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GRAPHENE QUANTUM DOTS AS A REDUCING REAGENT AND STABILIZER FOR GREEN SYNTHESIS OF SILVER NANOPARTICLES: TOWARDS A HYDROGEN PEROXIDE AND GLUCOSE SENSOR

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ABSTRACT

In this work, we have developed a simple method for preparation of silver nanoparticles/graphene quantum dots hybrid (AgNPs/GQDs) using graphene quantum dots (GQDs) as reducing reagent and stabilizer. The synthesized GQDs and AgNPs/GQDs hybrid have been characterized by ultraviolet–visible spectroscopy (UV-Vis), photoluminescence (PL), X-ray diffraction (XRD) and transmission electron microscopy (TEM). Results indicated that mono-dispersed AgNPs were obtained with particles size around 30 nm. Based on the etching of silver nanoparticles by hydrogen peroxide (H_2O_2), we have constructed a colorimetric sensor for H_2O_2 and glucose sensors basing on the use of AgNPs/GQDs hybrid as capture probe and signal probe. The fabricated sensors performed excellent sensitivity and selectivity, high reproducibility for H_2O_2 and glucose detection with a low detection limit of 100 nM and 0.1 mM for hydrogen peroxide and glucose, respectively.

Keywords: graphene quantum dots, silver nanoparticles, green synthesis, glucose sensor, hydrogen peroxide detection.

1. INTRODUCTION

Diabetes mellitus is one of the most common non-communicable diseases globally, and its related complications result in increasing disability, reduced life expectancy, and enormous health costs for virtually every society. Therefore, the efforts to develop various sensors for fast and reliable glucose monitoring for the diagnosis of diabetes have received continuous interest, for which enzyme-based glucose sensors have been extensively explored [1-2]. Currently, glucose sensors have been fabricated basing on the use of two enzymes: glucose oxidase as specific enzyme (capture probes) and the second is peroxidase for signal measurement (signal probes). However, natural enzymes (such as peroxidase) in organisms are proteins composing of hundreds of amino acids that can catalyze chemical reactions. It has been widely applied in

various fields because of their high substrate specificity and catalytic efficiency. Moreover, their catalytic activity can be easily affected by environmental conditions such as acidity, temperature and inhibitors. Furthermore, high costs of preparation, purification and storage also restrict their widespread applications [1-4]. Therefore, many nanomaterials with unique peroxidase-like activity have been discovered, including magnetic nanoparticles and their composites [1, 5, 6], cerium oxide nanoparticles [7], silver nanoparticles [2] and carbon-based nanomaterials [8-10]. These nanostructured materials as peroxidase mimetic show unparalleled advantages of low cost and stability over natural enzymes [1].

In this work, we have synthesized AgNPs/GQDs hybrid by using of GQDs as reducing reagent and stabilizer. We have found that, the AgNPs/GQDs have catalytic activity as peroxidase-like for degradation of H_2O_2, therefore a colorimetric sensor directed to H_2O_2 has been fabricated using AgNPs/GQDs as capture probe and signal probe. By combining of AgNPs/GQDs hybrid with glucose oxidase (GOx), a glucose sensor has also been generated.

2. MATERIALS AND METHODS

2.1. Synthesis of silver nanoparticles/graphene quantum dots hybrid (AgNPs/GQDs) using GQDs as reducing reagent and stabilizer

GQDs have been synthesized by hydrothermal method following previous report [11] with small a modification; urea has been used instead of thiourea. For synthesis of AgNPs, 100 µL of GQDs stock solution was added into 3 mL of D.I water; after that, 20 µL of 0.1 M $AgNO_3$ solution was added into the GQDs solution. The mixture was heated to 90 ^oC for 3h. AgNPs/GQDs have been formed in the solution then were cooled to room temperature. The AgNPs/GQDs solution has been stored at 4 ^oC for use. To prepare of AgNPs/GQDs detection probe solution, 250 µL of synthesized AgNPs/GQDs solution was pipetted into 10 mL of D.I water and the solution was stirred by vortex machine and stored at 4 ^oC for use.

2.2. Direct detection of hydrogen peroxide

1 mL of AgNPs/GQDs detection probe solution was pipetted into an eppendorf. Then, 100 µL of H_2O_2 solution was added. The mixture was stirred by vortex machine for 30 sec and then it was incubated at 40 ^oC in a water bath for 20 minutes. Then the UV-vis spectrum of solution was recorded. The optical density at 415 nm (OD_{415}) of the AgNPs/GQDs solution before and after addition of various H_2O_2 quantities was used to draw a calibration curve, i.e. $\Delta A/A_0$ vs. $[H_2O_2]$, here:

$$\Delta A = 100*(A_0 - A_C)/A_0 \quad (1)$$

where A_0 and A_C are OD_{415} of the AgNPs/GQDs solution before and after H_2O_2 addition, respectively).

2.3. Direct detection of glucose

100 µL of glucose oxidase (GOx) (2 $mg.mL^{-1}$) was added into 200 µL of glucose solution at various concentration. Then, the mixture was heated to 37 ^oC for 30 min. Then, 1 mL of AgNPs/GQDs solution was added. The mixture was stirred and incubated in a 40 ^oC water bath for 20 minutes. Finally, the reaction mixture was transferred to a cuvette for UV-vis absorbance measurement and optical density at wavelength of 415 nm was recorded.

3. RESULTS AND DISCUSSION

3.1. Synthesis of AgNPs/GQDs hybrid using GQDs as reduction reagent

The UV–vis absorption spectrum of GQDs (Fig. 1A, curve (i)) exhibited two distinct absorption peaks at about 220 and 345 nm, which were attributed to the π–π* transition of C=C and the n–π* transition of C=O, respectively. The fluorescence emission spectra of the GQDs were recorded and maximum fluorescence emission (~520 nm) was obtained with an excitation wavelength of 380 nm (Fig. 1A, curve ii).

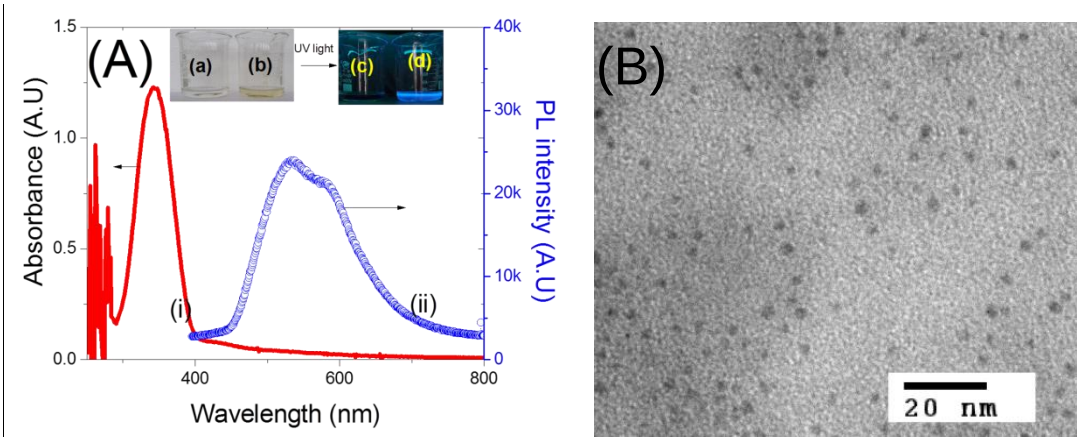

Figure 1. (A): (i) UV-vis spectra and (ii) PL spectra of GQDs (inset: color of (a,c) water and (b,d) GQDs under normal light and violet light); (B): TEM of GQDs.

Figure 1A (inset) indicated the color of water (a, c) and GQDs solution (b, d) under normal light (a, b) and violet light (c, d). It can be seen the emission at blue light of the GQDs solution under violet light (Fig.1A, inset d). Moreover, the emission wavelength showed a red shift with increasing excitation wavelength (data not shown). According to the TEM image (Fig. 1B), the synthesized GQDs were of spherical shape and monodisperse nanoparticles with size distribution in the range of 5 ± 2 nm.

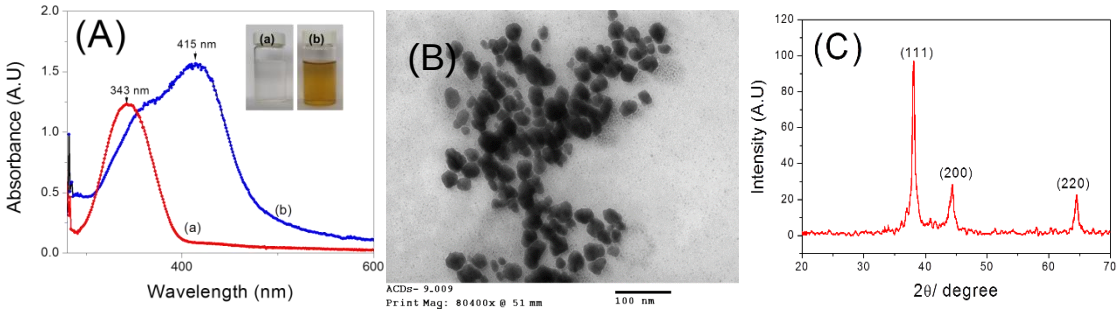

Figure 2. (A): UV-vis spectra of: (a) GQDs, and (b) AgNPs/GDQs (inset: the color of (a) GQDs and (b) AgNPs/GDQs solution, respectively); (B): TEM of AgNPs/GDQs; and (C): XRD of AgNPs/GDQs.

After heating at 90 ^{o}C for 3 hours, the mixture of $AgNO_3$ solution and GQDs solution turned dark yellow (Fig. 2A, inset (b)) indicating the formation of AgNPs in solution. As shown by the UV-vis spectra of AgNPs/GDQs (Fig. 2A, curve b), the new adsorption band at 415 nm is attributed to the specific surface plasmon of AgNPs, which is compared with UV-vis spectra of

GQDs only (Fig. 2A, curve (a) and inset (a)). TEM micrographs of AgNPs/GDQs (Fig. 2B) show that the AgNPs have a spherical shape, a smooth surface morphology and particle sizes from 5 nm to 40 nm. No aggregation of AgNPs was evidenced, which demonstrates the stabilizing role of GQDs. A diffractogram of AgNPs/GDQs is shown on Fig. 2C, which evidences the typical diffraction planes (111), (200), (220) of the *fcc* lattice of AgNPs. The diffractogram does not exhibit any diffraction peak of GQDs. These results confirmed the successful preparation of the AgNPs using graphene quantum dots (GQDs) as reducing reagent and stabilizer.

3.2. Colorimetric sensor for hydrogen peroxide detection

It is clearly shown that in-situ growth of AgNPs in the GQDs solution, which has resulted in a strong absorption band at 415 nm (Fig. 3A, curve a) a specific of surface plasmon of silver nanoparticles (AgNPs), responsible for the yellowish color of AgNPs/GQDs solution. An obvious color fading was observed in the presence of H_2O_2, more pronounced for increased H_2O_2 concentration (Fig. 3E, insert). This behavior provides a potential for quantitative detection of H_2O_2. Fig. 3A (curve b) shows the UV−vis absorption spectra of AgNPs/GQDs solution in the presence of H_2O_2. The color fading was attributed to the oxidation of AgNPs in the presence of H_2O_2, the standard potential of Ag^+/Ag being lower than that of H_2O_2/H_2O ($E^0_{Ag+/Ag}$ = 0.8 V < $E^0_{H2O2/H2O}$ = 1.77 V) in water at pH ~7. This reaction equation is described in equation (2) below:

$$(GQDs)Ag^0 + H_2O_2 \rightarrow (GQDs)Ag^+ + 2OH^- \quad (2)$$

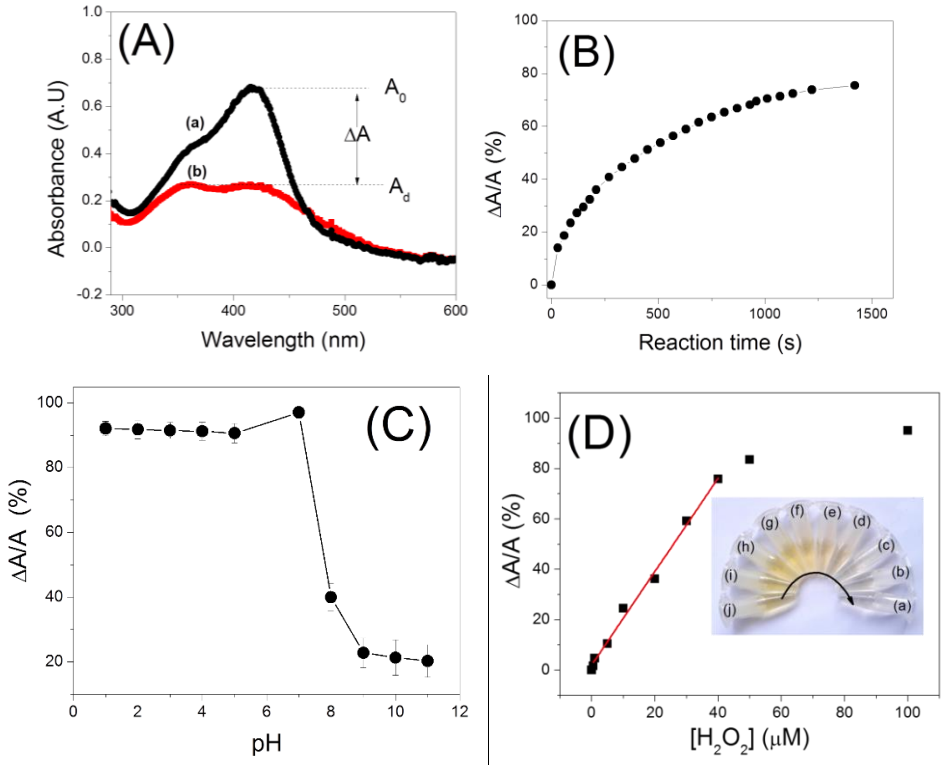

Figure 3. (A): UV−vis spectra of AgNPs/GQDs (a) before and (b) after addition 30 µM H_2O_2; (B): Plot ΔA/A (%) vs. reaction time; (C): Effect of pH on ΔA/A (%); (D): Corresponding calibration curve plotting $\Delta A*100/A_0$ versus H_2O_2 concentration (inset: digital photographs of AgNPs/GQDs solutions in the presence of (j to a) 0, 0.5; 1; 5; 10; 20; 30; 40; 50 and 100 µM H_2O_2, respectively).

The reaction time has been investigated using H_2O_2 concentration of 50 µM and plotting of ΔA/A (%) vs. reaction time (Fig. 3B). This result indicated the reaction can be finished after 20 minutes. Effect of medium (pH) on readout signal of the sensor has also been studied by detecting of 50 µM H_2O_2 in solution pH = 1 to pH =14 (Fig. 3C). These results indicated that a good medium for H_2O_2 detection is pH = 7 to pH = 7.5. The detection mechanism has been illustrated in previous report [2], which demonstrates the leading to a significant "oxidation-etching" of AgNPs with greatly reduced absorbance. Therefore, the concentration of Ag^0 in the AgNPs/GQDs solution is depleted, which explain the fading of the AgNPs/GQDs solution when H_2O_2 is added. Therefore, the H_2O_2 concentration can be quantified by monitoring the decrease in the AgNPs surface plasmon resonance at 415 nm. A linear calibration was obtained by plotting ΔA/A (%) vs. H_2O_2 concentration within a concentration H_2O_2 range from 0 to 50 µM following equation:

$$\Delta A/A\ (\%) = (2.1725 \pm 1.352) + (1.8525 \pm 0.065).C_{H2O2}(\mu M), \quad (3)$$

with R^2 = 0.9927. The detection limit was estimated around 500 nM (Fig. 3E). For immediate and qualitative detection, this reaction can also be monitored by naked eyes (Fig. 3E, inset).

3.3. Colorimetric sensor for glucose detection

Based on the sensitive of hydrogen peroxide sensor, we have designed a visual sensor for glucose detection by using glucose oxidase (GOx) enzyme to oxide of glucose to gluconic acid and H_2O_2 by equation (4):

$$\text{Glucose} + O_2 + H_2O \xrightarrow{\text{GOx}} \text{Gluconic acid} + H_2O_2 \quad (4)$$

H_2O_2 product from equation (3) can be recognized by AgNPs/GQDs as mentioned above. UV-vis spectra of mixture of AgNPs/GQDs detection probe solution and GOx mixed with glucose solution with different glucose concentration are shown in Fig.4A. It can be seen that absorption peak at 415 nm of AgNPs decreased by increasing of glucose concentration. It can be attributed to increasing of glucose concentration, GOx can oxide glucose to higher H_2O_2 concentration therefore AgNPs have been etched stronger, which can be observed by stronger decreasing of absorption band at 415 nm. From Fig. 4B, glucose concentration in samples can be quantified by monitoring the decrease in the AgNPs surface plasmon resonance at 415 nm. A linear calibration was obtained by plotting ΔA/A (%) vs. glucose concentration within a range from 0 to 16 mM:

$$\Delta A/A\ (\%) = (1.72 \pm 1.25) + (3.50 \pm 0.12).C_{glucose}(mM), \quad (5)$$

with R^2 = 0.9925. The detection limit was estimated around 0.1 mM (Fig. 4B).

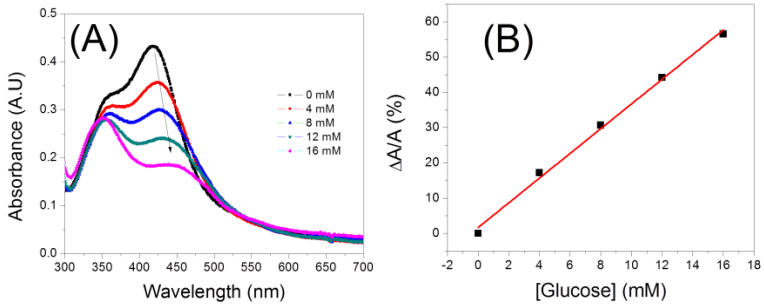

Figure 4. (A): UV−vis spectra of AgNPs/GQDs in presence of GOx + glucose solution with various glucose concentrations; (B) Calibration curve (ΔA/A (%) vs. glucose concentration) of the glucose sensor.

4. CONCLUSIONS

In summary, AgNPs have been successfully prepared by using GQDs as reducing agent and stabilizer. AgNPs/GQDs hybrid exhibits good performance for colorimetric detection of H_2O_2 and glucose. The simple fabrication procedure, effective discrimination ability, and low detection limit suggest that the proposed strategy may hold practical applications in environmental chemistry and biotechnology.

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