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CHARACTERISATION OF PEROXIDASE-LIKE ACTIVITY OF THERMALLY SYNTHESIZED GOLD NANOPARTICLES

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Abstract

The development of nanotechnologies has attracted a lot of interest since it brings new possibilities in the field of nanomedical applications, from diagnostic methods to targeted nanotransporters. Gold nanoparticles (AuNPs) belong to a group of nanomaterials with significant peroxidase-like activity. We have successfully synthesized AuNPs thermally over a range of temperatures from 20 to 100 °C. The AuNPs size varied from 17 to 37 nm and the zeta potential was found to vary from -32 to -44 mV, in which the maximum absorption maximum was detected at 529 nm. Peroxidase-like activity was monitored in the presence of TMB (1 mM) and hydrogen peroxide (10 mM). The proposed system based on peroxidase-like activity was further evaluated in immune detection of human IgG, in which anti-IgG antibody was modified with AuNPs.

Keywords: Gold nanoparticles, electrochemistry, spectrophotometry, enzymatic reaction, ELISA, nanomedicine

1. INTRODUCTION

With a strong development of nanotechnology and its subsequent applications in biology, there is significant interest in their application in nanomedicine in the field of drug delivery, imaging or biological separation techniques. Despite the fact that nanoparticles have shown adverse effects, they are almost biologically and chemically stable [1]. Gold nanoparticles (AuNPs) are used as a suitable tool in molecular biology experiments, especially for introducing genetic information into cells [2]. Furthermore, with the rapid development of nanotechnology, they are used as one of the major carriers of biomolecules in nanomedicine and biosensor applications [3, 4], including applications in experimental cardiology [4]. Peroxidase reaction participates in many of the biochemical pathways and biotechnological applications [5] due to the fact that some groups of compounds or nanomaterials may have enzyme activity (also peroxidase) [5-7]. As it is shown in **Figure 1**, since 2010, the number of outputs regarding with this research has dramatically increased.

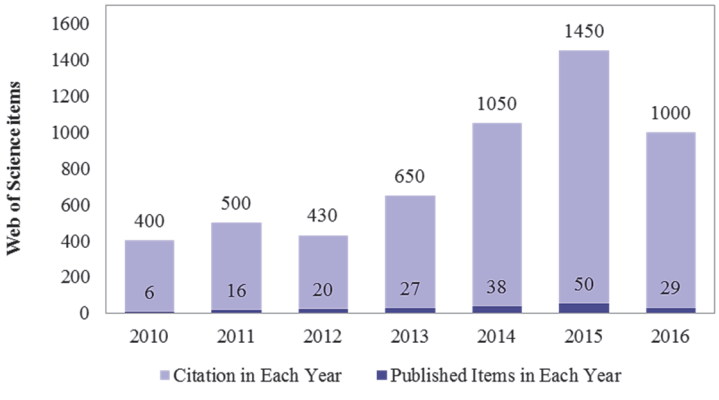

Figure 1 Web of Science - number of items for the keyword peroxidase-like activity, data evaluated between the years 2010 and 2016 (to the date of 5. 8. 2016)

Very interesting and attractive possibilities and applications of nanomaterials with peroxidase-like activity have been reported since. Peroxidase-like activity is not limited only to the type of nanomaterial; nanomaterials may have modifying effects on peroxidase-like activity. Graphene in combination with gold nanoparticles significantly enhances ~~their~~ peroxidase-like activity [8]. Other nanoparticles have been successfully reported and prepared, such as carbon-based AuPd bimetallic nanocomposite (AuPd/C NC) [9]. Special type of nanoparticles - nanoflowers - modified with amino acids exhibited peroxidase-like activity depending on the reaction mechanism of Fenton-like reaction [10]. Combination of nanomaterials with peroxidase-like and peroxidase activity leads to an enhancement of this activity too. Peroxidase activity has a wide range of practical applications. For example, the ability to catalyse the oxidation of organic substrates to reduce their toxicity and/or to produce a colour change, it could be used as a detection tool of heavy metals [11] or urea and urease [12]. The aim of the work was to synthesize AuNPs modified with trisodium citrate at different temperature profiles and to study their peroxidase-like activity. The fact that AuNPs have peroxidase-like activity poses the potential for their novel applications as ELISA.

2. MATERIAL AND METHODS

Reagents

Human IgG (H-IgG), goat anti-human IgG antibody (anti-IgG), HRP labelled goat anti-human IgG antibody (HRP-an- ti-IgG), and bovine serum albumin (BSA) were supplied by Sigma Aldrich. 3,3′,5,5′-tetramethylbenzidine (TMB) and sodium citrate dihydrate, hydrogen tetrachloroaurate(III) hydrate ($HAuCl_4 \cdot 4H_2O$), hydrogen peroxide (H_2O_2), hydroxylammonium chloride ($NH_2OH \cdot HCl$), sodium carbonate anhydrous (Na_2CO_3), sodium hydrogen carbonate ($NaHCO_3$), disodium hydrogen phosphate dodecahydrate ($Na_2HPO_4 \cdot 12H_2O$), sodium dihydrogenphosphate dihydrate ($NaH_2PO_4 \cdot 2H_2O$), acetic acid (HAc),and sodium acetate (NaAc) were purchased from Merck. All reagents were of analytical grade and used without any further purification.

Synthesis of Gold Nanoparticles (AuNPs) and AuNPs-anti-IgC

AuNPs were synthesized according to the reported method with slight modification [13]. Thermal synthesis of AuNPs was performed using a magnetic stirrer with heating under controlled temperature, stirring for 60 minutes and preventing evaporation of liquid. The anoparticles were characterized using the following equipment and approaches: ZetaSizer, spectrophotometry, fluorimetry, and electrochemistry. Preparation of AuNPs-anti-IgG conjugate followed. One ml of anti-IgG (1 mg/ml) was added into 10 ml of AuNPs suspension with pH at 9.0 (0.1 M Na_2CO_3 and 0.1 M HCl were used to adjust pH). The mixture was incubated at room temperature for 1 h. Then, 1 ml of 10% BSA solution was injected under stirring to block the unspecific binding of proteins to antibodies on AuNPs, followed by incubation at room temperature for 0.5 h. The conjugate was then centrifuged (16,000 rpm) at 4°C for 15 min, and the soft sediment was washed and re-suspended in PBS solution [14]. The blue colour development appeared after an incubation in 100 µl of 1 mM TMB and 10 mM H_2O_2 (15 min, 28°C). The absorbance at 652 nm was recorded by using a reader Infinite M200. For comparison, HRP-anti-IgG was used as a detection antibody to detect H-IgG in a substrate solution containing 1 mM TMB and 10 mM H_2O_2 (incubation for 15 min at 28°C). Prepared nanoparticles (20AuNPs, 40AuNPs, 60AuNPs, 80AuNPs, 100AuNPs) were characterized by different approaches including spectrophotometry (UV-3100PC, VWR Germany) and Infinite F50 (TECAN, Switzerland, fluorimetry (Cytation 3 Cell imaging multi-mode reader, TECAN, Switzerland) and electrochemistry. Electrochemistry: measurements were carried out in a volume of 1.0 ml; where the electrolyte was 0.2 M acetate buffer (pH 5). Parameters of voltammetry were as follows: initial potential −1.2 V; end potential –0.3 V; potential step 3 mV; and potential of deposition –1.0 V. Electrochemical measurements were performed using a minicomputer-connected potentiostat 910 PSTAT mini (Metrohm, Switzerland).

3. RESULTS

Preparation of nanoparticles is based on Tukevich Method for Gold Nanoparticle Synthesis [13]. Thermal synthesis at different temperatures (20, 40, 60, 80, and 100 °C) was used to prepare AuNPs and subsequently were modified with trisodium citrate. The AuNPs were characterized by available physical-chemical approaches. **Figure 2a** illustrates typical recordings of AuNPs prepared by thermal synthesis at different temperatures 20, 40, 60, 80, and 100 °C and the absorption spectra of individual types of AuNPs.

Size of AuNPs varied from 17 to 37 nm, zeta potential was between -32 and -44 mV; absorption maximum was recorded at 529 nm. Peroxidase-like activity was evaluated for individual AuNPs; all types exhibited significant peroxidase-like activity.

Figure 2B shows the ability of AuNPs to create a colour product with a characteristic blue coloration. AuNPs with TMB without hydrogen peroxide showed no colour reaction, TMB with only hydrogen peroxide (without AuNPs) showed no colour change too.

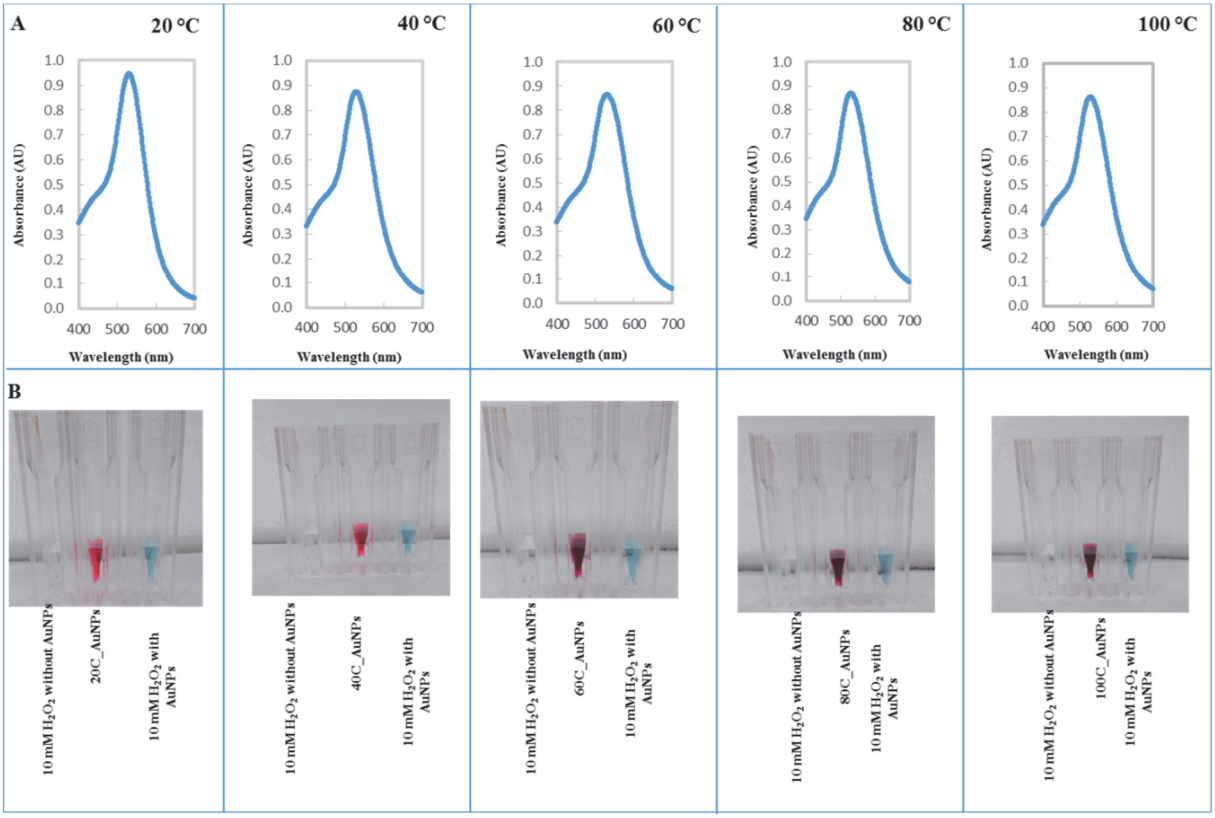

Figure 2 Typical recordings of AuNPs prepared by thermal synthesis at temperatures 20, 40, 60, 80, and 100 °C. A) Absorption spectra of individual types of AuNPs. B) Peroxidase-like activity of individual types of AuNPs in the presence of 1 mM TMB and 10 mM H_2O_2 (incubation 15 min at 24°C)

AuNPs in the presence of hydrogen peroxide oxidize TMB and produces a characteristic colour product at 652 nm. A characteristic absorbance curve showing the effect of gold nanoparticles on the oxidation of TMB in the presence of hydrogen peroxide is shown in **Figure 3** in which the absorbance maximum was detected at 652 nm. The AuNPs synthesized in-house at different temperatures exhibited similar signals. **Figure 3C** represents the dependence of the relative peroxidase-activity with time. The results indicate a positive response with the maximum signal in the time interval between 10 and 15 min. The effect of the amount of AuNPs on the peroxidase-like activity is represented in **Figure 3D**. A linear response is observed over the range from 3 to 200 ng/µl here y = 0.0013x - 0.0022 and R^2 = 0.9975.

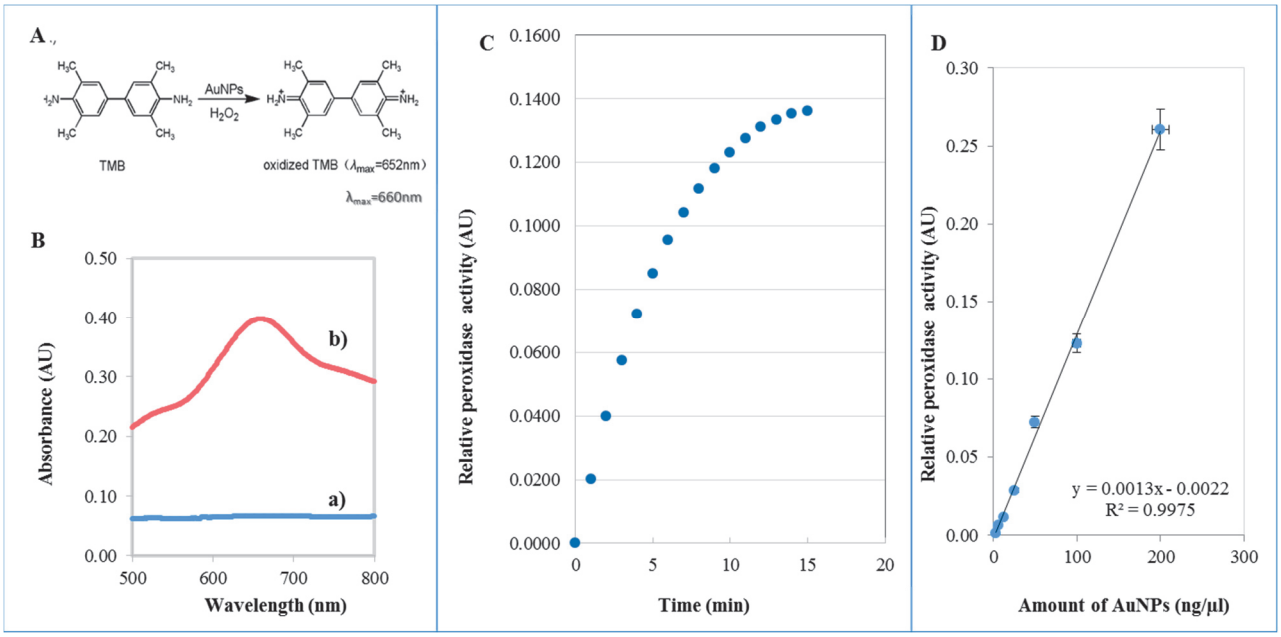

Figure 3 (**A**) Scheme of TMB oxidation by AuNPs. (**B**) Typical absorption curves of TMB a) before and b) after addition of hydrogen peroxide into a reaction mixture with AuNPs. The peroxidase-like activity of the AuNPs (**C**) Relative activity of 20AuNPs, (**D**) Effect of AuNPs concentration on relative activity (1 mM TMB and 10 mM H_2O_2, incubation 15 min at 24 °C)

We can expect a very broad application of nanomaterials in diagnostic procedures, such as ELISA, in the future. Next, we modified antibody with AuNPs to investigate immunoglobulin content in biological samples. Modified antibody were used in the ELISA method to verify possible application of AuNPs.

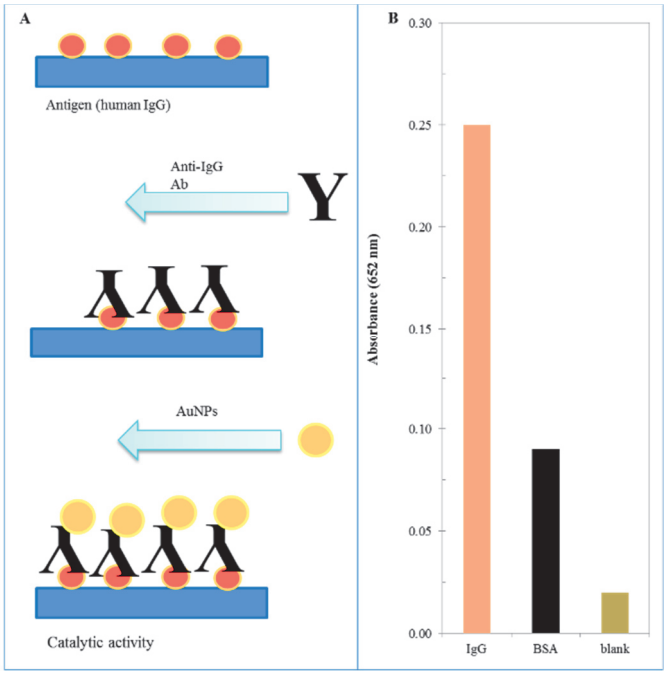

Figure 4 Immunoassay based on the peroxidase-like activity of AuNPs nanoparticles. Human IgG antigen was recognized by anti IgG antibody and detected by AuNPs. AuNPs conjugated to the antibody (**A**). A colour reaction was developed when the substrate TMB was added in the presence of H_2O_2, (**B**). Comparison between IgG, BSA and Blank at maximum absorbance 652nm

Figure 4a shows the proposed and developed immunoassay protocol based on the peroxidase-like activity of AuNPs. Antigen (IgG) was immobilized on the surface of the plate. In the first step of the reaction, the Antigen reacted with anti-IgG antibody labelled with AuNPs. The TMB oxidases in the presence of hydrogen peroxide which also change the colour.

Figure 4b represents a series of responses for IgG, in comparison with control sample (BSA) and TMB without IgG-AuNPs (Blank) at the maximum absorbance 652nm. The maximum response was obtained for IgG followed by BSA and the blank.

4. CONCLUSION

For the first time we have successfully reported a new method in which AuNPs were thermally synthesized and they exhibited peroxidase-like enzymatic activity in a wide range of concentrations from 3 to 200 ng/µl Moreover, AuNPs were used as a platform for antibody modification of anti-IgG as well as being successfully employed as a sensor for the detection of human IgG using a simple immune detection method without the application of proteins as enzymes.

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