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Research Article

Gold Nanoparticle Sensor for the Visual Detection of Pork Adulteration in Meatball Formulation

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We visually identify pork adulteration in beef and chicken meatball preparations using 20 nm gold nanoparticles (GNPs) as colorimetric sensors. Meatball is a popular food in certain Asian and European countries. Verification of pork adulteration in meatball is necessary to meet the Halal and Kosher food standards. Twenty nm GNPs change color from pinkish-red to gray-purple, and their absorption peak at 525 nm is red-shifted by 30–50 nm in 3 mM phosphate buffer saline (PBS). Adsorption of single-stranded DNA protects the particles against salt-induced aggregation. Mixing and annealing of a 25-nucleotide (nt) single-stranded (ss) DNA probe with denatured DNA of different meatballs differentiated well between perfectly matched and mismatch hybridization at a critical annealing temperature. The probes become available in nonpork DNA containing vials due to mismatches and interact with GNPs to protect them from salt-induced aggregation. Whereas, all the pork containing vials, either in pure and mixed forms, consumed the probes totally by perfect hybridization and turned into grey, indicating aggregation. This is clearly reflected by a well-defined red-shift of the absorption peak and significantly increased absorbance in 550–800 nm regimes. This label-free low-cost assay should find applications in food analysis, genetic screening, and homology studies.

1. Introduction

Detection of selective DNA sequences is the key step in non-aggregated, genetic screening [1–3], food analysis [4–7], environmental monitoring, and forensic investigations [8]. Most of the sequence detecting assays, available at hand, rely on polymerase chain reaction (PCR) followed by electrophoretic visualization of PCR products [4–6, 8]. Although the use of PCR effectively amplifies DNA from single copy to easily detectable quantities, it is an expensive technique in the platforms of reagent and instrumental costs [3, 9]. Moreover, authentication of PCR products may further need identification of specific sequences within it by RFLP analysis [4, 6], southern blotting or sequencing [8, 9]. Therefore use of PCR is unwarranted where sample scarcity is not a concern.

The distinct surface plasmon resonance (SPR) characters of aggregated and biodiagnostics GNPs are interesting as they can be monitored by absorption spectroscopy and also visually [3]. Researchers have long exploited these distinctive optical properties of colloidal GNPs for sensing specific oligonucleotide sequences to address a wide range of biological issues such as biodiagnostics, genetics, and food analysis [2, 3, 10–19]. However, those studies are limited to a cross-linking mechanism with synthetic probes and targets. A cross-link-based DNA detection scheme requires surface modification of GNPs to immobilize two DNA probes. The immobilized probes are further needed to be interlinked by a complementary target to realize aggregation [10, 16, 19].

Detection of nucleotide sequences by a noncross-linking method is particularly interesting [2, 3, 16]. It does not involve any modification chemistry and target hybridization is considerably fast. Li and Rothberg [2] pioneered this work by detecting selective sequences and single nucleotide mismatch in PCR amplified DNA with 13 nm-GNPs. Mismatch detection is a challenging but necessary task for the early

diagnosis of cancers and other hereditary problems [2, 3]. However, spectroscopic supports in their findings were not adequate. In our last report, we have shown 40 nm GNPs can be used for visual identification of specific sequences and mismatches in PCR-products and also in nonamplified genomic DNA. We validated our visual findings by absorption spectroscopy [3]. In the current report, we use 20 nm GNPs for visual identification of pork adulteration in meatball formulations. We demonstrate that 20nm colloidal particles produce more pronounced changes in color and absorption spectra than those of 40 nm counterparts. The absorption peak at 525 nm changes its position and appears in a new location between 555–580 nm depending on the degree of aggregation in 3 mM PBS (60 mM NaCl, pH 7.4). Stronger absorbance also remarkably appeared between 550–800 nm, making the identification more obvious. The detection limit (DL) of genomic DNA in heterogeneous mixture is also significantly reduced than that of 40-nm counterpart [3].

Meatball is a special type of restructured comminuted meat products [20, 21]. It is a favorite food in certain Asian countries such as Malaysia and Indonesia and also some European countries [20, 21]. Pork is a potential adulterant in beef and chicken meatballs due to its availability at cheaper prices. The mixing of pork or its derivatives in the Halal and Kosher foods is a serious matter as it is not permissible by the respective religious laws [20–22]. Unconscious consumption of pork may also ignite allergic reactions in certain individuals [4, 5]. Additionaly, its high content of cholesterol and saturated fats are a concern for people with diabetes and cardiovascular diseases.

2. Materials and Methods

2.1. Swine Specific Probe Design. A 25 nt swine probe (567-(5′)-TAC CGC CCT CGC AGC CGT ACA TCT C-(3′)-591) is designed by comparing *Sus scrofa* cytochrome b (cytb) gene (GenBank: GU135837.1) with *Bos taurus* (cow; GenBank: EU807948.1) and *Gallus gallus* (chicken; GenBank: EU839454.1) cytb genes by ClustalW multiple seq-uence alignment program (http://www.genome.jp/tools/clustalw/). NCBI BLAST (http://www.ncbi.nlm.nih.gov/nucleotide/blast) analysis against nonredundant nucleotide collection confirms the probe is unique for the pig as no other species shows similarities with it. The probe is purchased from the first BASE, Selangor, Malaysia.

2.2. Synthesis of Colloidal Gold Nanoparticles. Colloidal GNPs are synthesized by the citrate method described in bibliography [23]. The resultant particles are characterized by Hitachi 7100 transmission electron microscope (TEM) (Figure 1) and PerkinElmer Lambda 25 UV-vis spectrophotometer (Figure 2). The concentration and particles number are determined according to Haiss et al. [24]. All chemicals are procured from Sigma-Aldrich, USA, in the highest analytical grades and are used without further purification. All solutions are prepared in 18.2 MΩ water (Sartorius) immediately before use. All glass wares are cleaned with piranha solution and are oven dried prior to use.

2.3. Preparation of Meatballs and DNA Extraction. Meatballs are prepared according to Rahman et al. [21] either with pure or 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 (A_{260}/A_{280}) of all DNA samples used in all experiments is 1.95–2.0.

2.4. Detection of Single-Stranded and Double-Stranded DNA. In four separate vials, labeled as ((a)–(d); Figure 2)), 100 μL of 1.8 nM colloidal GNPs is taken. Thirty microliters (30 μL) of 25-mer single-stranded (ss-) and double stranded (ds-) oligoprobes of 100 nM (1st BASE, Malaysia) are added into vials (c) and (d). Volume in vial (b) is adjusted with water (18.2 MΩ). All vials, except dsDNA containing one (d), are incubated in a water bath at 50°C for 3 min to facilitate ssDNA adsorption onto GNPs [2]. Vial (d), which contains dsDNA, is incubated at 25°C to avoid temperature-induced dehybridization of the complementary strands [3]. Then 300 μL of 10 mM PBS (0.2 M NaCl, pH 7.4) is added into each tube except vial (a) where the volume is homogenized with water. All tubes are vortexed immediately. Colloidal suspension in PBS (b) and dsDNA (d) turns into grey-purple within 3 min or immediately. However, GNPs in DI water (a) and ssDNA exposed vial (c) remain undisturbed. They retain their characteristic pinkish-red color. After 10 min, sufficient water is added into each vial to adjust the final volume to 1 mL and is characterized by transmission electron microscopy (Figure 1) and absorption spectroscopy (Figure 2). Thus the final concentration of probe, GNPs, and PBS buffer is made to 3 nM, 180 pM, and 3 mM, respectively. Stability of ssDNA-incubated colloidal particles in 3 mM PBS is studied for seven days keeping them at 4°C and is found unchanged.

2.5. Pork Identification in Beef and Chicken Meatballs. In order to detect pork contamination in processed meat products, meatballs are prepared with emulsified mixed meats of pork-beef, pork-chicken, and chicken-beef binary mixtures in 1 : 1 (w/w) ratios. Pure meatballs are formulated with pure meats of each species under identical conditions. After 20 min of cooking in boiling water, DNA extractions are performed. One hundred microliters (100 μL) of mixed genomic DNA (300 μgml^{-1}) is taken in vials ((b)–(d); Figure 3)). Equal portion of pure genomic DNA of pork, beef, and chicken is taken in vials (a), (e), and (f). All tubes are exposed to 30 μL of 100 nM swine probe (25 nt; inset of Figure 3) at 95°C for 3 min to allow denaturation. All mixtures are cooled down to 50°C for 2 min to favor

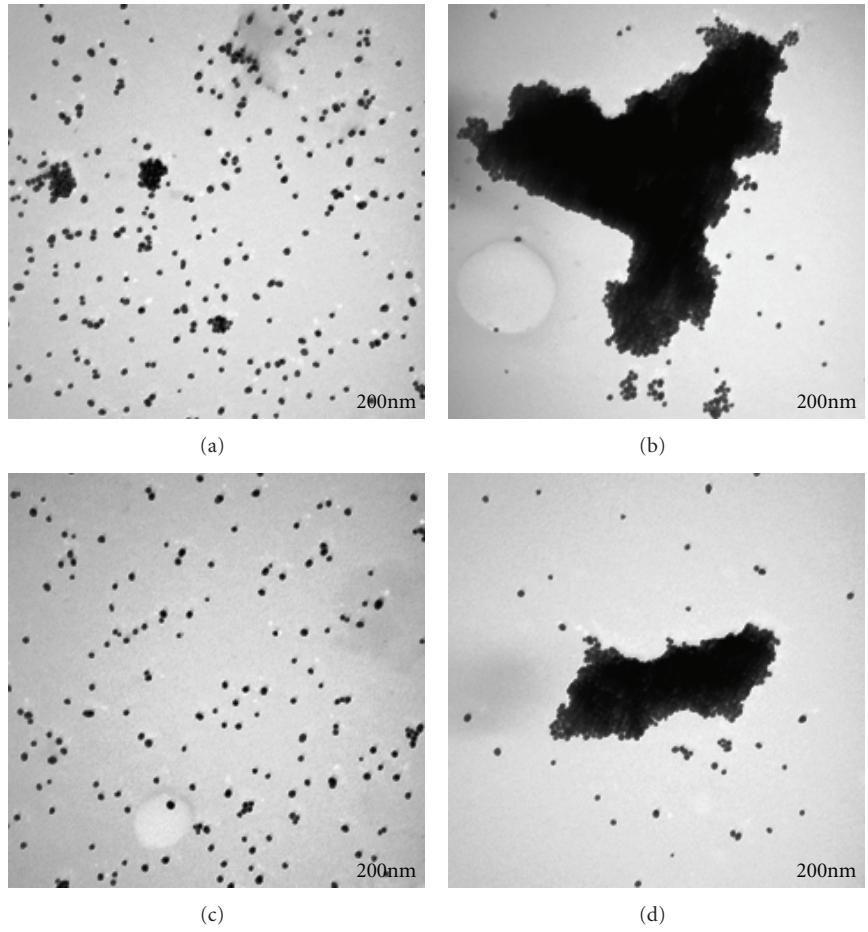

Figure 1: TEM images of colloidal particles before and after salt-induced aggregation. Shown are 180 pM gold colloids in DI water (a), in 3 mM PBS (b), in 3 mM PBS after 3-minute incubation in 3 nM ssDNA probe at 50°C (c), and in 3 mM PBS after the same-time incubation in equimolar 25-bp dsDNA at 25°C (d). All images are shown at a magnification of 100,000 times.

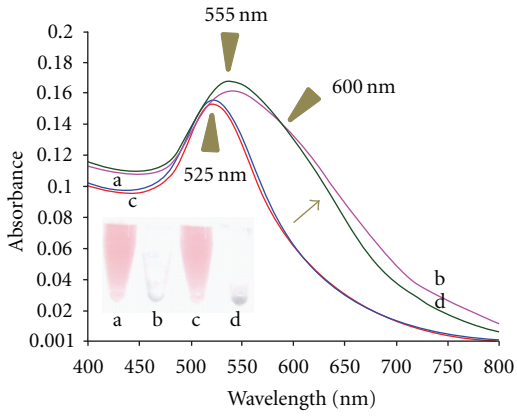

Figure 2: Absorption spectra of aggregated and non-aggregated GNPs. Shown are absorption spectra of 180 pM gold colloids in DI water (blue curve (a)), in 3 mM PBS (pink curve (b)), and in equimolar PBS after incubation with 3 nM ssDNA probes (red curve (c)), and with the equimolar dsDNA probes (green curve (d)). The vials in the inset shows the color photographs of the solutions in DI water (a), PBS buffer (b), PBS buffer plus ssDNA (c) and PBS buffer plus dsDNA (d).

perfectly matched annealing and mismatched nonannealing. Subsequently, 100 μL of 1.8 nM gold colloids is added to each vial and mixed for 2 min by mild shaking to allow adsorption of unhybridized probe onto GNP-surfaces. Finally, 300 μL of 10 mM PBS is added to induce aggregation of colloidal particles. All the swine DNA containing vials ((a)–(c)), either in pure (a) or mixed forms (b) and (d), immediately turn into purple-grey. However, the rest of the vials ((d)–(f)) that contain other species (chicken or beef) retain the characteristic color of colloidal particles. The final volume is adjusted to 1 mL with water and is characterized by absorption spectroscopy. Thus the final concentration of GNP, probe, genomic DNA and PBS is made to 180 pM, 3 nM, 30 μgmL^{-1}, and 3 mM.

2.6. Determination of LOD. To determine LOD, raw pork and beef are mixed in a ratio of 1 : 99, 3 : 97, 5 : 95, 10 : 90, and 15 : 85 (w/w). All mixtures are emulsified and meatballs are prepared. DNA is extracted from cooked meatballs of each formulation. One hundred microliters (100 μL) of mixed DNA (400 μgmL^{-1}) is taken into five separate vials ((a)–(e); Figure 4)). All vials are exposed to 15 μL of 100 nM

Sus scrofa cytb 567(5′) TAC CGC CCT CGC AGC CGT ACA TCT C (3′) 591

Probe (5′) TAC CGC CCT CGC AGC CGT ACA TCT C (3′) 25 nt

Bos taurus cytb 567 (5′) CAT AGC AAT TGC CAT AGT CCA CCT A (3′) 591

Gallus gallus cytb 567 (5′) CGC AGG TAT TAC TAT CAT CCA CCT C (3′) 591

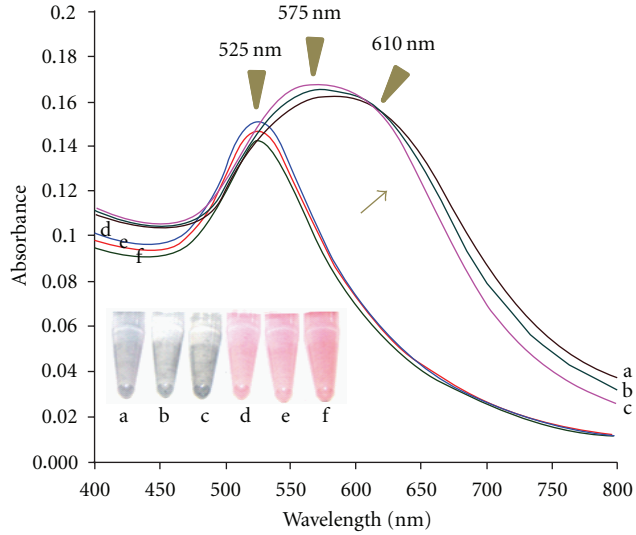

FIGURE 3: Identification of swine DNA in mixed meatballs. Vials ((a)–(f)) represent color of GNPs in genomic DNA extracted from meatballs prepared with pure pork (a), 1 : 1 (w/w) mixtures of pork-beef (b), pork-chicken (c), chicken-beef (d), pure beef (e), and pure chicken (f). The corresponding absorption spectra are labeled by respective alphabets. All vials are incubated at 95°C for 3 min and annealed at 50°C for 2 min before adding the colloidal particles and PBS. The top inset is the comparison of probe sequences with shown species. Mismatch bases are demonstrated by red.

swine probe (25 nt; Inset of Figure 3) at 95°C for 3 min and then annealed at 50°C for 2 min. After that 50 μL of 1.8 nM gold colloids is added to each vial and incubated for 2 min with mild shaking. Finally, 100 μL of 10 mM PBS is added to each vial. Vials ((a)–(c)) retain the pinkish-red color of monomeric GNPs with an increasing trend of fading. The fading of color proportionates the portion of pork in each vial. Vials (d) and (e) clearly turn into purple-grey, indicating clumping of colloidal particles. The final volume is made to 1 mL with water and is characterized by absorption spectroscopy. Thus the final concentration of probe, GNPs, mixed genomic DNA, and PBS is made to 1.5 nM, 90 pM, 40 μgmL^{-1}, and 1 mM. The concentration of swine DNA in vials ((a)–(e)) is calculated to be 0.4, 1.2, 2.0, 4.0, and 6.0 μgmL^{-1}.

3. Results and Discussion

3.1. Characterization of Gold Nanoparticles and Detection of DNA. The formation of gold nanoparticles is confirmed by TEM images (Figure 1) and UV-vis spectra (Figure 2). The size of the particles (diameter: 20 ± 5 nm) is assigned according to previously established methods [2, 3]. TEM images revealed that most of the particles are spherical in shape and homogeneously distributed throughout the bulk solution in water (a) and in ssDNA incubated 3 mM PBS (c), clearly showing particle isolation. A minor fraction of the particles are appeared in small groups sitting side by side or one on another in water (a), showing a very low level of aggregation in DI water. This is consistent with the findings of Li and Rothberg [2]. Negative coatings of citrate ions on GNP-surfaces electrostatically repel one other, keeping them separated. ssDNA adsorbed onto GNPs surfaces by van Waals interactions and adds negative charges on GNP surfaces with the exposed phosphate groups [2, 3]. Thus the GNPs are stabilized against salt-induced aggregation when they are previously exposed to ssDNA [3].

However, the huge aggregates of GNPs become obvious after the addition of salts (3 mM PBS; b) that induces clogging of particles by screening the repulsive negative charges on particle surfaces [2]. Particles aggregation is also found in dsDNA, containing 3 mM PBS (d). However, the size of the aggregates appeared to be smaller. This is probably due to the partial protection provided by a small fraction of ssDNA which is frequently present in dsDNA solution [3].

Unlike ssDNA, dsDNA cannot protect the particles from salt-induced aggregative stresses [2, 3]. This is contrary to the conventional wisdom as both of them are highly negatively charged due to the constituent phosphate back-bone. However, when the nitrogenous bases of uncoiled ssDNA face the

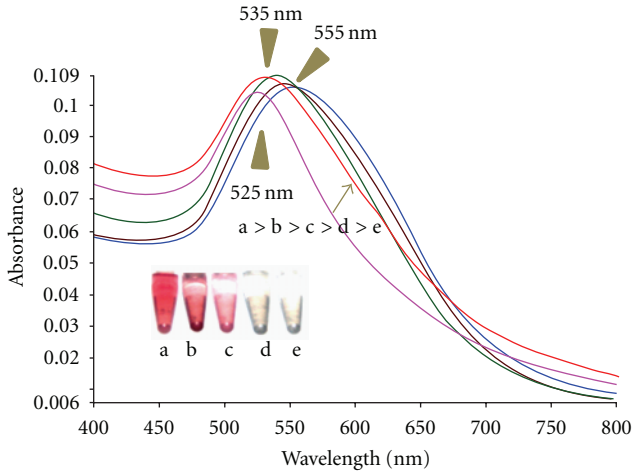

FIGURE 4: Determination of LOD for pork in ready-to-eat beef meatballs. In the inset, vials ((a)–(e)) demonstrate the color of gold nanoparticles in 1% (a), 3% (b), 5% (c), 10% (d), and 15% (e) pork DNA extracted from processed pork-beef meatballs. The corresponding absorption spectra are shown with alphabetical labels. The LOD is shown to be 10% ($4\,\mu\text{gmL}^{-1}$) of swine DNA in mixed meatball preparation (vial (d) and spectrum (d)).

citrate-coated GNPs, they adsorb onto their surfaces, adding negative charges and enhancing intermolecular repulsion. On the other hand, dsDNA is highly stable and seldom uncoils to expose constituent bases [2].

Figure 2 shows the UV-vis spectra of isolated and aggregated 20 ± 5 nm-GNPs in DI water (a), and in PBS (b). The color of colloidal GNPs are very sensitive to the degree of their aggregation which can be easily induced by adding electrolytes such as salts [2]. The aggregated and non-aggregated forms of the particles can be easily distinguished by absorption spectroscopy and also visually [2, 3]. The monomeric sol exhibits pinkish red-color in DI water (a) and produces an intense surface plasmon resonance (SPR) peak at 525 nm. This is consistent with the previously reported findings [2, 3, 23, 25]. The particles aggregate immediately in 3 mM PBS (60 mM NaCl, pH 7.4) as shown in TEM image (Figure 1(b)). This is reflected by a visually detectable dramatic change in color from pinkish-red (a) to grey-purple (b).

The visually detected changes in color is strongly supported by the remarkable features in UV-vis spectrum ((spectrum (b)) of the aggregated particles. The collective plasmon peak is intensified and appears in a new position between 550 and 580 nm (Figures 2–4), depending on the degree of aggregation and concentration of GNPs. The position of this peak is more and more red-shifted with an increment of particles clumping and particle concentration. The absorption is significantly increased throughout the 550–800 nm regimes, a feature that is indicative of particles coagulation [2]. These features in absorption spectra show strong relevance with the pioneering work of Li and Rothberg [2] and Ali et al. [3]. His group detected specific sequences in PCR products by a non-cross-linking method using 13 nm GNPs. They studied the temperature and length-dependent adsorption of ssDNA on colloidal particles and observed collective plasmon peak of aggregated 13 nm particles near 700 nm. In our last report, we did not observe any collective peak of aggregated 40 nm gold particles. However, we reported strong absorption between 600–800 nm in aggregated form. As the optical properties of GNPs are size dependent [2, 3, 12, 13], the new position of the collective plasmon peak of 20 nm particles between 550–580 nm is acceptable.

We observe that 20 nm GNPs do not change color in PBS (the inset of Figure 2(c)) if they are previously exposed to sufficient (3 nM) ssDNA at a reasonable temperature (50°C). Temperature is implicated to break down secondary structure of ssDNA and facilitates their adsorption onto GNP-surfaces by van der Waals interactions [2]. Thus the water-exposed phosphate groups of ssDNA add negative charges on particle-surfaces and protect them from salt-induced aggregation. dsDNA is highly negatively charged as phosphate groups on their back-bone are exposed to aqueous media and nitrogenous bases are shielded interior by the helical structure. Consequently, it does not adsorb onto the negatively charged GNPs [2]. Thus the particles do not get any support from dsDNA to survive in salt-induced stress in PBS. This is clearly revealed by the drastic changes in color (d) and absorption spectrum (d). This is also confirmed by relevant TEM image of Figure 1(d).

3.2. Detection of Pork Adulteration in Mixed Meatballs. In order to detect pork adulteration in beef and chicken meatballs, we design a 25 nt swine probe that bears full matching with swine cytb and 13 nt and 14 nt mismatching with the bovine and chicken cytb genes (Figure 3: inset). Thus the mismatching with bovine and chicken genes is 52% and 56%, respectively. The presence of mismatch bases has a remarkable effect on hybridization [2, 3, 9, 12, 13, 16]. Mismatches reduce melting temperature (Tm) significantly, making hybridization difficult [2, 3]. Therefore, it is highly unlikely for the probe to hybridize with bovine and chicken DNAs that contain more than 50% mismatch nucleotides at or near temperature where perfectly match hybridization is possible [2, 3]. Consequently, the probe should be available to interact with GNPs if it is annealed with bovine and chicken genes at or near its melting temperature (64°C).

We mix the probe with an excess of pure and mixed genomic DNAs of pork, beef and chicken extracted from ready to consume meatballs of respective species as shown in Figure 3. We denature the mixtures at 95°C to induce strand separation of all genomic DNAs [4–8]. Afterwards, we cool down the mixtures to 50–60°C to allow complementary base-pairing between the strands and the probe. Previous studies demonstrated shorter DNA hybridizes before the longer counterparts due to steric reasons [2]. Thus the limited probe should be completely engulfed by the excess genomic DNA if it bears complementary targets within it. However, the probe does not hybridize with mismatch bearing targets if they are not forced to do so by the excessive reduction of annealing temperature.

The inset of Figure 3 clearly shows that the probe is consumed completely by the pork DNA in pure (vial (a)) or

mixed forms (vials (b) and (c)). Thus the colloidal particles in all pork containing vials ((a)–(c)) experience clogging upon the addition of salts as they are not protected by the ssDNA probe. This is clearly depicted by the dramatic change of color from pinkish-red to grey purple. The UV-vis spectra demonstrate a huge red-shift of ~100 nm and appearance of a collective plasmon peak at 575 nm, confirming the visually determined result. However, probe is not engulfed at all by the huge mismatch (>50%) containing beef or chicken DNA either in pure (vials (e) & (f)) or mixed formulation (vial (d)). Thus the probe is available in vials ((d)–(f)) to adsorb onto GNPs surfaces to provide them withstanding strength in salt solution. Consequently GNPs of these vials do not undergo aggregation upon the addition of equimolar PBS. This is clearly revealed by their retention of characteristic pinkish-red color and plasmon peak of isolated colloidal particles at 525 nm.

3.3. Determination of LOD. The absorption spectra and visually detected color of GNPs in various percentages of pork containing beef meatballs are shown in Figure 4.

It is very clear from visually observed results as well as spectroscopic data that 1% pork containing vial (a) retains almost 100% original color of colloidal particles (pink curve: spectrum (a)). However, original pinkish-red color of GNPs in 3–5% pork containing vials ((b) and (c)) considerably disappeared, reflecting partial aggregation. This is confirmed by the appearance of collective plasmon peak near 535 nm and considerably stronger absorption between 550 and 650 nm ((red curve: spectrum (b) and green curve: spectrum (c)). On the other hand, 10% and 15% pork containing vials ((d) and (e)) change color from pinkish-red to purple-grey simulating aggregation. Absorption spectra of vials (d) and (e) display the collective surface plasmon features of 20 nm aggregated particles between 550 and 700 nm with a collective plasmon peak near 555 nm. Concentration of swine DNA in 10% pork containing vial is $4\,\mu\text{gmL}^{-1}$. Thus the determined LOD is $4\,\mu\text{gmL}^{-1}$ swine DNA in processed beef meatballs. It is observed that some of the particles (~3–5%) retain their colors in vials (d) and (e) that contain 10% and 15% swine DNA. These are most likely the unconsumed probe-bound particles that withstand the salinity stresses.

3.4. Efficacy and Limitation of the Current Assay. The current assay directly determines swine-specific sequences in a population of nonamplified mixed genomic DNA. The mixed population of genomic DNA is obtained from meatballs, prepared with the emulsified meats of chicken, beef, and pork. The method is capable of detecting target sequences just by visually observed color change of GNPs. The visually determined results are sufficient to make a concrete decision. However, it can be further authenticated by a relatively inexpensive and easily available absorption spectroscopy. This eliminates any sort of color blindness errors that may arise from visual findings. Sensitivity of the assay is also improved as revealed by a low LOD ($4\,\mu\text{gmL}^{-1}$).

Both the color and absorption spectra of 20 nm GNPs are more remarkable than the earlier report [3], making them a more suitable candidate for the analysis of targets in processed meat products. In earlier report, we have shown absorption peak of 40 nm colloidal particles at 530 nm fall down commensurating the degree of aggregation [3]. However, the current study has shown the absorption peak of 20 nm particles change its position and appears in a new position proportioning particle clumping. Absorption between 550–800 nm regimes is also significantly increased following aggregation. Thus a well-defined change in the peak position of aggregated and non-aggregated particles can be easily detected avoiding any ambiguity. These features probably make 20 nm counterparts more sensitive than 40 nm particles.

The LOD of the assay is higher than that of the real-time and conventional PCR [5–7]. However, PCR-based methods need comparatively longer targets which are reported to break down during the chemical and physical stresses of food processing, causing template crisis in PCR assay [9]. On the other hand, the present assay uses DNA target (25 nt) that is comparable with the size of a typical PCR-primer [5–7]. As shorter targets are more stable than the longer one [7], the method can be applied to analyze highly degraded samples where PCR may lose its candidacy. The probe deign is also much simpler than that of a PCR assay. Moreover, PCR-electrophoresis is a clumsy technique and sometimes needs self-authentication by RFLP-analysis [4], sequencing, or blotting [8]. The LOD of the assay can be decreased by using increased amount of DNA mixtures to ensure sufficient targets for the probe. Using increased amount of targets is not problematic in food analysis because here sample scarcity is not a concern.

The presence of single stranded nucleic acids (DNA or RNA) interferes with target detection by sticking to GNPs and interfering particle aggregation. However, by using appropriate purification technique [26], the single-stranded nucleic acid can be easily removed from the degraded samples.

The method cannot provide quantitative information of the target DNA. TaqMan fluorogenic probe can detect, quantify, and amplify specific sequences by real-time PCR without the need of electrophoresis and blot analysis [7]. However, the TaqMan probe, real-time PCR, and the master-mix used in real-time PCR are highly expensive and ordinary laboratories cannot afford them. On the other hand, UV-vis spectroscopy is available in most laboratories and can authenticate the visually identified results of colloidal gold.

4. Conclusion

A rapid (less than 10 min), reliable, and cheap method for the selective detection of target DNA sequences in processed meat products is developed. It does not need any instrument or surface modification chemistry and directly detects target DNA in nonamplified mixed genomic DNA. The procedure is very simple and relies on the color change of 20-nm GNPs following salt addition. The visual finding is solid and can be further confirmed by an inexpensive, available, and reliable technique, absorption spectroscopy which incurs

only the instrumental cost and reusable cuvette. The use of absorption spectroscopy increases sensitivity and eliminates any sort of color-blindness error or ambiguity in visual detection by producing well-defined bands of aggregated and non-aggregated colloidal particles. The assay needs a shorter probe whose design is simpler than PCR primers. The method is applicable to analyze extensively degraded sample which may not be possible by PCR which require longer targets.

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