

# [Determination of sodium thiopental using gold nanoparticles](https://assignbuster.com/determination-of-sodium-thiopental-using-gold-nanoparticles/)

Development of a new colorimetric method for the determination of sodium thiopental using gold nanoparticles

Sodium thiopental (sodium pentothal) is in a group of drugs called barbiturates. this barbiturate commonly used anesthetic induction agents in man and animals because recovery is rapid and it has the advantage of having very little or no side effects[1]. It is used for intensive-care patients with head injuries to control convulsions and reduce raised intracranial pressure[2]. As a resultmonitoring of theserum concentrations is important in this patient population.

Several analytical procedures have been reported for the quantitative determination of thiopental. Among these high-performance liquid chromatography (HPLC) are more popular. HPLC assays are not completely reliable, and do not have the short process-time required in most of the above-mentioned indications[3, 4]. other methods are available for determining thiopental including stripping voltammetry[5], membrane sensors[6], capacitive chemical sensor [7], gas chromatography (GC)[8], spectrophotometric and spectrophotofluorometric[9, 10]. Donald et al[11]reported that, after the usual 4. 8 mg/kg induction doses, thiopental concentration in serum as a function of time varies between 10 mg/L to 25 mg/L during 50h. As stated before most of these currently used methods for sodium thiopental detection usually need expensive and complicated instruments and are time-consuming, making on-site and real-time thiopental detection difficult. Therefore, it is important to develop a simple reliable and highly sensitive method for on-site and real-time detection of sodium thiopental.

Recently, gold nanoparticles (NPs) explored for metallic NP-based colorimetric detection have attracted considerable attention due to biocompatibility, stability, and high extinction coefficients[12]. gold nanoparticles present size-dependent optical properties owing to the surface plasma resonance(SPR)[12]. The color of the colloidal Au NPs can be readily and precisely changed via aggregation of Au NPs. Au NPs were widely applied in colorimetric detection of several analytes such as protein, DNA, metal ions and small molecules[ ].

In this study, we used gold nanoparticles as a colorimetric probe for sensitive and selective detection of sodium thiopental. The gold nanoparticles were prepared using the classical citrate method [12]. thiopental on the surface of AuNPs displaced the stabilizing citrate ions because thiol group of sodium thiopental tends to readily adsorb onto the surface of colloidal gold via chemisorptions-type interactions. The thiopental capped Au NPs were stable at basic and neutral conditions . Puntes et al [13] have studied the stability of cationic gold nanoparticle bioconjugates as a function of pH and the presence of citrate in solution. The pH of an aqueous solution of thiopental-Au NPs was varied by direct addition of citrate buffer. the thiopental-Au NPs can be aggregated by adding certain amounts of citrate buffer due to the electrostatic attraction between amino group contained in thiopental molecular and citrate ion on the surface of Au NPs, the amino group of the thiopental would be positively charged at the given pH value and they would therefore interact electrostatically with the negative charges of the citrate molecules. Thus forcing the aggregation of the conjugated Au NPs and subsequently resulting in the color change from wine red to purple or blue color. So that we detected it by UV–Vis spectrophotometer and paptode techniques and contrast both methods. First time at 2004 paptode was developed in Dr. Abbaspour group for speciation of iron(II) and iron(III) and the full range pH monitoring [14]. Then it was used for the determination of dopamine [15], hydrazine [16]. In paptode, conventional ï¬‚ atbed -scanner (as a nondestructive detector) was used to acquire the analytical parameters for quantitative determination of analyte that occurs via colorimetric reaction. The estimated re ï¬‚ ection density, as an analytical parameter, is obtained from an area of the sensing zone of spots using the average Red (R), Green (G) and Blue (B) channel. Degrees of the color of the spots are found to be proportional to the concentration of the testedanalyte.

Experimental section:

Reagents:

HAuCl4. 3H2O, trisodium citrate and citric acid were purchased from Sigma. Thiopental was obtained from Biochemie (Kundl, Austria) and zinc sulfate purchased from Fluka All solutions were prepared with ultrapure water

Apparatus and software:

The colorimetric study of NPs were performed by means of a Shimadzu 1601PC UV–Vis spectrophotometer (Kyoto, Japan)from 300 to 700 nm. Also a Canon scanner were used to record the color changes in paptode technique. The paptode Cells were built by creation of the holes (i. d 1. 5 cm) in the sheet of plexiglas (thickness 0. 9 cm). We used by photoshop Cs6 software to convert the recorded pictures of color of cells to RGB (Red, Green and Blue) and L\*a\*b data. The morphology and size of the nanoparticles were characterized by a transmission electron microscope (TEM model CM10; Philips). The X-Ray diffraction (XRD) patterns were obtained by using a D8 ADVANCE type (BRUKER-Germany) with Cu-Kα radiation (λ= 0. 1542 nm). Powder XRD patterns were taken in 0. 02° steps at 1 s per step. All the experiments were carried out at room temperature(25 ± 2 C)

Synthesis of citrate-stabilized Au nanocrystals:

Nanoparticles of noble metal were prepared by classical citrate method[12]. the10ml of 0. 014M of trisodium citrate dehydrate solution was added quickly to the 100ml of boiling solution of 0. 5mM of HAuCl4. 3H2O under magnetic stirring. The stirring was continued until a dark red color was observed (around 20 min) and the maximum absorbance of AuNPs solution was centered at 520 nm

Sample preparation:

Fresh human blood samples (2. 0 mL) were obtained from volunteers of the local hospital. After letting sample stand for 60 min at room temperature we centrifuged at 4000 rpm for 10 min. The supernatant was used as the source of the serum. We used zinc sulfate method as a deproteinization technique[]: we vortex-mix for 10s of the 10ml of serum sample and 150mg zinc sulfate, then we centrifuged the mixture at 3000 rpm for 20 min. The supernatant, which excluded protein, was used for further analysis.

Procedures for the detection of sodium thiopental:

In a typical detection of sodium thiopental, different amounts of thiopental solution were added to the above XmlAu NPs solutions at room temperature. we proceeded to study the behavior of the conjugated system by modifying the pH . To investigate the effect of pH of the buffer solutions on thiopental detection, 0. 5 mL of 0. 1 M buffer solution (citric buffer solution in the pH range of 3. 0–6. 0 ) was added in mixture of thiopental and Au NPs solution. The obvious color change was observed with the naked eye and the absorbance spectra and scanning images of the solution were recorded 1 min after the addition of citrate buffer. In spectroscopy technique , The concentration of sodium thiopental was quantified by the absorption ratio (A670/A520).

Results and discussion

Citrate was chosen as the stabilizer for AuNPs because it is negatively charged, and can act as a stabilizingagent to disperse AuNPs in aqueous solutions. The Au NPs after synthesis showed a surface plasmon resonance (SPR) band at 405 nm (Fig. 1a). the addition of sodium thiopental doesn’t led to a color change of Au NPsin ultrapure water, although the thiol group of sodium thiopental tends to readily adsorb onto the surface of Au NPs. The pH of AuNPs solution in present of sodium thiopental is 10. 2 and Puntes et al[13]reportedthat the presence of charged molecules insolution may induce NPs aggregation by bridging particlestogether. It was observed that multiple electrostatic interactions between the conjugates mediated by cross-linking species led to an effective strong bond and consequently to irreversible aggregation and precipitation. So that at the given pH value , charge of thiopental can be change and thenthe color of the colloidal thiopental-Au NPs can be changed to blue (broad band above 600 nm).\*Scrutiny of pH/Concentrate diagrams of citrate and thiopental shows that at the pH of between 5 to 7 , charge of citrate and thiopental can benegative and neutralfig S1. But when sodium thiopental add to AuNPs solution, the S- group in the sodium thiopental provides a strong affinity for gold. So that orbital of thiol group of thiopentalinvolved for Au NPs surface and when pH change from 10. 2 to 6 , the amino group of the thiopental would be accepted H + and get positive charge. In present of excesscitrate at the pH of 6 , thiopental-AuNPscan be aggregated via electrostatic attraction between the citrate ions and the thiopental. So that in this study we used citrate buffer solutionfor control of pH( in the pH range of 3. 0–6. 0) and source of citrate (as a bridging factor). The aggregation mechanism of Au NPs is illustrated in Fig. 1.

Optimization pH and time

we proceeded to study the behavior of the conjugated system by modifying the pH( 7. 1-5. 4). The pH of an aqueous solution of0. 00001M thiopental capped AuNPs was varied by direct addition of 0. 05Mcitrate buffer to the solution andThe UV-Vis spectrum wasmonitored and the extinction ratio of absorbance at 600 nm to 420 nm (A600/A410) is plotted against the pH inFig. 3A. The thiopental-capped Au NPs were stable at basic and neutral conditions. When the pH of the solution was below the 6. 4 , Au NPs agglomerated. the aggregation was solely due to the bridging citrate between the amine functionality. Onthe basis of this optimization experiment, the pH was set to 6. 2 to achieve a best aggregationFig. 3A. When the pH was decreased immediately from 5. 4 after the addition of the citrate buffer scatteringwasobserved. Fig. 3A illustrates theabsorption spectra of AuNPs at different pH value.

At the concentration of sodium thiopental as 0. 00001M, the extinction ratio ofA650/A520 at room temperatureexhibited a rapid increaseduring the first 1. 5min, then increased gradually from 1 min to 18 min and then remained constantFig 3B. Thus, the detection time was chosen as 20 min.

We choseto use the absorbance ratio at 500 and 600 wavelengths to quantify thecolor of the system, thecolor change at various sodium thiopental concentrations were monitored byUV/Vis spectroscopyfig4A. Quantitative analysis was performed by monitoringthe absorbanceat 1minute after the addition of citrate buffer Fig4B . The linear range, detection limit and reproducibilityof the method were evaluated under the optimumconditions. Thecalibration curve for sodium thiopental was linear in two ranges of( ……. To …… and…… to ……) with correlation coefficients 0. 9981 and0. 9979, respectively. The Experimental detection limit has been obtained as 2µM. The relative standard deviation(R. S. D.) for1. 0×10−8M thiopental measurementwas2. 7% (n= 11)Fig4A . when thiopental concentrationincreased above 0. 0005M, scattering was observed fig3B because thiopental polymerized white citrate molecule. So that we tried paptode techniques to resolve thisproblemFigS1. Although the higher concentrations of sodium thiopental was determined by paptode, but the limit of detection was rather high (LOD 10 µM) in comparison to the spectrophotometric method. The detailed procedure for sodium thiopental determination by the paptode method is explained in supporting information.

To test the selectivity of the above method for sodium thiopental, we testing the response of the assay to some potential interference species and structurally similar to the sodium thiopental such as…………. in optimum condition and different concentration . the results areshown in bar diagramFigure 8 . red barsexhibit Color changes of the solution in thepresence of various interference species at concentrations of 10mMand bluebars exhibit Color changes in presence ofinterference species at real concentration in serum ( 1M cysteine, 2M………), The maximum absorption wavelength of AuNPs did notchange in the presence of the tested species, Except for cysteineat concentrations of 10mM. Therefore, AuNPs had good selectivity for sodium thiopental detection in optimum condition in the serum.

Colorimetric detection of sodium thiopental in serum:

To validate the reliability of the proposed method for sodium thiopental detection in real samples, The unknown amounts of thiopental were added to thethree different human serum samples before samplespre-treatment . Detecting of sodium thiopental in a serum is not easy because of the serum constituents. the color of the Au NPs was not stable by the addition of the blank serum. So that it mustdiluted ten times. As regardsthe calibration curve for detection thiopental by this methodand dilution of serum and thiopental concentration in serum as a function of time varies after the usual 4. 8 mg/kg induction doses [] , we can detect sodium thiopental in human serumbefore 3 hour. samples were determined by both the AuNP-based method reported herein and the standard addition method. Satisfactory results and recoveries as shown in Table 2. The satisfactory results obtained indicate that proposed sensors can be applied to real sample assays.

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