ANTIBODY-LABELED GOLD NANOPARTICLES BASED IMMUNOSENSOR FOR THE DETECTION OF THYROXINE HORMONE

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ABSTRACT

Immunosensors are used to provide simple, quick and effective way of detecting analyte for diagnosis of a number of diseases. This work reports a novel optical immunosensor based on thyroxine antibody labeled gold nanoparticles (anti-T4/C-AuNPs) for the sensitive detection of Thyroxine (T4) hormone. T4 hormone is a reliable diagnostic biomarker present in the human blood serum that is necessary for normal neural development and cellular metabolism. The free T4 hormone, a major key player of total biologically active T4 hormone, in serum regulates the patient's thyroid function. The immunosensor was synthesized by using the amine functionalized cysteamine capped gold nanoparticles (C-AuNPs) of size 36 nm approximately. The T4 specific antibody (anti-T4) was covalently attached to C-AuNP by EDC-NHS (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride and N-hydroxysuccinimide) cross-linking chemistry. This anti-T4/C-AuNP bioconjugate was used for the analysis of free thyroxine hormone in samples. The proposed method exhibited high performance immunosensors having a linear range of 0.52pg/ml to 65.1pg/ml and the detection limit of 9.11pg/mL.

KEYWORDS

Gold nanoparticles; Thyroxine; Absorbance spectra; Aggregation; Surface plasmon resonance; Cysteamine
INTRODUCTION

Thyroxine (3, 5, 3, 5 -tetraiodothyronine,) is a major hormone secreted from the thyroid gland of human being. T4 hormone is known to regulate number of biological processes along with cellular metabolism and crucial for the development of nervous system. It has been estimated that more than 99.9% of total T4 hormone present in the blood bind to the transport proteins i.e. thyroxine-binding globulin (TBG), thyroid binding prealbumin (TBPA), and albumin (Alb) are inactive. Thus only 0.02-0.04% of T4 is present in metabolically active free (unbound) form. This active portion moves into the body tissue that utilizes T4. T4 is also converted into another thyroid hormone i.e. triiodothyronine (T3), therefore T4 is the indicator of any hormonal change in thyroid dysfunction. For this reason, the measurement of free T4 hormone concentration is prominent to analyze the thyroid status of patients in clinical diagnostics.

The various analytical techniques such as high performance liquid chromatography coupled mass spectrometry (HPLC-MS), time-resolved immunoassay, dialysis, ultra-filtrations, polyacrylamide gel filtration, radioimmunoassay (RIA), enzyme-linked immunosorbent assay (ELISA), mass spectrometry, bioluminescent immunoassay, chemiluminescent immunoassay (CLIA), electrochemical immunoassay, surface plasmon resonance (SPR) has been employed for the determination of T4. However, these methods being the direct methods are complicated, time-consuming and require sophisticated non-portable instruments and expertise. Light sources (such as aequorin) used in bioluminescence assays have relatively very low light output result in less efficiency. ELISA is time-consuming as it involves multiple incubation and washing steps. RIA is associated with the handling of harmful radioactive labels, expensive waste disposal and limited half-life. Electrochemical sensors are temperature dependent, requires maintenance of electrolyte and working electrode. As an alternative, immunosensors are dynamic and versatile technique for antigen detection as they are fast to analysis, less costly, easy to operate and portable, and have similar sensitivity.

Biosensors utilizing gold nanoparticles stimulate the great interest in researchers due to their unique inherent properties. Gold colloids are the most explored class of noble metal nanoparticles and provide excellent platform for the development of biosensor. They posses all the favorable characteristics such
as easy preparation, high surface area to volume ratio with excellent biocompatibility, conductivity, high electron density, easy surface modification, and size and shape dependent optoelectronic properties, required for the good and efficient biosensor\(^{23,24}\). Gold colloids are the only class of metal nanoparticles which exhibit both the incident and scattering of the incident light\(^{25}\). Agglutination or aggregation of stable gold nanoparticles is the basic principle of gold nanoparticles based immunosensor either by decrease in absorption intensity (due to depletion of stable nanoparticles) or by the broadening and formation of secondary peak at higher wavelength (due to formation of aggregates). These nanoparticles or their composites (AuNP-QDs\(^{26}\), AuNPs-carbon nanotubes\(^{27}\), AuNP-graphene composites\(^{28}\) etc) are used for the different detection platforms such as colorimetric\(^{29,30}\), FRET (Fluorescence Resonance Energy Transfer)\(^{31,32}\), SPR (Surface Plasmon Resonance)\(^{33,34}\), and SERS (Surface Enhanced Raman Scattering) assay\(^{35-38}\) and electrochemical detection\(^{39-41}\).

In this work, we reported a thyroxine immunosensor based on antibody labeled gold nanoparticles for the sensing of thyroxine for the detection of several thyroid disorders. First time the gold nanoparticles were explored as an optical sensing platform for the thyroxine detection. The cysteamine capped gold nanoparticles (C-AuNPs) were synthesized by sodium borohydride reduction methods. As cysteamine posses both sulphur and amine group in its structure, therefore it is frequently used as a stabilizer for AuNPs and linker for antibody immobilization on gold surface. Thyroxine antibody (anti-T4) was covalently immobilized on C-AuNPs using EDC-NHS coupling chemistry. This anti-T4/C-AuNP bioconjugate was then further investigated for the detection of thyroxine hormone. In the presence of T4 hormone, due to the negligible morphological changes in C-AuNPs, there is a change in SPR angle only which results in decrease in the absorbance intensity at \(\lambda_{\text{max}}\) 528 nm without any red shift\(^{42}\). Therefore, it can be emphasized that the antigen-antibody complex formation over C-AuNP leads in the reduction of stable C-AuNPs. The phenomenon of immunosensing for the detection of thyroxine hormone is schematically represented in the Fig.1.

**MATERIALS AND METHODS**

**Materials**

Thyroxine antibody (Origin: mouse) was received from GeneTex. Hydrogen tetrachloroaurate trihydrate (H AuCl\(_4\) .3H\(_2\)O) was purchased from Loba Chemie. Cysteamine, 1-ethyl-3-(3-
dimethylaminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS), sodium borohydride, bovine serum albumin, thyroxine (T4), triiodothyronine (T3) was and procured from Sigma Aldrich and used without any further purification. Millipore water (18 MΩ cm) was used in all the experiments. The experiments were conducted at room temperature.

Preparation of solutions

The thyroxine and triiodothyronine were mixed in 4M ammonium hydroxide in methanol solution and thyroxine antibody (anti-T4) was diluted in phosphate buffer saline (Sodium chloride 7.650 g/L, Disodium phosphate 0.724 g/L, Dipotassium hydrogen phosphate 0.210 g/L, pH 7.4). Both the solutions were stored at 4 °C. 2 mg/mL Bovine serum albumin was (BSA, 98%) prepared in distilled water and used for the blocking of non-specific binding sites.

Synthesis of cysteamine capped gold nanoparticles

Cysteamine capped gold nanoparticles were synthesized by the protocol given by Sharma et. al43. 400µL of 213mM cysteamine was mixed with 40mL of 1.42mM of HAuCl₄ and vigorously stirred for 20 minutes in the dark at room temperature. After homogeneous mixing, 10µL of 10mM sodium borohydride was quickly added to the solution. The mixture was vigorously stirred for another 30 minutes in same conditions. The color of the solution changes from pale yellow to characteristic wine red color ensuring the formation of cysteamine capped gold nanoparticles (C-AuNPs). This wine red color of colloidal gold nanoparticles is arise from the collective oscillation of conduction band electrons44,45. This solution was stored at 4°C and used in subsequent reactions without any purification.

Bioconjugation of antibody with C-AuNP

The bioconjugation of antibody with C-AuNPs was carried out using EDC-NHS cross linking chemistry. 1µL of 2.2µg/µL thyroxine antibody (anti-T4) was treated with 5µL of 52mM EDC and 5µL of 192mM NHS for the activation of -COOH in anti-T4 to easily bind with amine modified gold nanoparticles. Activated anti-T4 was mixed with 1mL of C-AuNPs under stirring condition that lead to the formation of strong covalent amide bond. After 30 minutes of stirring, 30µL of 2mg/ml BSA was introduced to the solution to block the non-coated surface of gold nanoparticles. The anti-T4 bound gold nanoparticles were collected by centrifugation at 10,000 rpm for 20 minutes followed by washing.
with distilled water. The pellet was collected and stored at 4°C. The concentration of thyroxine antibody required for gold surface modification was optimized separately.

**Sensing of thyroxine hormone**

For T4 detection, anti-T4/C-AuNPs were dispersed in PBS solution of pH 7.4 and 90µL of anti-T4/C-AuNPs was added to each well of microtitre 96-well plate. A range of concentration of thyroxine hormone was prepared and 10µL of each concentration was added to the well. After 15 min of incubation time, the absorbance was recorded using Spectra Max i3X microplate reader.

**Characterization**

UV–visible absorption measurements were recorded at room temperature in the wavelength region 200-800 nm using Spectra Max i3X microplate reader. X-ray powder diffractometer (XRD) with Cu-Kα radiation (Bruker AXS D8) was used to identify the crystalline phase of as synthesized C-AuNPs. Surface morphology of as synthesized cysteamine capped gold nanoparticles was examined using cold field-emission scanning electron microscopy (SEM) [Hitachi SU1080] coupled with EDX. The hydrodynamic size and surface charge of as synthesized cysteamine capped gold nanoparticles were measured by dynamic light scattering (DLS) zetasizer [ZS90 series]. Centrifugation was carried out using Eppendorf Centrifuge 5804 R. Attenuated total reflection (ATR)-FTIR spectra of cysteamine capped gold nanoparticles and antibody labeled gold nanoparticles were recorded with Perkin Elmer - Spectrum RX-FTIR mounted with a diamond crystal ATR unit. The colloidal solution was cast onto the diamond crystal, dried and subsequently characterized.

**RESULTS AND DISCUSSION**

**XRD study**

The crystallinity of as synthesized C-AuNPs was confirmed by XRD shown in Fig. 2. The peaks at 2θ = 38.16°, 44.60° and 64.71° correspond to the FCC metallic gold diffraction (JCPDS 00-001-1172). Other peaks at 2θ = 27.90°, 31.60°, 32.64°, 35.31°, 43.16°, 55.81° and 57.59° shows the crystal planes of cysteamine hydrochloride.

**Optical study**

The cysteamine capped gold colloidal solution was synthesized by the reduction of gold salt with sodium borohydride in the presence of cysteamine. This colloidal solution obtained by sodium borohydride reduction was wine red in color. Fig. 3 shows the UV absorption spectra of cysteamine
capped gold nanoparticles. The UV-vis absorption spectra of cysteamine capped gold nanoparticles solution exhibit a surface plasmon resonance absorption band at 524 nm and is a feature of 36 nm cysteamine capped gold nanoparticles.

The concentration of C-AuNPs was calculated and is given below:

Calculation of average number of gold atoms per nanoparticle

C-AuNPs are spherical shaped nanoparticles with diameter 36 nm and have a uniform fcc structure. The average number of gold atoms per nanoparticle was calculated by Eq. (1), D is the diameter of the nanoparticles.

\[ N = 30.89602 \times D^3 = 1441203.84 \]  

Determination of molar concentrations of colloidal solution

Molar concentration of the colloidal solution was calculated by dividing the total number of gold atoms over the average number of gold atoms per nanoparticle Eq. (2). It is assumed that there is 100% conversion of gold (III) to gold atoms.

Where,

\[ N_{\text{total}}: \text{Initial amount of gold salt added to the reaction} \]

\[ N: \text{Average number of gold atoms per nanoparticles, calculated from equation (1)} \]

\[ V: \text{Volume of reaction solution (in liters)} \]

\[ N_A: \text{Avogadro’s constant (6.022} \times 10^{23}) \]

\[ C = \frac{N_{\text{total}}}{NVN_A} = 1.56 \times 10^5 \text{pM} \]  

The molar concentration of the as prepared cysteamine-capped gold nanoparticles was calculated to be approximately 1.56*10^5 pM.

The absorption and scattering properties of gold nanoparticles is greatly influenced by the size and shape of nanoparticles, and also dependent on the refractive index of the surrounding medium. After the binding of large polymeric anti-T4 molecules, there is a dramatic increase in the C-AuNP size. It resulted in the shifting of absorbance wavelength from \( \lambda_{\text{max}} 524 \text{ nm} \) to \( \lambda_{\text{max}} 528 \text{ nm} \) as observed in UV absorption spectra. Along with shift, another peak of antibody absorption is noticed in between 240 - 280 nm (inset graph of fig.3) which ensures its presence in C-AuNPs surface. Absorbance spectra of supernatant were also recorded to determine the concentration of antibody required for C-AuNPs.
surface modification. A peak at 280 nm confirms that the above antibody concentration is sufficient for 3 mL C-AuNPs surface modification Fig. S1 (Supporting Information).

Optimization studies

For in vivo or in vitro biomedical applications, study the behavior of gold nanoparticle at different concentration of electrolyte (NaCl) and pH are the important aspects to check its stability. The high ionic strength of the biological media and the presence of electrolyte can result in nanoparticle aggregation. The effect of NaCl on C-AuNPs was optimized. Different molar solutions of NaCl were prepared first. 10 µL solution of NaCl of each concentration was mixed with 90 µL C-AuNPs, shaken and incubated for 10 mins and UV-Vis absorbance spectra recorded. It has seen that the as synthesized C-AuNPs were highly stable upto $10^{-2}$ M NaCl concentration (Fig. 4a).

In 2018, C. F. Ponce et al. reported that the cysteamine capped gold nanoparticles are stable at pH<9. While at pH>9, C-AuNPs undergo aggregation. Moreover, the absorption spectrum of C-AuNPs shows a narrow LSPR band at $2<\text{pH}<9$, after which, the spectrum starts to show evidences of C-AuNPs aggregation\textsuperscript{49}. Relying on this survey and from future perspectives to let this proposed T4 detection assay be utilized for in-vivo application, the optimized pH value for the further experimentation was selected as pH 7.4.

Fig. 4b shows the UV-Vis absorbance spectra of C-AuNPs mixed with different molar concentration of EDC and NHS required for antibody functionalization to bind with the amino groups present on C-AuNP surface without affecting nanoparticles stability. It has been observed that no aggregation of gold nanoparticles occurred upto 0.52mM EDC and 1.92 mM NHS concentration. And above this concentration, nanoparticles were aggregated. Hence, 0.52mM EDC and 1.92 mM NHS concentration was used for the antibody functionalization.

FTIR study

The FTIR spectra of the C-AuNPs (black line) and anti-T4/C-AuNPs (red line) are shown in Fig. 5. The peak at 677 cm\(^{-1}\) and 831 cm\(^{-1}\) is due to gold-thiol (S=O) linkage. The peaks at 892 cm\(^{-1}\), 939 cm\(^{-1}\) and 3312 cm\(^{-1}\) are associated with N-H of cysteamine. The sharp band at 2926 cm\(^{-1}\) would be ascribed to the C-H stretching of cysteamine\textsuperscript{50}. After immobilization of anti-T4/C-AuNPs, appearance of new peak at 814 cm\(^{-1}\) and 1556 cm\(^{-1}\) are associated with N-H stretching and N-H bending of amide.
bond, respectively. The peak at 1454 cm$^{-1}$ is assigned to the action of amide (C–N). The peak at 1649 cm$^{-1}$ can be associated with the C=O stretching of amide bond and peak at 1701 cm$^{-1}$ is due to C=O stretching of carboxylic acids. These results verify the bioconjugation of thyroxine antibody to C-AuNPs.

**FESEM with EDX**

FESEM images show the plain and uniform morphology of spherical shaped cysteamine capped gold nanoparticles with an average particle size of about 36 nm (Fig. 6a). The presence of nitrogen (N) and gold (Au) peak in EDX data reveals the successful capping of cysteamine on gold surface (Fig. 6b).

**Hydrodynamic size and Zeta potential**

The hydrodynamic size and surface potentials of the as synthesized cysteamine capped gold nanoparticles are shown in Fig. 7. The hydrodynamic size of as synthesized C-AuNPs is found to be 141 d.nm. and zeta potential was positive (+8.8 mV) because of presence of positively charged amine (-NH$_3^+$) groups of cysteamine onto gold surface. The hydrodynamic diameter of this electric double layer in C-AuNPs is 3 to 4 times greater than the actual C-AuNPs FESEM size (36nm). This occurrence is might be due to the ionic combination of positively charged C-AuNPs and negatively charged Cl$^-$ ions present in impurities, resulting in the formation of NH$_3^+$Cl$^-$ and affect the “apparent” diameter of the particles.

Since, it has been reported that the zeta potential is always lesser than the surface potential and is inversely proportional to the diameter of this electric double layer (therefore the hydrodynamic size). As the hydrodynamic size of C-AuNPs is very larger therefore, the net zeta potential of C-AuNPs is found to be very less (e.g. +8.8 mV). The C-AuNPs were stabled against aggregation due to the electrostatic repulsion between positively charged gold nanoparticles.

**Sensing of thyroxine**

To determine the sensitivity of the assay, C-AuNPs were mixed with different concentration of T4, incubated for 15 mins and optical properties of C-AuNPs were observed under optimized conditions (Table 1). With increase in T4 concentration, the intensity of red color decreases gradually (Fig. 8b). The result was also confirmed by the UV–Vis spectroscopy: as the concentration of T4 increases from 0.52pg/mL (0.8pM) to 65.1pg/mL (100pM), intensity of longitudinal plasmon absorbance band (LPAB) decreases at $\lambda_{528}$. This might be due to the destabilization of gold nanoparticles during antigen-
antibody complex formation. Instead of shoulder formation at higher wavelength with change in red color of gold nanoparticles to blue, decrease in LPAB and color intensity is noticed and this phenomenon were also reported in some other studies\textsuperscript{57,58}.

The variation of absorbance at 528 nm (I\textsubscript{0}-I) with T4 hormone concentration is shown in Fig. 8a. Triplicate experiments were carried out at room temperature (25±2 °C) to determine the deviation in UV-Vis absorbance spectra. The limit of detection was expressed as LOD=3Sa/b, where Sa is the average standard deviation and b is the slope of the calibration curve (or sensitivity). These experiments evidenced a standard deviation of ± 8.277x10\textsuperscript{-3} in the change in absorbance (I\textsubscript{0}-I) intensity at 528nm. Plot of I\textsubscript{0}-I and concentration shows liner relation (R\textsuperscript{2} = 0.98301) (Fig. 8c and 8d). According to signal to noise ratio (S/N=3), the limit of detection was calculated to be 9.11 pg/mL (14pM). This data is in complete agreement of the fact that this proposed method could be applied for the optical detection of T4 in picomolar range.

\textit{Evaluation of selectivity}

Study of interfering compounds in an assay is the key parameter for determining its applicability. To investigate the selectivity, the nanoprobe was exposed to the similar structural candidate of thyroxine hormone, i.e. triiodothyronine (T3). As shown in Fig. 9, the absorbance spectra of anti-T4/C-AuNPs was measured in the absence and presence of T4 and T3 hormone under the identical conditions. The concentration and the incubation time were remaining same in both the cases. Hence, antibodies are very specific in nature. Therefore, no aggregation and decrease in absorbance intensity was observed when nanoprobe was treated with T3 as compared to T4.

\textit{Comparison between the proposed immunosensor and the other methods}

In this paper, the sensitivity of proposed immunosensor is compared with other immunosensors and assays, which was shown in Table 2. From Table 2, it is cleared that the detection limit of proposed sensor is quite similar with the other assays and assay time required for sensing is almost very less than most of the other sensors. SPR based immunosensor and fluorescent sensor offers less assay time of about 5 mins but the limit of detection is found to be higher compared to this work. Also, fabrication of the reported sensors require multiple incubation and washing steps which makes it a complex process whereas our proposed assay is a simple process that uses one step detection platform for thyroxin.

\textbf{Conclusion}
In total, the cysteamine capped gold nanoparticles of average diameter of 36nm and surface charge +8.8 mV were successively synthesized using sodium borohydride reduction method. Further, the as synthesized cysteamine capped gold nanoparticles bioprobe were labeled by thyroxine antibody with using EDC-NHS cross-linking chemistry. A simple, fast and sensitive immunosensor has been developed for the biosensing of thyroxine hormone based on decrease in longitudinal plasmon absorbance intensity of C-AuNPs with increase in T4 concentration. The developed immunosensor is found to detect T4 in the concentration range of 0.52 pg ml$^{-1}$ (0.8pM) to 65.1 pg ml$^{-1}$ (100pM), with limit of detection of 9.1pg/ml. Our studies indicate that antibody labeled gold nanoparticles is an appropriate choice for use in clinical diagnostics.

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Table 1 Optimized experimental conditions for T4 detection using cysteamine capped gold nanoparticles

| Parameter                                | Optimized value                  |
|------------------------------------------|----------------------------------|
| Type of metal NPs                        | Cysteamine-capped gold nanoparticles |
| Antibody estimated for NPs               | 2 µg/µL                          |
| Time required to perform measurements    | 15 mins                          |
| Phosphate buffer solution                | 0.01M                            |
| pH                                       | 7.4                              |
| Temperature                              | 25±5                             |
Table 2 Performance of proposed assay compared with other developed methods

| S.No. | Sensing assay | Material | Linear range | Detection limit | Assay time (in mins) | Reference |
|-------|---------------|----------|--------------|-----------------|---------------------|-----------|
| 1.    | Surface plasmon resonance (SPR) based immunosensor | Self assembled monolayer (SAM) of poly(ethylene) glycols (PEG) aromatic dialkanethiols was employed for fabricating sensor surface | 1 ng mL\(^{-1}\) to 1000 ng mL\(^{-1}\) | 1.0 ng mL\(^{-1}\) | 5 | 3 |
| 2.    | CLEIA* | FITC* labeled antibody coated magnetic nanoparticles and HRP* labeled t4 analog | 1.59–122 pmol L\(^{-1}\) | 0.16 pg ml\(^{-1}\) | 90 | 4 |
| 3.    | Electrochemical immunosensor | • Cascade catalysis of cytochrome c (Cyt c) and glucose oxidase (GOx) as signal amplified enhancer.  
• Multi-functionalized magnetic graphene sphere as signal tag. | 0.05 pg mL\(^{-1}\) – 5 ng mL\(^{-1}\) | 0.015 pg mL\(^{-1}\) | 40 | 22 |
| 4.    | Amperometric | Graphene oxide tuned with gold nanoparticles and thiolated β-cyclodextrin | 0.65 ng mL\(^{-1}\) to 9.11 ng mL\(^{-1}\) | 0.65 ± 13.02 ng mL\(^{-1}\) | _ | 59 |
| 5.    | Impedance | Multi-thiol SAM attached with selective T4 antibody | 1 ng mL\(^{-1}\) to 5 ng mL\(^{-1}\) | 1.5 ng mL\(^{-1}\) | 30 | 60 |
| 6.    | Fluorescent sensor | L-cysteine modified ZnS quantum dots/ CTAB* | 13 ng mL\(^{-1}\) to 26.04*10\(^{11}\) ng mL\(^{-1}\) | 48.3 ng mL\(^{-1}\) | 5 | 61 |
| 7.    | Electrochemical sensor | Molecularly imprinted polymer modified silver electrode | 0.010–17.2 ng mL\(^{-1}\) | 0.0060 ng mL\(^{-1}\) for L- and 0.0062 ng mL\(^{-1}\) for d-thyroxine | _ | 62 |
| 8.    | Optical immunosensor | Anti-T4 labeled gold nanoparticles | 0.52 pg/ml to 65.1 pg/ml | 9.1 pg mL\(^{-1}\) | 15 | This work |

*CLEIA: Chemiluminescence enzyme immunoassay  
*CTAB: Cetrimonium bromide  
*FITC: Fluorescein isothiocyanate  
*HRP: Horseradish peroxidase
Captions to figure

Fig. 1 Schematic illustration of T4 detection using anti-T4 labeled gold nanoparticles. (a) Synthesis of C-AuNPs through borohydride reduction, (b) Antibody functionalization of C-AuNPs, and (c) Detection of T4 using anti-T4/C-AuNPs nanoprobe.

Fig. 2 XRD spectra of C-AuNPs

Fig. 3 UV-Vis absorbance spectra of (a) C-AuNPs and (b) C-AuNPs/anti-T4 (Inset graph showing anti-T4 absorption in anti-T4/C-AuNPs nanoprobe and photographic image of C-AuNPs and nanoprobe)

Fig. 4 UV absorption spectra and photographic image of C-AuNPs with different concentration of (a) NaCl and (b) EDC-NHS

Fig. 5 FTIR analysis of C-AuNPs (black line) and C-AuNPs/anti-T4 (red line)

Fig. 6 (a) SEM images of well dispersed C-AuNPs and (b) their EDX data with elemental composition

Fig. 7 (a) DLS size characterization and (b) zeta potential of C-AuNPs

Fig. 8 (a) Absorbance spectra and (b) photographic image of anti-T4/C-AuNPs in the presence of different thyroxine hormone concentrations i.e. 0.8pM (0.52 pg ml\(^{-1}\)), 0.9pM (0.58 pg ml\(^{-1}\)), 1pM (0.65 pg ml\(^{-1}\)), 10pM (6.51 pg ml\(^{-1}\)), 20pM (13.02 pg ml\(^{-1}\)), 40pM (26.04 pg ml\(^{-1}\)), 60pM (39.06 pg ml\(^{-1}\)), 80pM (52.08 pg ml\(^{-1}\)) and 100pM (65.1 pg ml\(^{-1}\)), (c) and (d) showing linear calibration plot between A528 and thyroxine concentrations in the range 0.8pM (0.52 pg ml\(^{-1}\)) to 100pM (65.1 pg ml\(^{-1}\)).

Fig. 9 UV-Vis absorption spectral change of anti-T4/C-AuNPs in the absence and presence of 80pM T4 and T3.
Fig. 1
Fig. 2
Fig. 3
Fig. 4
Fig. 5
Fig. 6
Fig. 7

(A) Size Distribution by Intensity

C-AuNPs = 141 d.nm.

(B) Zeta Potential Distribution

C-AuNPs = +8.8 mV
Fig. 8
Fig. 9