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Fluorescence Spectrophotometric Determination of 6-Thioguanine in Biological Sample

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Abstract

Present work describes the fluorescence spectroscopy determination of (6-TG) using silver nanoparticles (AgNPs). The acridine could be electrostatically adsorbed to the surface of the citrate-stabilized silver nanoparticles. Upon addition of 6-TG, the acridine is replaced from the surface of AgNPs which induce the aggregation of AgNPs. Under optimum condition, the fluorescence intensity enhance showed the linear relationships with 6-TG concentration range from 0.05 × 10^{-8} M to 4.0 × 10^{-7} M. The detection limits (3s) and % RSD for 6-TG was occurred to be 9.6 nM, ± 2 % respectively. Proposed work has been successfully applied for the determination of 6-TG in biological sample such as urine sample.

Keywords- Fluorescence spectroscopy, silver nanoparticles, acridine, 6-Thioguanine.

1. Introduction

6-Thioguanine (6-TG) is similar to guanine which is one of the basic components of the nucleic acid [1,2]. Due to its chemotherapeutic properties 6-TG is often used as life saving drugs in acute myeloid leukemia and some other pathological condition [3]. The plausible dose dependent side effects include bone marrow depression [4]. For such health issues, numerous techniques are used like high-performance liquid chromatography (HPLC) [5], Surface-enhanced Raman scattering [6], mass spectrometry [7], localized surface plasmon resonance [8] etc. These methods have their own demerits and limitations due to complication time consuming, Costly and poor sensitivity. Thus for the determination of 6-thioguanine, a simple rapid method is required.

Silver nanoparticles (Ag NPs) has been extensively used as highly sensitive fluorescent probe for the determination of biomolecules [9] and drugs [10] due to nanoparticles (AgNPs) are promising nonmaterial it can be employed in many applications because it exhibit unique optical, chemical and electronic properties.

In present work the 6-TG was determined by use AgNPs as quencher tool for the fluorescence intensity. Acridine is an example of cationic dye, thus it adsorb to the surface of the citrate capped AgNPs through electrostatic interaction. Formation of an Acridine-AgNPs conjugate is preceded by neutralization of charge.

2. Materials and methods

2.1 Materials and instrumentation.

All reagents used were of analytical grade. Silver nitrate (99.8%) purchased from Qualigen, Fischer Scientific, Mumbai, India, Acridine, trisodium citrate and authentic samples of 6 TG was purchased from Sigma Aldrich. The pH has been adjusted in 1.0 M concentration of Sodium hydroxide and hydrochloric acid. Fluorescence spectra of all of the samples recorded using a G9800A Fluorescence Spectroscopy. Absorption

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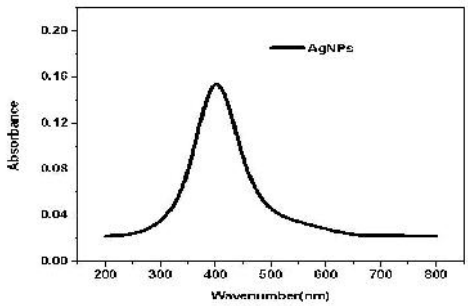
spectra measure by a UV-1800 UV-Visible spectrophotometer. The morphologies of AgNPs were performed on High-Resolution Transmission electron microscopy.

Figure 1. Absorption spectrum of AgNPs (λmax=419nm)



2.2 Procedure

Citrate stabilized AgNPs has been synthesized according to previously reported method a pale yellow color appeared. As shown in figure 1 AgNPs exhibits one absorption peak at 419 nm [11], A solution containing appropriate concentration of 0.1 M acetate buffer (pH~5.5) was mixed with 1.1 nM synthesized AgNPs. Further appropriate concentration of 1.5×10^{-5} Acridine was added to bring the total volume adjusted at 5 mL with deionized water in 5 ml volumetric flask. Afterward, a different concentration of 6-TG was added. The fluorescence emission intensity (λ_{em}) of the mixture was measured in the absence and present of 6-TG at 525 nm. The calibration and analysis depends on the differences between the two signals.

3. Result and discussion

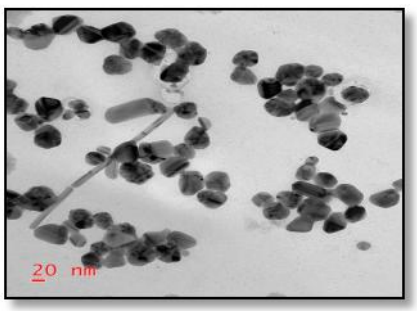
3.1 Fluorescence recovery in the Acridine /AgNPs system caused by 6-TG inducing AgNPs aggregation

The quenching of the fluorescence intensity of dye by AgNPs is due to fluorescence resonance energy transfer mechanism. The synthesized citrate capped AgNPs having negatively charged surface acridine is positively charged species it gets absorbed surface of the AgNPs due to electrostatic interactions. The fluorescence intensity of acridine proficient quenched. The efficiency quenching was found that good signal to noise ratio when applied the determination of any analytes. The quenching efficiency is analyzed by the stern-volmer equation.

$$F/F0 = 1 + K_{SV}[Q] \quad (1)$$

Where F_0 and F respectively represent the fluorescence intensity in the absence and presence of AgNPs, K_{SV} is quenching constant and [Q] indicates molar concentration of qunencher [12]. Fig 4B shows the Stern-Volmer plot for quenching of fluorescence of Acridine by AgNPs. The K_{SV} calculated starting linear plot at lower concentration of AgNPs. A HR-TEM result image of Acridine-AgNPs in the present of 6-TG is shown in Fig 2, that also provided convincing evidence supporting that the aggregation of AgNPs was greater in the presence of 6-thioguanine is due to the surface charge neutralization [13].

Figure 2 HR-TEM image of the AgNPs



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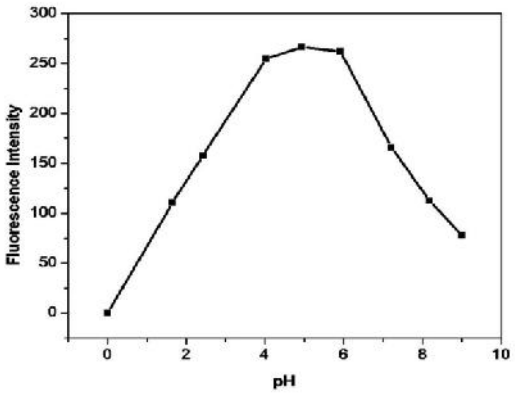
4. Optimization of experiment

In proposed Acridine-AgNPs system, the fluorescence intensity is strongly influenced by various parameters such as pH, concentration of AgNPs, Acridine, and buffer solution and incubation time. The effect of Acridine was investigated in the different appropriate concentration which is essential to get adsorbed on the surface of the AgNPs. In our study, the result shown that the intensity of fluorescence of OA was magnificently quenched at concentration of 4.0×10^{-7} M. The pH play important role in aggregation of the system [14]. Hence, the effect of pH was investigated in the range from 2.0–10.0 by addition 1.0 M N NaOH or HCL. The Acridine-AgNPs mixture solution in the present of 6-thioguanine was mentioned. The highest response was obtained pH~5.5 the further studies as shown in fig 3. The effect of the concentration of AgNPs on the fluorescence recovery toward 6-TG has been also studied in the range of 1.0×10^{-8} M to 3.5×10^{-8} M. The concentration of AgNPs was increased with the enhanced fluorescence intensity and constantly achieved a maximum intensity at 2.0×10^{-8} M which is optimum for whole experiment.

Figure 3 Effect of pH on determination of 6-TG under optimized condition



4.1 Determination of drugs.

The experiment testing process is the intensity of fluorescence of Acridine-AgNPs increased gradually with increasing the concentration of 6-thioguanine in the range of 0.05×10^{-8} M to 4.0×10^{-7} M. The concentration of drug 2.5×10^{-7} was finalized as optimum concentration for use in the assay as shown in Fig 4. The detection limit was 9.6 nM. The relative standard derivation of the method was ± 2.0 % for five independent measurements of 6-thioguanine, subsequently it can be concluded that the cluster of Acridine-AgNPs and its response for detection of 6-thioguanine is highly valuable.

Figure 4. Fluorescence spectra of Acridine–AgNPs system in the presence of different concentrations of 6-TG

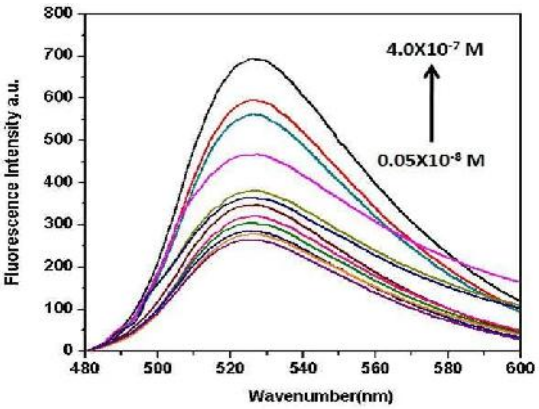


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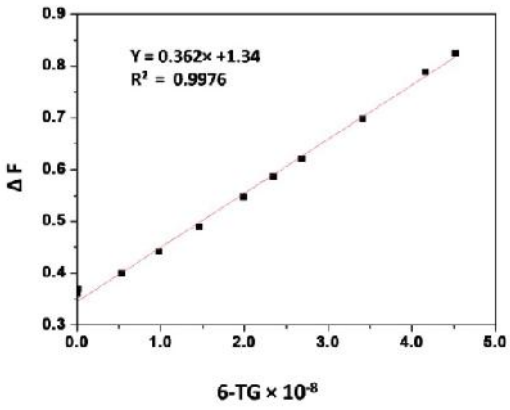
Figure 5 Calibration curve of 6-TG under optimized condition



5. Application

The optimized Acridine/AgNPs system is applied for the determination of 6-TG in real samples such as urine by standard addition method as shown in Table 1 in which recovery, RSD was calculated. The samples were collected from the private pathology lab, Raipur, Chhattisgarh, India. Matrixes present in urine and blood samples cause greater interference in determination of 6-TG by fluorescence spectrophotometric method. The recoveries were obtained in the range of 96.2%~102.6%. Suggest that developed method could be feasibility and potentially be used to the determination of 6-TG.

Table 1 Determination of 6-TG in urine sample under optimized condition

Sample	Added	Found (μM)	Recovery %	RSD (±%)
Urine	0.05	0.049(±.04)	99.4 %	± 0.5678 %
	0.5	0.521(±.02)	101.3 %	± 0.4231 %
	1.0	1.041(±.01)	102.6 %	± 1.1610 %

6. Conclusion

In present work a novel fluorescent probe has been developed for the determination of 6-TG. The Acridine-AgNPs system was effectively recovered when 6-TG was added due to aggregation of AgNPs induced by 6-TG, made the AgNPs surface free from Acridine. The complete mechanism has been proved by HR-TEM analysis. The proposed work was successfully applied for the determination of 6-TG in real samples such as urine.

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