Visualization Detection Based on Gold Nanoparticles

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Abstract. The studies focusing on gold nanoparticles (AuNPs) is developing rapidly of late years. AuNPs are of great importance in biological and chemical researches and applications. Thanks to their special optical properties, AuNPs are widely applied in the visualization detection of biochemcials. By controlling the morphology and sizes, AuNPs can be suitable for the detections of various conditions and targets. In this review, synthetic methods aiming to obtain AuNPs with uniform morphology and sizes are summarized. The applications of AuNPs in the field of visualization detection on three different targets, including latent fingerprints and pathogens, are discussed. The results are promising and could be benefit to further investigations on the field of visualization detection.

Keywords: Gold Nanoparticles, Synthetic Methods, Visualization Detection.

1. Introduction

Nanotechnology derived from the discovery and emphasis of the matters under nanometer scales.

The famous physician Richard Feynman first addressed the key point of nanoscale and stressed the importance of studying into the little scale in 1959 during his renowned speech: “There’s plenty of room at the bottom”. Nanoscience and nanotechnology including quantum theories were later widely known to the world after the speech from Feynman. Sizes of materials are critical in the new field. Studies and facts lead to the same conclusion that the physical properties of nanomaterials were decided by the sizes. Quantum theories put forward two principles to explain it. The first one is the quantum size effects, which indicates that electronic level around metal Fermi level will become discrete. This is achieved by the characteristic widths of the atoms being significantly less than the dimensions of the free exciton of the bulk of atoms [1]. The second one is the single-electron transitions phenomenon. When a scanning probe and a nanoscale object with thickness of 10-15 nm get close enough, it will lead to Coulomb blockade, creating a single electron tunnelling phenomenon [2]. These two phenomena together with many other derivative effects contribute to the different electrical and optical properties of nanoparticles. Quantum size effects and single-electron transitions also provide a well-organized theory system for the following researches as well as an instruction for the analysis and construction of nano-sized materials.

Nanomaterial can be described as the material that could be measured in nanometers in at least one of its dimensions or an entity that contains such entities [3]. It can be regarded as a special kind of materials, which shows a series of distinct properties compared with the macro materials, such as electrical property, mechanical property, optical property, et al. These unique properties are mostly illustrated and even predicted by the quantum theories in nanoscience and nanotechnology. Therefore, it has triggered an enormous growth of activities in applied research of nanomaterials. Currently, nanomaterials can also be seen in the new frontier of many other interdisciplinary subjects. Gold nanoparticles (AuNPs) is one of these promising materials. In recent years, AuNPs have been an increasingly hot topic for researches focusing on nanomaterials. The number of studies focusing on the properties and applications of AuNPs is rising hugely [4]. Researchers are devoted into looking for more various properties of AuNPs. There are already multiple applications. With strong light absorption and low toxicity, the optical properties of AuNPs are remarkably different from those of bulk gold, making them a promising kind of nanomaterials for new biomedical applications. For example, the gold particles glitter variedly with the change of the sizes, as it is shown in Figure 1 [5]. In macro scale, bulk gold has the most common color which is named after itself, “gold”. As the
diameter decreases from 500 nm to 30 nm, gold particles changes from crimson to blue. When it
reaches a range of 30 nm to 3 nm, the color of AuNPs turns into red and is somehow transparent
because it is too small. Then, for the gold clusters with a diameter less than 1 nm, the color becomes
orange and the clusters show no metallic properties. Finally, a single gold atom is completely
colorless with the diameter of 0.1 nm. Due to the fascinating color-change abilities, AuNPs show
promising applications in the detection field. It is of importance to take a sight into these trends and
summarize the important information for further development. However, visualization detection
based on the special optical properties, as one of the most significant applications for AuNPs is still
lack of systematic summary.

Figure 1. The colors of gold particles vary a lot from macro to nano [5]

This work focuses on AuNPs that can be used for visualization detection. Firstly, the non-cytotoxic
synthetic approaches of AuNPs with uniform morphology and sizes were reviewed. Then,AuNP-
dependent visualization detection of biochemicals were summarized, including latent fingermarks
and pathogens. Along with the insight and analysis of these branches of applications, the review tries
to give some inspirational ideas in the field of AuNPs applications.

2. Main Body

2.1. The synthesis of AuNPs

The synthesis of AuNPs is critical for the applications. AuNPs with uniform morphology and sizes
are more conducive to its application in visual detection. The synthetic approaches can often be
separated into two types: chemical method and biological method. Both of them are now mostly based
on the bottom-up approach, which means that the AuNPs are constructed from atoms aggregating to
nucleus through self-assembly process, and then the nucleus grows into clusters and then
nanoparticles.

Chemical method, which is also named the chemical reduction method, is the most widely used
one for AuNPs preparation. Since J. Turkevich first put out the idea of synthesizing colloidal gold in
1951, this method is named after him [6]. After decades of years, the synthetic method has been
greatly improved. In recent studies, the procedures are shown in Figure 2 [4]. This synthesis starts
with the reduction of chloroauric acid by certain reductant such as sodium citrate. Then, in the nearly boiling solution, the nucleus takes place and Au atoms aggregate into clusters to form non-stabilized AuNPs. In the next stage, there are stabilizers such as tri-sodium citrate, to stabilize AuNPs and can also functionalize the AuNPs in order to suit in various acquirements for the certain application situations.

**Figure 2.** The synthesis procedures of AuNPs’ chemical reduction method [4]

Based on the original Turkevich method, more synthetic methods are developed. These new methods achieve a better control of AuNPs size by adding certain reagents, changing the pH or temperatures, introducing of the nucleus, et al. Here are some research examples. In order to overcome the cytotoxic problem of the commonly used stabilizers, cetyltrimethylammonium bromide and tri-some citrate, A. Kumar et al. introduce taurine as the stabilizer [7]. They can control the sizes and the shapes of AuNPs through alternating temperatures precisely. In addition, Lv et al. come up with the method of simultaneously introducing chloroauric acid, ascorbic acid and 4-(2-Hydroxyethyl)-1-piperazinyl-ethanesulfonic acid to the solution [8]. It shows that along with the increase of the concentration of ascorbic acid, the process of the nucleus formation speeds up and the average diameter of the AuNPs thus decreases. Meanwhile, the surface-enhanced Raman spectroscopy properties of AuNPs are enhanced by this method, which makes it better for visualization detections. Photochemical synthesis is another method which based on the decomposition of water molecule to form radicals that can take place of the traditional reductants. In order to produce AuNPs more efficiently, V. Costa Bassetto et al. develop flash light synthesis from photochemical methods [9]. They introduce polyvinylpyrrolidone together with chloroauric acid into the solution, and then use a xenon flashlamp to generate intense pulsed radiation to make the chloroaucic acid reduce to AuNPs with the decomposition of polyvinylpyrrolidone.

Biological method has also aroused a wave of research interest. Compared with chemical method, it exhibits environmentally friendly characteristics without extra ligands as stabilizers. Biological approaches can also fall into two types: biological creatures-based method and biological derivatives-based method. The former directly add biological creatures or part of them into the reaction system, while the latter makes use of biological synthesized derivatives to achieve a green synthesis of AuNPs.

For biological creatures-based method, the most commonly applied creatures are the plants. The basic principle of this method still is that the biochemical reductants in the plants turns Au(III) into Au atoms. And due to the different properties of the reductants, the use of different parts of different plants results in various morphology and sizes of AuNPs. For example, N.R. Chowdhury et al. make use of cacao extract to synthesize spherical AuNPs [10]. It is based on the oxalic acid contained in cacao as a reductant and also a stabilizer. AuNPs’ sizes are controlled through the alternation of cacao concentration, which induce the decrease of the average diameters of AuNPs from 35 ± 10 nm to 7 ± 4.2 nm. For AuNPs of bigger sizes, D.S. Bhagat et al. develop another plant-based synthesis with Citrus limonum leaf extract and ultrasonication technique [11]. They succeed in synthesizing uniform
spherical AuNPs with sizes of 40 nm to 90 nm. The synthetic method can be used in various fields including quantitative analysis. Also, the approaches applied are all simple, environmentally friendly and economical, which is potential for low-cost synthesis.

Biological derivatives are also used in the synthesis of AuNPs. A. Madhusudhan et al. develop an ultrafast synthetic method with cellulose nanocrystals and microwave techniques [12]. They obtain the spherical AuNPs with diameters of $8 \pm 5.3$ nm, controlled by the concentration of cellulose nanocrystals in the solution system. H.M. Ibrahim et al. introduce a synthetical method based on carboxymethylchitosan as reductants and polyvinyl alcohol (PVA) which help with the aggregation of gold clusters into AuNPs [13]. The AuNPs are well-formed spheres due to PVA nanofibers with diameters ranging from 15 nm to 25 nm. The low cytotoxicity of the AuNPs allows them to be utilized in various applications in vivo and in vitro.

2.2. Visualization Detection of Biochemicals

Along with the synthesized AuNPs with uniform morphology and sizes, the visualization detection of biochemicals can be achieved. It is due to the surface plasmon resonance (SPR) and the surface-enhanced Raman scattering (SERS) properties of AuNPs. When the distance between AuNPs or the configuration of AuNPs are changed, the plasmonic resonance wavelength of maximum absorption are also changed. Due to the SPR phenomenon from visible to near-infrared spectra range, the color of the AuNPs solution varies correspondingly [14]. Thus, the color changes of AuNPs can even be observed by naked eyes. With the multiple optical properties, AuNPs have been introduced to many detection fields. Therefore, in this work, the visualization detection applications are classified based on the target biochemical samples including latent fingermarks and pathogens.

2.2.1 Visualization of Latent Fingermarks

Nowadays, the requirement of a stable and safe living condition from the citizens has been emphasized along with the great progress of human society and civilization. In the area of criminal investigation, the detection of fingermarks is of great importance. Although there have been lots of modern physical or chemical fingerprint-detection techniques targeting on various substrates such as paper, plastic, wood, human skin..., researchers have developed the multi-metal deposition technique (MMD) and single-metal deposition technique (SMD) both based on metal nanoparticles. AuNPs are of great importance in both of the two techniques.

MMD is based on colloidal gold and is applied to detect the latent fingermarks both on different types of surfaces. The MMD process can be simply described as two parts: the first is to treat the sample in the solution of AuNPs; and the second is to use physical developers (usually silver physical developer, Ag-PD) to enhance the image of aggregated AuNPs on the fingermarks. However, the experimental conditions strongly affect the outcome of MMD techniques. M. Sametband et al. functionalize AuNPs with hydrophobic $n$-alkanethiols which could greatly enhance the intensity and clarity compared to the mere AuNPs [15]. X. Spindler et al. started from another sight, abandon Ag-PD enhancement and take use of the interaction between amino acids and anti-L-amino acid antibodies functionalized AuNPs [16]. The method is efficient in detecting the aged fingermarks on the non-porous samples. In addition, N. Jaber et al. first come up with the idea of reversed method for fingermarks detection on papers [17]. They have conjugated AuNPs with bifunctional reagents which has both a sulfur group bonding with AuNPs and an acyl pyridazine binding with paper cellulose. Thus, the functionalized AuNPs will attach to paper substrate but not the traditional fingermarks and then Ag-PD is applied to precipitate on the AuNPs to enhance the trace. Finally, the fingermarks can be observed with dark paper substrate.

MMD is efficient but complicated to handle. Therefore, a more convenient method named SMD is created. A. Becue et al. develop a one-step method with cyclodextrins functionalized AuNPs, which can efficiently bind the molecules in solution [18]. Thus, only one solution bath is needed for the detection of fingermarks without the second step in MMD and this approach can be named as SMD. D.M Gao et al. use sodium borohydride to reduce the Au(III) with glucose as a stabilizer [19]. With only one bath of non-porous samples in the glucose functionalized AuNPs solution, the latent
fingermarks could be detected quickly and clearly. Also, there is a so-called SMD II method, which uses AuNPs to first deposit on the latent fingermarks and then applies AuNPs again to enhance the deposited fingermarks. G. Kolhatkar et al. present SMD detection on transparent glass substrates based on trisodium citrate dihydrate synthesized AuNPs as the reagent for SMD II [20]. They investigate the luminescence enhancement by stereomicroscopy and detect SERS whose results confirm the feasibility of the method.

2.2.2 Visualization of Pathogens

The applications of AuNPs in the visualization detection of pathogens attract much interest of researchers. Recently, because of the widespread COVID-19, a quicker and more effective detection for the virus SARS-CoV-2 is required. There are basically 2 detection methods: antigen detection and RNA detection.

The AuNP-based antigen detection has been investigated deeply. For SARS-CoV-2, the specific antigen could be the spike protein (S protein) or the nucleocapsid protein (N protein). For S protein detection, X.H. Chen et al. functionalize AuNPs with biotinylated nanobodies (S protein receptor binding domain for SARS-CoV-2) and achieve a rapid optical detection by color changes in the solution under LED light source [21]. Their method allows for a quick test within 5-20 min. M.J. Bistaffa et al. design an immunoassay approach which is capable of detecting S protein under trace levels [22]. They first make a conjugation of AuNPs, methylene blue (MB) and ultrathin silica shell. Then the conjugation of AuNPs is functionalized with antibodies to form a nanoprobe. On a cover slide conjugated with antibodies, they put the flow of antigens and nanoprobes in order. Finally, the nanoprobes are attached to the high concentration part, resulting in SERS which can be quickly and precisely detected. Also, P.B. da Silva et al. use AuNPs functionalized with S protein antibodies to achieve a quick and cheap detection within 0.5 h by dynamic light scattering (DLS) [23]. DLS spectrum is altered by the bioconjugation between functionalized AuNPs and S proteins. In this approach, the limit of detection is quite low and the selectivity is proved to be effective. Also, it is suitable for samples of large volumes, providing an economical detection approach.

For RNA detection, M. López-Valls et al. introduce a naked-eye method [24]. They first use clustered regularly interspaced short palindromic repeats (CRISPR) protein to recognize RNA of SARS-CoV-2 and start the RNase activity. Thus, the single-stranded RNA oligonucleotides on the functionalized AuNPs will be cut off and the AuNPs will aggregate together, inducing a color change in solution. However, the method still needs the extraction process of RNA and remains complicated to handle. M. Alafeef et al. develop a diagnosis detection method without the extraction of RNA of SARS-CoV-2 [25]. They functionalize the AuNPs with antisense oligonucleotides (ASOs), which is a specific reporter for SARS-CoV-2 N-gene. If the specimens with SARS-CoV-2 are mixed into the ASOs-conjugated AuNPs solution, the aggregation of AuNPs will take place and induce the color change of the solution, providing a quick and naked-eye detection.

3. Conclusions

In this work, the applications of AuNPs on visualization detection were investigated. The synthetic methods of AuNPs of different sizes and morphologies were discussed. Three important applications of AuNPs on visualization detection are reviewed: fingermarks and pathogens.

This work may also provide some promising ways for further investigations. Due to the inner optical properties of AuNPs, the aggregation or surface changes of functionalized AuNPs can be observed by naked eyes or spectra-methods. Functionalized AuNPs, which conjugated with a variety of biochemicals, could greatly enrich their application fields. This efficient and concise method promises an optimistic outlook in the field of visualization detection.
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