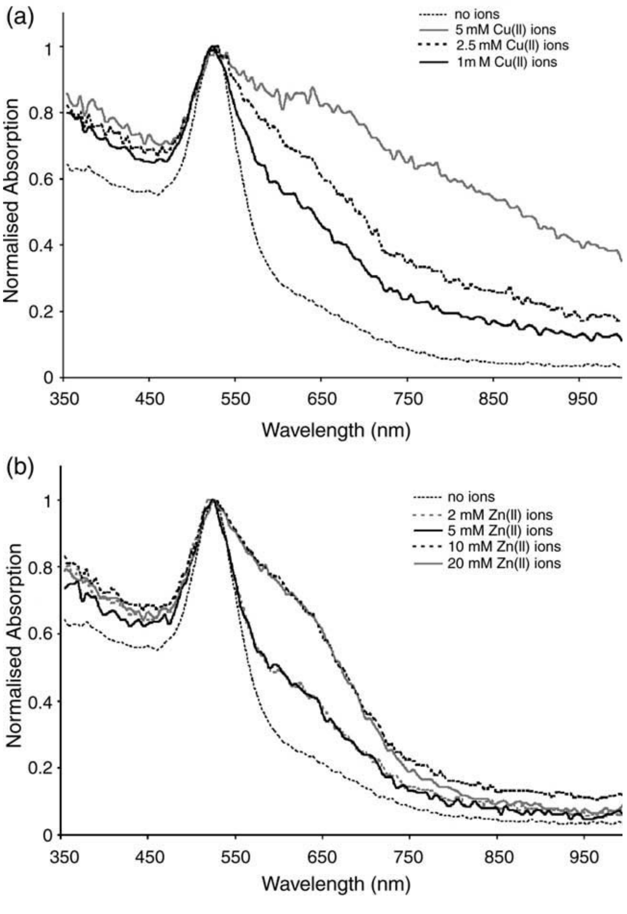

Fig. 5. (a) Effect of exposure to varying concentration of Cu(II) ions to chitosan treated nanoparticles on their optical absorption spectra. Unlike the untreated case (Fig. 2a), the spectral shape for every sample is clearly distinguishable. This effect is clearly visualized in (Fig. 6a). The concentration dependant variation in the spectral features is probably due to absorption of Cu(II) ions by chitosan molecules, thereby changing the dielectric properties of the media surrounding the nanoparticles. (b) Variation of the optical absorption spectra due to exposure to varying concentration of Zn(II) ions. The variation is not as uniform as in case of Cu(II).

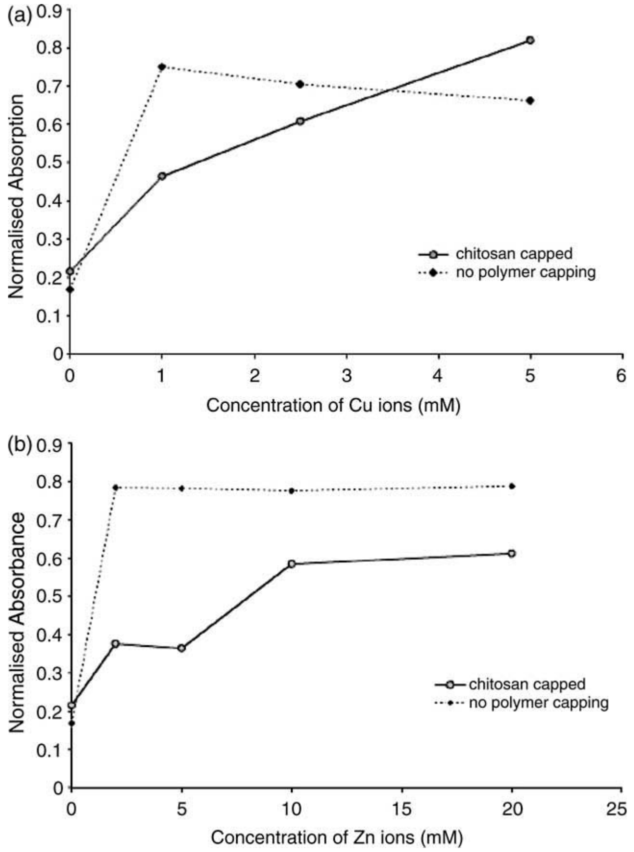

Fig. 6. (a) A comparison of changes in normalized absorption value at 650 nm, due to varying concentration of Cu(II) ions between chitosan treated and as-prepared gold nanoparticles. Chitosan treatment makes the response approximately linear, while untreated nanoparticles tend to agglomerate even under exposure to low concentrations of ions. Hence estimation of the concentration of Cu(II) ions in the solution is much simplified when the nanoparticles are capped with chitosan. (b) Comparison of optical absorption of 650 nm light for varying concentration of Zn(II) ions in the colloid, show that predicting the concentration of Zn(II) is simplified upon surface treatment of gold nanoparticles.

change in the optical absorption is observed (Fig. 5a and b). The change in absorption spectra is very clear in case of copper(II)sulfate (Fig. 5a) where the width of the plasmon resonance peak progressively increases with increase in concentration of Cu^{2+} ions. The variation of the normalized absorbance of light of 650 nm wavelength with increase in concentration of both the analytes is plotted in Fig. 6a and b. The effect of capping the nanoparticles with chitosan is apparent since it provides an approximately linear response with the increase in concentration of analytes. The plot suggests that chitosan capped gold nanoparticles can be used not only to detect the presence, but also to indicate the concentrations of the analytes present in the sample.

4. Conclusions and perspectives

In summary, a novel strategy for detection of heavy metal ions in water has been developed employing 20 nm gold particles capped with a biopolymer called chitosan. Polymer capping of nanoparticles serves a two-fold purpose, that of stabilization and surface functionalization for application as sensors. Chitosan is widely used as a chelating agent for removal of heavy metal contaminants in wastewater. This property of chitosan has been effectively used to demonstrate the detection of low concentrations of heavy metal ions like Cu^{2+} and Zn^{2+}. A relatively simple characterization tool like UV–visible absorption spectrum is found to be sufficient to observe the concentration levels of the analyte. Further modification of the attached chitosan molecules has the promise to achieve high-specificity sensors for various applications. Apart from applications in colorimetric pollution sensors, chitosan capped gold nanoparticles may have biology-oriented applications because it was found that chitosan shows selectivity in attachment to certain kinds of bacteria [23]. Future experiments will be directed towards this aspect of chitosan capped gold nanoparticles.

Acknowledgements

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