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Gold nanoparticles based enzyme biosensor for the detection of chloramphenicol

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Abstract

Chronic use of chloramphenicol (CAM) antibiotic leads to anaemia and bone marrow suppression resulting in 40 – 50% mortality. Hence, there is a need to develop an economical, fast and convenient method to detect CAM in milk, honey, shrimp and other aquaculture products. In the current method, coenzyme A was used to indirectly quantify CAM (since it is the cofactor product of the acetylation reaction of CAM). Coenzyme A (CoASH) was used to stabilize gold nanoparticles which were characterized by studying their extinction spectra. The reductant concentration and synthesis time were optimized. With optimized parameters the proposed system could detect CoASH up to 0.1 nM in buffer, with a linear range of detection from 0.1 μM to 1 mM.

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Keywords: Chloramphenicol; gold nanoparticles; enzyme biosensor; coenzyme A; chloramphenicol acetyltransferase

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1. Introduction

Chloramphenicol (CAM) is a broad-spectrum antibiotic, chronic use of which leads to non-regenerative anaemia, aplastic anaemia and bone marrow suppression. The conditions are dose independent and irreversible, prognosis is poor and mortality is 50% in adults and 40% in neonates [1]. Most countries have banned CAM from animal food production, maximal permissible levels being 0.3 parts per billion (ppb) in milk, and many have established zero tolerance policy, due to resistance issues and adverse health effects. However, it has been detected in milk, honey, shrimp and other aquaculture products [1, 2, 3], with many establishing zero tolerance policy, due to resistance issues and adverse health effects. However, it has still been detected in milk, honey, shrimp and other aquaculture products. In the past years, several serious concerns have been raised by European Union (EU) on export of antibiotic-containing honey to EU countries from India. The existing screening methods for CAM are time-consuming, laborious and expensive [4]. Hence, there is a need to develop a cost-effective, fast and convenient method to detect CAM. In the present work, CoASH was used to develop a biosensing technique for CAM. Chloramphenicol acetyltransferase enzyme catalyzes the acetylation of CAM, wherein CoASH is one of the products. CoASH was used to synthesize gold nanoparticles (AuNPs). The hypothesis is illustrated in Figure 1. Addition of higher quantities of this cofactor led to an increase in absorbance as well as wavelength shift in extinction spectra of nanoparticles signifying higher synthesis and improved protection of the AuNPs. The reductant concentration and synthesis time were optimized, followed by ultrasensitive detection of coenzyme A.

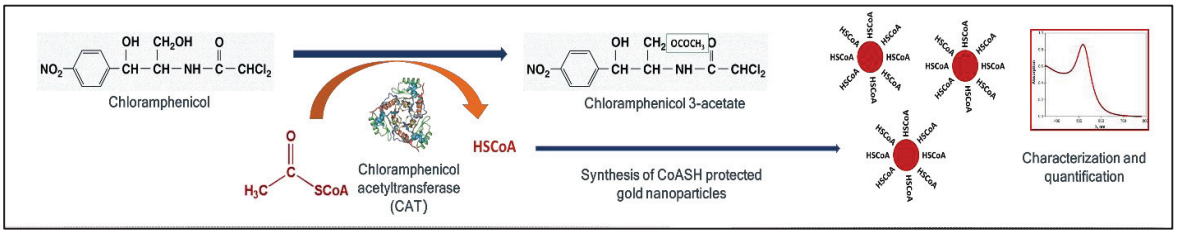

Fig 1. Schematic of the biosensing principle for CAM using CoASH assisted gold nanoparticle synthesis

Nomenclature

CAM	Chloramphenicol
CoASH	Coenzyme A
AcCoA	Acetyl coenzyme A
$NaBH_4$	Sodium borohydride
AuNPs	Gold nanoparticles

2. Methods

As mentioned in the introduction, during acetylation of CAM, AcCoA is converted to CoASH. To detect CoASH, the molecule was used to protect AuNPs synthesized by reducing gold(III)chloride with sodium borohydride ($NaBH_4$) under rapid stirring. Freshly prepared $NaBH_4$ was injected into a magnetically stirred solution of gold(III)chloride and CoASH in the buffer, required for the enzyme reaction. The synthesized nanoparticles were quantified by measuring their peak absorbance using a UV-Vis spectrophotometer. The reductant concentration was optimized in the range (0.1 to 0.3 mM $NaBH_4$) and synthesis time was optimized for 1, 2, 3 and 4 hours. Standard curve was prepared using different concentrations of CoASH. The detection limit and linear range was determined from the extinction spectra of the AuNPs.

3. Results and discussion

Optimization experiments have indicated that 0.3 mM $NaBH_4$ and 3 hours of synthesis could synthesize nanoparticles very efficiently. The results are shown in Figures 2 and 3. When CoASH concentrations were varied, it was seen that addition of higher quantities of CoASH led to an increase in absorbance as well as wavelength shift in extinction spectra of nanoparticles signifying higher synthesis and improved protection of the AuNPs. At optimized conditions, the proposed system could detect CoASH up to 0.1 nM in buffer, with a linear range of detection from 0.1 µM to 1 mM (Figure 4).

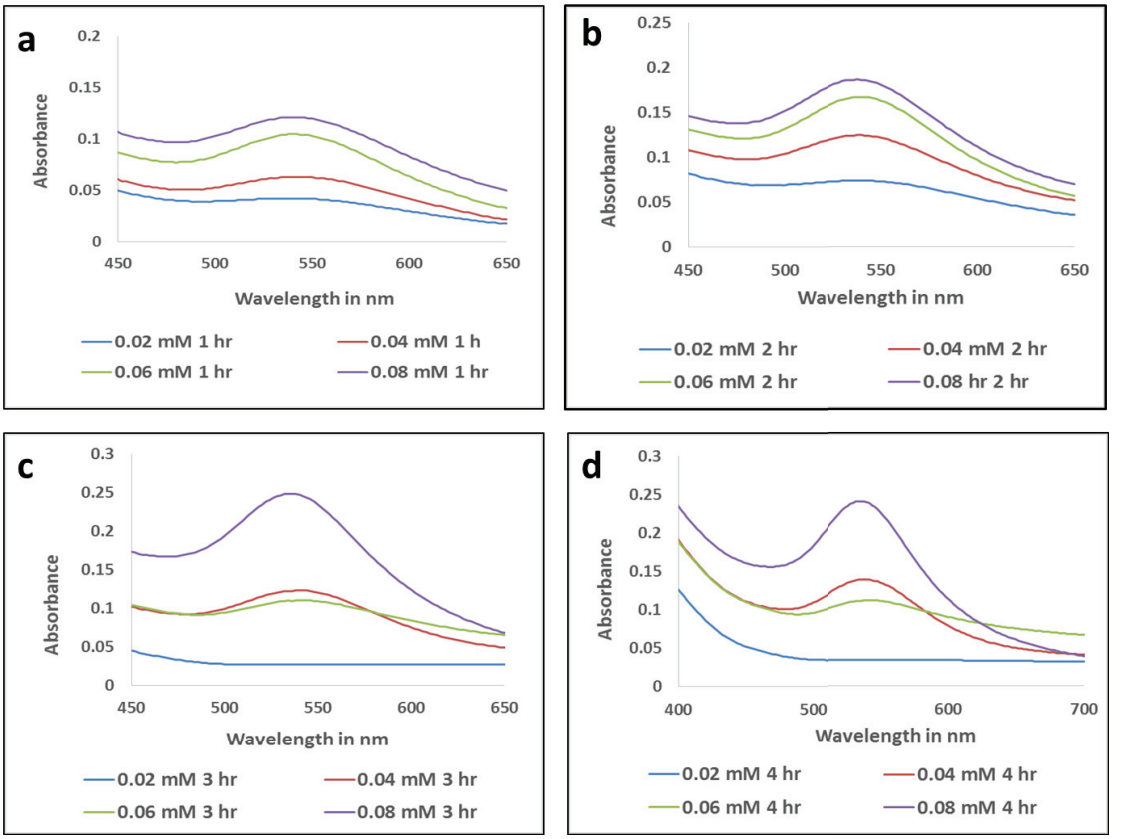

Fig. 2. Optimization of time of synthesis (a) 1 hour; (b) 2 hours; (c) 3 hours and (d) 4 hours with four concentrations of CoASH. It was seen that after 3 hours there was no significant change in the peak absorbance of the AuNPs.

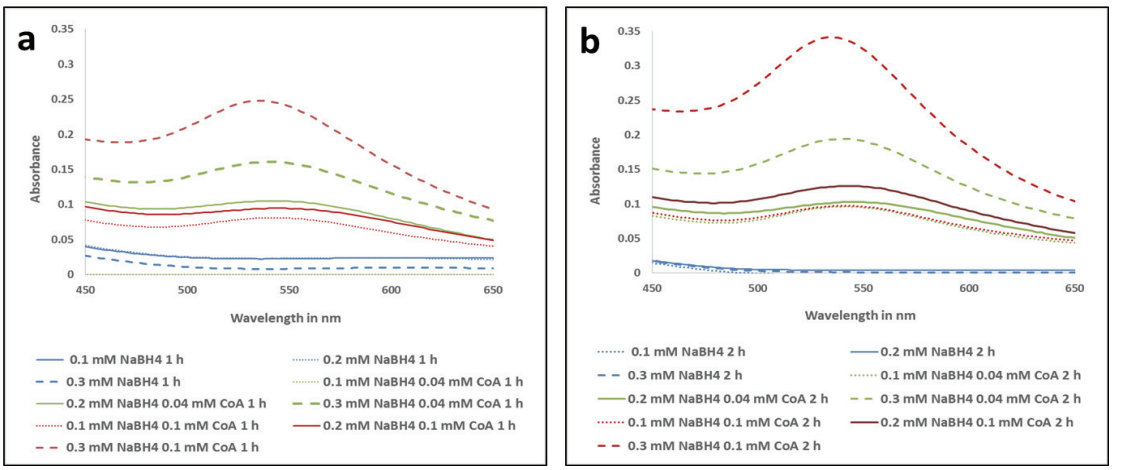

Fig. 3. Optimization of $NaBH_4$ concentration (0.1 mM, 0.2 mM and 0.3 mM) with different concentrations of CoASH (0 mM, 0.04 mM and 0.1 mM for (a) 1 hour and (b) 2 hours synthesis time. It was seen that after 0.3 mM sodium borohydride showed most efficient synthesis in all cases.

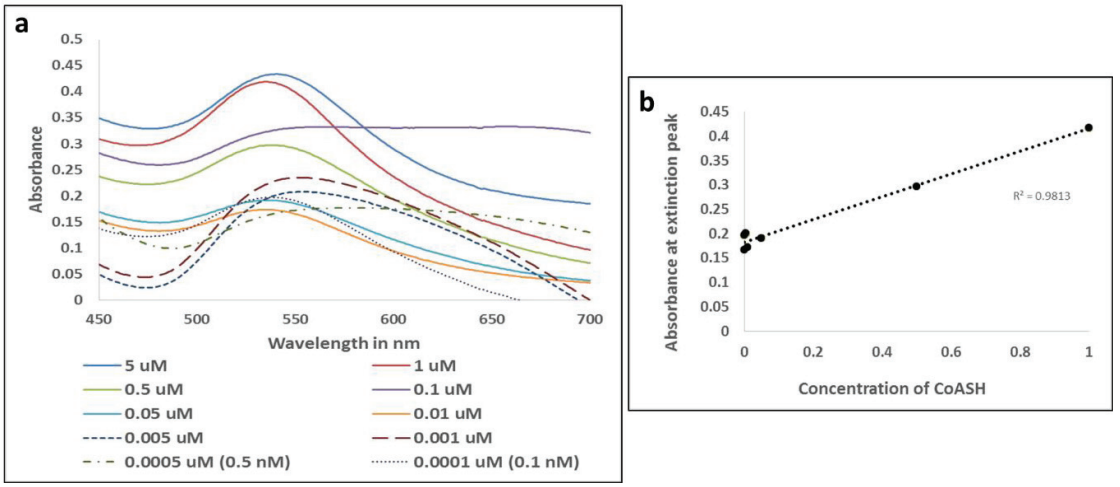

Fig. 4 (a) .CoASH assisted synthesis of AuNPs, where CoASH concentrations are in nanomolar range showing high sensitivity of the proposed method. (b) Linear plot of extinction peak values of AuNPs against CoASH concentrations

Gold nanoparticles have been widely used in biosensing because of easy chemical synthesis and excellent optical properties [5, 6]. Both these characteristics have played a pivotal role in the current biosensing format. Synthesis of $NaBH_4$ reduced AuNPs does not require heating, reflux conditions or several chemical reagents. However, the synthesized particles are not surface stabilized, as such they tend to aggregate within seconds of synthesis, with a visible colour change from dark red to purplish blue. As such there needs to be a protecting agent to form stable AuNPs, and polymers and surfactants are widely used for this purpose. Thiol (-SH) groups have been known to bind to AuNP surfaces protecting it from aggregation [7]. Since CoASH has a free thiol group, it was used in the present work to stabilize the AuNPs, and the extent of stable nanoparticle synthesis can be easily determined spectrophotometrically, due to the distinct extinction spectra of AuNPs. Using this principle, different stabilizer (analyte CoASH) concentrations yielded nanocolloid solutions of different concentrations, rendering the detection quantitative. Although, as low as 0.1 nM CoASH could synthesize detectable AuNPs, linearity could be observed in the range 0.1 μM to 1 mM of CoASH. The synthesized particles were found to be stable for six months.

4. Conclusion

CoASH was detected upto ultrasensitive levels using gold nanoparticles synthesis. Such a biosensing format can be an indirect method to quantify chloramphenicol, through its enzymatic conversion. The method uses simple steps, is rapid and analyte can be estimated spectrophotometrically. It can be extrapolated to the efficient and sensitive detection of several other compounds whose enzymatic transformations involve CoASH.

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References

[1] Shukla P, Bansode FW, Singh RK. Chloramphenicol toxicity: A review. J Med Med Sci 2011: 2: 1313-1316.

[2] Vora VR, Raikwar MK. Determination of chloramphenicol and thiamphenicol residues in fish, shrimp and milk by ESI-LCMSMS. Int J Agr Food Sci Tech 2013: 4: 823-828.
[3] Islam MJ, Liza AA, Reza AHMM, Reza MS, Khan MNA, Kamal M. Source identification and entry pathways of banned antibiotics nitrofuran and chloramphenicol in shrimp value chain of Bangladesh. Eurasia J Biosci 2014: 8: 71-83.
[4] Samsonova JV, Cannavan A, Elliot CT. A critical review of screening methods for the detection of chloramphenicol, thiamphenicol and florfenicol residues in foodstuffs. Crit Rev Anal Chem 2012: 42: 50-78.
[5] Sharma R, Ragavan KV, Abhijith KS, Akanksha, Thakur MS. Synergistic catalysis by gold nanoparticles and metal ions for enhanced chemiluminescence. RSC Adv 2015a: 5: 31434-31438.
[6] Sharma R, Ragavan KV, Thakur MS, Raghavarao KSMS. Recent advances in nanoparticle based aptasensors for food contaminants. Biosens Bioelectron 2015b: 74: 612-627.
[7] Häkkinen H. The gold–sulfur interface at the nanoscale. Nature Chemistry 2012: 4: 443-455.