A highly sensitive resonance Rayleigh scattering assay for detection of Hg(II) using immunonanogold as probe

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Material Preparation:

Apparatus and Reagents. A model of Cary eclipse fluorescence spectrophotometer (US Varian, Inc.) was used to record the RRS spectra and the intensity, with PMT volt=500 v, excited slit=emission slit=5 nm, emission filter=1% T attenuator, λ_{ex}-λ_{em}=$\Delta\lambda$=0. A model of 79-1 magnetic stirrer with heating (Zhongda Instrumental Plant, Jiangsu, China), a model of SK8200LH ultrasonic reactor (Shanghai Kedao Ultrasonic Instrument Co., Ltd., with frequency of 59 kHz), and a model of FEI Quanta 200 FEG Field environmental scanning electron microscope (SEM) (Netherlands), a model of NaNo-ZS90 nanoparticle and zeta potentiometric analyzer (Malvern, U.K.), and a model of thermostatic water bath (Shanghai No. 5 Medical Apparatus Plant, Shanghai, China) were used.

Methylmercury chloride (CH$_3$ClHg)**,** 6-mercaptonicotinic acid (MNA), mercuric chloride (HgCl$_2$), 3,3',5,5'-tetramethylbenzidine (TMB), bovine serum albumin (BSA), ovalbumin (OVA), Freund's complete and incomplete adjuvants, polyethylene glycol (PEG4000) were purchased from Sigma Chemical Co. (St Luis, Mo. USA). Cell medium and fetal calf serum was from Minhai (Lanzhou, China). Mouse SP2/0 myeloma cell was bought from the Cell Bank of Chinese Science Academy (Shanghai, China). BALB/C mice were purchased from Experimental Animal Center of Sichuan University (Chengdu, China). Monoclonal antibody (diluted the 100 μL mAb with water to 2.0 mL) was prepared by coupling the new lignad 6-mercaptonicotinic acid (MNA) with both methylmercury chloride (CH$_3$ClHg) and carrier protein, and 1.0 mg/mL mercury ions

coating antigen (CH$_3$Hg-MNA-OVA) were prepared by ourself. HAuCl$_4$.4H$_2$O was purchased from the National Pharmaceutical Group Chemical Reagents Company, China. A 1.00×10^{-2} mol/L Hg^{2+} standard solution was prepared by HgCl$_2$. A 0.20 mol.L^{-1} Na$_2$HPO$_4$ and a 0.10 mol.L^{-1} citric acid (C$_6$H$_8$O$_7$) were used to prepare Na$_2$HPO$_4$-citric acid buffer solution(PBCS) with different pH value. 1.0 % tri-sodium citrate, 0.20 mol.L^{-1} K$_2$CO$_3$, 0.10 mol.L^{-1} HCl and 10.0 % KCl were used. All the reagents were of analytical grade except explained and all the solutions were prepared with double distilled water.

The structure of 6-mercaptonicotinic acid (MNA)

Molecular structure of CH$_3$Hg-MNA

Preparation of nanogold. Nanogold (NG) was prepared by sodium borohydride reduction procedure. Into a clean conical bottle containing 35 mL water, 0.5 mL 1.0 % HAuCl$_4$.4H$_2$O and 3.5 mL of 1.0% sodium citrate solution were added under the stirring and mixed them well. Then, 4.0 mL 0.5 mg/mL NaBH$_4$ solutions were added slowly under the stirring, and the color of the

solution changed from dark red to red black and then turns red, at last no longer change. After

continued to stirring for 10 min, it was diluted to 50 mL with water. The NG concentration was

58.0 mg/L Au, with a size of 10 nm.

Adjustment of the pH of NG solution. Because the combination of NG and mAb is done by

physical force, the successful combination depends on the pH. In this experiment, we used a RRS

technique to test the influence of different pH values on NG labeling. A 0.40 mL NG in tube was

adjusted to different pH value, and then 50 μL of mAb (1:1000) was added. After 5 min, 15 μL of

10 % KCl solution was added to the tube, and diluted to 2.0 mL. We then used spectrofluorometer

to determine the RRS intensity at 580 nm. Table 1 indicated that when the pH<7.0, the mAb do

not stabilize the nanogold. When the pH is in 9.0-11.0 the intensity decreased and the system

stabilize because coating of the nanogold by the mAb prevented NG aggregation by the KCl. Thus,

a pH 9.0 was chosen for use.

Table 1 Influence of different pH value of the NG labeled antibody

pH	6.0	7.0	8.0	9.0	10.0	11.0	12.0
$I_{580\,nm}$	443.5	233.0	205.4	158.9	163.5	154.7	288.2

Selection of the ratio between NG and mAb. We added different amounts of mAb (1:1000) to

0.40 mL NG solution of which the pH had been adjusted to 9.0. After 5 min, we added 15 μL of

10 % KCl solution to each tube and mixed well. After 2 h, we measured the RRS intensity of each

tube. Table 2 showed that the RRS intensities were stronger in the tubes with 20–40 μL of mAb

than that with the 60-80 μL. When the amounts of antiserum were greater than 200 μL, the RRS

intensities were small and remained stable. Thus, 200 μL was the minimum amount of antiserum

that stabilized 0.40 mL NG solution. The actual amount of antiserum was 100 μL when 0.4 mL

NG was labeled at this experiment.

Table 2 Influence of amounts of mAb of the NG labeled antibody

mAb (μL)	0	20	40	60	80	100	120	180	200	300
$I_{580\ nm}$	552.1	471.5	125.1	69.5	69.7	54.8	47.6	44.6	27.2	25.4

Preparation of immunonanogold. During magnetic stirring, we added 125 μL of mAb

(1:1000) slowly to 5 mL NG with pH 9.0 that adjusted by 0.10 mol.L^{-1} K$_2$CO$_3$ and 0.10 mol.L^{-1}

HCl solution, keeping the dropping time for 5 min and stored at 4 °C. The ING concentration was

54.6 μg.mL^{-1} Au. The nanogold was wrapped by mAb, and the scanning electron microscopy
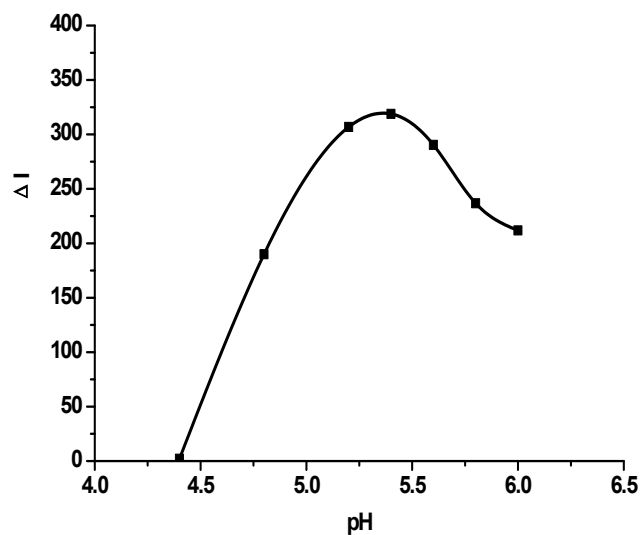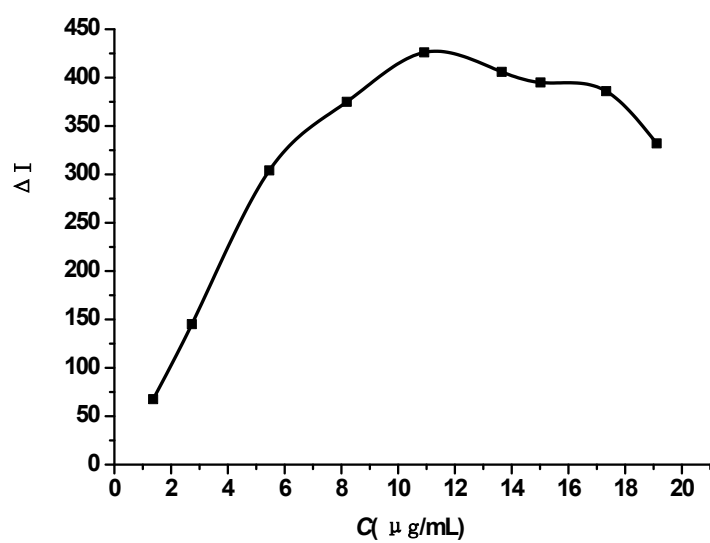
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Supplementary Figures:
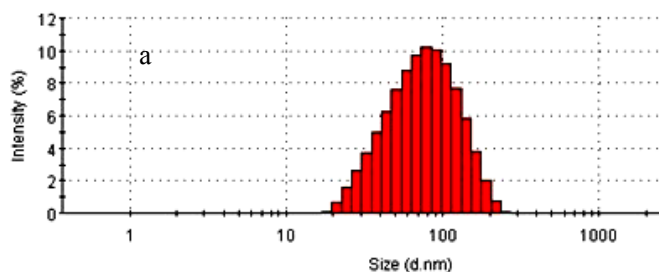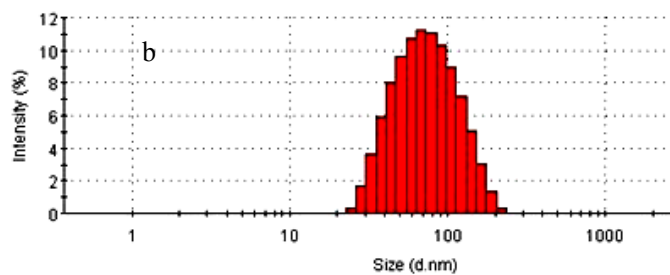
ESI,† Fig. S1 Laser scattering graph of the system

(a) pH 5.4 Na_2HPO_4- citric acid -10.9 $\mu g.mL^{-1}$ ING-37 ^0C -15 min; (b) a+ 1.5 $\mu mol.L^{-1}$ Hg^{2+}.

ESI,† Fig. S2 Effect of pH on ΔI_{580nm}

Na_2HPO_4-citric acid- 10.9 $\mu g.mL^{-1}$ ING -5.0 $\mu mol.L^{-1}$ Hg^{2+}

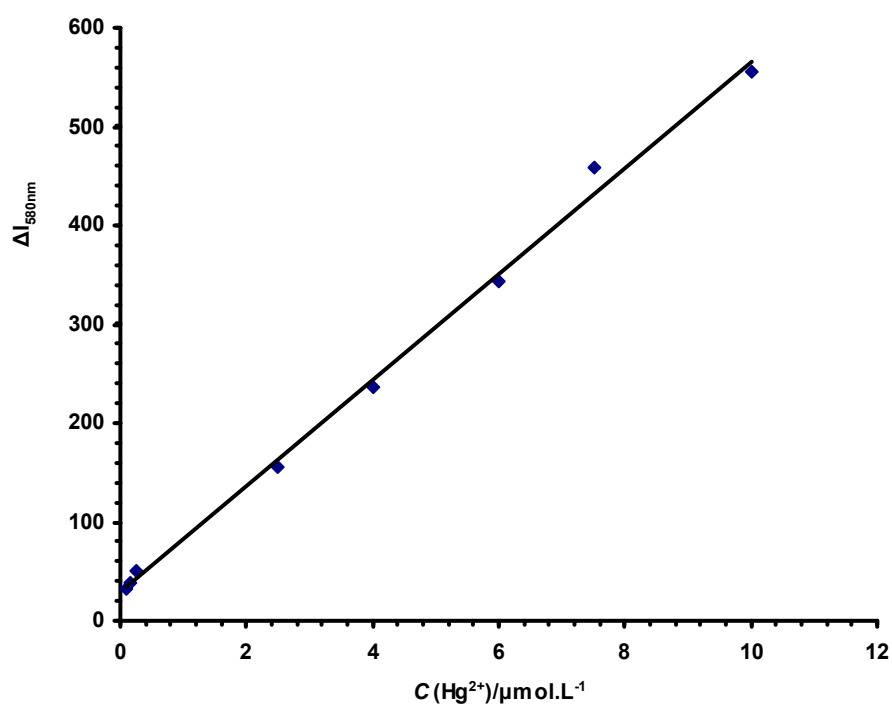**ESI,† Fig. S3** Effect of ING concentration ΔI_{580nm}

pH 5.4 Na_2HPO_4-citric acid-5.0 $\mu mol.L^{-1}$ $Hg^{2+,}$
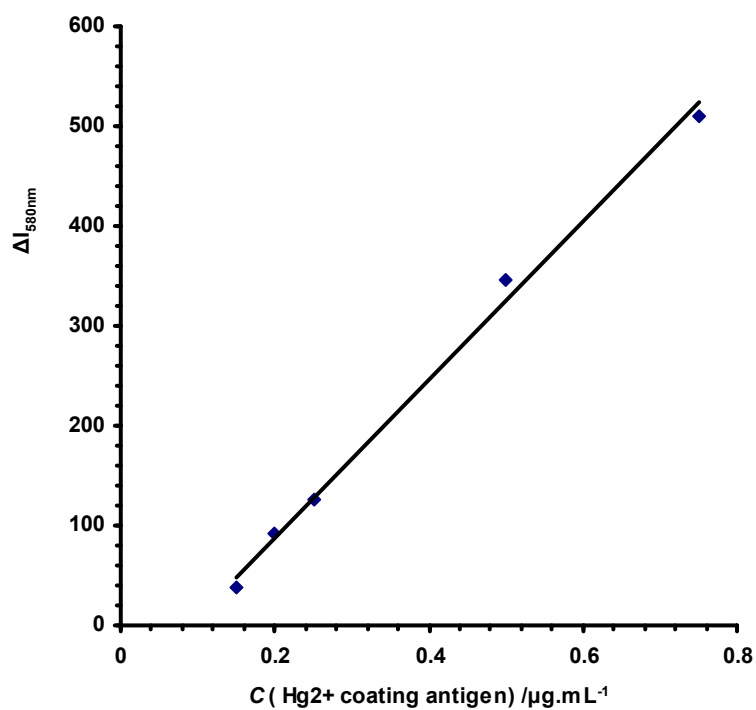
ESI,† Fig. S4 Working curve for the Hg^{2+} coating antigen

ESI,† Fig. S5 Working curve for Hg^{2+}

Supplementary Tables:

ESI,† Tab. S1   Results for the determination of Hg^{2+} in samples

Sample	Single value (μmol/L)	Average (μmol/L)	RSD （%）	added (μmol/L)	Recovery (%)
1	2.3，2.5，2.4，2.6，2.6	2.48	5.3	2.5	99.2
2	2.4，2.5，2.7，2.6，2.4	2.52	5.2	2.5	100.8
3	2.4，2.5，2.6，2.6，2.5	2.52	3.3	2.5	100.8