# Determination of Clenbuterol by Colorimetric Sensing using Gold Nanoparticles

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**Abstract:**

Foodstuff superiority and safety are directly significant to people's life and health. Incidents about unsafe food occur frequently. Clenbuterol (CB) incidents, related rapidly rising global cancer deaths and sportsperson performance showing lots of causality due to failures in detection. Clenbuterol is a β-adrenergic drug used for asthma treatment typically employed as a bronchial, particularly in the case of chronic illness. Colorimetric method is an economic and a highly sensitive method for the detection of outline for clenbuterol based on gold nanoparticles. The key point to the gold nanoparticles-based visual detection assay is to control dispersion and aggregation of colloidal nanoparticles by targets of interest, which usually relies on affinities between gold nanoparticles and targets by showing visual colour change.Thus the concentration of CB could be determined with the naked eye or a UV/Vis spectrometer.

***Keywords:*** *Colorimetric sensing, Clenbuterol, gold nanoparticles, asthma*

## 1. Introduction:

Clenbuterol is a sympathomimetic amine used by patients with breathing disability (such as Asthma) as a bronchodilator to make breathing easier. β –agonists drugs can be easily deposited in human beings after meat consumption which may cause many serious health issues such as cardiovascularand central nervous diseases [1].Due to this serious side effect, many countries have forbidden the use of β–adrenergic drug in stockbreeding[4]. In the last few years,colorimetric sensor methods are getting attention consideration due to visual observation and simple experimental procedure. Colorimetric method is extremely economical and sensitive method for the detection of clenbuterol by using gold nanoparticles (GNPs).Hydrogen-bonding interaction between clenbuterol causes aggregation of GNPs and a consequent color change of GNPs[7].This paper explains the visual detection assay of clenbuterol using GNPs. The experiment is to control dispersion and aggregation of colloidal nanoparticles by targets of interest, which usually relies on affinities between gold nanoparticles and targets. The degree of dispersion or aggregation can be visualized through the change of the solution colour or the precipitation of nanoparticles from the solution.

Clenbuterol is a β2 agonist with some structural and pharmacological similarities to Epinephrine and Salbutamol, but Clenbuterol is more effective and persistent as a stimulant and heat producing the drug. It causes an increase in aerobic capacity, CNS stimulation, and an increase in BP and Circulation of oxygen in human beings [1].Clenbuterol also a prospective drug promoting major and sustaining improvements in the levels of oxygen-carrying in the body and aerobic capacity. It can even encourage the central nervous system and target beta-2 receptors that promote the stimulation of muscle growth and improving protein storage in muscles [3]. Clenbuterol ha the benefit of a unique place in sports medicine. It is commonly used as a drug to lose body fat and weight while retaining muscle mass and body strength gains [16]. It is also used by athletes who are diagnosed with exercise-induced pulmonary haemorrhage (EIPH).



Various analytical techniques for the determination of β –agonist drug such as clenbuterol, salbutamol etc have been reported, including Liquid Chromatography Spectroscopy (LC), Gas Chromatography(GC), ELISA[3] and electrochemical detection [2]. Gold nanoparticles exhibit attractive properties in electrode modification by improving the electrode conductivity and enhancing the analytical sensitivity and selectivity.

## 2. Objectives:

The objective of the proposed research work is to develop a technique to detect and quantify clenbuterol using gold nanoparticles. The purpose of the research also reduces the expense of detection of clenbuterol which may be useful for industrial as well as the analytical development of pharmaceutical sectors. This review begins with a summary of the appealing properties and various applications of Clenbuterol and briefly summarizes recent advances in using gold nanoparticles to detect different kinds of targets including nucleic acids, proteins, metal ions and small organic molecules. To investigate the practical application of this colorimetric method, the detection of the human urine sample was carried out by the standard addition

method according to most relative publications [7-12]. Colorimetry, HPLC and other analytical instruments will be used for the collection of data for detection of clenbuterol using gold nanoparticles**.**

## 3. Experimental Reagents and Chemicals

### 3.1. Preparation of Nitrophenol stabilized Gold Nanoparticles

$HAuCl_4.XH_2O$ (Tetrachloroauric acid) was obtained from Sigma Aldrich. Millipore Distilled Purified water was used to conduct experimental works. The neem broth was used for reduction of $Au^{3+}$ ions to $Au^0$. In this research, 0.2 ml of broth was added to 50 ml of $10^{-3}$ M aqueous chloroauric acid (HAuCl4) solution. The colour of the solution was changed to a cherry red colour within an hour (50 minutes).At a higher concentration of the extract, a large number of isomorphic spherical gold nanoparticles of 15-18nm in size could be seen in the transmission electron micrographs [15].

### 3.2. Chemicals and Reagents

All chemicals used were analytical reagent grade and the solutions were prepared with Millipore purified distilled water. Clenbuterol hydrochloride tablets were purchased from Medson pharmaceuticals, India.$HAuCl_4.XH_2O$ (Tetrachloroauric acid),ethanol from Sigma Aldrich and few other chemicals for supporting experiments from a reputed company were used as obtained.



### 3.3. Apparatus

3.3.1. Glassware

All glassware such as conical flask, measuring cylinder, test tubes burette, beaker, petridishes were thoroughly washed and rinsed with purified water followed by drying properly for the experiment. The experiments are carried out by using re-usable flat bottom glass cuvettes for absorbance mode.

3.3.2. Spectrophotometer

Absorption spectra data were obtained using a UV/Vis spectrophotometer called Color Eye Microprocessor-based Digital Colorimeter (Advanced Version [Fig.:-1]), Invitro Biotech Ltd (IBL).This digital colorimeter is a microprocessor photometer with 9 LED-based wavelengths (410, 430,470,490,520,540,580,610& 640nm) within visible range. Dynamic light scattering (DLS) data were collected on Zetasizer Nano ZS instrumentation (Malvern Instruments Ltd.).


*Fig 1 Color Eye Microprocessor-based Digital Colorimeter (Advanced Version)*

3.3.3. HPLC(High-performance liquid chromatography or high-pressure liquid chromatography)

The experiment was done by Waters 2695 Separations Module using PDA 996 and data interpretation by the software called Empower 2. This is a technique in analytical chemistry used to separate, identify, and quantify each component in a mixture.

## 4. Results and Discussions

4.1. Colorimetric sensor

Here in the experiment, three different concentrations of GNPs and blank absorbance's were observed at a different wavelength as shown in fig.3.In the absence of Clenbuterol, the solution of GNPs appears red and displayed an intense surface plasma band at 520 nm. While in the presence of Clenbuterol, the absorbance of GNPs at 520 nm decreased and a new peak appeared at about 640 nm by changing the colour red to Purple blue as shown in fig.2. Change in colour is easily observed by naked eyes and quantify the clenbuterol concentration with solution b i.e. 0.1µM CBGNPs solution.

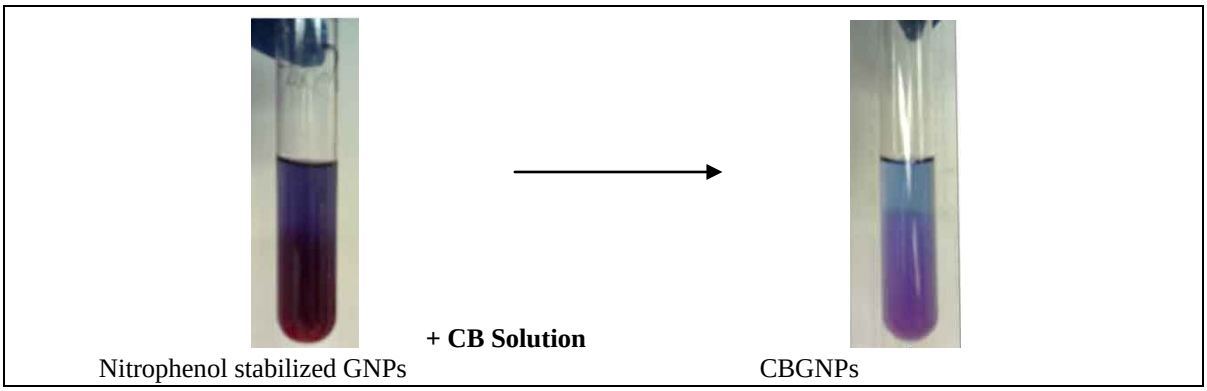


*Fig. 2 Solution showing Visual colour change red (Nitrophenol stabilized GNPs) to Purple blue (CBGNPs) showing the presence of clenbuterol.*

**Based on Beer-Lambert Law: Converting Absorbance to Transmittance**

**$A = 2 - \log_{10} \%T$**

*a:- 0.05µM

b:- 0.1µM

c:- 0.3µM

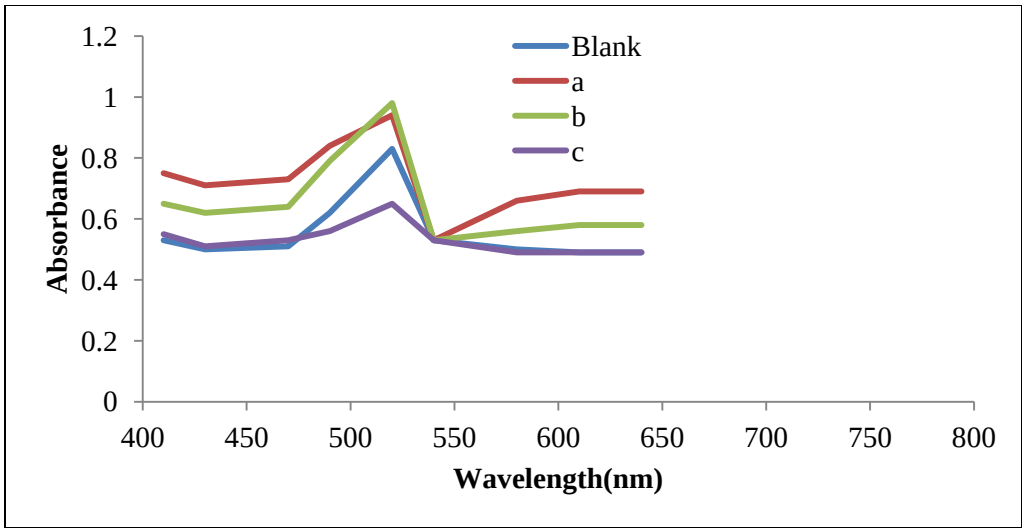


*Fig. 3UV–Vis Spectra of CBGNPs in the presence of different concentrations at different wavelength of visible range (410, 430,470, 490, 520, 540, 580, 610 & 640nm)*



Matrix calculation of absorbance Vs transmittance as per colorimeter or UV-Vis Spectrophotometer will be captured and illustrated with different concentrations of CB solutions.Data analysis was carried out on the basis of the statistical planwith proper scientific evidence with respect to mechanisms to assure the quality of the study – e.g. control of bias, safe storage of data.

### 4.2. HPLC:

The highperformance Liquid chromatography experiment is done byWaters 2695 Separations Module using PDA 996. The software called Empower 2 was used data collection to show accuracy, precision, linearity, robustness and calibration curve of the solution. Multiple samples with unknown concentrations are measured and graphed, which allows the solution to be determined for samples concentration by interpolation on the graph as shown in fig.4.

**Optimized Chromatographic Conditions:**

On the basis of the planned strategy, a series of experiments were conducted to establish the optimum analytical conditions for the detection of CB.

| | |
|---|---|
| Stationary phase | : Zorbax Sb-C8 (4.6×250mm) 5µ |
| Column temperature | : 38ºC |
| Wavelength | : 245nm |
| Mobile phase ratio | : Methanol: water (78:22 v/v) |

Flow rate : 1.0ml/min

Injection volume : 10µl

Run time : 10min

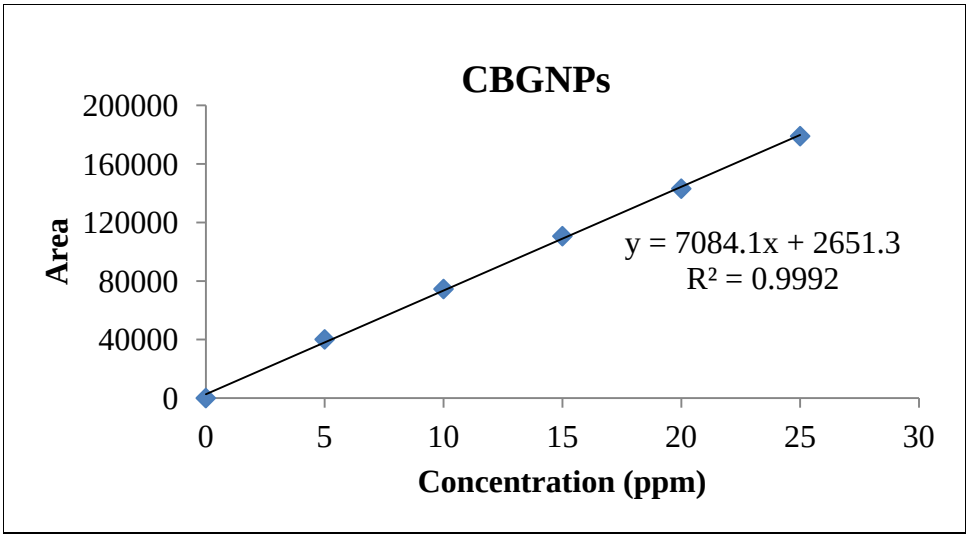


*Fig. 4 Calibration curve showing the area of the peak of different concentrations of CBGNPs (6 samples), the concentrations of CBGNPs in a different sample are determined by identifying where on the standard curve.*



Based on ICH guidelines, HPLC method linearity is normally based on 5 concentration levels between 70% and 130% of the nominal concentration sampled 3 times each. The laboratory effort for this process can be extensive, especially when the method is required to quantify several substances simultaneously and to be individually prepared. So only one stock solution is prepared and subsequently dilutes it to the different concentration levels. A dilution method has the effect of randomizing potential sources of error (such as incorrect weighing for one of the standards, wastage, spillage etc) and to save time. This is a fast way of preparing standards, but the major drawback is that any error in the stock solution will be carried through to the diluted standards. The value of this exponent for a given detector has been termed the response index of the detector and has been used to define the detector linearity.

*Table 1 Peak Results*

| S. No. | Name | RT | Area | Height | USP Tailing | USP Plate count | Injection |
|---|---|---|---|---|---|---|---|
| 1 | CB | 3.339 | 40088 | 8646 | 1.12 | 7502 | 1 |
| 2 | CB | 3.324 | 74513 | 14236 | 1.12 | 7686 | 1 |
| 3 | CB | 3.349 | 110612 | 20956 | 1.11 | 7691 | 1 |
| 4 | CB | 3.345 | 143067 | 25380 | 1.11 | 8325 | 1 |
| 5 | CB | 3.338 | 178937 | 29790 | 1.08 | 8038 | 1 |
| 6 | CB | 3.379 | 110249 | 20635 | 1.10 | 7434 | 1 |

Statistically, it is recommended to take 6 samples are taken to determine the value of the solvent. The retention time of different samples are little varying but under a range of acceptance due to optimized chromatographic condition and auto-scaled chromatogram is shown in fig.5.The retention time is taken as the elapsed time between the time of injection of a sample and the time of elution of the peak maximum of that sample, which is a unique feature for identification purposes[14].

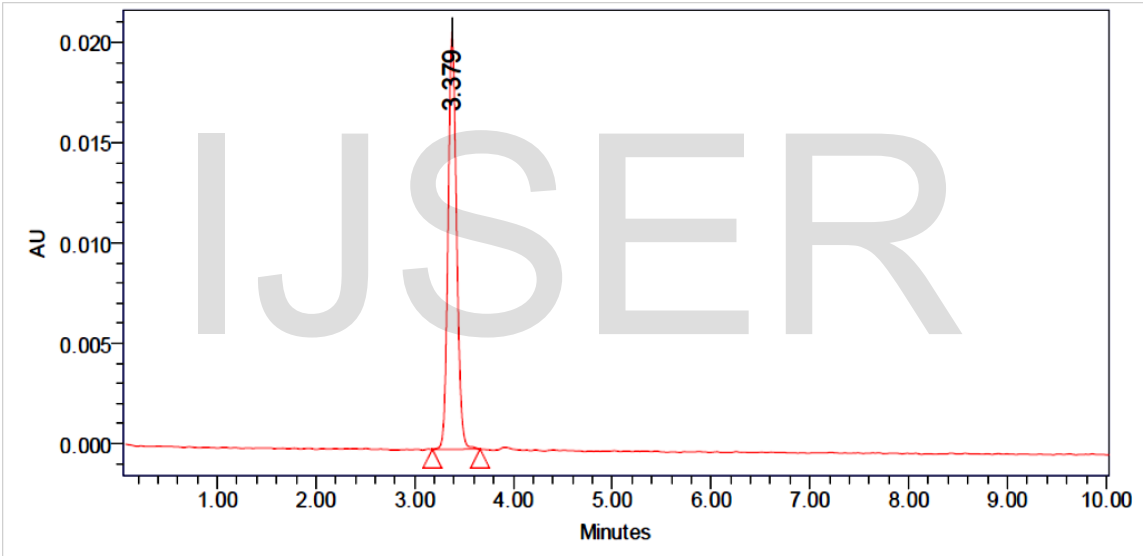



*Fig. 5 Auto scaled chromatogram of CBGNPsshowing a sharp peak as RT 3.379min*

This experiment is summarized with visual detection and quantification of clenbuterol using GNPs. Several researchers and experiments had already been done for determination of clenbuterol using different nanoparticles and catalyst as mentioned in Table 2. With these, all references and literature reviews this experiment is different as the nanoparticles generated for the purpose of research work are by phytoextraction i.e. green GNPs eco-friendly.

*Table 2Comparison of detection limits on GNPs based probes towards colorimetric detection of Clenbuterol*

| Method of detection | Probes | Adopted Strategy | Linear range | LOD by linear regression | LOD by Naked eyes | Ref |
|---|---|---|---|---|---|---|
| Electrochemical | Au electrode modified ZnSQDs | None | 0.01~10 $ng.mL^{-1}$ | 5.5 pg $mL^{-1}$ | NA | 2 |
| Colorimetric | Citrate stabilized GNPs | Melamine Presence | 0.028 µM ~0.28 µM and 0.28~1.4 µM | 28 µM | 0.87 µM | 6 |
| Colorimetric | Cetyltrimethyl - ammonium chloride stabilized GNPs | None | 2.34 ~ 600 µM | 0.722 µM | 2.3 µM | 7 |
| Colorimetric | Cysteamine Modified GNPs | CLB stirred in Advance | NA | NA | 0.05 µM | 8 |
| Colorimetric | Nitrophenol stabilized GNPs | CB stirred in Advance | $5x10^{-8}$ to $1x10^{-5}$ mol/L | $5x10^{-8}$mol/L | 0.1nmol/L | This work |

NA: Not available



## 5. Conclusions:

This experimental work shows very economic and highly sensitive detection of CB using gold nanoparticles. The hydrogen bonding interaction CB rapidly induces the aggregation of GNPs and colour change of GNPs from red to purple-blue. Thus the concentration of CB could be determined with the naked eye or a UV–vis spectrometer. Results showed that the absorption ratio (A640/A520) was liner with the logarithm of CB concentration range from$5x10^{-8}$ to $1x10^{-5}$ mol/L with a correlation coefficient of 0.999.The visual detection of CB using GNPs is also helpful to quantify the element in biomedical applications either pathology or analytical lab by using a colorimeter. Detection of clenbuterol in human urine and other biological excretions even in below traceable limits. The detection procedure expenses can be reduced since the instrument cost is much lesser than the existing procedures in the laboratory

## 6. Recommendation

As the pollution is rising will leads to more breathing disability, which can easily lead to the comeback of CB as it will easily detect at lower expenses & quicker. In the upcoming days, Nanoparticles will be much cheaper and highly available for all purposes which will help this methodology of detection of CB as a quick response at worthy expenses. Side effect evaluation &upgradation can be easily prescribed to Asthma &breathing disability patients similar to Diabetes nowadays (Glimepiride and many more).

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