现代环境污染控制理论与技术

Theory and Technology of
Modern Environmental Pollution Control

蒋治良 / 主编

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前　言

环境保护已成为当前和未来的一项全球性重大课题之一。近来，环境污染如 PM2.5 和重金属污染已引起我国政府的高度重视。因此，运用现代科技新技术新方法，开展环境污染控制新理论与新技术研究具有十分重要的意义。

广西师范大学环境与资源学院的前身是广西师范大学计算分析测试中心，历经化学与生命科学学院、环境与资源学系。近年来，我们以珍稀濒危动植物生态与环境保护省部共建教育部重点实验室、广西环境污染控制理论与技术重点实验室、广西重点学科——环境科学、广西高校特色专业——环境科学建设为契机，锐意创新，追求卓越，形成了环境分析化学、污染控制、环境资源开发与利用、环境生态、环境地理 5 个较稳定的、颇具特色的、较高水平的研究方向，取得了一批在国际国内具有一定影响的研究成果。共获得国家自然科学基金项目 13 项，广西自然科学基金创新团队项目、重点项目、广西千亿元重大科技攻关工程项目等省级项目 11 项；在 *Environmental Science & Technology*，*Analytical Chemistry* 等国内外高水平刊物发表论文 500 余篇，其中 SCI，EI，ISTP 论文 160 余篇，SCI 一区和二区论文 23 篇；获省部级以上科技奖 7 项，其中一等奖 1 项，二等奖 3 项，三等奖 3 项；获得授权的中国发明专利 31 件；出版著作 5 部；等等。

本书共分 5 篇。第一篇环境分析与监测，论述了有关纳米材料制备及其在环境污染物免疫分析、适配体分析中的应用，以及重金属和持久性有机污染物的共振瑞利散射光谱分析和表面增强共振拉曼散射光谱分析；第二篇污染控制，主要论述了有关印染废水、糖蜜酒精废水、电镀废水等的催化湿式氧化、超临界水氧化、吸附、吸附-微波催化、吸附-氧化再生、光催化、高级还原、机械化学等处理新工艺、新技术；第三篇资源开发与利用，主要论述了有关天然产物提取新工艺、新技术和农产品废弃物的综合利用，包括广西特色植物中的活性天然产物的分离、鉴定、衍生、生理活性研究，及甘蔗渣、稻壳、沸石的综合利用等；第四篇环境生态，主要论述了有关土壤重金属污染特征及其生物修复新技术，包括退化或污染生态系统的综合评估、重金属污染土壤的生物修复、矿业废弃地的生态恢复、受污染自然水体的生物修复，及生态脆弱区的生物多样性保护等；第五篇环境地理，论述了有关石漠化综合治理模式和桂林市低碳旅游发展研究。

在该书编辑出版过程中，得到环境与资源学院老师们的大力支持，得到国家自然科学基金（21267004，21165005，21075023，21267005，41161057，21166005，51108100）、广西自然科学基金（2013GXNSFF016003）、珍稀濒危动植物生态与环境保护省部共建教育部重点实验室基金和广西环境污染控制理论与技术重点实验室基金等的资助，在此一并致谢！由于编写时间仓促，错漏或不妥之处，恳请同人批评指正。

蒋治良

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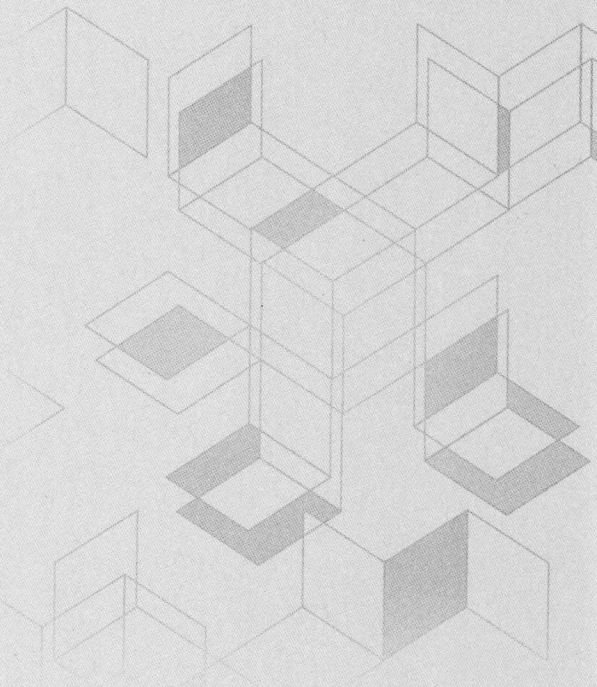
目 录

C O N T E N T S

第一篇　环境分析与监测



Resonance Scattering Spectral Detection of Trace Hg(II) Using Aptamer Modified Nanogold as Probe and Nanocatalyst

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Abstract

Single strand DNA (ssDNA) was used to modify 10 nm nanogold to obtain an aptamer modified nanogold resonance scattering (RS) probe (AussDNA) for detection of Hg^{2+}. In the presence of NaCl, Hg^{2+} interacts with AussDNA to form stable Hg^{2+}-ssDNA complexes, and release nanogold particles that aggregate to larger nanogold clusters causing the RS intensity at 540 nm enhanced linearly. On those grounds, 1.3-1667 nmol/L Hg(II) can be detected rapidly by the aptamer modified nanogold RS assay, with detection limit of 0.7 nmol/L Hg(II). If the larger nanogold clusters were removed by membrane filtration, the excess AussDNA in the filtrate solution exhibited catalytic effect on the new Cu_2O particle reaction between NH_2OH and Cu(II)-EDTA complex at 60℃. The excess AussDNA decreased with the addition of Hg^{2+}, which led Cu_2O particle RS intensity at 602 nm to decrease. The decreased RS intensity ($\Delta I_{602\,nm}$) had linear response to Hg^{2+} concentration in the range of 0.1 to 400 nmol/L, with detection limit of 0.03 nmol/L Hg^{2+}. This aptamer modified nanogold catalytic RS method was applied for the detection of Hg^{2+} in water samples, with sensitivity, selectivity and simplicity.

Key words

Hg^{2+}; Aptamer; Nanogold probe; Nanocatalysis; Cu_2O particle; Resonance scattering assay

Introduction

Mercury has been widely recognized as one of the most hazardous pollutants and highly dangerous elements due to its accumulative and toxic properties in the environment. Accumulative properties of mercury may cause serious harm to animals and human beings [1, 2]. Thus, simple, rapid, sensitive and selective detection of mercury is of great significance for biochemistry, environmental science and medicine. Although several modern

analytical techniques for mercury detection have been established, such as atomic absorption spectrometry[3], atomic fluorescence[4,5], and inductively coupled plasma mass spectrometry[6], most of them are time-consuming or/and require an expensive equipment. Therefore, it is still a challenge to develop rapid and inexpensive methods to monitor total mercury, with new technique and principle. Recently, chemosensors based on molecular recognition have started to emerge in very important research area within the field of supramolecular chemistry, which allows the detection of guest ions by binding-induced changes in spectroscopic or electrochemical properties. Among many Hg^{2+}-selective chemosensors developed so far, chromogenic receptors are especially attractive because the substrate determination can be carried out by the "naked-eye" without the use of instrumentation[7-9]. For example, Yan's research group reported a simple twisted intramolecular charge transfer chromogenic chemosensor for rapid and selective detection of up to 7.5×10^{-6} M Hg^{2+}, with 4,7-substituted 2, 1, 3-benzoxadiazole derivatives[9]. Aptamers are artificial nucleic acid ligands[10,11], showing specific binding affinity for metal ions, small organic molecules, peptides and proteins and even whole cells or microorganisms. Due to their considerable advantages, such as improved temperature stability and shelf life, ease in conjugation to various molecules, and adaptability to various targets, aptamers have become a potential alternative to antibody[12]. Based on T-T mismatches, aptamers were applied to the detection of Hg^{2+} by naked-eye and colorimetry, with high selectivity[13-15]. Resonance scattering (RS) spectroscopy was a new, simple, rapid and sensitive spectral technique[16-21], and it has been utilized for detection of inorganic and organic substances such as metal ions, antigens and DNA, combined with ion-associated complex reaction, and inorganic catalytic reaction, enzyme catalytic reaction, and immuoreaction[16-24]. However, there is no aptamer modified nanogold RS method for detection of Hg^{2+}, based on aptamer modified nanogold reaction and aptamer modified nanogold catalytic reaction. In this paper, we introduce two low-cost, rapid, sensitive and selective RS bioassays for detection of Hg^{2+}, combining AussDNA-Hg^{2+} reaction, membrane filtration and catalytic effect of AussDNA on the reaction of NH_2OH-Cu(II).

Experimental

Apparatus and reagents.

A Model Cary Eclipse fluorescence spectrophotometer (Varian Company, Palo Alto, CA), was used to record the RS spectra by means of synchronous scanning excited wavelength λ_{ex} and emission wavelength λ_{em} ($\lambda_{ex}-\lambda_{em}=\Delta\lambda=0$) and the RS intensity. A model SK8200LH ultrasonic reactor (Kedao Ultrasonic Instrument Limited Company, Shanghai, China), model YGL-16G high-speed centrifuge (Shanghai Anting Science and Technology Instrument Plant, Shanghai, China), model H-600 transmission electron microscope (Electronic Stock Limited Company, Japan), model JLM-6380LV scan electron microscope (Electronic Co Ltd, Japan), model HH-S thermostated water bath (Dadi Automatic Instrument Plant, Jiangsu, China),

and model NaNo-ZS90 particle sizer and Zeta potentiometer analyzer (England) were used. Membrane filtrate apparatus was showed in Figure 1.

Single strand DNA used in this paper was purchased from Sanbo Yuanzhi Biotechnology Limited Company, Beijing, China. The sequence of mercury ion aptamer is 5'-TTTCTTCTTTCTTCCCCCCTTGTTTGTTGTTT-3', and a control DNA sequence is 5'-AAACGGCAAACATCCCCCCAAGTAAGAAGAAA-3'. $HgCl_2$ was purchased from Guangzhou Chemicals Factory, China, and was used to prepare a 1.00 mmol/L Hg^{2+} standard solution with water. The Hg^{2+} working solution was prepared freshly. A 1% $HAuCl_4$ solution (National Pharmaceutical Group Chemical Reagents Company, China), 1.0 % sodium citrate, 0.5 M NaCl, 0.21 M $CuSO_4$, 0.21 M EDTA, 0.3 M NaOH, 0.12 M NH_2OH. HCl, and 0.20 M Na_2HPO_4-NaH_2PO_4 buffer solution were used. Cu(II)-EDTA solution was prepared with 10 mL 0.21 M $CuSO_4$ and 10 mL 0.21 M EDTA and 80 mL 0.30 M NaOH. A 58.0 μg/mL nanogold in size of 10 nm was prepared by the improved trisodium citrate-reduced procedure[20,25]. All the reagents were analytical grade and the used water was doubly distilled water.

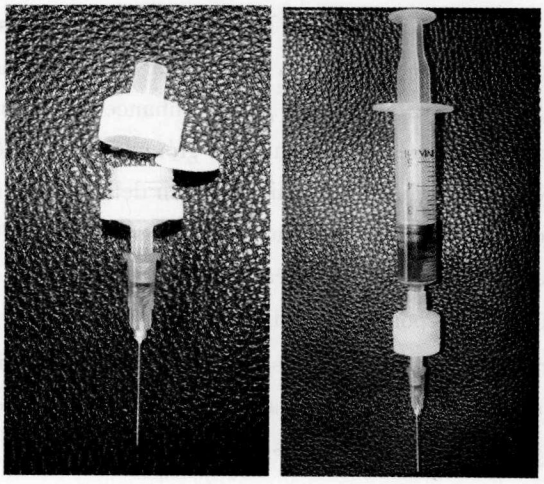


Figure 1 Apparatus of membrane filtration

Left: Including PVC filter, membrane and pinhead

Right: Injector with the filter

Preparation of AussDNA probe.

The suitable ratio of ssDNA and nanogold was examined. The following procedure was recommended. A 1.8 mL 0.17 μM ssDNA solution was added to 37.5 mL 58.0 μg/mL nanogold solutions while stirring. The concentration, counted as nanogold, was 55 μg/mL AussDNA.

Procedure.

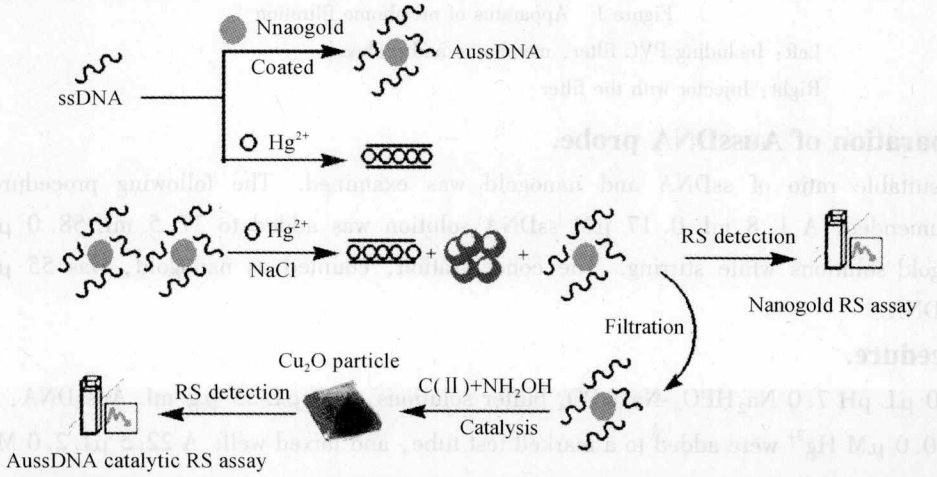
A 400 μL pH 7.0 Na_2HPO_4-NaH_2PO_4 buffer solutions, 400 μL 55 μg/mL AussDNA, a 180 μL 10.0 μM Hg^{2+} were added to a marked test tube, and mixed well. A 22.5 μL 2.0 M NaCl

was added, diluted to 1.5 mL, and mixed well. After about 6 min, the RS intensity at 540 nm ($I_{540\ nm}$) was recorded. A blank $(I_{540\ nm})_b$ without Hg^{2+} was recorded and the value of $\Delta I_{540\ nm} = I_{540\ nm} - (I_{540\ nm})_b$ was calculated. The mixture can be filtrated by cellulose nitrate membrane in size of 150 nm. The filtrate solution was used as follows.

A 1.0 mL the Cu(II)-EDTA solution, 50 μL the filtration solution (or gold nanoparticles), 200 μL 0.12 M NH_2OH were successively added to a 5.0 mL marked test tube, then the solution was diluted to 3.0 mL and reacted 6 min at 60℃. Finally, the test tube was cooled in tap water. The RS intensity at 602 nm ($I_{602\ nm}$) was recorded. A blank $(I_{602\ nm})_b$ without Hg^{2+} was recorded and the value of $\Delta I_{602\ nm} = (I_{602\ nm})_b - I_{602\ nm}$ was calculated.

Results and discussion

Single strand nucleic acids were found to conjugate on nanogold particles, resulting in the uncoiling of the oligonucleotides and stabilizing the nanogold against aggregation under high salt concentration[14]. When 10 nm nanoglod particles conjugated with ssDNA to form AussDNA by means of electrostatic, hydrophobic and intermolecular forces, the size of the AussDNA was same as the nanoglod. Upon addition of Hg^{2+} to the system of AussDNA-salt, very stable double strand T-T mismatches formed[14, 25-27], and larger nanogold clusters produced, which led nanogold cluster RS intensity to enhance. The enhanced RS intensity was linear with respect to Hg^{2+} concentration change. Based on those grounds, 1.3–1667 nmol/L Hg^{2+} can be detected simply, rapidly, selectively and sensitively, with detection limit of 0.7 nmol/L Hg^{2+}. Then the larger nanogold clusters are removed by the membrane filtration. The excess of AussDNA in the filtrate is used as the catalyst to form Cu_2O particles in NH_2OH-$[Cu(EDTA)]^{2+}$ reaction. Corresponding to Cu_2O RS intensity of 602 nm can be measured. With Hg^{2+} concentration increasing, the excess AussDNA in the filtration solution decreased, which leads Cu_2O particles decreasing in the nanocatalytic system, and RS intensity at 602 nm decreased (Scheme 1). Based on our results, we propose that this assay can be used for ultratrace Hg^{2+} detection.



Scheme 1 Principle of both RS assays for Hg^{2+}

AussDNA RS assay.

In pH 7.0 Na_2HPO_4-NaH_2PO_4 buffer solution and in the presence of 0.025 M NaCl, the AussDNA showed weak RS signal as in Figure 1a. Upon addition of Hg^{2+}, it interacted with ssDNA to form very stable double strand T-T mismatches, and release nanogold particles that aggregate to large nanogold clusters in mean size of 300 nm causing the RS signal increase at 540 nm. Thus, a wavelength of 540 nm was chosen for use. Under the selected conditions, the increased RS intensity at 540 nm is linear to the Hg^{2+} concentration. On those grounds, a rapid and sensitive aptamer-nanogold RS assay was proposed for detection of Hg^{2+}.

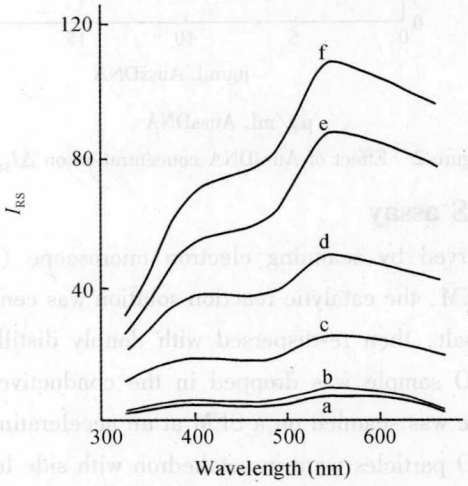
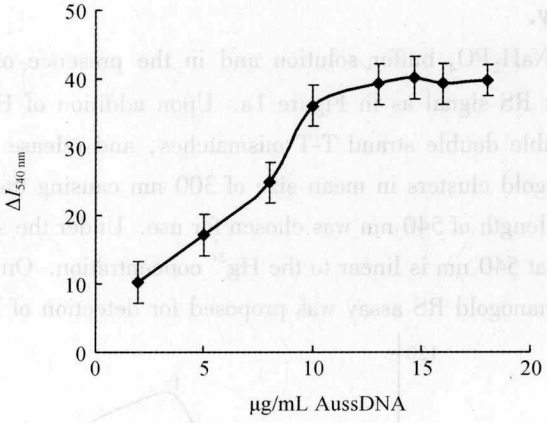


Figure 1 RS spectra of AussDNA-Hg^{2+} system

a: pH 7.0 NaH_2PO_4-Na_2HPO_4-14.7 μg/mL AussDNA-0.03 M NaCl b: a+0.133 nmol/L Hg^{2+} c: a+333.33 nmol/L Hg^{2+} d: a+666.67 nmol/L Hg^{2+} e: a+1333.33 nmol/L Hg^{2+} f: a+1666.67 nmol/L Hg^{2+}.

The ssDNA and a control DNA were tested for the detection of Hg^{2+}. Results showed that the control DNA can not be used to detect Hg(II), and the ssDNA was chosen for preparation of Hg^{2+} probe (AussDNA). Results of transmission electron microscope indicated that nanogold particles were 10 nm spheres. After conjugating with ssDNA, the particles did not change in the size and shape. Upon addition of Hg^{2+}, large nanogold clusters were formed in the presence of salt.

Effect of pH value, AussDNA and NaCl concentrations on the $\Delta I_{540\ nm}$ were investigated respectively. When pH was in the range of 6.0–8.5, and AussDNA was greater than 13 μg/mL (Figure 2), the $\Delta I_{540\ nm}$ was biggest. Thus, pH 7.0 Na_2HPO_4-NaH_2PO_4 buffer solutions, and 14.7 μg/mL AussDNA were chosen for use. Strong electrolyte is known to cause the aggregation of nanogold particles in aqueous solution[25]. The effect of NaCl concentration on $\Delta I_{540\ nm}$ was examined. The $\Delta I_{540\ nm}$ was stable and highest when NaCl concentration was greater than 20 mmol/L. Thus, 30 mmol/L NaCl was chosen. Using the AussDNA RS probe, trace Hg^{2+} can be assayed simply and rapidly.



μg/mL AussDNA

Figure 2 Effect of AussDNA concentration on $\Delta I_{540\ nm}$

AussDNA catalytic RS assay

Cu_2O particles were observed by scanning electron microscope (SEM). To eliminate the interference of salt with SEM, the catalytic reaction solution was centrifuged at 10000 rpm for 5 min to remove the mass salt, then re-dispersed with doubly distilled water in the ultrasonic apparatus. 50.0 μL Cu_2O sample was dropped in the conductive adhesive, and let to dry naturally. Then the sample was scanned on a SEM at an accelerating voltage of 15 kV. Figure 3 indicates that mass Cu_2O particles were in octahedron with side length of about 1 μm.

The product of $CuSO_4$-EDTA-NaOH-NH_2OH-nanogold catalytic system was obtained, and was used to do X-ray diffraction with scanning range of 10.0–80.0° (2 Theta), step size of 0.017° (2 Theta), scanning step time of 10.3 s, anode material of Cu, current of 40 mA, voltage of 40 kV. As in Figure 4, the diffraction peaks at 29.533°, 36.386°, 42.330°, 52.508°, 61.382°, 73.585° and 77.403° showed that there is Cu_2O in the product. The two weak peaks at 43.337° and 50.501° were owing to Cu(0). The peak at 36.386° was strongest that showed the main product was Cu_2O particles.

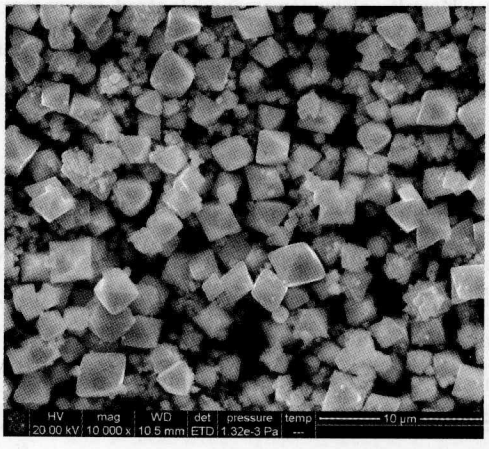


Figure 3 Scanning electron microscopic images of Cu_2O particles

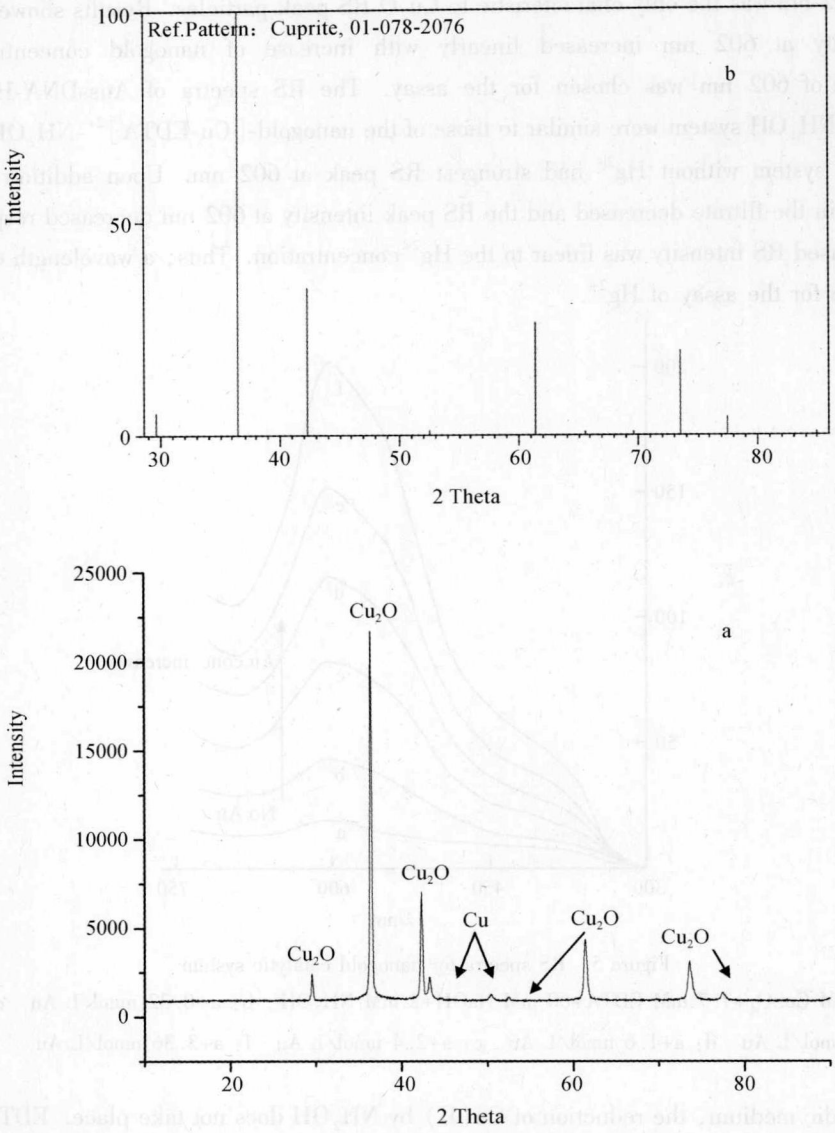


Figure 4 X-ray diffraction pattern

a: the product of $CuSO_4$-EDTA-NaOH-NH_2OH-nanogold system b: Cu_2O

The system of $[Cu(EDTA)]^{2+}$-NH_2OH exhibited a very weak synchronous scattering peak at 602 nm. When Au or AussDNA was added into the system, the Cu_2O particles formed, which caused the scattering intensity at 602 nm enhanced obviously (Figure 5). The studies of inorganic nanoparticles in liquid phase have indicated that three factors, the light source of the apparatus, absorption of free molecule and the RS effect of particles, caused synchronous scattering peaks. The apparatus exhibited the strongest emission at 465 nm, the absorption of free molecule such as unreacted Cu(II) can be neglected since the absorption value of the uncatalytic reaction system at 450–850 nm was less than 0.1. Thus, the scattering

peak at 602 nm was the only characteristic to Cu_2O RS peak particles. Results showed that the RS intensity at 602 nm increased linearly with increase of nanogold concentration. A wavelength of 602 nm was chosen for the assay. The RS spectra of AussDNA-Hg^{2+}-[Cu-EDTA]$^{2+}$-NH_2OH system were similar to those of the nanogold-[Cu-EDTA]$^{2+}$-NH_2OH system. The blank system without Hg^{2+} had strongest RS peak at 602 nm. Upon addition of Hg^{2+}, AussDNA in the filtrate decreased and the RS peak intensity at 602 nm decreased respectively. The decreased RS intensity was linear to the Hg^{2+} concentration. Thus, a wavelength of 602 nm was chosen for the assay of Hg^{2+}.

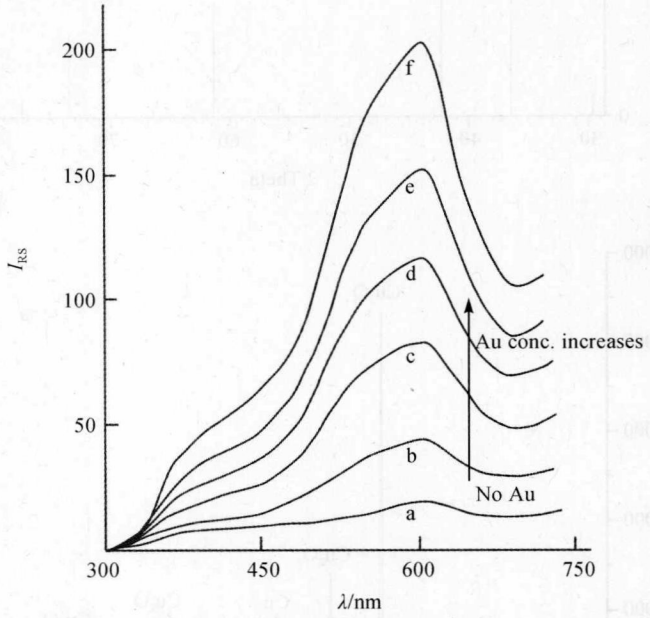


Figure 5 RS spectra for nanogold catalytic system

a: 7 mM $CuSO_4$+7.7 mM EDTA+60 mM NaOH+2 mM NH_2OH b: a+0.32 nmol/L Au c: a+0.96 nmol/L Au d: a+1.6 nmol/L Au e: a+2.4 nmol/L Au f: a+3.36 nmol/L Au

In acidic medium, the reduction of Cu(II) by NH_2OH does not take place. EDTA is used to stabilize Cu(II) in NaOH medium, which allows Cu(II) to be reduced slowly to Cu_2O particles. Upon addition of nangold catalyst, the particle reaction enhanced considerably. The effect of nanogold concentration on the [Cu-EDTA]$^{2+}$-NH_2OH particle reaction was studied by RS technique. The enhanced change of RS intensity at 602 nm (ΔI_{RS}) was linear to the change in nanogold concentration (Table 1). As the nanogold size reduced, the slope value of the regression equation increased, and a lower detection limit can be achieved. The 10 nm nanogold could be easily prepared and the detection limit of 10 nm nanogold catalytic system was lower than that of 30 nm and 50 nm nanogold catalytic systems, so 10 nm nanogold was chosen for preparation of AussDNA. In addition, the stability of the ΔI_{RS} value was considered. After cooled by tap water, the ΔI_{RS} hold constant within 60 min to make the recording freely.