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Gentamicin induced formation of gold nanoparticles as an assay protocol for its detection

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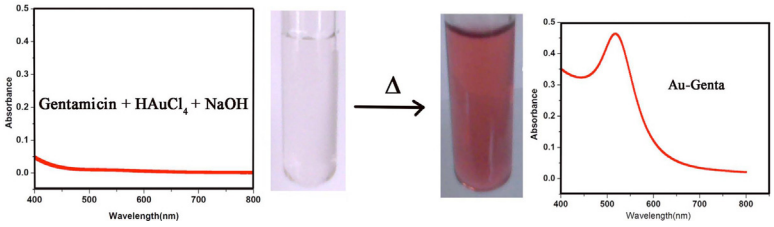
HIGHLIGHTS

- A simple and versatile optical method for the detection and estimation of gentamicin.
- The methodology relies on the gentamicin induced formation of gold nanoparticles.
- The method is devoid of complex derivatization steps normally followed for gentamicin detection.
- The method can be used for gentamicin detection in complex fluids like urine.

GRAPHICAL ABSTRACT



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ABSTRACT

A quick method for the visualization and estimation of gentamicin up to a concentration of 10 mg L^{-1} is formulated. Since gentamicin lacks any chromophore or fluorophore, direct spectro photometric or fluorometric estimation cannot be used for the detection of this widely used antibiotic. Here we explored the ability of gentamicin to reduce gold ions into gold nanoparticles. The formation of the wine red colored gentamicin stabilized gold colloid (Au-genta) from the colorless aqueous gentamicin solution made it possible its detection with both naked eye and spectrophotometrically. This method does not need any tedious chemical derivatization or fluorescent labeling steps or the use of any costly instrumental analysis techniques. It also offers comparatively better sensitivity (2.5 mg L^{-1}) than the other reported methods (390 mg L^{-1} for gentamicin derivatization with ninhydrin and 1000 mg L^{-1} with *o*-phthalaldehyde).

1. Introduction

Gentamicin is a broad spectrum aminoglycosidic antibiotic used to treat many types of bacterial infections [1]. The drug being a mixture of C_1, C_2, and C_{1A} (Chart 1), is highly active towards both gram positive and gram-negative species including *E. coli*, *Proteus*, *Salmonella*, and *Penicillin* resistant *Staphylococcus* strains. Gentamicin is widely recommended to treat severe purulent infection caused by even resistant gram-negative flora. In many instances, the drug shows effectiveness when other antibiotics fail. In parallel to its potentialities, the drug also has some side effects which are, to a large extent, is dose dependant. It is therefore, extremely important to determine its concentration particularly releasing out of formulations used in bone surgery, dressings etc.

Since gentamicin does not contain any chromophore or flurophore, indirect method is required for its optical estimation. Chemical derivatization or fluorescent labeling is used prior to its analysis [2–4]. Most of the reported methods for the estimation of gentamicin are based on the derivatization with *o*-phthaldialdehyde [5–8]. Derivatization methods are cumbersome and in many cases the sensitivity is reported to be too low

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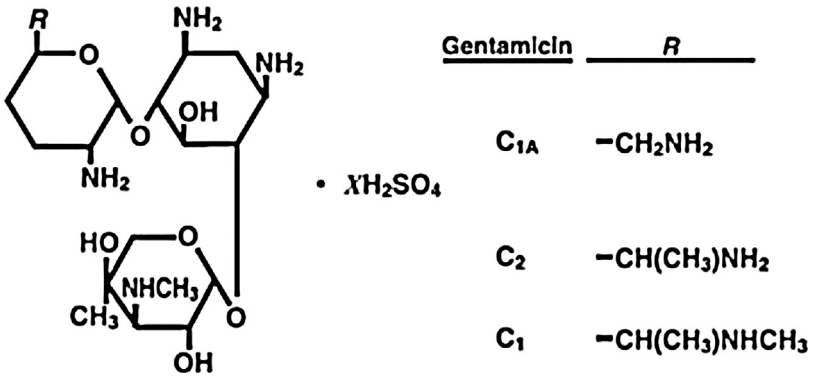

Chart 1. Structure of gentamicin.

(390 mg L^{-1} for gentamicin derivatization with ninhydrin [9] or 1000 mg L^{-1} when reacted with *o*-phthalaldehyde [10]) for intracellular concentration and pharmacokinetic studies. Kaale et al. have reported a capillary electrophoresis analysis of gentamicin sulphate with UV detection after pre-capillary derivatization with 1, 2-phthalic dicarboxaldehyde and mercaptoacetic acid [11]. HPLC in conjunction with detectors such as fluorescence, evaporative light scattering and mass spectrometer is the widely employed tool for its estimation. Such systems are not only very expensive but also needs lengthy procedures.

The automated assay procedures e.g., radio immuno assay (RIA) etc., are very specific and sensitive, they need expensive equipments, a labour intensive procedure and provision for use and disposal of radioactive materials [12].

Didamony et al. reported an indirect spectroscopic method for the estimation of gentamicin sulphate based on their oxidation by potassium permanganate in sulphuric acid medium and subsequent determination of unreacted oxidant by reacting it with appropriate dyes [13]. The methodology is lengthy and is less accurate.

Efforts have been expended to stabilize nanoparticles with varied entities including polymers and also to synthesize nanoparticle-polymer composites [14,15]. In recent years, several studies have been emerged on the use of antibiotic conjugated gold nanoparticles (AuNPs) as potential antibacterial agents [16,17]. Many studies have reported the use of blue aggregated mixture of AuNPs and antibiotics including gentamicin [18,19]. Nano vehicles are created by assembling antibiotics onto AuNPs synthesized by citrate reduction. With a view to avoid intermediate steps and chemicals, one pot synthesis of AuNPs using antibiotics has also been reported [20]. Rai et al. have reported one step synthesis of AuNPs and their capping with cefaclor, a second generation antibiotic [21]. These studies show that antibiotics, playing the dual role of reductant and capping ligand, still possess their antibacterial properties reflecting the lack of significant structural variation to these molecules during the process of nanoparticles creation. These reports again show that spherical AuNPs having typical surface plasmon resonanace (SPR) absorption can be generated easily by treating with antibiotics. We reasoned that this reaction can be used for the detection and quantification of an antibiotic of interest without the tedious process of derivatization and the employment of expensive tools.

Herein, we demonstrate a one step and easy method for the estimation of gentamicin by generating gentamicin stabilized gold nanoparticles and recording their SPR absorption intensity which varies proportionally with the drug's concentration. The results emerged from this report shows that this protocol can be used as an assay method for determining the concentration of gentamicin in studies where there is a need for its quantification.

2. Experimental

2.1. Materials

Gold chloride and gentamicin were purchased from Sigma–Aldrich, Bangalore, India. All other chemical used were of analytical grade and obtained from Merck India Ltd., Mumbai, India.

2.2. Preparation of gentamicin stabilized gold nanoparticles (Au-genta)

Different concentrations of aqueous gentamicin sulphate solutions were prepared ranging from 2.5 to 320 mg L^{-1}. To 2 mL of each solution 200 μL of 1 M NaOH and 500 μL of $HAuCl_4$ (5 mM) were added and kept at a boiling water bath for 5–10 min. The solutions turned wine red indicating the formation of gentamicin stabilized gold nanoparticles (Au-genta).

2.3. Calibration curve using Au-genta formed with different concentrations of gentamicin

The SPR absorbance maximum of the Au-genta formed with different concentration of gentamicin is plotted against the corresponding concentrations of gentamicin to get a calibration curve. The unknown concentration of the test sample of gentamicin can be calculated from the curve using the SPR absorbance maximum of the Au-genta colloid formed from the test sample.

2.4. Instrumental

The UV–Visible absorption spectra of the Au-genta NPs, was recorded using Varian, Cary 100 Bio UV–Visible spectrophotometer (Melbourne, Australia). Fourier Transform Infra Red (FTIR) spectra of the gentamicin powder, Au-genta and citrate stabilized AuNPs, were recorded in the range 600–4000 cm^{-1} on a Nicolet 5700 FTIR Spectrometer (Nicolet Inc., Madison, USA) using a Diamond ATR accessory. The technique of Dynamic Light Scattering (DLS), (Malvern Instruments Ltd., UK) was used for the determination of the size of the nanoparticles. All measurements were performed at 25 °C. Transmission Electron Microscopic (TEM) images were obtained on a Hitachi, H 7650 microscope (Hitachi, Tokyo, Japan). The colloidal solution was deposited onto a 200 mesh copper grid coated with a formvar film and dried overnight.

To confirm the precision and recovery of the probe each set of experiment was carried out in triplicate and similar results within the maximum error of 2–3% were obtained.