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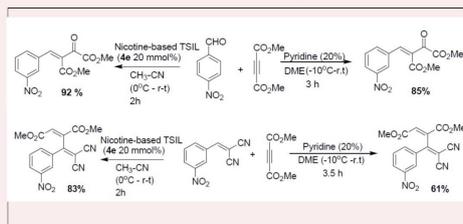
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A tidy laboratory
means a lazy chemist.
-- Jöns Jacob Berzelius (Swedish
chemist, 1779-1848)



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Tannic Acid Based Gold Nanosensor for Selective Detection of Hg²⁺ and Pefloxacin

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Abstract

Tannic acid capped gold nanoparticles with a mean diameter of 20nm were efficiently prepared by the green synthetic route. The size and morphology of gold nanoparticles were investigated by transmission electron microscopy and atomic force microscopy. The gold nanoparticles were effectively applied for the development of a sensing system that can detect and extract Hg²⁺ ions as well as Pefloxacin in a single step due to the strong interaction of gold nanoparticles with Hg²⁺ ions and pefloxacin. The sensing ability of gold nanoparticles was deliberated for the selective removal of Hg²⁺ ions and pefloxacin using UV visible absorption. The UV visible absorption of tannic acid capped gold nanoparticles was satisfied by different metal salts and drugs. The synthesized nanoparticles showed stability within a pH range of 3-12 and to heating at 100 °C for 5 minutes. Quenching of the peak and precipitation is observed with various concentrations of brine solutions. Job's plot analysis shows the binding stoichiometry to be a 1:1 ratio. This newly designed sensor offers a simple low-cost alternative tool for recognition and extraction of Hg²⁺ and pefloxacin.

Keywords: Tannic Acid, Gold Nanosensor, Detection Of Hg+2 Ions, Detection Of Pefloxacin, Gold Nanoparticles Synthesis

INTRODUCTION

Gold nanoparticles have long been deliberated as possible drug delivery automobiles appropriate for therapy. Among all of these arrangements, one of the most significant elements is emerging procedures to properly functionalize the nanoparticles to have the preferred effects. Numerous reports exist of gold nanoparticles functionalized through the distinguished gold–thiol covalent bond [Jadzinsky et al, 2007]. Gold nanoparticles are attractive for a number of reasons in addition to their brilliant surface functionalization chemistry. They can be definitely manufactured in a variety of shapes including core-shell, rod-like, spherical with sizes ranging from 1 nm to more than 100 nm [Jadzinsky et al, 2007; Stewart et al, 2008; Xia et al, 2011]. The electronic and optical properties of gold nanoparticles are highly dependent on shape and size [Tao et al, 2008; Vigderman et al, 2012], which has led to many biomedical uses [Slaughter et al, 2010; Grzelczak et al, 2008]. In addition, gold nanoparticles have generally shown themselves to be nontoxic, although there is some sign that non-covalently functionalized particles are not completely benevolent [Zhao et al, 2011; Elif et al, 2018]. There

are numerous approaches for using gold nanoparticles as a drug encapsulation electrostatic adsorption, drug delivery vehicle and other non-covalent assemblies [Kim et al, 2009].

Tannic acid, a polyphenolic plant extract, used as both the stabilizing and reducing agent. It is typically hydrolyzed tannin, a mixed gallotannin contain gallic acid esters of glucose, which has been used for the biomimetic progression of inorganic nanoparticles [Chen et al, 2007]. It has anti-cancer, antioxidant and antimutagenic properties, decreases serum cholesterol, triglycerides and lipogenesis by insulin [Nakamura et al, 2003]. It prevents nanoparticles from agglomeration and results in smaller particles by higher capping efficacy [Zhang et al, 2009]. It is a biologically active molecule possessing antiviral, antimicrobial activities and plays an important role as a protective agent against diseases and damages [Chen et al, 2003; Sahiner et al, 2015]. It contains multiple phenolic hydroxyl groups which are related to its biological activity while these hydroxyl groups also form complex structure through hydrogen bonding [Perelshtein et al, 2014].

However, in this paper, we focused only on the direct

covalent interaction of several types of drugs and metal salts with gold nano-conjugate for their sensing behavior.

RESULTS AND DISCUSSION

Synthesis of nanoparticles

The stock solution of tannic acid (0.5mM) was prepared by dissolving tannic acid in methanol. Then 0.5mM aqueous ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$) solutions were stirred for 30 minutes with different ratios of the stock solution of TA in the round bottom flask. After reaction completion, precipitation followed by different instrumental techniques like FT-IR, AFM and TEM analysis and UV.

Visible spectroscopy

The UV spectra of the Au nanoparticles at different ratios of the gold solution and TA were taken. The results of UV spectra (Fig-1) showed that 10:1 (10mL Gold solution and 1 mL Tannic acid solution) gives the best results with λ_{max} at 528 nm.

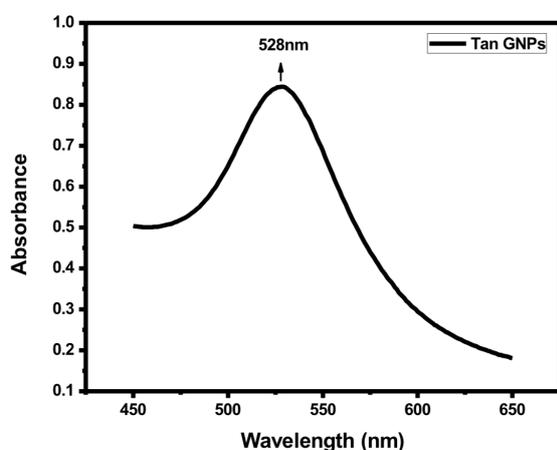


Figure 1. UV-Visible spectrum of synthesized gold nano particles at 568nm

FTIR of Tannic Acid and its nanoparticles

FT-IR spectroscopy of the pure TA and its Au nanoparticles was carried out to find some evidence about the interaction TA and Au nanoparticles. The FTIR spectrum of Tannic acid shows clear peaks in the regions 3400-3100, 1800-1650, 1650-1500 and 1200-1100 cm^{-1} corresponding to the stretching frequencies of $-\text{OH}$, $\text{C}=\text{O}$, $\text{C}=\text{C}$, and $\text{C}-\text{O}$ functional groups respectively. It has already been found that due to the interaction of the ligand with metal in

nanoparticles the peaks of the corresponding functional groups are shifted [34], weekend or even disappeared [35]. It is clear from the FTIR spectrum of the Tannic acid stabilized gold nanoparticles that the peaks in the stretching regions of $-\text{OH}$ (3400-3100 cm^{-1}), $\text{C}=\text{O}$ (1800-1650 cm^{-1}), $\text{C}=\text{C}$ (1650-1500 cm^{-1}) and $\text{C}-\text{O}$ (1300-1100 cm^{-1}) are predominantly weekend in intensity indicating interaction of these groups with the metal (Gold) nanoparticles. The FTIR spectrum of the gold nanoparticles stabilized by tannic acid indicates the involvement of hydroxyl, carbonyl, aromatic as well as ether functional groups in the stabilization of gold nanoparticles (Fig-2a and 2b).

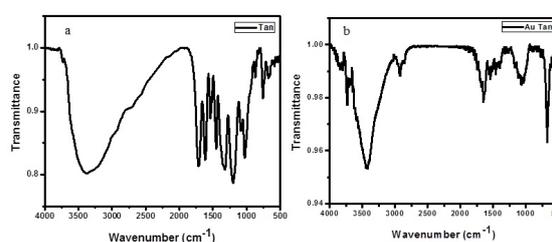


Figure 2. a) The FT-IR spectrum of tannic acid b) FT-IR spectrum of synthesized gold nanoparticles with tannic acid

TEM analysis

Size distribution with quite asymmetrical of average size 20 nm as shown in the histogram for AuNPs, was determined by TEM image (Fig-3).

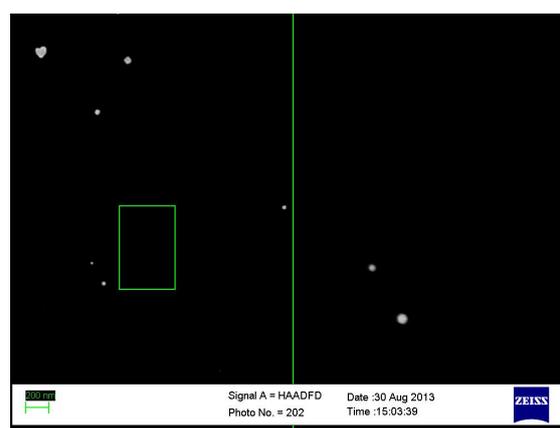


Figure 3. TEM images of gold nanoparticles with tannic acid (right side is 5x magnified according to left side)

AFM images of TA Stabilized gold nanoparticles

The morphology was further analyzed with AFM. Reduced AuNPs clearly showed a flat single layer of tannic acid stabilized nanosheets with an average height of 20 nm (Fig-4).

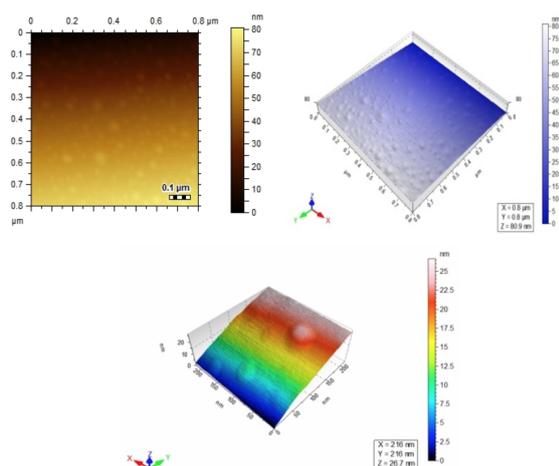


Figure 4. AFM images of gold nanoparticles with tannic acid

Optimization of Method

Sample pH value

Within a pH range 2-12, the nanoparticles showed resistance but precipitation is detected below pH 2 and above pH 12 which is indicated by UV Spectra (Fig-5).

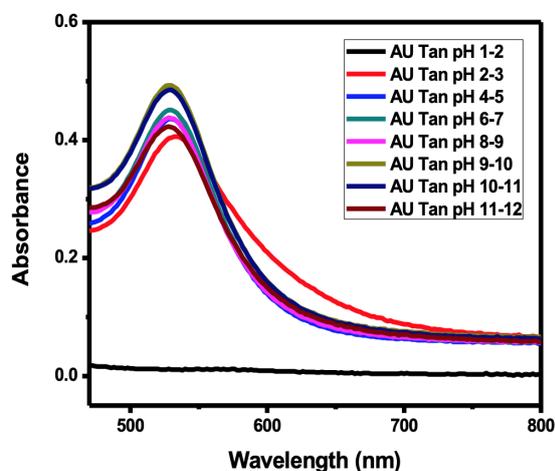


Figure 5. Effect of pH on Au-Tan nanosensor

Temperature

AuNPs were boiled for 5 minutes and the peak was observed which shows that tannic acid stabilized nanoparticles are heat sensitive (Fig-6). This may be due to the fact that heat cause changes in the surface charges resulting in the coagulation of particles into larger and also changes in light scattering.

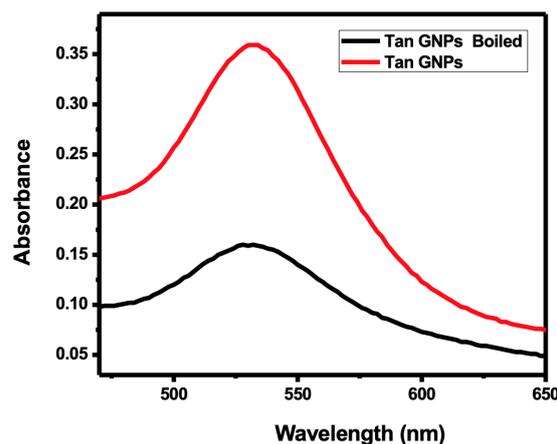


Figure 6. Effect of temperature on Au-Tan nanosensor

Salt Concentration

Tannic acid stabilized gold nanoparticles showed a shift to higher wavelength with brine solution ranging from 1mM to 5M as shown by the UV-Visible spectrum (Fig-7). The molecules associated with the nanoparticle surface show a double layer of charge that inhibits nanoparticle from aggregation. The local environment strongly affected the surface of nanoparticles which is dynamic in nature. Various situations will disturb particles in different ways. The environment of the double layer of higher salt will deteriorate and originate from the accumulation of nanoparticles. In solution form, the Brownian motion of nanoparticles is dependent on temperature. Particles permanent precipitation/accumulation is caused due to frequent collision if particle concentration is high. Proper stabilizing agent must be applied to the surface of particles that reduces adjacent particles repulsion in order to keep particles in dispersed solution form.

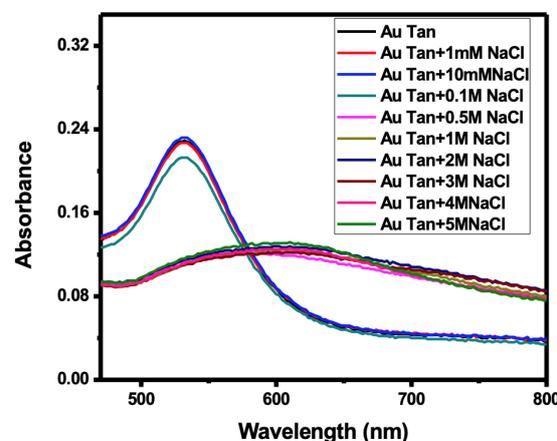


Figure 7. Effect of salt concentration on Au-Tan nanosensor

Optical studies

After click reaction on the surface of tannic acid gold nanoparticles, the intensity of target ion (Hg^{2+}) is quenched by gold nanoparticles. Tannic acid gold nanoparticle solution shows an effective switch-off response toward Hg^{2+} ion at 1mM (Fig-8).

The reason for this might be due to tannic acid which is composed of gallic acid and glucose molecules and has the ability to release freely reactive hydrogen atom. The hydroxyls group in tannic acid may also be responsible for reducing chloroauric acid. During reduction process, the carboxylic acid group (COOH) that exists in TA may lose their hydrogen atom to become carboxylate ion (COO^-). The COO^- ion so formed may be attached to the surface of metal ion to act as a surfactant and stabilized metal nanoparticles by electrostatic stabilization.

The main reason why we chose to use TA as a reducing agent in this study is its water solubility, and high extinction coefficient and its ability to form ion association complexes with Hg^{2+} .

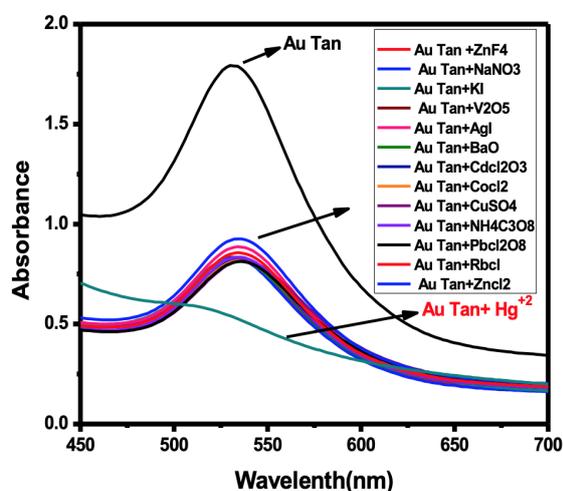


Figure 8. UV-Visible spectrum of Au-Tan nanosensor with different metals salts

Acid-base behavior

The optimum pH range for Hg^{2+} detection was 4.5, as in this range sensor showed maximum absorption (Fig-9). Such extensive pH ranges make it very expressive during various applications, such as industrial trade analysis, physiological course, and detection of ions of wastewater, etc.

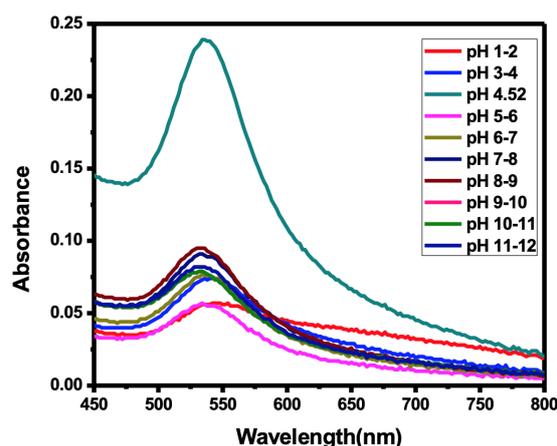


Figure 9. Effect of pH on Au-Tan nanosensor with Hg^{2+}

Competitive metal sensing studies

Competitive metal ions such as Hg^{2+} , Zn^{2+} , Ag^+ , Co^{2+} , Ba^{2+} , V^{5+} , Pb^{2+} , K^+ , Cu^{2+} , Cd^{2+} , Rb^+ , Na^+ and NH_4^+ were added to the TA AuNPs solution shows that most metal ions do not have response toward TA AuNPs. However, the intensity of TA AuNPs was slaked obviously after the addition of Hg^{2+} (Fig-10). Hg^{2+} exhibits an excellent selectivity over other metals ions. Upon addition of other metals ions, no clear spectral changes were detected.

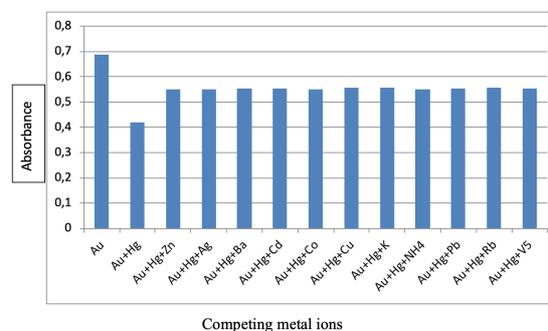


Figure 10. Competitive behaviour of Au-Tan nanosensor with different metal ions

Temperature affect

Under various environmental conditions, the nanoparticles solution was found to be stable. The temperature effect was studied in order to check the stability of nanoparticles. The complex at elevated temperature showed no precipitation as was observed in absorption spectra and Plasmon peak did not shift. On the basis of dominant electronic dephasing reduced absorbance can be described which includes electron-electron interactions as higher electronic temperatures do not only lead to a faster electron-electron scattering

rate but should also increase the electron-surface and electron-defect scattering. Electron velocity depends upon its temperature and its state energy. Under elevated temperature, the nanoparticles solution was found stable (Fig-11). Plasmon peak doesn't show shifting, reduce absorbance, and precipitation.

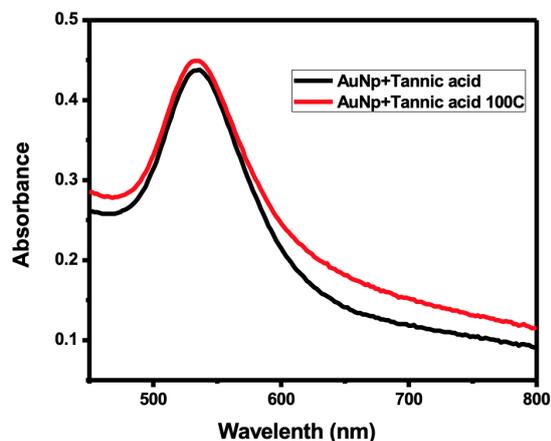


Figure 11. Effect of temperature on Au-Tan nanosensor with Hg²⁺

Titration studies

To examine the sensitivity of TA AuNPs towards Hg²⁺, the solution of TA AuNPs was titrated with an aqueous solution of Hg²⁺ (Fig-12a). Upon the addition of Hg²⁺, a distinct absorption band centered at 528 nm was observed. From the absorption titration profile of tannic acid stabilized AuNPs versus Hg²⁺, maximum absorption was detected in the presence of Hg²⁺ up to 0.9 mM. The absorption spectrum was gradually quenched without wavelength shift upon the continuous addition of Hg²⁺ (Fig-12b).

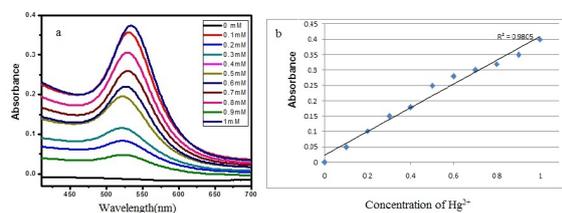


Figure 12. a) Titrimetric study of Au-Tan nanosensor with Hg²⁺ b) Absorption at 528 nm as a function of the Hg²⁺ concentration. The linear regression for Hg²⁺ R²=0.980

Job's plot

Job's method was made to determine the stoichiometry between metal ion and ligand. The job's plot experiment was conducted by changing the

concentration of both metal ion and host. Stock solutions of 0.5mM Hg²⁺ and 0.5mM TA AuNPs were used for this experiment. Initially, 10.0ml of TA AuNPs were taken, and the absorption spectrum was recorded. The procedure was repeated for 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 and 0:10 (TA AuNPs : Hg²⁺) mixtures. The stoichiometry of complexes was determined from the plot absorbance versus mole fraction. The job plot exhibited the formation of a 1:1 complex between TA AuNPs and Hg²⁺ at a mole fraction of about 0.5 (Fig-13).

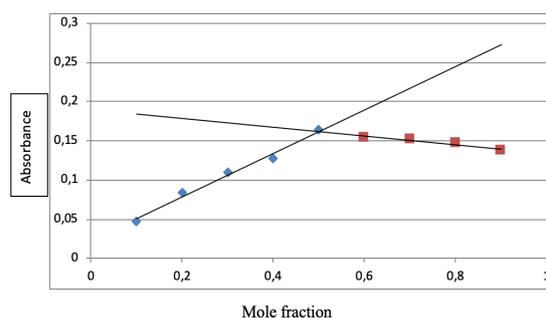


Figure 13. Job's plot for Au-Tan nanosensor with Hg²⁺

Drug Sensing

Absorption Measurements

Absorption was performed by the addition of a small amount of drug into a solution of the proper amount of AuNP Tannic acid solution. The AuNPs TA displayed an absorption band at 538 nm, while with the addition of pefloxacin the absorption showed at 554nm, which was considerably quenched in the presence of pefloxacin (Fig-14)

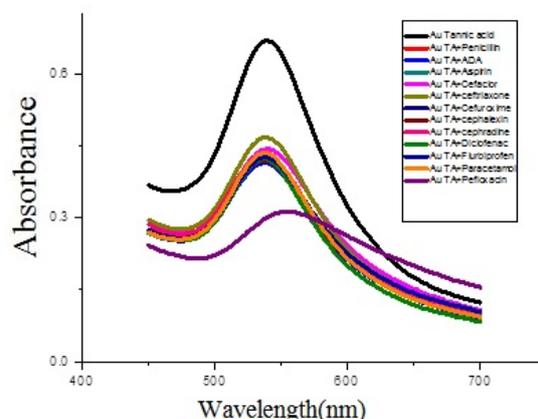


Figure 14. UV- Vis spectra of Au-Tan nanosensor after adding pefloxacin and different drug solutions.

Competitive study

To obtain the absorption properties of AuNP TA toward drug solutions, the absorption changes were investigated with different drugs. From the absorption spectra, it can be seen that there is quenching in the absorption intensity of pefloxacin with AuNP TA which could be clearly distinguished from the other drug solutions (Fig-15).

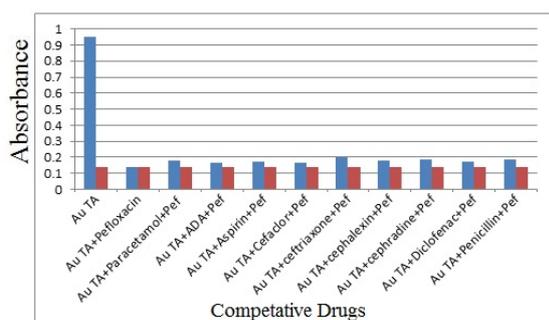


Figure 15. Absorption intensity of Au-Tan nanosensor after adding pefloxacin with various drug solutions

Stoichiometry

Job's method of continuous variation was used to establish molar ratio between pefloxacin and AuNPs TA. In this method, varying volume ratios of AuNPs TA and pefloxacin solution with identical molar concentration were used in such a way that final volumes of mixed solution remain the same. Solution absorbance was measured and plotted against drug mole fraction. These measurements show that 1:1 complex was formed. Further, the binding affinity of AuNP TA for pefloxacin drug was examined through an absorption titration experiment in which different concentrations of pefloxacin drugs were added to the solution of AuNP TA. Upon the addition of pefloxacin to the solution of AuNP TA, absorption quenching occurred and the absorption intensity at 552 nm clearly increased (Fig-16, Fig-17).

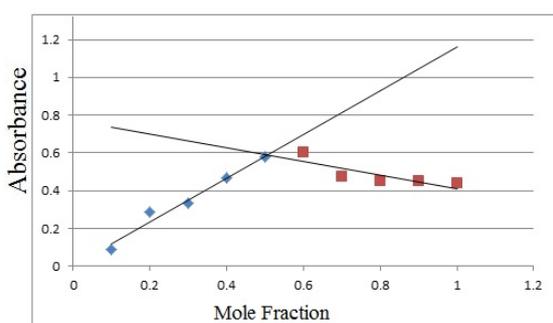


Figure 16. Job's plot for Au-Tan nanosensor and pefloxacin

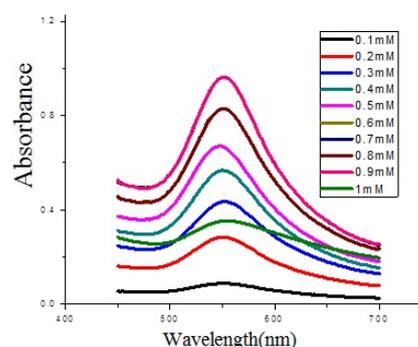


Figure 17. Effect of absorption spectra of Au-Tan nanosensor with the addition of different concentrations of pefloxacin

Effect of temperature

Under optimized conditions mixture of pefloxacin and AuNPs TA were heated at 100 °C up to 1 hour showed no change in absorption spectra. From absorption spectra, it was confirmed that 100 °C heating for an hour remains stable. Broad-spectrum of pefloxacin and AuNPs Ta curve shows that surface plasmon resonance absorption of the mixture is around 560 nm. Due to strong interaction between Au Nanoparticles and TA results broad absorption band of nanoparticles. (Fig-18).

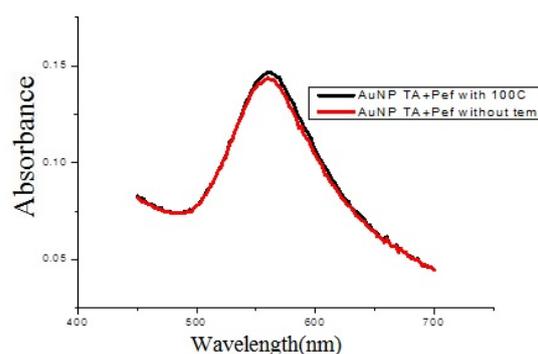


Figure 18. UV-Vis spectra of heat on the stability of Au-Tan nanosensor after adding pefloxacin.

Effect of pH

At its natural pH tannic acid is to be known as a weak reducing agent. At alkaline pH tannic acid reduces silver nitrate into silver nanospheres rapidly. Optimized conditions were employed by varying different pH to understand the role of pH as shown in

the figure. Tannic acid has a pKa value between seven and eight depending on its extent of dissociation and is known to partially hydrolyze under mild acidic/basic conditions into glucose and gallic acid units (Fig-19).

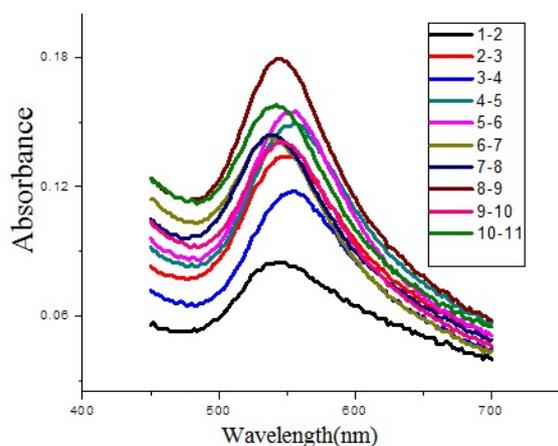


Figure 19. Effect of pH on stability of Au-Tan nanosensor after adding pefloxacin

EXPERIMENTAL

General information

Tetrachloroauric (III) acid trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$) was purchased from Merck. For pH measurement, a digital pH meter (Oakton, Eutech) model 510 fitted with a glass working electrode and reference Ag/AgCl electrode was used. Shimadzu UV-240, Hitachi U-3200 spectrophotometer having 1cm path length was used for UV-Visible spectra recording. Shimadzu IR-460 spectrometer was used for FT-IR spectra recording. Freeze-dried Au TA KBr 1:1 mixture was pressed into a pellet. At 120k on Zeiss Libra transmission AFM images were taken.

Preparation of metal ions solution

Throughout experimental procedures, deionized water was used. Analytical grade salts were used for the preparation of metal ion solutions. For the preparation of different pH solutions, a suitable amount of NaOH/HCl was used under adjustment by pH meter. Stock solutions (1 mM) different commercial drugs and salts of Hg^{2+} , Zn^{2+} , Ag^+ , Co^{2+} , Ba^{2+} , V^{5+} , Pb^{2+} , K^+ , Cu^{2+} , Cd^{2+} , Rb^+ , Na^+ and NH_4^+ were prepared in deionized water.

Preparation of drug solution

The drug solutions were prepared from their drug salts. The stock solutions (1mM) of the salts such as

paracetamol, ADA, aspirin, cefaclor, ceftriaxone, cephalexin, cephradine, diclofenac, and penicillin were prepared in deionized water.

Synthesis of Au-Tan-nanoparticle

For the preparation of gold nanoparticle, first of all, 1% and 5% aqueous stocks of chloroauric acid and TA were prepared respectively. Then the molarities of HAuCl_4 and TA were calculated. The gold chloride concentration was varied between 0.2 to 2 mM by taking a gold chloride solution. The TA to gold molar ratios were varied ranged by adding a suitable amount of TA from the 5% aqueous stock to the 0.2 – 2 mM solutions of boiling gold chloride. The boiling was continued for 2 minutes. The solutions were stirring for 20 to 30 minutes depending on the appearance of ruby red color. TA was used as the reducing as well as a stabilizing agent [31].

CONCLUSION

Tannic acid capped gold nanoparticles with a mean diameter of 20 nm were synthesized and characterized through TEM, AFM, and UV-Visible spectroscopy. Nanoparticles were effectively applied for sensing behavior toward metal ions and toward drugs. Using UV-Visible spectroscopy it was deliberated that the nanoparticles showed selective removal of Hg^{2+} ions and pefloxacin. UV visible absorption of tannic acid capped gold nanoparticles showed satisfactory with different metal salts and drugs. Nanoparticles showed stability at pH ranging between 3-12 and for 5 minutes at 100 °C. Quenching of the peak and precipitation is observed with various concentrations of brine solutions. Job's plot analysis shows the binding stoichiometry to be a 1:1 ratio. This newly designed sensor offers a simple low-cost alternative tool for recognition and extraction of Hg^{2+} and pefloxacin.

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