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Spectrophotometric Determination of 4-Hydroxy-2-mercapto-6-methylpyrimidine Based on Aggregation of Colloidal Gold Nanoparticles

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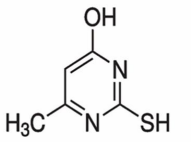
We report herein the development of a highly sensitive colorimetric method for the detection of 4-hydroxy-2-mercapto-6-methylpyrimidine (MTU) which acts as an anti-thyroid drug utilizing citrate capped gold nanoparticles (Au-NPs). This thiol-containing molecule exhibits intriguing affinity with Au-NPs. The reactivity involves the displacement of the citrate shell by the thiolate shell followed by intermolecular electrostatic interactions or hydrogen-bonding between the thiolate shells. The interparticle interactions depend on ionic strength, pH and Au-NPs concentration of the solution. The interparticle interactions lead to a small change in the plasmon band around 521 nm and the formation of a new red shifted band. The calibration curve is derived from the ratio of the absorption intensity changes at 650 nm to the changes at 520 nm. It was linear in the concentration range of 5.0×10^{-7}-2.75×10^{-6} M. The detection limit (3σ) for MTU was found to be 1.9×10^{-7} M.

Keywords: Gold Nanoparticle, 4-Hydroxy-2-mercapto-6-methylpyrimidine, Colorimetric detection

INTRODUCTION

MTU (Scheme 1) is a drug from thiouracil antithyroid family which inhibits thyroid hormone synthesis and is generally used to remedy hyperthyroidism in gestation and liver disease. In addition, MTU has been also applied to gain higher weight in animals. Since remaining of thyreostatic drugs in edible tissues of animals could be a risk for human health, their use in animal products was prohibited in the European Union in 1981. Based on these reasons, the need for an analytical procedure capable of determining MTU is of great importance [1].

The majority of the currently available methods relating to the detection of MTU are based on chromatographic separation employing various detection techniques. These include liquid chromatography using UV, electrochemical and mass spectrometry detection [2-3], thin layer chromatography [4-5] and gas chromatography with nitrogen-phosphorus, flame photometric, electron capture or mass-selective detection [6-7]. Most of these methods include a derivatisation step, either for alteration of the analytes into volatile compounds convenient for GC analysis [8-9], or for reducing their polarity and increasing their molecular mass for LC separation [10-11].



Scheme1. Schematic illustration of MTU structure

Although these methods can well detect antithyroid drugs, their practical applications are limited because of their intrinsic disadvantages, such as requiring wrapped instrumentation, expensive biological reagents or serious sample preparation.

Au-NPs have received immense attention for potential biological analysis, in recent years, due to the surface plasmon (SP) resonance phenomena, which is responsible

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for their extraordinary size and shape related optical properties [12-13]. Au-NPs have high extinction coefficients and the wavelength and intensity of their surface plasmon resonance are highly dependent on the dielectric constant of the medium. These properties have led to their applications in analysis of medicines through colorimetric assay [14,15].

Herein, we have developed a simple and highly sensitive colorimetric method for the detection of 4-hydroxy-2mercapto-6-methylpyrimidine based on the aggregation of citrate capped spherical Au-NPs, through employing the strong affinity characteristic of the thiol group towards the surface of the Au-NPs. The effects of impressive parameters, including concentration of Au-NPs, ionic strength and pH have been studied to find the optimal condition with highest sensitivity and selectivity.

EXPERIMENTAL

Reagents and Apparatus

4-Hydroxy-2-mercapto-6-methylpyrimidine (MTU, 98%), and all other chemicals, such as hydrogen tetra-chloroaurate ($HAuCl_4$, 99%), and sodium citrate (99%), were purchased from Merck and were used without further purification. For the preparation of all samples, water purified with cartridges from Millipore (Milli-Q) to a resistivity of 18 MΩ was used. UV-Vis spectroscopy was performed on a Lambda 25 (Perkin-Elmer, USA) spectrophotometer with the use of a 1.0 cm glass cell. Measurements of pH were made with a Denver Instrument Model of 270 pH meter equipped with a Metrohm glass electrode. Size distributions of the particles were obtained using Zetasizer Viscotec 802 at ambient temperature. Transmission electron microscopy (TEM) images were recorded with a Philips MC 10 TH microscope at an acceleration voltage of 100 KV.

Preparation of Au-NPs

Citrate-capped Au-NPs with an average diameter of about 13.1 ± 1.3 nm were synthesized for the first time by Turkevich *et al.* [16-17] in which a 50 ml solution containing 1 mM of $HAuCl_4$ was prepared and heated under reflux. At the boiling point, 5 ml of 38.8 mM trisodium citrate was added to this solution under strenuous stirring. Reflux was continued for 30 min, during which the color changed to deep red, indicating the formation of Au-NPs. The solution was left to cool at room temperature and then was stored at 4 °C for further consumption. According to Beer's law and the extinction coefficient of Au-NPs (13 nm) at 520 nm, the particle concentration for the resulted solutions was estimated to be 15 nM [18].

Determination of 4-Hydroxy-2-mercapto-6-methyl-pyrimidine

For the determination of MTU, the solutions containing 3 ml of as-prepared Au-NPs and 7 ml of Milli-Q water were prepared in volumetric flasks. Then, 50 µl of different concentrations of MTU were added to 2 ml of the above solution. To understand when the reaction is complete, the absorbance spectra were recorded by 3 min intervals and then the ratio of the absorption intensity changes versus time was studied and the measurements were performed at time that the reaction was almost complete.

RESULTS AND DISCUSIONS

Molecules with thiol functional group have high affinity to the surface of Au-NPs that can be described by the hard–soft acid-base theory [19]. Displacement of the citrate group shell with thiol-containing pyrimidines, such as MTU, induces the aggregation process, due to the hydrogen binding between pyrimidines. As a result of the aggregation, the surface plasmon resonance absorption of Au-NPs is decreased around 521 nm, associated with the formation of a new band at longer wavelengths. This is due to the near-field coupling in the resonant wavelength peak of the interacting particles [20].

Figure 1A shows TEM images of Au-NPs. The right image shows Au-NPs before aggregation and the left image shows Au-NPs after the addition of certain quantity of MTU. TEM images demonstrate the process of aggregation of Au-NPs in the presence of MTU. Figure 1B clearly shows the effect of aggregation on the optical properties of the Au-NPs. DLS images in Fig. 1C show size distribution of Au-NPs before and after the addition of certain quantity of MTU. These images show that after the addition of analyte to Au-NPs, the diameter of nanoparticles begins to increase, in a way that the average diameter changes from

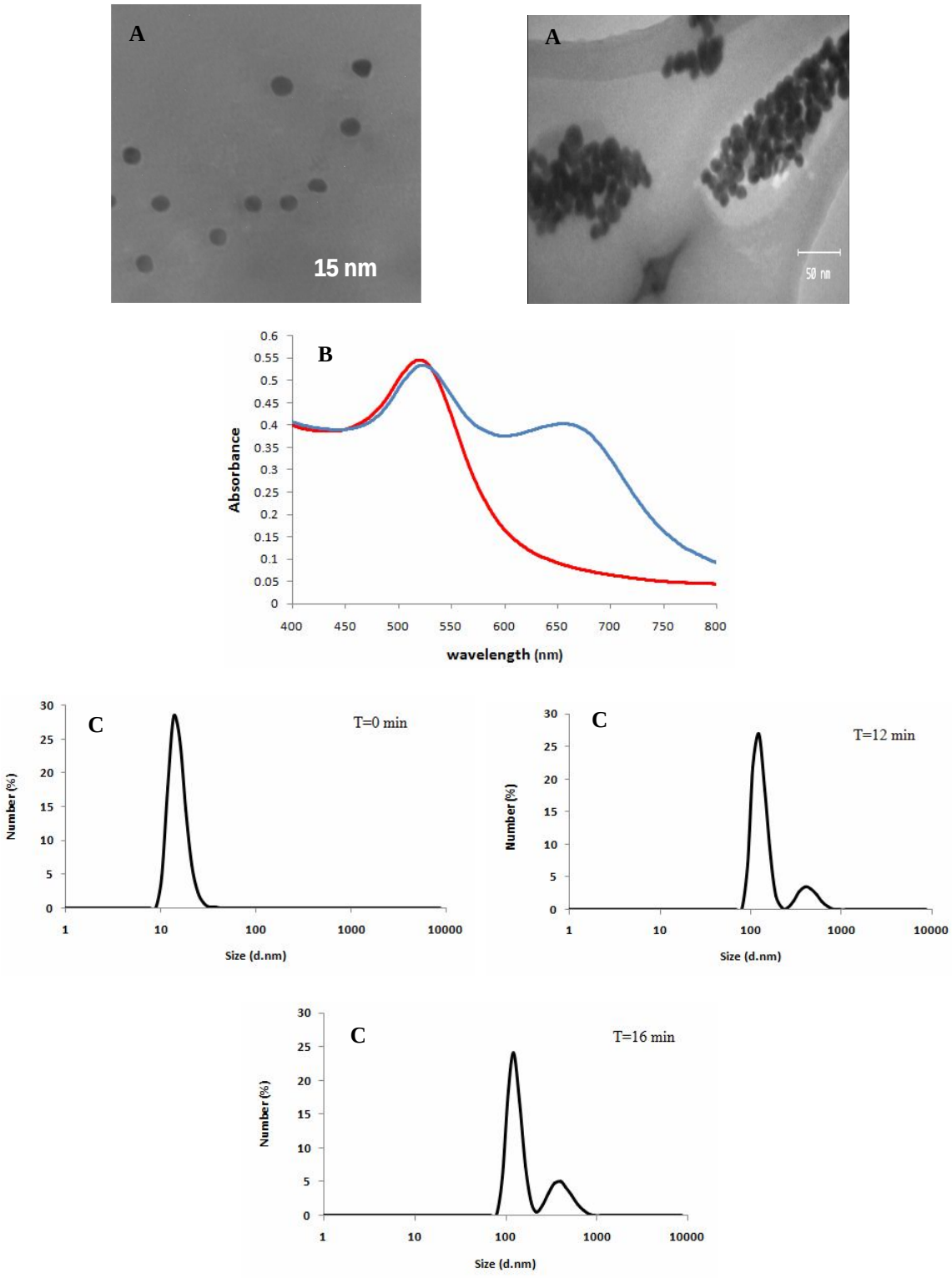


Fig. 1. (A) Typical TEM images; (B) corresponding UV-Vis absorption spectra; and (C) size distribution of the Au NPs before and after the addition of certain quantity of MTU.

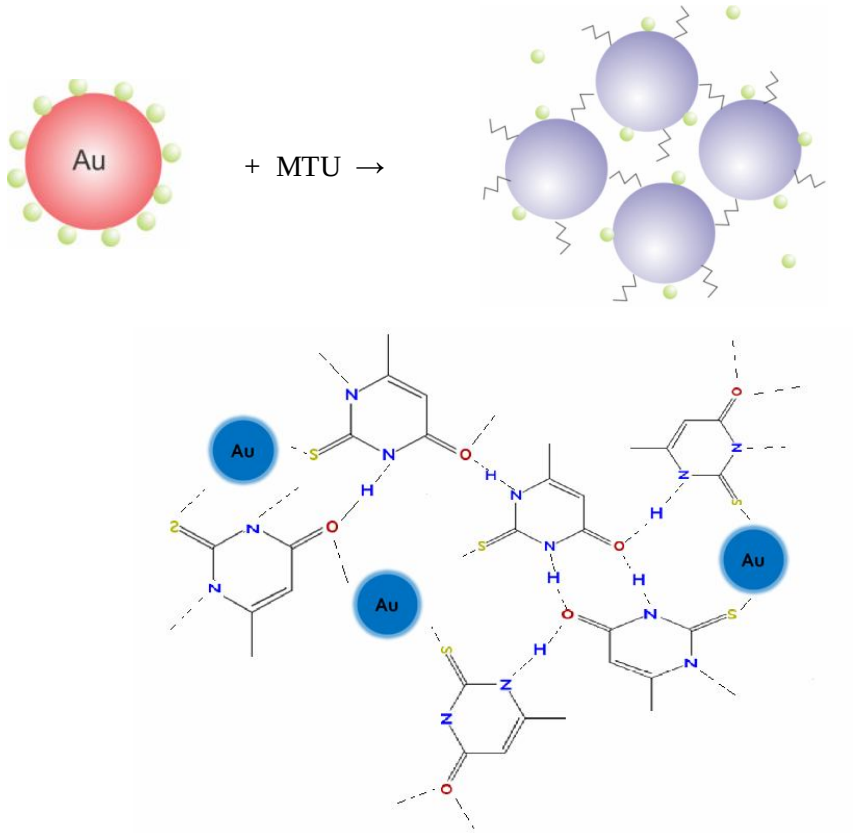
13 nm to 122 nm and then remains constant. To find the optimum analytical conditions for detection of MTU, the effects of the critical parameters, including ionic strength, concentration of Au-NPs and pH were investigated.

Ionic strength has a crucial role in the aggregation process. In previous work carried out in our research group, we observed that in the absence of strong electrolytes (low ionic strength) Au-NPs did not undergo the aggregation, even at the high concentration of analytes. Also, it was found that by increasing the ionic strength above a certain limit, Au-NPs were aggregated even in the absence of analyte. Therefore, some controlled experiments were conducted to optimize the concentration of NaCl in which aggregation only occurs in the presence of our target analyte [21]. In contrast, our research revealed that in the absence of strong electrolytes, MTU had the ability to constrict electrical double-layer and consequently, Au-NPs undergo the aggregation, even at low concentrations of MTU.

Effect of pH

Because of the presence of hydroxyl and pyrimidine, pH is another critical parameter that should be taken into consideration. As shown in Scheme 2, hydrogen-bonding between the thiolate shells is the main factor responsible for aggregation of Au-NPs in the presence of MTU. As shown in Fig. 2A, the analyte is present in two forms; form a in the acidic solution and form b in the alkaline solution. Actually, in acidic environment, the rate of aggregation increases due to the increase of hydrogen bonds between molecules of the analyte and in the alkaline solution, the rate of aggregation decreases due to the repulsion between the negative charges on the surface of Au-NPs. Based on the fact that the biological pH is 7 and since the aggregation of Au-NPs is appropriate at this pH, we chose this pH for the analyte (Fig. 2B).

As mentioned earlier, the Au-NPs have been coated with citrate ions. These ions have three carboxyl groups and one



Scheme 2. Schematic illustration of the aggregation mechanism for MTU

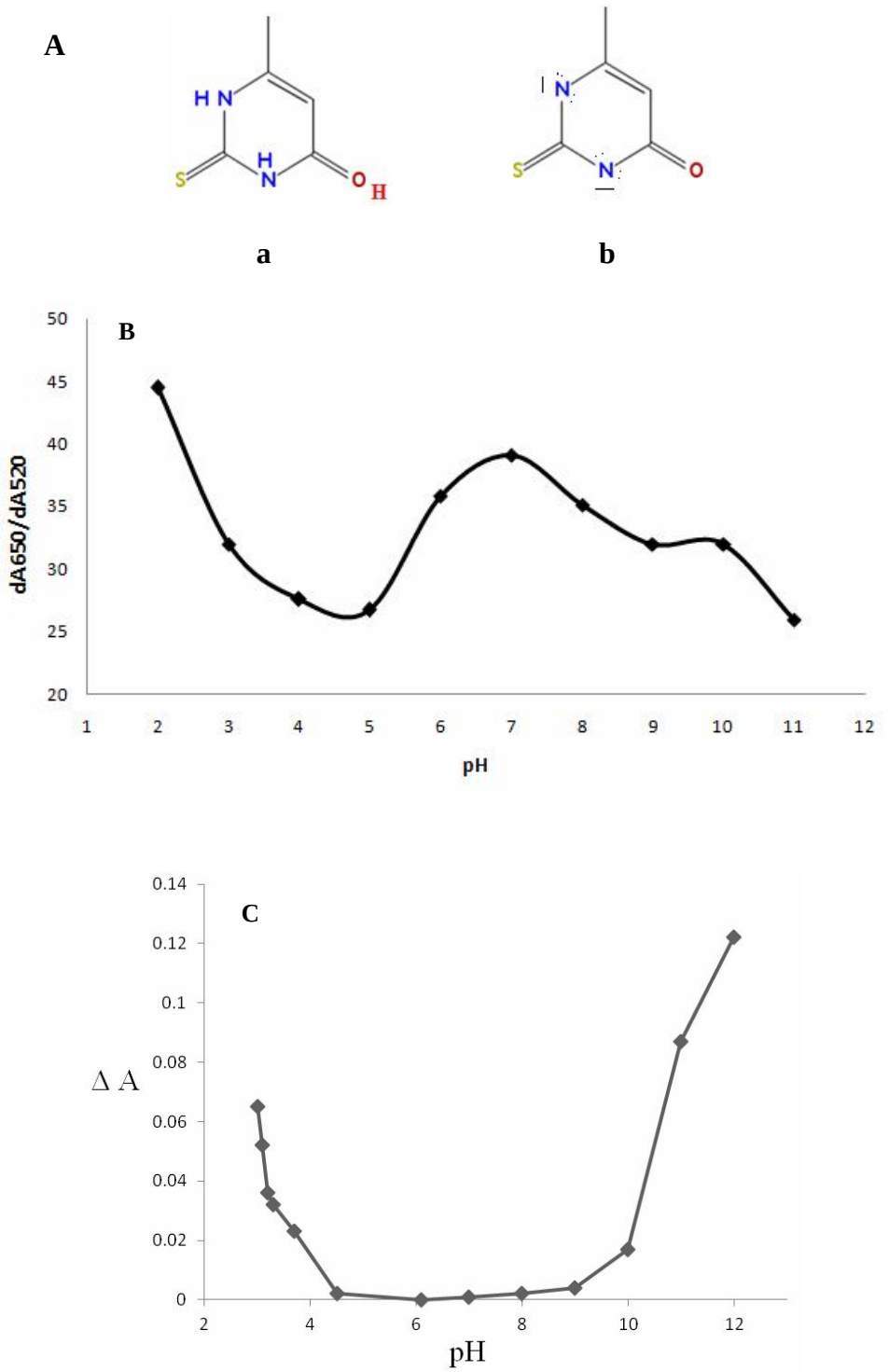


Fig. 2. (A) Schematic illustration of the structures of MTU in a) acidic and b) alkalic solution; (B) The effect of the pH of analyte solution on the self-aggregation of Au-NPs in the presence of analyte; and (C) effect of pH on the self-aggregation of Au-NPs in the absence of analytes.

group of hydroxyl. By changing the pH of Au-NPs solution, these functional groups can catch or lose a proton. In addition, the changes in the surface charge of Au-NPs, has a direct effect on the stability of Au-NPs. As shown in Fig. 2C, the synthesized Au-NPs are stable in the pH range from 4.5 to 10. Due to the fact that the initial pH of synthesized Au-NPs is found to be about 6, we chose this pH for Au-NPs.

Effect of Concentration of Au-NPs

To reach the highest sensitivity and selectivity of the method, we surveyed the effect of the concentration of Au-NPs on the aggregation process in the presence of 2 μM MTU. As shown in Fig. 3, for MTU, at concentrations of less than 4 nM the rate of aggregation increases with a decrease in the concentration of Au-NPs. However, at concentrations of higher than 5 nM the rate of aggregation decreases with an increase in the concentration of Au-NPs. Meanwhile, higher concentrations of Au-NPs suffer from a limited linear range of detection. Therefore, based on the preliminary experiments, a concentration of 4.5 nM of the Au-NPs at the final solution was selected for further experiments, which benefits from both acceptable linear range and reasonable time to complete the detection process.

Analytical Figures of Merit

The linear range for determination of MTU was assessed under the investigated optimum conditions. Figure 4 shows a linear relation between the ratio of dA650/dA520 and concentration of MTU in the range of 0.5-2.75 μM. The lower limit of detection for determination of MTU at a signal to noise ratio of 3 (3σ) was 0.19 μM. The study of precision, which was made with five independent experiments, revealed relative standard deviation (%RSD) of 1.8% for the determination of 2 μM of MTU.

Interference Study

In order to evaluate the selectivity of the proposed method, an analysis of a standard solution of MTU (2 μM) was performed under the given optimum conditions and in the presence of starch, sillicst and different amino acids. As shown in Fig. 5, a wide range of the presented species does not interfere, even at concentrations 50 times higher than those of the analytes.

Real Sample Analysis

The proposed method was applied for determining MTU in serum containing tablets matrix (Matrix: mannitol; sorbitol; arsil; calcium hydrogen phosphate; corn starch). Certain amounts of MTU were spiked into the real sample, and its concentration was determined. The results given in Table 1 show the potential and feasibility of the developed method for determination of MTU in the real samples.

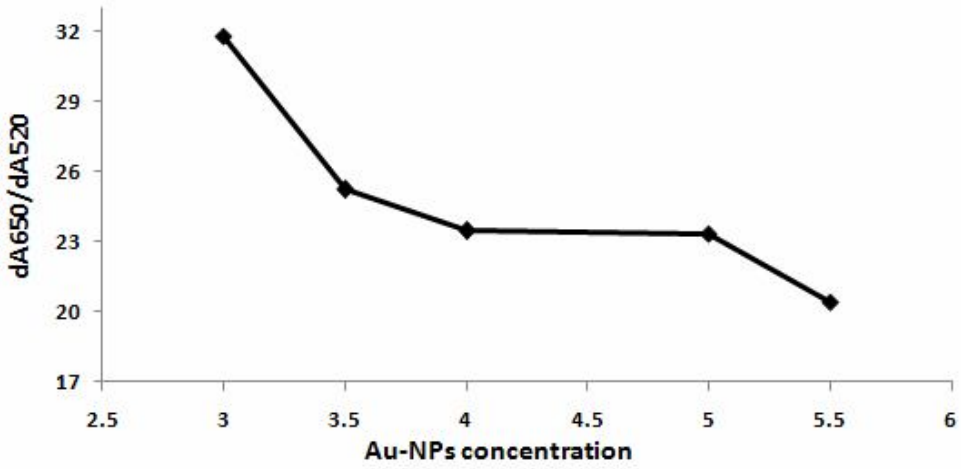


Fig. 3. Effect of Au-NPs concentration on aggregation rate in the presence of 2 μM of MTU.

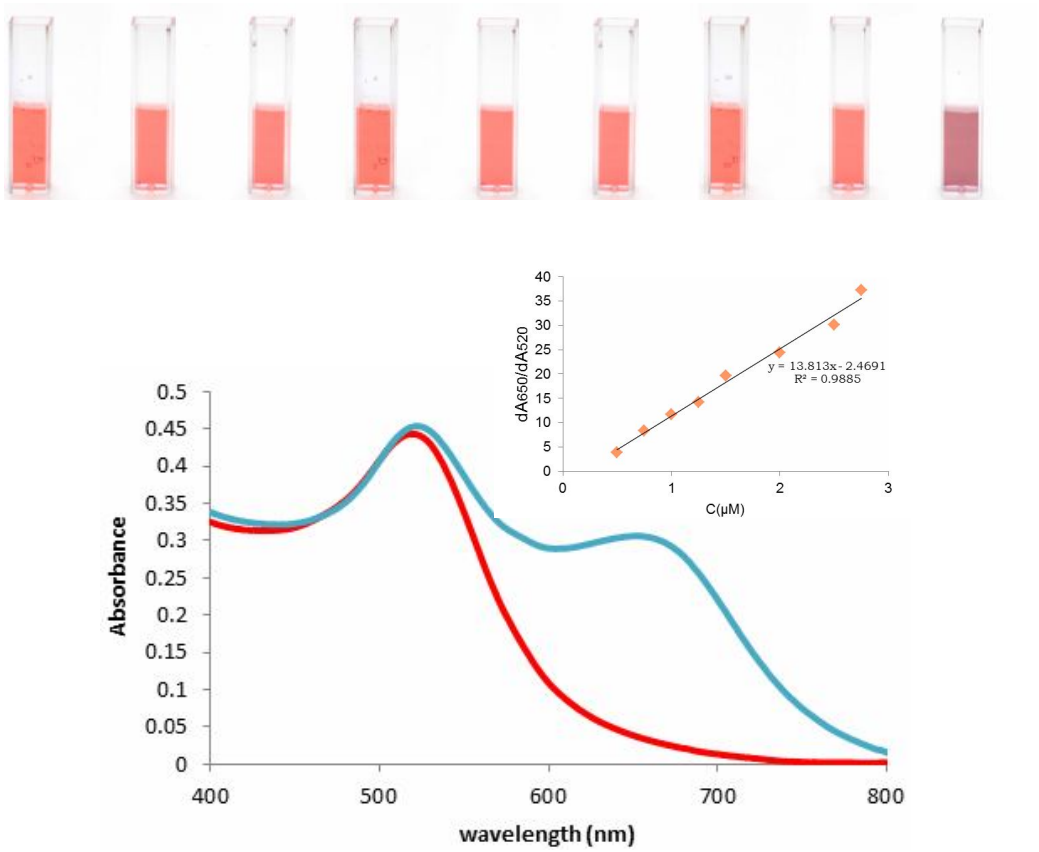


Fig. 4. A typical absorption spectra and digital photos of Au-NPs upon adding certain quantity of MTU (the insets are corresponding calibration curve in the range of 0.5-2.75 μM).

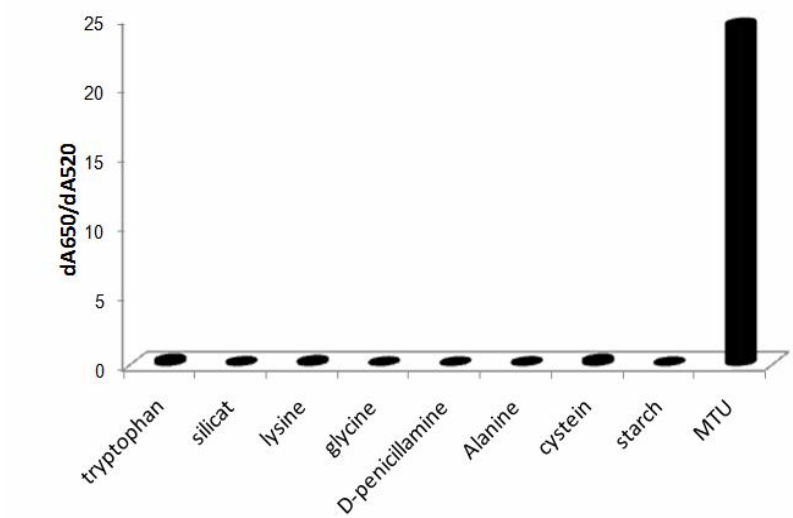


Fig. 5. The dA650 nm/dA520 nm value of solution in the presence of 0.1 mM tryptophan; silicat; lysine, glycine; D-penicillamine; alanine; cystein; starch and 2μM MTU.

Table 1. Determination of MTU in Serum Containing Tablets Matrix (Matrix: Mannitol; Sorbitol; Arsil; Calcium Hydrogen Phosphate; Corn Starch)

Analyte	Added (μ M)	Founded (μ M)	Recovery (%)	RSD
MTU	1.0	0.91	96.0	1.7
	1.5	1.48	98.7	1.5

CONCLUSIONS

A new spectroscopic technique for the determination of MTU has been developed. The method is based on the aggregation of Au-NPs in the presence of MTU. The analytical figures of merit of the proposed method are comparable even improved to the chromatography and electrochemical methods. Low cost, high stability and reproducible detections are some other remarkable advantages of the proposed method.

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