Immonocontrolling Graphene Oxide Catalytic Nanogold Reaction and Its Application to SERS Quantitative Analysis

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ABSTRACT: The gold nanoreaction between HAuCl4 and H2O2 is very slow at 50 °C, and the nanoenzyme of graphene oxide (GO) greatly catalyzes the nanoreaction to form gold nanoparticles (AuNPs) with high SERS activity in the presence of Vitoria blue 4R (VB4r) molecular probes, strong resonance Rayleigh scattering (RRS), and surface plasmon resonance (SPR) absorption effect. With the increase of GO, the SERS, RRS, and SPR absorptions were enhanced linearly due to the formation of more AuNPs. The rabbit antibody of human chorionic gonadotropin (RHCG) strongly adsorbed on the GO surface to inhibit its catalysis. Upon addition of human chorionic gonadotropin (HCG), the RHCG is separated from the GO surface due to the formation of HCG-RHCG specific immunocomplexes, which led to the recovery of GO catalysis. Using the new strategy of immunocontrolling GO catalysis, three types of resonance methods including SERS, RRS, and surface plasmon resonance (SPR) absorption have been developed for detection of HCG.

INTRODUCTION

Nanoparticles such as gold and graphene oxide not only are of enzyme property but also have high SERS activity. Since the Fe3O4 nanoenzyme was reported,1 research interest has risen rapidly, and it has been involved widely in different fields such as materials, physics, chemistry, biology, medicine, and environmental sciences. Compared with the natural bioenzyme, nanoenzymes have high stability and high catalytic activity, are cheap, and have other advantages over bioenzymes, especially to avoid the characteristics of bioenzymes of instability and variability. This unique property enhanced the application prospect of nanoenzymes in process catalysis and enzyme kinetics. Thus, these nanoparticles with catalytic activity have important significance in analytical chemistry. At present, the analytical application is mainly involved in the detection of heavy metal ions and biological molecules,7−8 based on nanoenzyme catalytic colored reactions. Seok et al.2 detected mercury ions, which based on the principle of ssDNA magnetic nanoparticles can inhibit the H2O2 oxidation of phthlate to amine with no color, and 5–75 μmol/L of Hg(II) caused the color deepening. Lien et al.3 used fluorescence enhancement to detect thrombin for H2O2 oxidation of Amplex reagent by thrombin transforming protein-mediated Bi-AuNPs, and Jiang et al.4 reported a simple nanogold catalytic spectrophotometric method for 2–10 nmol/L of glucose. Surface-enhanced Raman scattering (SERS) is due to some molecules adsorbed on a rough surface of the nanoparticles that caused the Raman scattering signal-enhanced phenomenon.9−12 It not only sensitively detects the concentration of molecules adsorbed on the nanosurface but also gives rich information on the molecular structure and has been widely used in materials, chemicals, polymer materials, biological, environmental protection, and other fields.13,14 According to the use of molecular probe markers, SERS can be divided into SERS marking technology and free-label SERS technology. SERS free-label technology is directly detected by the Raman signal itself or use of dye molecules to probe the Raman signal, according to the Raman fingerprints of the material to be quantitatively or qualitatively analyzed, with some advantages such as simple, fast, and direct use of the sample Raman characteristic signal without additional marking processing of the sample, which avoids the destruction of the sample, and these merits attract much attention.15,16 In addition, we have known that stable and highly SERS-active nanosol substrates are very important to SERS quantitative analysis. Although some gold nanosol substrates prepared by citrate and NaBH4 were used, the stable and highly SERS active nanosol substrate and nanoreaction with SERS activity will be explored still in SERS quantitative analysis, by means of green nanocatalytic synthesis.
Metal-free catalysts are newly emerging green catalytic materials that have attracted much attention in recent years for their advantages of high efficiency, environmental friendliness, and economy in many industrial catalytic processes. An important type of inorganic metal-free catalyst is nanocarbon materials that have demonstrated superior catalytic performance to traditional metal catalysts in many fields. Metal-free carbon-based catalysis has become one of the most promising research directions in nanomaterials and catalysis. The carbon material itself is used as the catalyst, and no metal is loaded or added; therefore, the active sites for the reaction are the defective structure or functional groups on the carbon surface. Graphene oxide (GO) is a kind of new carbon material with excellent performance such as good catalysis, high specific surface area, and abundant surface hydroxyl groups. In recent years, it has become a hot spot due to its unique physical, chemical, and biological characteristics. He et al. reported that GO prepared by the Hummers method could catalyze the hydrolysis of soybean isoflavones. In the nanoanalysis, Wang et al. established a resonance Rayleigh scattering (RRS) method to detect HSA, using GO as a probe. The SERS effect of GO was studied by Hao et al. The SERS properties of GO/Au/Ag composites were better than those of pure gold and silver nanoparticles. Banchelli’s research group found that GO–Ag composite nanoparticles were more effective than silver nanomaterials when used as a substrate in SERS analysis. Wang et al. used Cu²⁺-ion-modified graphene oxide nanoparticles as a heterogeneous catalyst, mimicking functions of horseradish peroxidase for the chemiluminescence detection of H₂O₂ and glucose. The dispersed Co₃O₄ nanoparticle-decorated crumpled graphene microsphere (CGM) possessed intrinsic peroxide-like activity and could catalytically oxidize 3,3′,5,5′-tetramethylbenzidine by H₂O₂ to produce a typical blue product and can be used to detect 30–140 μM ascorbic acid colorimetrically. An amperometric sensor was established for the detection of 0.1–43 μM indole-3-acetic acid, based on the hemin/reduced graphene oxide (hemin/rGO) composite with peroxidase-like activity. Rapid detection of sarcosine is a key requirement for both diagnosis and treatment of disease. A simple and sensitive colorimetric nanocomposite platform was reported for rapid detection of 0.73 μM sarcosine, based on the GO catalysis of the colored reaction of 1, 2-naphthoquinone-4-sulfonic acid sodium salt (NQS) that functionalized the GO nanocomposite through π−π stacking. At present, there are no reports about GO catalytic nanoparticle reaction with SERS activity and its application in nanoanalysis. HCG is a glycoprotein secreted by the placenta trophoblast cells, and it is an important medical diagnostic marker of pregnancy. It also is one of the important markers of clinical diseases, and its content is closely related to some diseases, such as gestational trophoblastic disease, germ cell tumors, and Down syndrome. In addition, the quantitative detection of HCG is of great significance to the analysis of clinical medicine and the abuse of stimulants. Immunoassay is a sensitive and selective analytical technology and was greatly paid attention by analysts. The detection methods are mainly immunoassays such as electrochemical, electrochemiluminescence, chemiluminescent, chemiluminescence resonance energy, fluorescence, resonance Rayleigh scattering, enzyme, and radioassay. Among them, the electrochemical immunoassay method has high sensitivity, but the operation is complex; the cost of fluorescence immunoassay is low, but there is a fluorescence quenching effect. Radioimmunoassay is widely used, but there are radiation hazards. Immunogold assay was the most mature and most widely used method for rapid detection of HCG, but this method can only detect whether or not HCG is present, and it is difficult to analyze the content. However, the application of SERS monitoring the GO catalytic oxidation–reduction nanoparticle reaction, the regulation of the GO catalytic activity by immune reaction, and its application in nanoanalysis have not been reported. In this paper, a new SERS quantitative analysis method was developed for HCG.

Figure 1. Scheme of the immunecontrolling GO catalytic activity–SERS detection of HCG. (a) GO catalyzed the formed AuNPs with strong SERS. (b) RHCG inhibited the nanocatalytic reaction with weak SERS. (c) HCG recovered the nanocatalysis to form AuNPs with strong SERS.
RESULTS AND DISCUSSION

Analysis Principle. Nanocatalytic reaction is an important route for analytical signal amplification and sensitivity improvement. The new nanoparticle catalytic reaction of $\text{H}_2\text{O}_2^-$−$\text{HAuCl}_4$ nanoparticle was investigated and used in the resonance scattering spectral analysis. The potential difference of +0.307 V indicates the reaction could take place. In fact, the reaction is very slow in the absence of nanocatalyst. Therefore, the uncatalytic reaction system exhibits weak SERS signal due to low concentration of AuNP as substrate, in the presence of VB4r molecular probes. We have known that GO containing abundant surface $\pi$ electrons, the $\text{AuCl}_4^-$ and $\text{H}_2\text{O}_2^-$, can be adsorbed on the GO surface, and the electron transfer of the AuNP reaction was enhanced greatly by means of the $\pi$ electrons. The produced small AuNPs could also act as nanocatalysts to speed the AuNP reaction. More AuNPs formed in the nanocatalytic system, and the SERS signal increased linearly with GO concentration. According to SERS theory, the SERS intensity ($I_{\text{SERS}}$) is related to incident laser intensity ($I_{\text{in}}$), molecular probe concentration ($C_M$), and the enhancement factor ($E_f$) of substrate physical properties such as size and shape of nanoparticles and the degree of aggregation, etc.47−49 The difficulty in obtaining highly stable and reproducible SERS signals renders SERS a qualitative or semiquantitative detection technique. Although some methods such as internal standard have been used to correct SERS intensity variations induced by the variations in the physical properties of SERS substrate, the process is complicated, and the internal standard is uneasy to obtain. Using highly stable and reproducible nanosol as SERS substrate, simple and accurate SERS quantitative analysis methods could be developed, and the SERS signals depend on not only the $C_M$ but also the nanosol concentration ($C_N$); that is, $I_{\text{SERS}} = K_1 \times I_{\text{in}} \times E_t \times C_M = K_2 \times I_{\text{in}} \times X_A \times C_N$. When the experimental conditions hold constant, the $K$ is a constant; the $I_{\text{SERS}}$ is linear to $C_N$ and the nanocatalyst GO concentration ($C_{\text{GO}}$) is linear to $C_N$, according to catalytic kinetics. Thus, the $I_{\text{SERS}}$ is linear to $C_{\text{GO}}$ that could be detected by SERS technique, as in Figure 1a. The RHCG has high affinity and specificity for antigen, and it can be easily adsorbed to the GO surfaces through electrostatic attraction that leads to weakening of GO catalysis (Figure 1b). When the HCG is present in solution, the RHCG selectively recognizes and tightly binds to HCG to form

![Figure 2. SERS spectra of the immunecontrolling GO catalytic system. (a) From low to high, the curves of the 13.33 ng/mL RHCG + 50 ng/mL GO + 0.15 mmol/L HCl + 2.5 mmol/L $\text{H}_2\text{O}_2$ + 6.3 μmol/L $\text{HAuCl}_4$ + 0.25 μmol/L VB4r system are 0, 0.33, 0.67, 1.67, 3.33, 6.67, 10, and 13.33 ng/mL HCG, respectively. (b) From low to high, the curves of the 13.33 ng/mL RHCG + 50 ng/mL GO + 0.167 mmol/L HCl + 0.34 mmol/L TCA + 5.6 μmol/L $\text{HAuCl}_4$ + 0.25 μmol/L VB4r system are 0, 0.67, 1.67, 3.33, 6.67, 10, 13.3, and 20 ng/mL HCG, respectively. (c) From low to high, the curves of the 20 ng/mL RHCG + 100 ng/mL GO + 0.5 mmol/L HCl + 50 mmol/L GS + 5.6 μmol/L $\text{HAuCl}_4$ + 0.33 μmol/L VB4r system are 0, 0.33, 1, 2, 4, 6, 10, and 13.33 ng/mL HCG, respectively.](https://doi.org/10.1021/acsomega.7b01335)
immunocomplexes that escape from the GO surface and the GO catalytic activity recovery, and the SERS signal enhanced linearly due to the formation of more active AuNPs as substrate (Figure 1c). Thus, a new SERS method was developed for the determination of trace HCG, with high sensitivity and selectivity.

**SERS Spectra.** For the immuno-controlling system of HAuCl₄ − H₂O₂, the VB₄r was used as a SERS probe; the main SERS peaks showed at 435 cm⁻¹, 803 cm⁻¹, 1197 cm⁻¹, 1203 cm⁻¹, 1398 cm⁻¹, and 1615 cm⁻¹; the assignment of those SERS peaks was examined (Table S1); and the intensity increased linearly at 1615 cm⁻¹ with the increase of HCG concentration (Figure 2a). For the immunocontrolling system of HAuCl₄−TCA and HAuCl₄−GS, the SERS peaks showed at 434 cm⁻¹, 804 cm⁻¹, 1201 cm⁻¹, 1292 cm⁻¹, 1388 cm⁻¹, and 1618 cm⁻¹, and the SERS intensity increased linearly at 1613 cm⁻¹ with the increase of HCG concentration (Figure 2b, 2c). In the three analytical systems, the HAuCl₄−H₂O₂ system is the most sensitive and most stable and was chosen for SERS detection of HCG. The SERS spectra of H₂O₂−HAuCl₄−GO nanocatalytic system were recorded (Figure S1A). The SERS intensity at 1617 cm⁻¹ increased linearly with the increase of GO nanocatalyst concentration. Similarly, small AuNPs also exhibited strong catalysis of the HAuCl₄−H₂O₂ reaction from the SERS spectra (Figure S1B). The SERS spectra of the RHCG−GO−H₂O₂−HAuCl₄ system showed that the SERS intensity decreased linearly with the increase of RHCG concentration (Figure S1C), and RHCG has strong inhibition on the catalysis.

**RRS Spectra.** RRS is a sensitive spectral technique to determine trace metal and organic compounds such as protein and DNA, and it is also a good and sensitive tool to investigate nanoparticle reaction and was selected to study the AuNP nanoreaction. The as-prepared AuNPs exhibited strong catalysis on the HAuCl₄−H₂O₂ reaction that indicated that formed small AuNPs in the reaction process also have catalysis, in which there are two RRS peaks at 300 and 540 nm (Figure 3a). The RRS spectra of GO−HAuCl₄−H₂O₂, RHCG−GO−HAuCl₄−H₂O₂, and RHCG−HCG−GO−HAuCl₄−H₂O₂ nanocatalytic systems were recorded. All systems exhibited two RRS peaks at 300 and 540 nm (Figure 3c−3d), and GO, RHCG, and HCG have catalysis, inhabitation, and recovery.

Figure 3. RRS spectra of the GO and AuNP nanocatalytic system. (a) From low to high, the curves of the 0.33 mmol/L HCl + 2.8 μmol/L HAuCl₄ + 2.5 mmol/L H₂O₂ system are 0, 38.7, 96.7, 154.7, and 2320 ng/mL AuNP, respectively. (b) From low to high, the curves of the 0.15 mmol/L HCl + 2.5 mmol/L H₂O₂ + 6.3 μmol/L HAuCl₄ system are, 0, 5, 12.5, 25, 37.5, 50, and 75 ng/mL GO, respectively. (c) From high to low, the curves of the 50 ng/mL GO + 0.15 mmol/L HCl + 2.5 mmol/L H₂O₂ + 6.3 μmol/L HAuCl₄ system are 0, 0.67, 1.67, 3.33, 6, 9, 13.3, and 16.67 ng/mL HCG, respectively. (d) From low to high, the curves of the 35 nmol/L RHCG + 50 ng/mL GO + 0.15 mmol/L/HCl + 2.5 mmol/L H₂O₂ + 6.3 μmol/L HAuCl₄ system are 0, 0.67, 1.67, 3.33, 6, 9, 13.3, and 16.67 ng/mL HCG, respectively.
catalysis, respectively. The RRS peak at 300 nm was chosen for detection of HCG, with high sensitivity.

**SPR Absorption Spectra.** The SPR absorption spectral technique is a simple and low-cost tool to examine some nanoparticles such as AuNPs in solution and was chosen for the AuNP reaction. For the GO−HAuCl₄−H₂O₂ nanoreaction (Figure 4a), the product of AuNPs exhibited a SPR absorption peak at about 520 nm, and these results indicated that the formed small AuNPs in the reaction process could also catalyze the nanoparticle reaction; that is, there is self-catalysis in the system. The spectra of the RHCG−GO−HAuCl₄−H₂O₂ and RHCG−GO−HAuCl₄−H₂O₂ systems (Figure 4c) showed that the catalytic activity of the GO nanoenzyme inhibited by RHCG and HCG recovery the GO activity, and the absorption value at 530 nm could be used for detection of HCG selectively (Figure 4d).

**GO Catalysis and Its Mechanism.** The three spectral techniques including SERS, RRS, and SPR absorption were...
used to study the AuNP reaction of HAuCl₄−H₂O₂. Results (Table 1) showed that the SERS and RRS intensity and SPR absorption value increased linearly with the GO catalyst concentration increasing, and the SERS is most sensitive with the biggest slope in the linear equation. The AuNPs with size of 8 nm also exhibited catalysis of the AuNP reaction, but it is less than the activity of GO. However, when RHCG concentration increased, the SERS and RRS intensity and SPR absorption value decreased linearly. The reason was that they could be attached to the surface of the GO nanocatalyst by intermolecular forces, to block the contact between the catalyst and the reactants and inhibit the catalytic activity.

The heterogeneous electron transfer of sp² carbons occurs at the edges and defects and not at the basal plan of graphene sheets. Oxygen-containing groups on the GO surface and the edges and defects and not at the basal plan of graphene attached to the surface of the GO nanocatalyst by redox to form more AuNPs (Figure 5).

**Optimization of Analysis Conditions.** For the RHCG−HCG−GO−H₂O₂−HAuCl₄−VB4r system, the analytical conditions, including GO, RHCG, HCl, HAuCl₄, H₂O₂, and VB4r, the reaction temperature, and time (Figure S2) were optimized, respectively. The effects of GO concentration on the ΔI₁₆₁₆ cm⁻¹ were investigated, and a 50 ng/mL GO was selected to use. The effects of RHCG concentration on ΔI₁₆₁₆ cm⁻¹ were investigated, and when it reached 13.33 ng/mL, the value of ΔI₁₆₁₆ cm⁻¹ was the largest; therefore, 13.33 ng/mL was selected. The dosage of HCl was optimized, and when the concentration of HCl was 0.15 mM/L, ΔI₁₆₁₆ cm⁻¹ reached the maximum value. A value of 0.15 mM/L HCl was chosen. When the H₂O₂ concentration reached 2.5 mM/L, the value of ΔI₁₆₁₆ cm⁻¹ was the largest, and was selected for use. The reaction temperature, and time were examined. A reaction time of 8 min at 50 °C, giving the largest ΔI₁₆₁₆ cm⁻¹ was selected for use. GO also catalyzed the gold nanoparticle reaction of TCA−HAuCl₄ and the RHCG−HCG−GO−TCA−HAuCl₄−VB4r system could be used for SERS detection of HCG. The conditions of the RHCG−HCG−GO−TCA−HAuCl₄−VB4r system were optimized (Figure S3). A 13.33 ng/mL RHCG, 50 ng/mL GO, 0.34 mM/L TCA, 5.6 μm/L HAuCl₄, 0.167 mM/L HCl, and 0.25 μm/L VB4r and a reaction temperature of 60 °C for 10 min were selected for use. The analytical conditions of the RHCG−HCG−GO−TCA−HAuCl₄−VB4r system were examined (Figure S4). A 0.5 mM/L HCl, 100 ng/mL GO, 20 ng/mL RHCG, 50 mM/L GS, 5.6 μm/L HAuCl₄, and 0.33 μm/L VB4r and a reaction temperature of 75 °C for 20 min were selected for use.

**Working Curve.** For the system of RHCG−HCG−GO−H₂O₂−HAuCl₄−VB4r, the SERS effect was enhanced with increasing HCG concentration, and the SERS intensity increased due to the nanocatalyst GO concentration increasing, and all three energy spectral peaks are at 1.7, 2.1, and 9.7 keV for the Au element.
Δ$I_{1617 \text{ cm}^{-1}}$ had a good linear relationship with HCG concentration in the range of 0.25–10 ng/mL, with a linear equation of $\Delta I_{1617 \text{ cm}^{-1}} = 101.6C + 31.8$, a correlation coefficient of 0.9905, and a detection limit of 0.07 ng/mL, and the RRS and Abs working curves were also obtained. For the system of RHCG–HCG–GO–TCA–HAuCl$_4$–VB$_4r$, the linear range is

Figure 6. TEM and ED of the nanocatalytic analysis system. (a) 35 nmol/L RHCG + 50 ng/mL GO + 0.15 mmol/L HCl + 2.5 mmol/L H$_2$O$_2$ + 6.3 μmol/L HAuCl$_4$. (b) a + 2.5 ng/mL HCG. (c) a + 10 ng/mL HCG.

Table 2. Comparison of the Immunocontrolling GO Catalytic Reaction: Spectral Methods for HCG

| system          | methods | detection range ng/mL | regress equation                  | coefficient | LOD ng/mL |
|-----------------|---------|------------------------|-----------------------------------|-------------|-----------|
| H$_2$O$_2$−HAuCl$_4$ | SERS    | 0.2–13.3               | $\Delta I_{1617 \text{ cm}^{-1}} = 101.6C + 31.8$ | 0.9905      | 0.07      |
| H$_2$O$_2$−HauCl$_4$ | RRS     | 0.5–16                 | $\Delta I = 83.7C + 108$          | 0.9847      | 0.20      |
| H$_2$O$_2$−HauCl$_4$ | Abs     | 1.0–18                 | $\Delta A = 0.0308C + 0.036$      | 0.9619      | 0.50      |
| TCA−HAuCl$_4$    | SERS    | 0.67–20.0              | $\Delta I_{1615 \text{ cm}^{-1}} = 43.1C - 50.8$ | 0.9972      | 0.22      |
| GS−HAuCl$_4$     | SERS    | 0.67–26.67             | $\Delta I_{1618 \text{ cm}^{-1}} = 55.2C + 36.4$ | 0.995       | 0.25      |
0.67–20 ng/mL HCG, with a linear equation of $\Delta I_{1615 \text{ cm}^{-1}} = 143.1C - 50.8$, a coefficient of 0.9972, and a detection of 0.5 ng/mL. For the system of RHCG–HCG–GO–GS–HAuCl4–VB4r, the linear range is 0.67–26.67 ng/mL HCG, with a linear equation of $\Delta I_{1618 \text{ cm}^{-1}} = 55.2 + 36.4$, a coefficient of 0.9995, and a detection limit of 0.25 ng/mL. From Table 2, we can see that the SERS system of HAuCl4–H2O2 is the most sensitive, which was chosen for sample detection. Although the sensitivity of the absorption method is inferior to the SERS and RRS methods, the cost is lowest. The sensitivity and cost of the RRS method are between the SERS and RRS methods.

Interference. The effect of the coexisting substances on the system for the SERS detection of 10 ng/mL HCG was investigated. The tested common interfering ions and amino acids, IgG and IgM, did not interfere with the determination when the relative error was within 10% (Table S2). It indicated that this nanocatalytic SERS method had good selectivity.

Analysis of Samples. Five serum samples of women were offered by the No.5 People’s Hospital of Guilin, Guangxi, China, and a 1.0 mL sample was diluted to 100 mL with water before determination. The following operations were according to the procedure of the RHCG–HCG–GO–H2O2–HAuCl4–VB4r system. In addition, recovery tests were performed. The results (Table S3) show that the recoveries were in the range of 96.40–98.76%, and the RSDs were in the range of 0.93–3.97%. The obtained results were not obviously different from clinical diagnosis values of the No.5 People’s Hospital, so the results demonstrate that the method was accurate and reliable.

**CONCLUSIONS**

First, the GO–H2O2–HAuCl4 nanocatalytic particle reaction was studied in detail by SERS, RRS, and SPR absorption techniques. Then, the antibody protein adsorbed on the surface of GO nanoparticles, which blocked the binding of the nanoenzyme to the reactants and inhibited its catalytic action, and the enhancement of catalytic effect led to the increase of SERS effect when the antigen was added. Finally, according to this principle of immunecontrolling GO activity, a new SERS method for HCG was established. Furthermore, other immunoreactions would combine with the GO catalysis to develop the SERS detection platform.

**EXPERIMENTAL SECTION**

**Apparatus.** A model of DXR smart Raman spectrometer (Thermo Company, United States) with laser wavelength of 633 nm and power of 3.0 mW, a model of Cary Eclipse fluorescence spectrophotometer (Varian Company, United States), and a model of TU-1901 double-beam UV–visible spectrophotometer (Beijing General Instrument Co., LTD, China) were used.

**Reagents.** A 0.50 mg human chorionic gonadotropin (HCG, Beijing Boosen Biotechnology Co., Ltd.) freeze-dried powder was dissolved in 1.0 mL of water and then diluted to 10 mL to obtain a 50 μg/mL HCG standard solution. The solution was diluted to a solution of 1 μg/mL before use. A 0.1 mg/mL rabbit antibody of HCG (RHCG, Beijing Boosen Biotechnology Co., Ltd.), 84 μmol/L (1%) HAuCl4·4H2O (Sinopharm Chemical Reagent Co., Ltd.), 0.1 mol/L H2O2, 1% trisodium citrate (TCA, Guangdong Shantou Xilong Chemical Factory), 0.5 mol/L glucose (GS), 0.1 mol/L HCl, 0.3 mol/L CH3COOH, and 0.1 mmol/L Victoria blue 4R (VB4r) were prepared. Graphene oxide (GO) was prepared by the Hummer procedure, and 1 mg of GO was dissolved in 100 mL of water by means of ultrasound to obtain a concentration of 10 µg/mL of GO. AuNPs with size of 8 nm were prepared by the NaBH4 procedure. All reagents are analytically pure, and the water was double-distilled.

**Procedure.** We put a moderate amount of GO, RHCG, and HCG into a 5 mL test tube and mixed well. Then, H2O2, HCl, and HAuCl4 were added into the test tube, diluted to 1.5 mL and mixed well, reacted in a water bath for a certain time, and cooled with tap water. Finally, molecular probes of VB4r were added, diluted to 2 mL, and mixed well. The mixture was transferred into a quartz cell, and we recorded its SERS spectra. The SERS peak intensity $I_{1615 \text{ cm}^{-1}}$ and the blank ($I_{1615 \text{ cm}^{-1}}$) without HCG were recorded, and the $\Delta I_{1615 \text{ cm}^{-1}} = I_{1615 \text{ cm}^{-1}} - (I_{1615 \text{ cm}^{-1}})^0$ was calculated.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.7b01335.

SERS spectra, curves of optimization condition, Table of assignment of SERS peaks, influence of the coexisting substances and analytical results (PDF)

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**Notes**

The authors declare no competing financial interest.

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