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Simple Colorimetric DNA Biosensor Based on Gold Nanoparticles for Pork Adulteration Detection in Processed Meats

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Abstract: In order to develop simple sensing system for pork adulteration in processed meats, the DNA biosensor based on 20 nm gold nanoparticles (AuNPs) with aggregation property has been developed as a simple colorimetric detection of target DNA. Adsorption of single-stranded (ss) DNA on AuNPs protects the particles against salt-induced aggregation. However, mixing and annealing of a 27-nucleotide (nt) ssDNA probe on AuNPs with denatured DNA of different processed meats differentiated between perfectly matched and mismatch hybridization at a critical annealing temperature (55 °C). The AuNPs change color from red to purple, in 10 mM phosphate buffer saline (PBS). At a hybridizing temperature (55 °C), non-target mismatched DNA provided hybridization products allowing probe to be free and adsorbed to AuNPs. This prevented AuNPs from salt-induced aggregation as the color still red. In matched DNA, hybridization would occurred, allowing probe to be occupied. This facilitated AuNPs from salt-induced aggregation and induced colorimetric change of particles from red to purple. These signals could be observed easily with naked eye. This label-free DNA nanobiosensor should find applications in food analysis and other DNA based screening.

Keywords: Colorimetric sensors, DNA biosensor, Gold nanoparticles, Pork adulteration, Processed meats.

1. Introduction

Detection of specific sequences of DNA has important applications in medical and diagnosis [1, 2], food analysis [3, 4] and forensic study [5]. Usually, the detection of DNA specific sequence via hybridization has been perform after the DNA target has been amplified by polymerase chain reaction (PCR). PCR is widely used to selectively amplify a segment of longer DNA from as little as a single copy to easily detectable quantities. The application of PCR addresses sensitivity issues and requires sample purification steps [3]. For these main features, PCR amplification has become a mandatory step for the analysis of genomic DNA. Although, there are many sensing approaches available without PCR amplification, such as surface plasmon resonance (SPR) [6], fluorescent microarrays [7], semiconductor [8], nanoparticle or polymer based biosensors [9, 10]. Besides PCR, most assays identify DNA specific sequence via hybridization of an immobilized DNA probe toward a target DNA after the target has been linked with a label or tag [11]. DNA detection schemes that avoid labeling or tagging such as surface plasmon resonance [12], ellipsometry [13], and lately, an assay using functionalized gold nanoparticles [14, 15] have been developed. Generally, these approaches use surface chemistry functionalization and usually require sophisticated instrumentation system for detection of hybridization even. On the other hand as reported in the literature, there is a difference in electrostatic properties of single- and double-stranded DNA (ssDNA and dsDNA) that can be used to design a colorimetric sensor to detect DNA hybridization [16, 17, 18]. Here, the color change derives from colloidal gold particle aggregation, with no modification of the gold or the ssDNA probe or target DNA is required. Furthermore, hybridization is completely separate from detection so that it can be done under optimal conditions and makes the method more efficient and effective.

The color change derives from colloidal gold particle aggregation occurs due to ssDNA and dsDNA have different electrostatic properties, which resulted the distinction on their surface plasmon resonance (SPR) characters of aggregated dsDNA–AuNPs and disperse colloidal ssDNA-AuNPs, which are interesting as they can be monitored by colorimetry and also observed easily by naked eye [11-19]. The essential difference arises as ssDNA can uncoil to expose its bases sufficiently, whereas dsDNA has a stable double-helix structure that presents the negatively charged phosphate backbone [20]. Typically, in solution AuNPs are stabilized by adsorbed negative ions (e.g., citrate ions) whose repulsion prevents the strong van der Waals attraction between AuNPs from aggregation [21]. In dsDNA, repulsion between its charged phosphate backbone and the adsorbed citrate ions dominates the electrostatic interaction between AuNPs and dsDNA so that dsDNA will not adsorb. While in ssDNA, is sufficiently flexible to uncoil its bases partially, they can be exposed to the AuNPs. Under these conditions, the negative charge on the backbone is sufficiently distant so that attractive van der Waals forces between the bases and the AuNPs are sufficient to make ssDNA to stick to the gold, thus make them still as colloidal nanoparticles. However, this mechanism is not applied with dsDNA, due to the duplex structure does not permit the uncoiling needed to expose the bases, and thus make them in aggregated nanoparticles [11].

In this article, the selective adsorption of ssDNA on AuNPs was employed as a basic mechanism for calorimetric detection of specific DNA sequence in processed meat, i.e. meatballs. This is due to the fact that adsorption of ssDNA stabilizes AuNPs against aggregation at high concentrations of salt [11-13]. Since, the color of AuNPs is determined principally by SPR, and affected by aggregation of AuNPs drastically [22], therefore, we used this mechanism to design a simple colorimetric sensor for detection of DNA hybridization. The sensor can be used for sequence-specific detection of unlabelled DNA by using unmodified commercially available 20 nm AuNPs [23, 24]. The DNA nanobiosensor was applied to detect pork DNA in processed meat (i.e. pork in beef and chicken meatballs) as a pork adulteration issue in meatballs increases currently. The sensor is easy to implement for visual detection and successfully applied to detect hybridization between ssDNA probe and it's complementary. The assay also directly detected pork DNA in genomic DNA mixtures extracted from processed meats, avoiding PCR amplification, electrophoresis and sample purification chemistry. Thus, it does not need any instrument or surface modification chemistry of nanoparticles and directly detects hybridization even in non-amplified mixed genomic DNA.

2. Materials and Methods

2.1. Chemicals

Design of porcine-specific oligoprobe was chosen using a 27-nt AluI-cut fragment (428–454 bp) of porcine (Sus scrofa) cytb gene (GenBank accession no. Gu135837.1 in NCBI data base) and used as a porcine-specific marker. This fragment demonstrated a high degree of polymorphism between the species and similarities within the species by NCBI-BLAST analysis against non-redundant nucleotide collections and also by ClustalW alignment analysis. The probe and synthetic targets (complementary, non-complementary) were supplied by the 1st Base, Malaysia. The probe and oligosequences are shown in Table 1. All other chemicals used were of analytical grade or of the highest purity available and were purchased from Sigma-Aldrich (USA). All glassware was cleaned thoroughly with freshly prepared aqua regia (3:1 (v/v) HCl/HNO_3) and rinsed thoroughly with Milli-Q water prior to use. Milli-Q water was used to prepare all the solutions in this study.

Table 1. Sequences of oligonucleotides used.

Name	Sequence (5'→ 3')
Probe	CTG ATA GTA GAT TTG TGA CCG TAG
Complementary target	CTA CGG TCA CAA ATC TAC TAT CAG
Non-complementary target	ACG TAA CTG CTG TGG CCT GGT CGC TGA

2.2. Preparation of Meatballs and DNA Extraction

In order to detect pork adulteration in processed meat products, meatballs are used as an example. Meatballs are prepared according to Kuswandi et al. [25] both with pure and mixed emulsified meats of pork, beef, and chicken, along with the addition of starch, seasonings, and salts in certain ratios. All the meatballs are cooked in boiling water for 20 min prior to DNA extraction. DNA extraction is performed from 100 mg of cooked meatball of each formulations using

MasterPure DNA Purification Kit (Epicenter Biotechnologies, USA) as per the manufacturer instructions. The DNA concentration is determined with a biophotometer (Eppendorf, Germany) based on triplicate readings. The purity (A260/A280) of all DNA samples used in all experiments is 2.0 ± 0.05.

2.3. DNA Nanobiosensors Preparation

A 27 nt pork specific probe (5')- CTA CGG TCA CAA ATC TAC TAT CAG -(3') was employed was used as porcine specific probe. A gold nanoparticles (AuNPs) 20 nm in size in water solution were purchased from Sigma-Aldrich (USA). The DNA probe–AuNPs binding was performed as follows. In brief, 30 µL of 27-mer ssDNA of 100 nM was added to a mixture of 100 µL of 1.8 nM colloidal AuNPs. After mixing, they were incubated in a water bath at 50 °C for 3 min to facilitate DNA probe adsorption onto AuNPs. The final products were loose red pellets and ready used as the DNA nanosensor.

In order to test that DNA probe was successfully adsorb onto AuNPs, the DNA nanosensor was tested by adding 100 µl of 10 mM PBS was added in vial (c) (inset Fig. 1), and 50 µl complementary target products (500µg/ml) were added in vial (d), where both contain 400 µl of the DNA nanobiosensor (100 nM). Finally, 100 µl of 10 mM PBS was added to vial (d) (inset Fig. 1). The final volume was adjusted to 1 ml with DI (deionized) water (18.2 MΩ) and the absorption spectra were measured by UV–vis spectroscopy (Ocean Optics® USB2000, Dunedin, FL, USA) along with absorption spectra of AuNP colloids in DI water (a), and in 10 mM PBS buffer (b) as shown in Fig. 1 as comparison.

2.4. Complementary and Non-complementary Target Detection

For hybridization and mismatch detection, 50 µl complementary target products (500µg/ml) and 400 µl of DNA nanobiosensor (100 nM) were added in vials (a) and (b) as given in Fig. 3. Equal volumes of non-complementary target and DNA nanobiosensor were added in vials (c) and (d) as given in Fig. 3 respectively. The mixtures were denatured at 95 °C for 3 min and then annealed at 55 °C for 2 min. Finally, 100 µl of 10 mM PBS was added to each vial (Fig. 3). The final volume was adjusted to 1 ml with DI water and the absorption spectra were measured by UV–vis spectroscopy.

2.5. Detection of Pork in Processed Meat Sample

In order to detect pork adulteration in processed meat products, meatballs were used as a model. The meatballs were prepared by emulsifying the mixed meatballs of pork-beef and pork-chicken, binary mixtures in 1:1 (w/w) ratios. Pure meatballs of each species (pork, beef and chicken) are formulated with pure meats under identical conditions. After 20 min of cooking in boiling water, DNA extractions are performed. Approximately 100 mg mixed and pure meatball samples from each specimen were autoclaved at 120 °C for 30 min at a pressure of 3 bar. By this treatment, all samples lost their normal textures and turned into a liquid broth. DNA extraction was performed as described previously. One hundred microliters (100 µl) of mixed genomic DNA (200 µg/ml) was taken in vials (a)–(c) as shown in Fig. 4. Equal amounts of pure genomic DNA (pork, beef and chicken) were taken in vials (d), (e) and (f) as shown in (Fig. 4). All vials were incubated with 250 µl of DNA nanosensor at 95 ◦C for 3 min and then annealed at 55 ◦C for 2 min. Finally, 100 µl of 10 mM PBS was added to each vial (Fig. 4). The final volume was made up to 1 ml with DI water and the spectra were characterized by absorption spectroscopy.

2.6. Determination of the Limit of Detection

To determine the LOD (limit of detection), pork meatball and beef meatball was mixed at concentration 1 %, 5 %, 10 %, 15 %, 20 % and 30 % (w/w) as well 100 % (pure pork). DNA extraction was performed from the mixed meatballs and 100 µl of mixed DNA (300 µg ml-1), extracted from the above specimens, was taken in six separate vials marked. All vials were incubated with 15 µl of (100 nM) DNA nanobiosensor (27-nucleotide shown in Table 1) at 95 °C for 3 min and then annealed at 55 °C for 2 min. Finally, 100 µl of 10 mM PBS was added to each vial. The final volume was made up to 1 ml with DI water and the absorbance intensity was measured at 525 nm by visible absorption spectroscopy. The concentration of porcine DNA in mixed samples at concentration 1 %, 5 %, 10 %, 15 %, 20 % and 30 % (w/w) is calculated to be 0.4, 2.0, 4.0, 6.0, 8.0 and 1.2 µg/ml respectively.

3. Results and Discussion

3.1. Characterization of DNA Nanobiosensor

The particle size (20 nm) was employed according to previously proposed methods [24, 26]. The vials in Figs. 1(a) and (c) revealed the distribution of disperse small particles in the bulk sample as shown by the red color. The aggregates (one on another) in vials were seen after addition of the salt (Figs. 1(b) and (d)) as shown by the purple color. Visible of absorption spectra of disperse colloidal (non-aggregated) and aggregated 20 nm AuNPs in DI water and 10 mM PBS are shown in Fig. 1. The aggregated particles exhibited a plasmon shift from 525 nm to 700 nm as seen in Figs. 2b and 2d while no such a change was observed in non-aggregated ones (Figs. 2a and 2c). Naturally, this surface plasmon resonance mode, which gives colloidal gold (non-aggregated), its characteristic red color, is well understood, as it depends on particle size and shape [3, 15, 16, 27-29]. However, the aggregated colloidal particles in this report lost the absorption peak at 525 nm in PBS as they turned from red to purple.

The visible spectra of AuNPs in PBS lost its absorption peak at 525 nm, which indicating aggregation as the red color turn to purple. The procedure for determining the critical concentration of salt can be found elsewhere [27]. The hybridization between complementary targets DNA toward porcine probe DNA could not prevent salt-induced aggregation of AuNPs. This was also confirmed by the loss of the 525 nm peak (spectrum (d) in Fig. 1). However, a slightly reduced level of aggregation was observed in the visible spectrum (Fig. 1, spectrum (d)). The aggregation reduction could be due to the presence of some ssDNA that not all react with complementary target during hybridization reaction, which might interfere with the aggregation. This was also reported elsewhere [19]. The spectrum of AuNPs immobilized with porcine probe ssDNA (DNA nanobiosensor) produced a less intense peak at 525 nm, indicating some degree of aggregation upon the addition of salt. This was revealed by the higher base line in the region of 620–800 nm in the absorption spectrum (Figs. 1b and 1d).

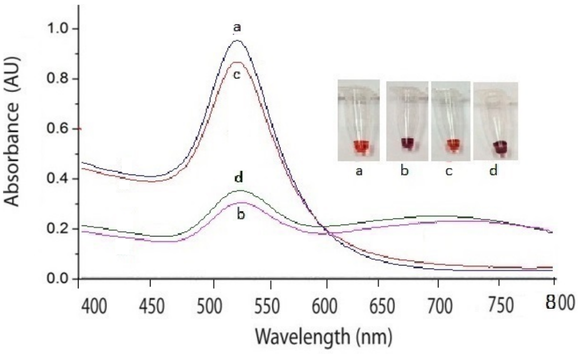


Fig. 1. Absorption spectra of disperse and aggregated AuNPs. Shown are absorption spectra of AuNP colloids in DI water (a), in 10 mM PBS buffer (b) and in 10 mM PBS buffer with porcine probe-AuNPs colloids (c), and in after reaction with complementary target DNA (d). Insets are the color photographs of AuNP colloids in DI water (a), PBS buffer (b), PBS buffer in porcine probe-AuNPs (c) and PBS buffer after hybridization between porcine probe-AuNPs with complementary target DNA (d).

3.2. Complementary and Non-complementary Target Detection

Colloidal AuNPs is bio-compatible and commercially available at reasonable prices for desirable sizes. Therefore, AuNPs used to identify species specific sequences in complementary target products before applied to detect genomic DNA, in order to proof the mechanism of the method that it could detect complementary and non-complementary target detection as described in the Fig. 2.

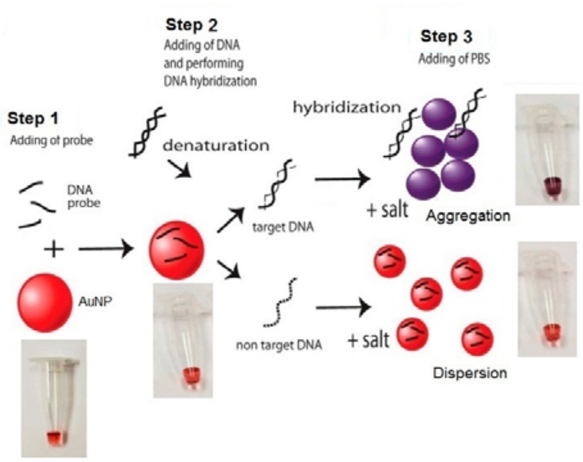


Fig. 2. Schematic of a colorimetric DNA detection based on AuNPs.

A 27-mers probe that had 100 % matches with complementary target product and mismatch with non-complementary target were used as given in Fig. 3 (see inset). The key to the successful DNA detection was the control of particle dispersion and aggregation with complementary target DNA products of interest and DNA probe. Addition of single-strand DNA probes to AuNPs as DNA nanobiosensor, as the first step allowed the adsorption of the probes on the surface of AuNPs via nitrogenous base/gold van der Waals forces [17]. Here, it was found that at a critical temperature (55 °C), porcine-specific probe (27-mer ssDNA) and its complementary target product was hybridized and could not prevent from salt-induced aggregation of AuNPs (vial (a) and (b) in the inset of Fig. 3), and it is used DNA hybridization detection. However, the porcine-specific probe was not hybridized from the non-complementary products and used as mismatch detection. Therefore, the detection of DNA hybridization and mismatch were observed after the addition of salt at a critical concentration (0.2 M NaCl), where hybridized probe and non-hybridized probes (27-mer ssDNA) interact with AuNPs to form aggregation and non-aggregation, respectively. This produces a rapid and drastic color change in the reaction mixtures, which can be detected by nude eye (vial (a) and (b) in the inset of Fig. 3) without the need of any instrumentation.

The visible spectra of AuNPs in PBS lost its absorption peak at 525 nm, which indicating aggregation as the red color turn to purple. In this case, it clearly observed that absorption at 525 nm gradually decreased, and between 620 and 800 nm increased, as the particles undergo aggregation. A slight variation in spectral shape and base line was observed between the samples, which could be explained for different dielectric properties of the solution due to different level of nucleic acids, proteins or other impurities [28, 29]. In addition, experimental errors (e.g. pipetting error etc.) could also contribute to this variation. However, this variation was not affected to our visual detection. Furthermore, visible absorption spectroscopy strongly supported the visual observation through the spectral intensity at 525 nm and 700 nm (Fig. 3).

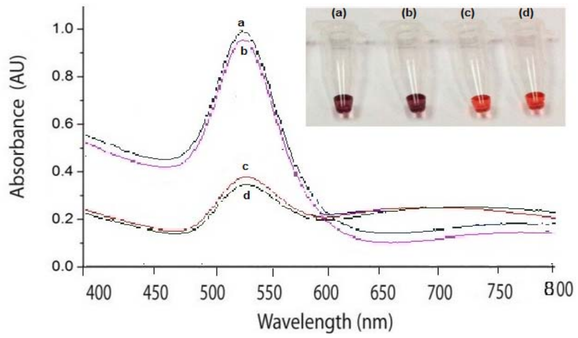
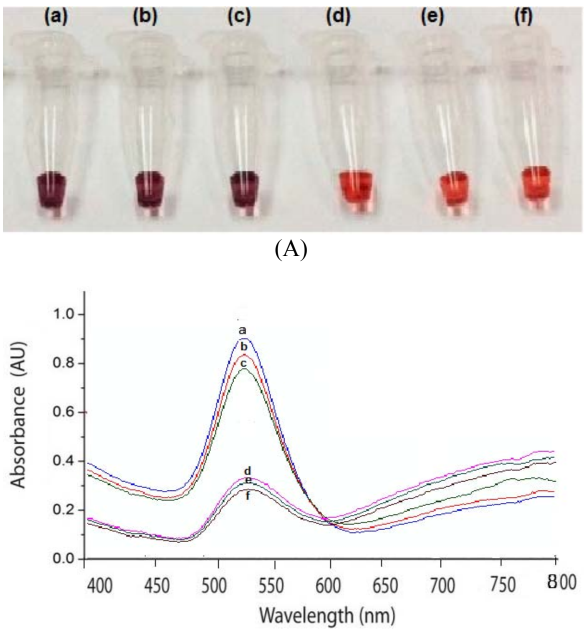


Fig. 3. Identification of complementary target as nucleotide hybridization and non-complementary target as nucleotide mismatch by porcine probe AuNPs. In the illustration vials (a) and (b) represent the color of gold colloids in complementary target product annealed with the porcine probe DNA at 55 °C, and vials (c) and (d) shows the same in non-complementary target products respectively, annealed with the same probe at the same temperatures. The sequence of the corresponding spectra is also shown alphabetically.

3.3. Detection of Pork in Processed Meat Sample

The porcine-specificity of the probes was confirmed by detecting porcine DNA in binary admixtures (1:1 w/w) of pork–beef, pork–chicken and beef–chicken DNA extracted from autoclaved mixed meatballs, and the results are given as shown in Fig. 4A and its corresponding absorption spectra are given in Fig. 4B. The purpose of autoclaving was to see whether partially degraded DNA could be detected by AuNPs, as several literature reports have demonstrated that such treatment breaks down genomic DNA into small fragments [8, 10]. In addition, a certain level of genomic DNA degradation is also obvious during physical and chemical processing of meat and processed meat products [8, 10]. Therefore, the procedure for sample preparation in heat-treated and pressurized processed meat mixture was appropriate to detect specific DNA sequences. Incubation of the nano-biosensor (27-mer ssDNA probe-AuNPs) with pure and mixed genomic DNA, clearly demonstrated that only the porcine DNA-containing vials (Fig. 4A (a)–(c)) changed colour from red to purple, reflecting hybridization of the probe to the porcine genomic DNA at 55 ˚C. No detectable change in color was observed in beef–chicken meatball mixture (Fig. 4A (d)) or pure genomic DNA of beef and chicken meatball (Fig. 4A (e) and (f)) indicating that the probe did not hybridize to mismatch containing genomic DNA under similar conditions. In addition, visible absorption spectroscopy (Fig. 4B) strongly supported the visually results, suggesting that the color change was real.



(A)

(B)

Fig. 4. Detection of porcine DNA in mixed meatballs (A). Vials ((a)–(f)) represent color of AuNPs in genomic DNA extracted from meatballs prepared with pure pork (a), 1 : 1 (w/w) mixtures of pork-beef (b), pork-chicken (c), chicken-beef (d), pure beef (e), and pure chicken (f). The corresponding absorption spectra are labeled alphabetically (B). All vials are incubated at 95 °C for 3 min and annealed at 55 °C for 2 min with 27 mer ssDNA-AuNPs before adding the 10 mM PBS.

In order to support the experimental findings, the comparison between the nucleotide sequence of cytb genes of these species (pork, beef and chicken) could be aligned with the probe by ClustalW alignment program. The number of mismatches and mismatched nucleotides of each species is shown in Table 2. Only the *Sus scrofa* (pork) species showed 100% matching, and other species (beef and chicken) showed 6 nucleotide mismatching (shown in red). It is reflecting the specificity of the designed probe used in this work.

3.4. Determination of LOD

The visible absorption intensity of 27 mer ssDNA-AuNPs in various percentages of pork–beef binary admixtures is shown in Fig. 5. It was very clear from the visually observed results, as well as the intensity of the absorbance data, that 1 % pork-containing vial still retained the absorbance intensity of original color of colloidal AuNPs. However, 5 % and 10 % pork containing vial reduced at 30-40 % color of AuNPs, reflecting partial aggregation. On the other hand, 20 % and 30 % pork containing vials changed color from red to purple demonstrating aggregation.

Table 2. The comparison between the nucleotide sequence of probe used toward the nucleotide sequence cytb genes of these species (pork, beef and chicken).

Species	Sequences	Mismatch	Genbank	Meat
S. Scrofa	CTA CGG TCA CAA ATC TAC TAT CAG	0	GU 135837.3	Pork
Pork	CTA CGG TCA CAA ATC TAC TAT CAG			
B. taurus	CAA CAG TCA CCA ACC TCT TAT CAG	6	EU 807948.1	Beef
G. gallus	CCA CCG TTA TCA CAA ACC TAT TCT CAG	6	EU 839454.1	Chicken

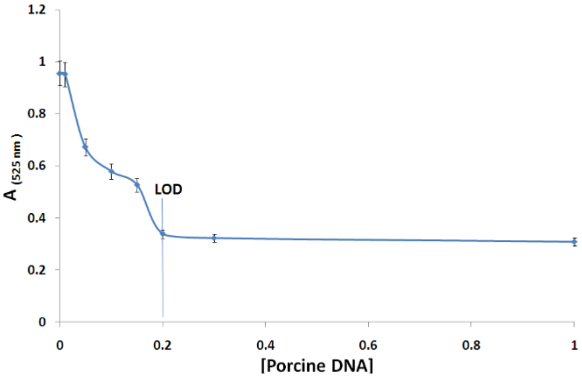


Fig. 5. Determination of LOD for pork in the beef meatballs at 525 nm, where the LOD is shown to be 20 % (6 µg/ml) of porcine DNA in mixed meatball preparation.

Absorption intensity reflected the surface plasmon features of 20 nm diameter of non -aggregated particles and reduced of the intensity, when aggregated particles formed at 525 nm. Concentration of porcine DNA in 20 % pork-containing vial was 6 µg/ml. Thus, the LOD was determined at 6 µg/ml porcine DNA for pork meatball in mixed samples. In this LOD, since the intensity of observed absorbance decrease was less than the 30 % pork-containing vial, meaning that not all the particles change color. These might be due to the fact that the unreacted ssDNA probe-bound particles tolerated the salinity stress.

3.5. Effectiveness and Limitation

The spectra of the results were measured by visual absorption spectroscopy. However, the assay could not provide quantitative information, since linear correlation (r) of the calibration curve from their absorption spectra was low (Fig. 5). The LOD of the assay was slightly higher compared to other [24]. However, ss-AuNPs-based sequence detection can reduce both the cost and time, and can be afforded by ordinary laboratories. UV–vis spectroscopy, which is available in most laboratories, can complement the spectra of the visually identified results of DNA nanobiosensor. In addition, the developed assay can be applied to detect specific sequences and mismatch without the need of PCR amplification if adequate sample is available. Currently, we are also developing this method as a chip based DNA nanobiosensor on the microwells for easily handle and simple measurement of genomic DNA detection. Besides using simple visual detection, in order to increases sensitivity and eliminates any color-error as well as low-cost, the measurement could be coupled with a conventional flatbed scanner as a reader for quantitative signal measurement as a novel “scanometric” chip-based detection system for genomic DNA detection.

4. Conclusions

A simple colorimetric sensor for rapid and reliable detection of specific sequences and nucleotide mismatch in non-amplified DNA employing aggregation properties of 20 nm AuNPs was developed. The DNA biosensor was label free, without surface functionalization, instrumental detection and was applied for detection pork DNA in processed meats, which really useful in food analysis and halal authentication. The DNA nanobiosensor was prepared by a simple and ease method. In addition, the procedure of measurement is very simple and relies on the color change of 20 nm AuNPs following salt addition. The visual finding is solid and can be further confirmed their spectra by an inexpensive, widely available and reliable technique, i.e. absorption visible spectroscopy. The use of absorption spectroscopy increases sensitivity and eliminates any sort of color-blindness error or ambiguity in visual detection by producing well defined spectrum of aggregated and non-aggregated colloidal AuNPs. Thus, the method was successfully identified porcine DNA, particularly in 20 % pork-containing samples without PCR amplification and just by using visual observation.

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References

[1]. S. B. Primrose, and R. M. Twyman, Principles of Genome Analysis and Genomics, 3rd Ed, *Blackwell*, Malden, MA, USA, 2003.

[2]. J. Rees, Complex disease and the new clinical sciences, *Science*, Vol. 296, No. 5568, 2002, pp. 698–701.

[3]. C. Murugaiah, Z. M. Noor, M. Mastakim, L. M. Bilung, J. Selamat, and S. Radu, Meat species identification and Halal authentication analysis using mitochondrial DNA, *Meat Science*, Vol. 83, No. 1, 2009, pp. 57–61.

[4]. N. Z. Ballin, F. K. Vogensen, and A. H. Karlsson, Species determination - can we detect and quantify meat adulteration?, *Meat Science,* Vol. 83, No. 2, 2009, pp. 165–174.

[5]. J. M. Butler, Forensic DNA Typing-Biology, Technology and Genetics of STR Markers, 2nd edition, *Elsevier*, NY, USA, 2005.

[6]. Nelson B. P., Grimsrud T. E., Liles M. R., Goodman R. M. and Corn R. M. Surface plasmon resonance imaging measurements of DNA and RNA hybridization adsorption onto DNA microarrays, *Analytical Chemisty*, Vol. 73, No. 1, 2001, pp. 1–7.

[7]. S. Sueda, J. L. Yuan and K. Matsumoto, A homogenouse DNA hybridization system by using a new luminescence terbium chelate, *Bioconjugation Chemistry*, Vol. 13, No. 2, 2002, pp. 200–205.

[8]. D. Garion, W. J. Parak, S. C. Williams, D. Zanchet, Micheel C. M. and A. P. Alivisator, Sorting Fluorescent Nanocrystals with DNA, *Journal of American Chemical Society,* Vol. 124, No. 24, 2002, pp. 7070–7074.

[9]. K. Sato, K, Hosokawa and M. Maeda, Rapid aggregation of gold nanoparticles induced by non-cross-linking DNA hybridization, *Journal of American Chemical Society,* Vol. 125, No. 27, 2003, pp. 8102–8103.

[10]. B. S. Gaylord, A. J. Heeger and G. C. Bazan, DNA hybridization detection with water-soluble conjugated polymers and chromophore-labeled single-stranded DNA, *Journal of American Chemical Society*, Vol. 125, No. 4, 2003, pp. 896–900.

[11]. B. D. Hames and S. J. Higgins, Gene Probes 1, *IRL*, New York, USA, 1995.

[12]. Brockman, J. M., Frutos, A. G. and Corn, R. M. Multi-step-chemical modification procedure to create DNA arrays on gold surface to study of protein DNA interaction with surface plasmon resonance imaging, *Journal of American Chemical Society,* Vol. 121, No. 1 1999, pp. 8044–8051.

[13]. R. M. Ostroff, D. Hopkins, A. B. Haeberli, W. Baouchi and B. Polisky, Thin film biosensor for rapid visual detection of nucleic acid targets, *Clinical Chemistry*, Vol. 45, No. 9, 1999, pp. 1659–1664.

[14]. Y. C. Cao, R. C. Jin and C. A. Mirkin, Nanoparticles with Raman spectroscopic fingerprints for DNA and RNA detection, *Science*, Vol. 297, No. 5586, 2002, pp. 1536–1540.

[15]. S. J. Park, T. A. Taton and C. A. Mirkin, Array-based electrical detection of DNA with nanoparticle probes, *Science*, Vol. 295, No. 5559, 2002, pp. 1503–1506.

[16]. H. Li and L. Rothberg, Colorimetric detection of DNA sequences based on electrostatic interactions with unmodified gold nanoparticles, *Proceedings of the National Academy of Sciences of the United State of America,* Vol. 101, 39, 2004, 14037-1409.

[17]. H. Li and L. Rothberg, Colorimetric detection of DNA sequences based on electrostatic interactions with unmodified gold nanoparticles, *Proceedings of the National Academy of Sciences of the United State of America,* Vol. 101, No. 39, 2004, pp. 14036–14039.

[18]. H. Li and L. Rothberg Label-free colorimetric detection of specific sequences in genomic DNA amplified by the polymerase chain reaction, *Journal America Chemical Society,* Vol. 126, No. 35, 2004, pp. 10958–10961.

[19]. M. E. Ali, U. Hashim, S. Mustafa et al., Nanoparticle sensor for label free detection of swine DNA in mixed biological samples, *Nanotechnology*, Vol. 22, No. 19, 2011, Article ID 195503.

[20]. V. A. Bloomfield, D. M. Crothers, Jr., and I. Tinoco, Nuclei Acids: Structures, Properties, and Functions, *University Science Books*, Sausalito, CA, USA, 1999.

[21]. R. J. Hunter, Foundations of Colloid Science, *Oxford Univ. Press*, New York, USA, 2001.

[22]. S. Link, and M. A. El-Sayed, Shape and size dependence of radiative, non-radiative and photothermal properties of gold nanocrystals, *Int. Rev. Phys. Chem*., 19, 2000, pp. 409–453.

[23]. F. Xia, X. Zuo, R. Yang, Y. Xiao, D. Kang, A. Vallée-Bélisle, X. Gong, J. D. Yuen, B. B. Y. Hsu, A. J. Heeger, and K. W. Plaxco, Colorimetric detection of DNA, small molecules, proteins, and ions using unmodified gold nanoparticles and conjugated polyelectrolytes, *Proceedings of the National Academy of Sciences of the United State of America,* Vol. 107, No. 24, 2010, pp. 10837–10841.

[24]. M. E. Ali, U. Hashim, S. Mustafa, Y. B. Che Man, and Kh. N. Islam, Gold Nanoparticle Sensor for the Visual Detection of Pork Adulteration in Meatball Formulation, *Journal of Nanomaterials,* 2012, pp. 1-7.

[25]. B. Kuswandi, K. A. Cendekiawan, N. Kristiningrum and M. Ahmad, Pork adulteration in commercial meatballs determined by chemometric analysis of NIR Spectra, *Food Measurements & Characterizations,* Vol. 9, No. 3, 2015, pp. 313-323.

[26]. A. Muangchuen, P. Chaumpluk, A. Suriyasomboon and Sanong Ekgasit, Colorimetric detection of Ehrlichia Canis via nucleic acid Hybridization in gold nano-colloids, *Sensors*, Vol. 14, No. 8, 2014, pp. 14472-14487.

[27]. G. Frens, Particle size and sol stability in metal *colloids, Colloidal Polymeric Sciences*, Vol. 250, No. 1, 1972, pp 736–741.

[28]. N. R. Jana, L. Gearheart and C. J. Murphy, Seeding growth for size control of 5−40 nm diameter gold nanoparticles, *Langmuir,* Vol. 17, No. 22, 2001, pp. 6782–6786.

[29]. A. A. Lazarides and G. C. Schatz, DNA-Linked Metal Nanosphere Materials: Structural Basis for the Optical Properties, *Journal Physical Chemistry B*, Vol. 104, No. 3, 2000, 460–467.
