



Original Research Article

Nanobiosensor based on Gold nanoparticles Probe for Aflatoxin B1 detection in food

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ABSTRACT

Keywords

Flow-through;
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AuNPs.

Aflatoxin B1 (AFB1) is potent genotoxic carcinogenic mycotoxins member affecting significantly human health. So, in the present work a dot-immunogold chromatography flow-through assay (DIGFA) was developed for detection of AFB1 in food and feed samples using colloidal gold nanoparticles (AuNPs). AuNPs are being extensively used in various applications due to its stability, controlled geometrical, optical, and surface chemical properties. AuNPs were synthesized by chemical reduction method and characterized by spectrophotometer, Dynamic Light Scattering (DLS), Electrophoretic Light Scattering (ELS), TEM and XRD. AuNPs-Anti AFB1 conjugates were designed by physical conjugation. Results showed that AuNPs can be used as a probe for AFB1 detection with acceptable sensitivity and specificity compared to HPLC technique. Constructed DIGFA sensor detects AFB1 with high sensitivity (5 ng/mL) which is validated by HPLC. Assay is rapid (test completion time is 2 min), reproducible and doesn't require any equipment.

Introduction

Fungal invasion of food can result in a marked deterioration in quality and often outright destruction. A more subtle, but even more compelling reason to control such fungi is that some species are capable of producing mycotoxins. The historical record of human and animal mycotoxicoses is a long one. In Africa, for example, aflatoxins, which are produced by *Aspergillus* species, have been implicated in human diseases including liver cancer, Reye's syndrome, and certain respiratory diseases (Sibanda et al., 1997).

Aflatoxins are secondary metabolites when ingested by animals and higher vertebrates cause diverse health effects and disease called aflatoxicoses (Adegoke and Letuma, 2013). A great economic losses produced by aflatoxins contamination when attacking different agricultural and dairy products (Cleveland et al., 2003). Studies have been shown that in the storage processes or cultivation of grains there exist different levels of contamination, especially with aflatoxin B1 (AFB1) (Caldas et al., 2002).

AFB₁ is a powerful genotoxin carcinogen for humans and many animal species, including rodents, non-human primates, and fish (E.C., 2012; Santacroce et al., 2008). The main target of this carcinogen is the liver, although tumors may also develop in other organs, such as the lungs, kidney and colon (Gelderblom et al., 1996). The current maximum residue levels for aflatoxins set by the European community (EC) are 20 µg/kg for AFB₁ and 40 µg/kg for total aflatoxins in groundnuts, nuts, dried fruits and cereals for direct human consumption (E.C, 2006).

Because AFB₁ is heat stable, the initial contamination levels could persist to the final product and finally reach the human or animal digestive tract. Therefore, to protect human and animal health, early prevention of contaminations of grains and animal feeds from this toxin is necessary. Current analytical procedures are based on the time-delayed analysis of AFB₁ by thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), over pressured-layer chromatography (OPLC), and enzyme-linked immunosorbent assay (ELISA) (E.C, 2006). These methods are laborious, use expensive equipment, require well-trained personnel and can only be employed in laboratories, although they have excellent detection limits ranging from 0.01~6 ng/L (E.C, 2006). Therefore, a rapid and easy-to-use detection method is required.

The Nanobiosensors applications for detection of pathogens from food samples is challenging because of the complex nature of food matrices (Bhunia, 2008). A lot of biosensor-based methods have developed and those are grouped as electrochemical, optical and thermo-metric (Bhunia, 2008).

Dot-immunochromatography Flow through assay strip (DIGFA) is a new

immunochromatographic technology combining chromatography with immunoassay and has great interest recently. Nanoparticles usually selected as the detector reagent, e.g., colloidal gold nanoparticles is most applied.

DIGFA become one of the commercial and widely-used immunoassays for rapid determination of mycotoxins (Kolosova et al., 2008; Lai et al., 2009; Xu et al., 2010) Among the rapid methods for screening of food contaminants, the 'flow immunoassay' (FIA) (also known as immunochromatographic assay or immunocolloid gold immunoassay, ICG) has recently attracted the interest of researchers and industry.

The recent development of nanobiosensors has stimulated their application also to aflatoxin analysis: Many examples are reported, like DNA biosensor (Tombelli et al., 2009), electrochemical immunosensor (Linting et al., 2012), electrochemical sensor (Liu et al., 2006), fluorometric biosensor (Carlson et al., 2000).

Advantages of nanobiosensor techniques are reduction of extraction, clean-up analytical steps and global time of analysis (1 min or only few seconds); possibility of online automated analysis; low cost; skilled personnel not required. On the other side, sensitivity should be enhance and their stability improved to allow long-term use. Because of the ease of use of these devices, many commercial systems continue to develop not only for aflatoxins, but also for all mycotoxins

The goal of this study was to develop a rapid and simple detection method that was based on one-step membrane-based competitive DIGFA. Preparation and characterization of colloidal gold-Pab conjugates specific to

AFB1 and its use in developing a competitive DIGFA for on-site assessment of AFB1 were studied.

Materials and Methods

Tetrachloroauric (III) acid (HAuCl_4), sodium hydrogen carbonate, sodium chloride, albumin bovine serum (BSA), Triton-X, sodium azide and Anti-Aflatoxin B₁ polyclonal (Pab) antibody were purchased from Sigma (St. Louis, MO, USA), sodium citrate tribasic dihydrate (Merk), AFB1 Axxora (Bioscience, USA). Phosphate buffered saline (PBS) (Lonza, Germany). 0.2 μm Nitrocellulose membrane (NCM) and filter paper grade 4 (Whatman, UK).

Synthesis of gold nanoparticles(AuNPs).

Preparation of gold nanoparticles (AuNPs) was carried out by the reduction of tetrachloroauric acid with trisodium citrate according to Frens method (FRENS, 1973). In brief; 50 mL aqueous solution of 0.03 mM HAuCl_4 in flask was heated until boiling with vigorous continuous stirring for 10 min. Then 0.5 mL of 1% trisodium citrate solution was added with stirring until the solution color turned bright red. The solution was left to cool to room temperature with stirring. AuNPs was characterized by UV-Vis spectroscopy, particle size analyzer, HR-TEM...etc.

Preparation of AuNPs-Anti-AFB1 probe

The conjugation process between AuNPs and AFB1 antibody is pH and concentration dependent, Inadequate conditions will lead to flocculation and precipitation of AuNPs (Horisberger, 1990). Anti-AFB1-Pab was conjugated to gold nanoparticles under variable pH and Pab concentration, to determine the optimal conditions.

Effect of Pab concentration

For determination of the optimum amount of Anti-AFB1-Pab needed to coat the surface of AuNPs, a series of solutions of anti-AFB1-Pab were prepared in various concentrations (0–100 $\mu\text{g}/\text{mL}$) and 10 μL of Anti-AFB1-Pab for each concentration added to each well in a 96-well microplates containing 100 μL colloidal gold adjusted to pH 7.4. The solutions left to react for 2 h. The necessary amounts of Pab and of colloidal gold solution were calculated by characterization using UV-Vis spectroscopy, DLS (particle size analysis) and ELS (zeta potential) to determine the minimum amount of antibody required to functionalize and stabilize AuNPs.

pH effect on the conjugation process

100 μL of colloidal AuNPs having different pH values (3.5 to 11 increasing by 0.5 units) has been added to a series of aqueous solutions (4 μL) of anti-AFB1 at a concentration of 100 $\mu\text{g}/\text{mL}$. The pH of the AuNPs suspension adjusted by using 0.1 N HCl or 0.2 M Na_2CO_3 . The mixtures were allowed to react for an additional 2 h at room temperature and under shaking. The optimum pH for monitored by absorbance at 520 and 580 nm (Wang et al., 2011) and studied by variation in particle size and zeta potential.

Preparation of AuNPs-Pab conjugates (Gold Probe) under the optimal conditions

The optimum colloidal gold-Pab conjugates were prepared as represented in scheme (Fig.1.) as follows (Ye et al., 2010):- 20 mL of colloidal gold, adjusted to pH 7.4, was mixed with 800 μL of anti-AFB1- Pab (100 $\mu\text{g}/\text{mL}$) and allowed to react for 2 h. The residual surface of the gold particles was

blocked by adding 2 mL of 1% BSA in PBS and stirring for 30 min, and then stored at 4 °C for 2 h. Then, the conjugates were centrifuged (SORVALL –MX-150 centrifuge, Hitachi, Japan) at 14,000 rpm at 4 °C for 40 min and the supernatant is discarded. The pellet was dispersed in 5 mL 0.01 M PBS containing 1% BSA, 2% sucrose and 0.05% sodium azide. Centrifuge once again at 14,000 rpm at 4 °C for 40 min. After removing the supernatant, the final volume was adjusted to 2 mL and the conjugates solutions were stored at 4 °C until usage.

Characterization of AuNPs and gold-Anti-AFB1 probe

UV-Vis spectroscopy is routinely used for AuNPs- size and concentration determination (Haiss et al., 2007). Changes in the wavelength of surface plasmon resonance (SPR) absorption band provides information about concentration, shape, particle size and dielectric properties of medium (Henzie et al., 2006). An ultraviolet-visible-near infrared spectrophotometer (Cary5000 UV-Vis-NIR, Varian Instrument, U.K) have been used in the present study.

Both of zeta potential and Particle size distribution was determined using Zetasizer Nano series device (Malvern Instruments, U.K) by dynamic light scattering (DLS) technique and electrophoretic light scattering (ELS) technique studies performed in an aqueous solution and the Smoluchowski approximation in software was used to calculate the zeta potentials. High resolution transmission electron microscope (HR-TEM) imaging was performed by Tecnai G20 (FEI, Netherlands) operating at 200 kV. Samples were prepared by dropping 20 µL of AuNPs solution onto Formvar carbon coated copper grids placed over filter paper. Grid was subsequently dried and imaged.

For phase analysis of AuNPs, X-ray diffractometer (XRD) (X'Pert Pro X-ray diffraction system, Panalytical, Netherland) was used to investigate the crystal structure of AuNPs. The AuNPs centrifuged at 8000 rpm and washed three times with deionized (DI) water then pallet was dried in oven at 60 °C. Diffraction pattern has been recorded over a 2θ range of 30° -100°. The diffractogram obtained was compared to the standard database of the International Centre for Diffraction Data (ICDD).

Dot-Immunochromatography Flow-Through Assay (DIGFA) assembly

DIGFA used in this study was assembled according to (Wang et al., 2011) with modification: - 0.2 µm NCM has been dipped in DI water for 2 h, then dry in vacuum oven at 50 °C for 30 min. Two µL AFB1-BSA solution (1 mg of AFB1-BSA dissolved in 1 mL 0.01 M PBS containing 3% DMSO) dropped on NCM and left for 15 min at room temperature for drying. 50 µL of 2% BSA (diluted in PBS) was dropped on NCM and left to 15 min for blocking the nonspecific binding sites. Finally, washing three times with 0.02% Triton-X (diluted in 0.01M PBS) and left for drying in vacuum oven at 50 °C for 30 min. NCM was dropped with 10 µL of AuNPs-Pab conjugate and left to react (Fig.2.). Finally, the appearance of a reddish dot indicated the AuNPs-Pab conjugate was sufficient.

AFB1 Analysis in food sample

Analysis of AFB1 added to tested food samples was extracted as follows: samples of 1 kg were thoroughly mixed and 100 g subsamples were ground to powder by grinder. Twenty grams of powder (added to 1, 2, 5, 10, 20 ng/g of AFB1 respectively) was mixed with 60 mL of CHCl₃

(chloroform) by blending in aware blender. Extraction followed by shaking for 30 min and filtered through Whatman 4 filter paper. 6 mL filtrate was dried at 65 °C and dissolved in 2 mL 0.01 M PBS (containing 20% methanol). Quantitative analysis of AFB1 in samples was performed by DIGFA by a protocol similar to that for determining the sensitivity of colloidal gold-Pab conjugates.

To test the efficacy of the DIGFA of AFB1 in food samples, (24 rice-, 24 peanuts- and 24 maize samples) were purchased from the market. Analysis of AFB1 in these samples was performed by DIGFA. The AFB1 positive samples also have been analyzed by HPLC (Agilent 1200, Agilent, USA). Gold probe was stored at 4 °C and used to assay the AFB1 standard solution at 21 day intervals in order to test the stability of the gold probe for DIGFA.

Results and Discussion

Synthesis of gold nanoparticles

Gold nanoparticles (AuNPs) was prepared according to Frens method 1973 (FRENS, 1973) .The UV-Vis spectrum (Fig.3.A) displayed sharp absorbance peak at 525 nm, which represent the characteristic Surface Plasmon Resonance (SPR) absorbance peak of spherical AuNPs (Aryal et al., 2006). While the Gaussian distribution shape of the absorbance peak suggesting formation of AuNPs with no aggregation, uniformity and excellent dispersion in agreement with Perezjuste et al (Perezjuste et al., 2005).

This approach has been confirmed by both Dynamic Light Scattering for particle size distribution and Transmission Electron Microscopic imaging.HR-TEM imaging (Fig.3.B) indicate with no doubt formation of spherical AuNPs with average particle size 32.3 ± 4.82 nm, the size of particles has

been measured using ImageJ software (version1.46r). Also, The corresponding Selected Area Electron Diffraction (SAED) pattern inset confirms formation of cubic crystal structure of AuNPs. Dynamic Light Scattering (DLS) measurements for particle size distribution analysis (Fig.3.C) display average hydrodynamic particle size 30.7 ± 8 nm with acceptable polydispersity index (PDI) which is equal to 0.106 indicating monodispersity and excellent dispersion of AuNPs solution. Electrophoretic Light Scattering (ELS) for zeta potential analysis of prepared AuNPs is equal to -39 mV which is due to encompassing surface of AuNPs with citrate-, (which act as a reducing agent and stabilizing agent), chloride and sodium anions. Zeta potential indicates degree of repulsion between nanoparticles, and thus can be used as a sign of stability of AuNPs. So, if the particles have low zeta potential (less than -25 values) then there is no hindrance technique for particles flocculation (Horisberger, 1990).

A typical XRD (Fig.3.D) pattern for AuNPs has been shown for phase formation and exhibits diffraction peaks (Bragg's reflections), which were indexed on the basis of face-centered cubic gold structure. The diffraction peaks (111), (200), (220), (311), (222) and (400) corresponding to 38.14° , 44.39° , 64.61° , 77.58° , 81.13° and 98.17° 2θ angles respectively. This is in agreement with reference pattern card no: (04-003-3089) and confirmed that synthesized AuNPs were of crystalline nature with face-centered cubic gold structure. Also, broadening of Bragg's peaks provide additional indication of the formation of gold in nano size. Finally, characterization results confirm the formation of spherical AuNPs with average particle size 30 ± 5.41 nm and have zeta potential -39 mV.

Preparation of Anti-AFB1-auNPs conjugate

Anti-AFB1-gold nanoparticles probe (AuNPs-Pab) was prepared by physical conjugation between AuNPs and polyclonal antibody of AFB1 (Pab) at optimum pH and optimum antibody concentration according to method described by (Ye et al., 2010) as mentioned in methodology section.

Determination of optimum Pab concentration

It is important to determine the optimum antibody concentration required to be loaded on AuNPs surface to avoid over excess or deficiency of antibody. The AuNPs- Pab conjugate was formed in solution as a result of balance between electrostatic repulsion force and London-van der Waals attraction force among the particles and antibody molecules (Ye et al., 2010).

Fig.4. (A) shows that AuNPs and AuNPs-Pab conjugates exhibit strong and narrow absorption peak at 525 and 531 nm respectively. After addition of Pab, SPR absorption band broadened, absorption intensity decreased and red shifted occurred due to interaction of Pab with AuNPs (Ye et al., 2010). The characteristic antibody peak exhibited at 280 nm is due to tryptophan group of Pab (Xiulan et al., 2005). Upon the conjugation process a decrease in the intensity of the characteristic antibody peak occurred.

The minimum shift in SPR absorbance (about 5 nm) have been reached at 40 μ g/mL (Fig.4.B) which provides stable conjugate without affecting AuNPs shape or suspension status. The anti-AFB1 concentration increases the stability of the AuNPs and no aggregation affect the conjugate, till it reaches the optimum

antibody concentration, then excess of the antibody will lead to increase of the size that reflect presence of aggregation in the conjugate (Fig.4.(C,D&E)).

Stable conjugate produced at pH 7.4 which gives the minimum SPR shift about 5 nm (Fig.5.B) accompanied by stable conjugate. Colloidal gold stabilized and coated by Pab and did not make any aggregation or flocculation (Fig.5.A) as appear in difference in Absorbance intensity at (A_{520} - A_{580}) (Ali et al., 2014; Xiulan et al., 2005). Therefore it is better to make reaction at the isoelectric point of antibody or little bit above the isoelectric point because pH affect particle size of conjugate as it shown in Fig.5.C

Fig.5. (D) shows that the maximum negative charge obtained at pH 7.4. The -ve charges surround the AuNPs increase until reach the isotonic point of the antibody pH= (7-8) at which the optimal physiological condition for the antibody interaction with AuNPs is obtained.

The results revealed that the conjugation process between anti-AFB1-Pab and AuNPs is very sensitive to pH and antibody concentration, as these affect the antibody affinity and antigenicity.

Validation of AFB1 in standard samples

The optimized DIGFA sensor was validated by testing its sensitivity using various concentrations of AFB1 (0, 1, 2, 5, and 10 ng/ml) in prepared buffer PBS/DMSO (70:30% V/V). For a negative sample there will be a visible red dot in the center of the membrane. The dot color intensity was visually compared to that of the negative control which showed the most intense red dot because AFB1 concentration is inversely related to color intensity.

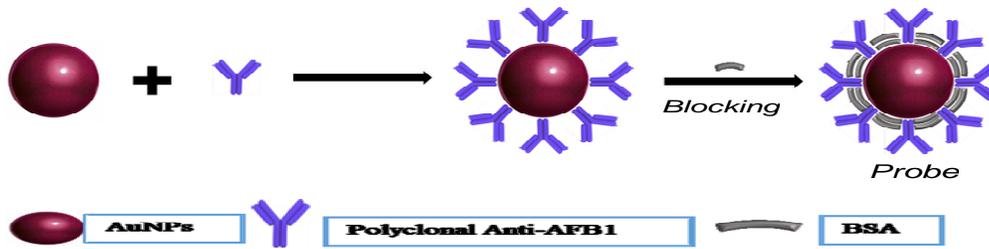


Fig.1. Schematic represent of conjugation process for probe preparation.

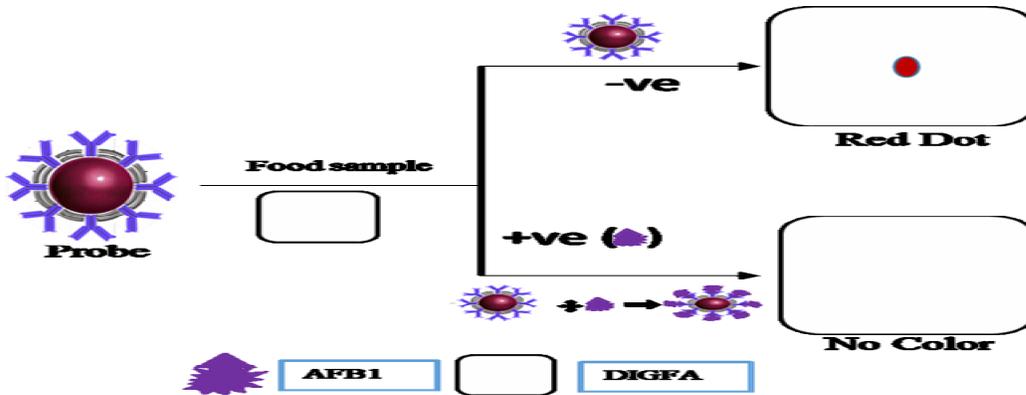


Fig.2. Schematic represent of food sample analysis and appearance. DIGFA

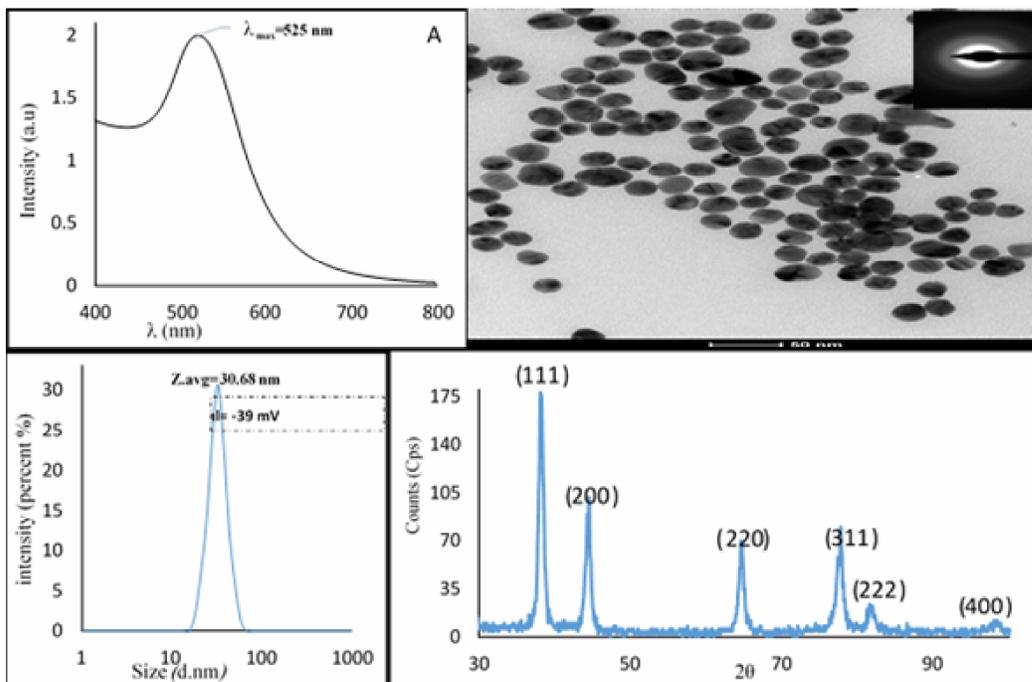


Fig. 3. (A) UV-Vis spectrum -, (B) TEM micrograph for AuNPs at high magnification with inset represents SAED pattern-, (C) Size distribution -, and (D) XRD pattern of AuNPs.

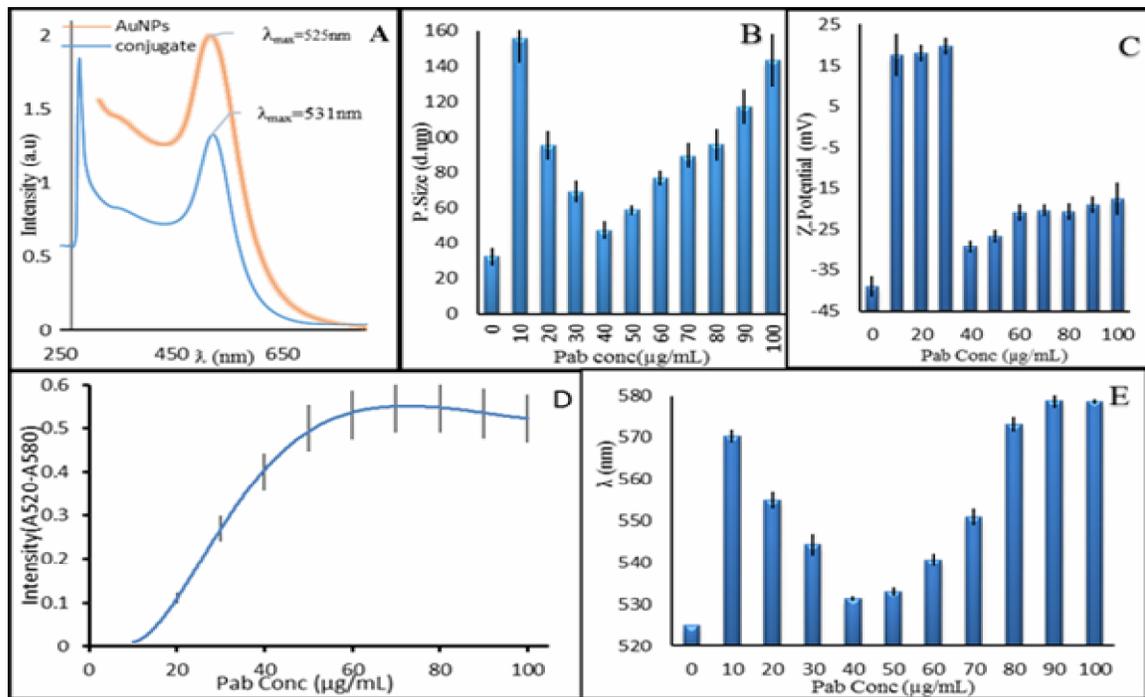


Fig. 4. (A) Comparison between UV-Vis spectrum of AuNPs and AuNPs-Pab conjugate, (B) Variation of particle size distribution- and (C) zeta potential with Pab concentration, (D) Effect of Pab concentration on the absorbance of Pab characteristic peak at 280nm and subtraction of absorbance intensity of conjugate at A_{520}/A_{580} , and (E) red shift SPR absorbance peak of AuNPs upon conjugation process. Error bars are standard deviation of the mean of the minimum of three different samples.

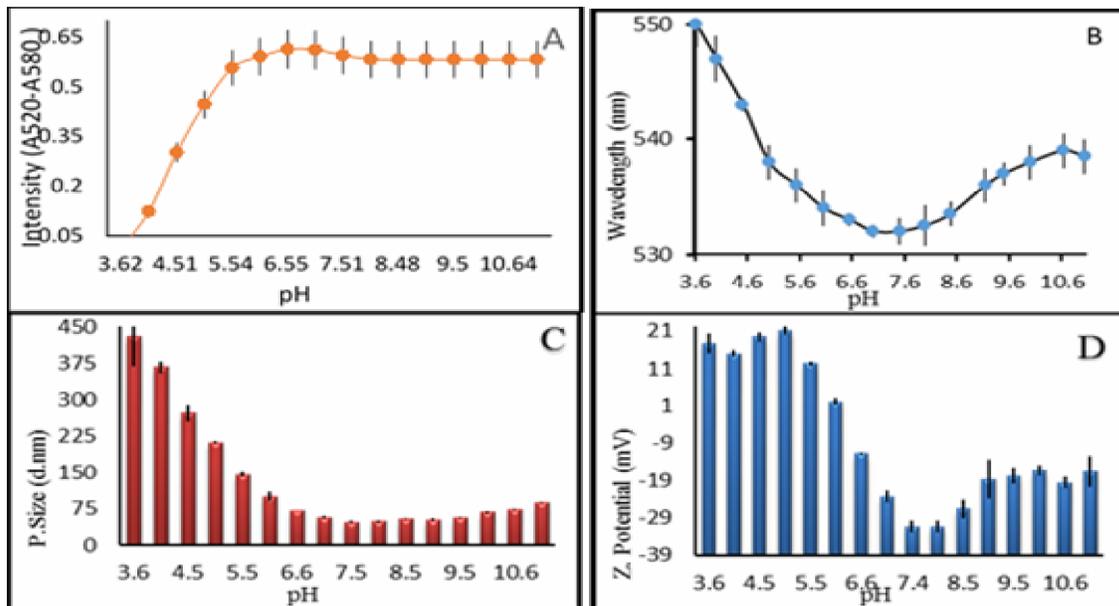


Fig. 5. (A). Effect of pH on red shift of AuNPs (A_{520}/A_{580}). (B) shift of absorbance peak of AuNPs conjugate with anti-AFB1-Pab, Effect of pH on particle size and zeta potential of conjugate. Error bars are standard deviation of the mean of the minimum of three different samples.

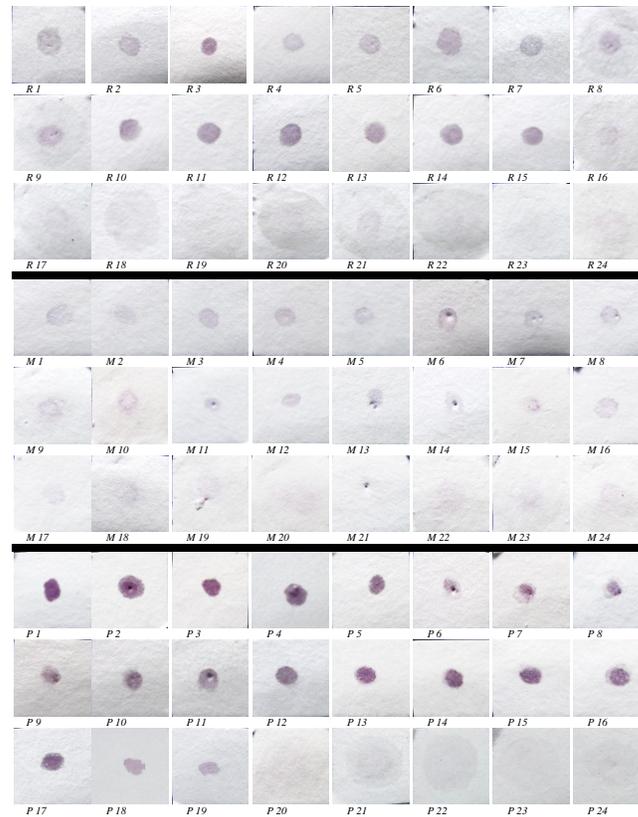


Fig.6. Food samples analyzed by DIGFA

Table.1 Comparison between DIGFA& HPLC

Sample type	No. of samples	DIGFA		HPLC	
		+ve	-ve	+ve	-ve
Rice	24	9	15	9	15
Maize	24	8	16	8	16
Peanuts	24	5	19	5	19

The smallest AFB1 concentration that resulted in no color development was considered as the minimum visual detection limit. Seventy two food and feed samples, including rice, peanuts and maize analyzed by DIGFA and results as shown in figure (6).

The results of the food samples tests showed that twenty two samples were positive and in agreement with that of HPLC as shown in table 1. AFB1 was spiked to buffered samples that were free from AFB1, confirmed by HPLC, and other samples of concentrations equal to 1,

2-, 5- and 10 ng/mL, respectively. The concentration of the antibody used in conjugation was 40 µg/mL, the sensitivity for AFB1 detection was 2 ng/mL (by visual observation). Each experiment repeated three times. Results showed that DIGFA results correlated well to HPLC results and it gives DIGFA sensitivity 100%, concentration of AFB1 in samples was 15-57 ng/mL. The probe was stable for 6 months at 4 °C.

Performance of the DIGFA depends on the following factors: - external chemical properties of gold nanoparticles, affinity of the detection antibody and intensity of formed dot as a response to AFB1 concentration. The similarity between the sensitivity of DIGFA and HPLC holds significant promise for rapid and accurate AFB1 detection in the field with advantages of no cost relative to other techniques, no requirement of any specific instruments. Easiest applicability, saving-time and material, no need to expert person to perform the analysis and single-step assays that require only the addition of a sample represent extra advantages of the present nanobiosensor. Finally Dynamic Light Scattering and Electrophoretic Light Scattering techniques can be used as characterization methods for optimizing the preparation of conjugate with minimum error compared with NaCl method which depend on person opinion.

References

- Adegoke, G.O., Letuma, P., 2013. Strategies for the Prevention and Reduction of Mycotoxins in Developing Countries, in: Mycotoxin and Food Safety in Developing Countries. InTech, pp. 123–136.
- Ali, M.A., Eldin, T.A.S., Moghazy, G.M. El, Tork, I.M., Omara, I.I., 2014. Detection of *E. coli* O157:H7 in feed samples using gold nanoparticles sensor. *Int.J.Curr.Microbiol.App.Sci* 3, 697–708.
- Aryal, S., Bahadur K. C, R., Bhattarai, S.R., Prabu, P., Kim, H.Y., 2006. Immobilization of collagen on gold nanoparticles: preparation, characterization, and hydroxyapatite growth. *J. Mater. Chem.* 16, 4642–4648. doi:10.1039/B608300E
- Bhunja, A.K., 2008. Biosensors and bio-based methods for the separation and detection of foodborne pathogens. *Adv. Food Nutr. Res.* 54, 1–44. doi:10.1016/S1043-4526(07)00001-0
- Caldas, E.D., Silva, S.C., Oliveira, J.N., 2002. Aflatoxins and ochratoxin A in food and risks to human health. *Rev. Saude Publica.*
- Carlson, M. a, Bargerion, C.B., Benson, R.C., Fraser, a B., Phillips, T.E., Velky, J.T., Groopman, J.D., Strickland, P.T., Ko, H.W., 2000. An automated, handheld biosensor for aflatoxin. *Biosens. Bioelectron.* 14, 841–848.
- Cleveland, T.E., Dowd, P.F., Desjardins, A.E., Bhatnagar, D., Cotty, P.J., 2003. United States Department of Agriculture—Agricultural Research Service research on pre-harvest prevention of mycotoxins and mycotoxigenic fungi in US crops. *Pest Manag. Sci.* 59, 629–642. doi:10.1002/ps.724
- E.C, 2006. Council of the European Union 2006a 7775/1/06 REV 1. Brussels.
- E.C., 2012. Commission Regulation (EC) No 1881/2006 of 19 December 2006

- setting maximum levels for certain contaminants in foodstuffs.
- FRENS, G., 1973. Controlled Nucleation for the Regulation of the Particle Size in Monodisperse Gold Suspensions. *Nature* 241, 20–22. doi:10.1038/10.1038/physci241020a0
- Gelderblom, W.C., Snyman, S.D., Abel, S., Lebepe-Mazur, S., Smuts, C.M., Van der Westhuizen, L., Marasas, W.F., Victor, T.C., Knasmüller, S., Huber, W., 1996. Hepatotoxicity and -carcinogenicity of the fumonisins in rats. A review regarding mechanistic implications for establishing risk in humans. *Adv. Exp. Med. Biol.* 392, 279–296.
- Haiss, W., Thanh, N.T.K., Aveyard, J., Fernig, D.G., 2007. Determination of Size and Concentration of Gold Nanoparticles from UV–Vis Spectra. *Anal. Chem.* 79, 4215–4221. doi:10.1021/ac0702084
- Henzie, J., Shuford, K.L., Kwak, E.-S., Schatz, G.C., Odom, T.W., 2006. Manipulating the optical properties of pyramidal nanoparticle arrays. *J. Phys. Chem. B* 110, 14028–14031. doi:10.1021/jp063226i
- Horisberger, M., 1990. Quantitative aspects of labelling colloidal gold with proteins in immunocytochemistry, in: Zulauf, M., Lindner, P., Terech, P. (Eds.), *Trends in Colloid and Interface Science IV SE - 32*, Progress in Colloid & Polymer Science. Steinkopff, pp. 156–160. doi:10.1007/BFb0115544
- Kolosova, A.Y., Sibanda, L., Dumoulin, F., Lewis, J., Duveiller, E., Van Peteghem, C., De Saeger, S., 2008. Lateral-flow colloidal gold-based immunoassay for the rapid detection of deoxynivalenol with two indicator ranges. *Anal. Chim. Acta* 616, 235–244. doi:10.1016/j.aca.2008.04.029
- Lai, W., Fung, D.Y.C., Yang, X., Renrong, L., Xiong, Y., 2009. Development of a colloidal gold strip for rapid detection of ochratoxin A with mimotope peptide. *Food Control* 20, 791–795. doi:10.1016/j.foodcont.2008.10.007
- Linting, Z., Ruiyi, L., Zajun, L., Qianfang, X., Yinjun, F., Junkang, L., 2012. An immunosensor for ultrasensitive detection of aflatoxin B1 with an enhanced electrochemical performance based on graphene/conducting polymer/gold nanoparticles/the ionic liquid composite film on modified gold electrode with electrodeposition. *Sensors Actuators B Chem.* 174, 359–365. doi:10.1016/j.snb.2012.06.051
- Liu, Y., Qin, Z., Wu, X., Jiang, H., 2006. Immune-biosensor for aflatoxin B1 based bio-electrocatalytic reaction on micro-comb electrode. *Biochem. Eng. J.* 32, 211–217. doi:10.1016/j.bej.2006.10.003
- Perezjuste, J., Pastorizasantos, I., Lizmarzan, L., Mulvaney, P., 2005. Gold nanorods: Synthesis, characterization and applications. *Coord. Chem. Rev.* 249, 1870–1901. doi:10.1016/j.ccr.2005.01.030
- Santacroce, M., Conversano, M.C., Casalino, E., Lai, O., Zizzadoro, C., Centoducati, G., Crescenzo, G., 2008. Aflatoxins in aquatic species: metabolism, toxicity and perspectives. *Rev. Fish Biol. Fish.* 18, 99–130. doi:10.1007/s11160-007-9064-8
- Sibanda, L., Marovatsanga, L.T., Pestka, J.J., 1997. Review of mycotoxin work in sub-Saharan Africa. *Food Control* 8, 21–29.

doi:[http://dx.doi.org/10.1016/S0956-7135\(96\)00057-6](http://dx.doi.org/10.1016/S0956-7135(96)00057-6)

- Tombelli, S., Mascini, M., Scherm, B., Battacone, G., Migheli, Q., 2009. DNA biosensors for the detection of aflatoxin producing *Aspergillus flavus* and *A. parasiticus*. *Monatshefte für Chemie - Chem. Mon.* 140, 901–907. doi:10.1007/s00706-009-0137-3
- Wang, J., Liu, B., Hsu, Y., Yu, F., 2011. Sensitive competitive direct enzyme-linked immunosorbent assay and gold nanoparticle immunochromatographic strip for detecting aflatoxin M1 in milk. *Food Control* 22, 964–969. doi:10.1016/j.foodcont.2010.12.003
- Xiulan, S., Xiaolian, Z., Jian, T., Zhou, J., Chu, F.S., 2005. Preparation of gold-labeled antibody probe and its use in immunochromatography assay for detection of aflatoxin B1. *Int. J. Food Microbiol.* 99, 185–194. doi:10.1016/j.ijfoodmicro.2004.07.021
- Xu, Y., Huang, Z.-B., He, Q.-H., Deng, S.-Z., Li, L.-S., Li, Y.-P., 2010. Development of an immunochromatographic strip test for the rapid detection of deoxynivalenol in wheat and maize. *Food Chem.* 119, 834–839. doi:10.1016/j.foodchem.2009.08.049
- Ye, Y., Zhou, Y., Mo, Z., Cheng, W., Yang, S., Wang, X., Chen, F., 2010. Rapid detection of aflatoxin B(1) on membrane by dot-immunogold filtration assay. *Talanta* 81, 792–798. doi:10.1016/j.talanta.2010.01.017.