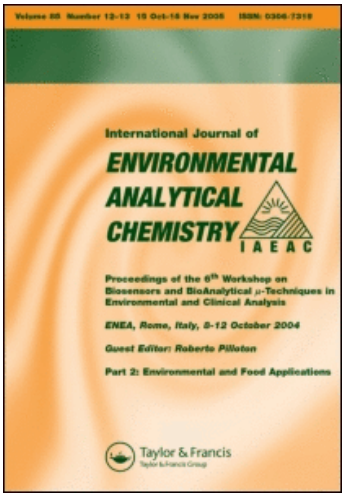

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A highly sensitive and selective immunonanogold resonance scattering spectral assay for sudan I

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A highly sensitive and selective immunonanogold resonance scattering spectral assay for sudan I

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A sensitive and selective resonance scattering spectral (RSS) assay was proposed for the determination of sudan I (SDI), using 10 nm nanogold to label the antibody against sudan I (anti-SDI Ab) to obtain a RSS probe for SDI. The immunonanogold reaction between nanogold-labelled anti-SDI Ab and SDI took place in pH 4.92 KH_2PO_4–Na_2HPO_4 buffer solution and in the presence of polyethylene glycol (PEG)-6000, and the intensity of resonance scattering peak at 580 nm decreased greatly. The decreased intensity $\Delta I_{580\,nm}$ was proportional to the concentration of SDI in the range of 0.23–45.0 ng mL^{-1}. The linear regression equation was calculated as $\Delta I_{580nm} = 1.20c + 2.01$ ($R = 0.9975$, $n = 6$), with a detection limit (3σ) of 0.13 ng mL^{-1}. The SDI in egg samples was assayed, with satisfactory results.

Keywords: sudan I; nanogold; immunonanogold; resonance scattering spectral probe

1. Introduction

Sudan I [1-(phenylazo)-2-naphthol], the synthetic dyestuff of azo compound, is proved to have potential carcinogenicity. Many countries in the world have prohibited companies from using it as a food additive. However, because of its constancy and stability of lustre, some illegal businessmen still use it to improve the sensory colour of goods [1,2]. At present, main methods for SDI include chromatography and chromatography-mass spectrometry. The extracting solution of samples was purified with polyamide, using methanol as mobile phase, sudan I, II, III and IV in the range of 1–150 ng mL^{-1} were determined by high performance liquid chromatography (HPLC), but the operation was time-consuming and has high costs [2]. Using methacrylic acid and 4-vinylpyridine as functional monomer and ethylene glycol dimthacrylate as crosslinker, SDI as the template was determined by HPLC. The method has a good selectivity, however, the course of washing steps for the complex was long [3]. Sudan I, II, III and IV were also separated by capillary electrophoresis and analysed by ultraviolet detector, with the detection limit of 96 ng mL^{-1}, but it took over 20 min to separate [4]. Using C_{18} as stationary phase, a mixed solution of methanol and formic acid as mobile phase, SDI in chilli powders was

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determined by HPLC-mass spectrometric technique. A 3–10 ng g^{-1} SDI was detected, with good accuracy [5]. According to reference [6], SDI in the range of 1.6–4.4 μg mL^{-1} in chilli powders was determined by capillary electrophoresis-mass spectrometric technique. For these methods, the chromatography mass spectrometry instrumentation is expensive, so the determination costs too much and the costs are hard to spread. Recently, Huang *et al*. found that sudan dyes reacted with silver nitrate to produce silver nanoparticles, resulting in the obvious plasmon resonance scattering signals at 452 nm, which was used to determine 0.2–2.4 μM SDI, with the detection limit of 3.2 nM [7].

Resonance scattering (RS) spectral analysis is sensitive, rapid and simple. It has been used to detect trace inorganic ions and organic compounds such as proteins and nucleic acids [8,9]. Most of them were based on the simple associated action between the protein and small molecule such as dye. However, their selectivity needs to be improved. In recent research, the liquid phase nanoparticles such as gold, silver and so on exist in the strong RS effect, but they have rarely been applied in immuno-analysis [10,11]. The gold nanoparticle labelling technique, with high labelling effectiveness and the tiny effect on the activity of protein, has become the fourth labelling technique besides radioactive isotope, enzyme and fluorescent labelling, but has rarely been applied in quantitative analysis [12–14]. Recently, we combined the RS spectral technique and gold labelling technique to analyse the special proteins in the human body such as apolipoprotein, fibrinogen and so on, with simplicity, rapidity and good selectivity [15,16]. However, there is no report about the immunogold RS spectral probe for small molecular hapten. Our study showed that SDI was diluted with polyacrylamide, the immunoreaction between gold-labelled SDI Ab and SDI took place in pH4.92 KH_2PO_4–Na_2HPO_4 buffer, and in the presence of PEG, ΔI_{RS} at 580 nm decreased linearly with the concentration of SDI added. So a sensitive and rapid immunogold-labelled RS spectral assay for SDI was established. It was tested for the assay of SDI in egg samples, with good selectivity, simplicity and rapidity.

2. Experimental

2.1 *Apparatus*

A RF-540 spectrofluorometer (Shimadzu, Japan) was used to record the RS spectra and measure the RS intensity. A H-600 transmission electron microscope (Electronic Stock Limited Company, Japan) was used to observe the appearance of gold nannoparticles. A TU-1901 double beams UV-visible spectrophotometer (Beijing Purkinje General Instrument Limited Company, China), a 79–1 magnetic heat beater (Zhongda Instrumental Plant, China), a Branson B3500S-MTH ultrasonic reactor (Binengxin Ultrasonic Instrument Limited Company, China) and a SYZ-550 quartz Sub-boiling machine (Jingbo Instrument plant, China) were also used.

2.2 *Reagents*

$HAuCl_4$ was obtained from National Pharmaceutical Group Chemical Reagents Company, China. Rabbit antiserum of sudan red I were prepared by standard immunisation procedure. SDI was modified to get a carboxylic group with different long-chain as shown in Figure 1. The modified SDI connecting with BSA as immunogen was injected into rabbit to obtain SDI antiserum, which was diluted to 20 times for labelling. A 0.30 μg mL^{-1} SDI working solution was prepared as follows, a 20 mL

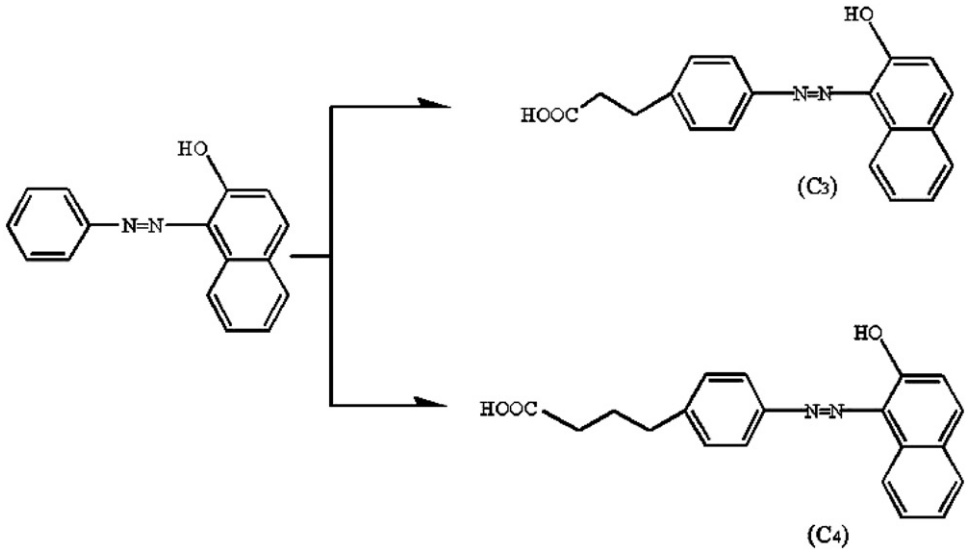

Figure 1. Sudan I and its derivatives structure.

1.50 μg mL^{-1} SDI and 0.10 g polyacrylamide (PAM) were mixed and dissolved by ultrasonic vibration and heating, then the mixture was diluted to 100 mL. A 0.067 mol L^{-1} Na_2HPO_4 and 0.067 mol L^{-1} KH_2PO_4 were used to prepare pH 4.49–9.18 buffer solutions according to a certain volume ratio. A 0.1 mol L^{-1} HCl, 0.1 mol L^{-1} K_2CO_3, 10.0% KCl and 1.0% trisodium citrate were prepared, and 3.0×10^5 μg mL^{-1} PEG-6000, PEG-4000, PEG-10000 and PEG-20000 were used. All reagents were of analytical grade and doubly distilled water was used.

2.2.1 *Preparation and identification of gold nanoparticles*

Gold nanoparticles, 10 nm size, were prepared by the improved trisodium citrate-reduced procedure [17]. They were identified by transmission electron microscope, absorption spectrum and RS spectrum. Briefly, 100 mL of doubly distilled water and 6.00 mL of 1.0% trisodium citrate were added in a conical flask and boiled for 5 min, then 1.00 mL of 1.0% $HAuCl_4$ solution was added into the boiled solution of trisodium citrate while stirring. The mixed solution was boiled for another 15 min, cooled, diluted to 100 mL and stored. First, the diameter and the appearance of gold particles were observed by transmission electron microscope, the diameter of the spherical gold nanoparticles was 10 nm. Second, the correlation of the size of gold particles (d) and the maximal absorption wavelength (λ_{max}) was calculated as: $d = (260\lambda_{max} - 1.34 \times 10^5)^{1/2}$, controlling λ_{max} as about 516 nm [18]. Third, the intensity at 580 nm $I_{580\,nm}$ increased with the size of gold particles. If the $I_{580\,nm}$ for 19.32 μg mL^{-1} colloidal gold solutions was controlled at 7.0 ± 2, about 10 nm nanogold can be obtained.

2.2.2 *Preparation of immunonanogold probe*

First, the labelling pH was adjusted and tested by the RS method: 1.0 mL of 57.96 μg mL^{-1} colloidal gold solution was added to each tube and adjusted to different pH by adding

0.10 mol L^{-1} K_2CO_3 or 0.10 mol L^{-1} HCl. Then 30.0 μL of anti-SDI Ab was added, respectively. After 5 min, 0.10 mL of 10.0% KCl solution was added, 2 h later, the solution was diluted to 3.0 mL. When the pH was less than 8.0, the $I_{580\,nm}$ enhanced greatly since gold particles aggregated. When the pH was more than 8.0, gold particles were coated by anti-SDI Ab and not aggregated by KCl solution. $I_{580\,nm}$ was stabilised. A pH of 9.0 was chosen for this assay.

Next, the amount of anti-SDI Ab was selected: 1.00 mL of the colloidal gold solution was piped in each tube and adjusted to pH 9.0 by adding 50.0 μL of 0.1 mol L^{-1} K_2CO_3, different concentrations of anti-SDI antiserum (0, 5.0, 10.0, 15.0, 20.0, 25.0, 30.0, 35.0, 40.0, 45.0 and 50.0 μL) were added, respectively. After 5 min, 0.10 mL of 10.0% KCl solution was added, mixed well and stored for 2 h. The solution was then diluted to 3.0 mL, and $I_{580\,nm}$ recorded. The results showed stronger RS intensities for the tubes with 0–20.0 μL of anti-SDI Ab than those in the tubes with 25.0–50.0 μL anti-SDI Ab. We choose 30.0 μL of anti-SDI Ab as the labelling amount for 1.00 mL of the colloidal gold solution and determined SDI in suitable conditions. When 25.0 μL of anti-SDI Ab was used as the minimum amount, some gold particles were not coated by anti-SDI Ab and not aggregated by KCl solution, the blank value was high and not stabilised. When 35.0 μL of antiserum was used, unnecessary anti-SDI Ab was left, the linear relationship between the concentration of SDI and RS intensity was bad and even disappeared. When we used 30.0 μL of antiserum, we got a good linear relationship. So 30.0 μL of anti-SDI Ab was chosen as the minimum use level to stabilise 1.0 mL of colloidal gold solution.

Third, we prepared the nanogold-labelled anti-SDI Ab: 90 mL of colloidal gold solution was adjusted to pH 9.0 by adding about 4.50 mL of 0.1 mol L^{-1} K_2CO_3 solution. While using magnetic stirring, 2.70 mL of anti-SDI Ab was added slowly, and stirred for 10 min, 1.65 mL of 3.0% PEG20000 as stabiliser was added and kept the last concentration at 0.05%. Later, the solution was stirred for another 30 min, and stored at 4°C. The gold-labelled antisera were not purified by centrifugation, and the results were consistent with those for the purified reagents.

2.3 *Procedure*

In 5 mL tubes, a certain volume of 0.40 mL pH 4.92 KH_2PO_4–Na_2HPO_4 buffer, 0.70 mL of gold-labelled anti-SDI Ab (calculated by the colloidal gold concentration, 52.8 μg mL^{-1}), certain volume of 0.30 μg mL^{-1} SDI and 0.35 mL of 300 mg mL^{-1} PEG-6000 were added in order, diluted to 2.0 mL, mixed thoroughly and incubated in an ultrasonic reactor (42 kHz) for 25 min at 20°C. Later, a low sensitive file and a longitudinal coordinate scale of 6 were chosen on an RF-540 spectrofluorometer. The synchronous scattering spectrum was obtained by means of synchronous scanning under the condition of $\lambda_{ex}-\lambda_{em}=\Delta\lambda=0$. The $I_{580\,nm}$ and $(I_{580\,nm})_b$ of the blank were measured. Then, $\Delta I_{580\ nm}=(I_{580\,nm})_b-I_{580\,nm}$ was calculated. A TU-1901 double beam UV-visible spectrophotometer was used to record the absorption spectrum.

3. Results and discussion

In pH 4.92 KH_2PO_4–Na_2HPO_4 buffer and in the presence of PEG, the gold-labelled anti-SDI Ab formed relatively stable aggregates as shown in Figure 2, the RS intensity at 580 nm was enhanced. Upon addition of SDI, the immunogold complex formed

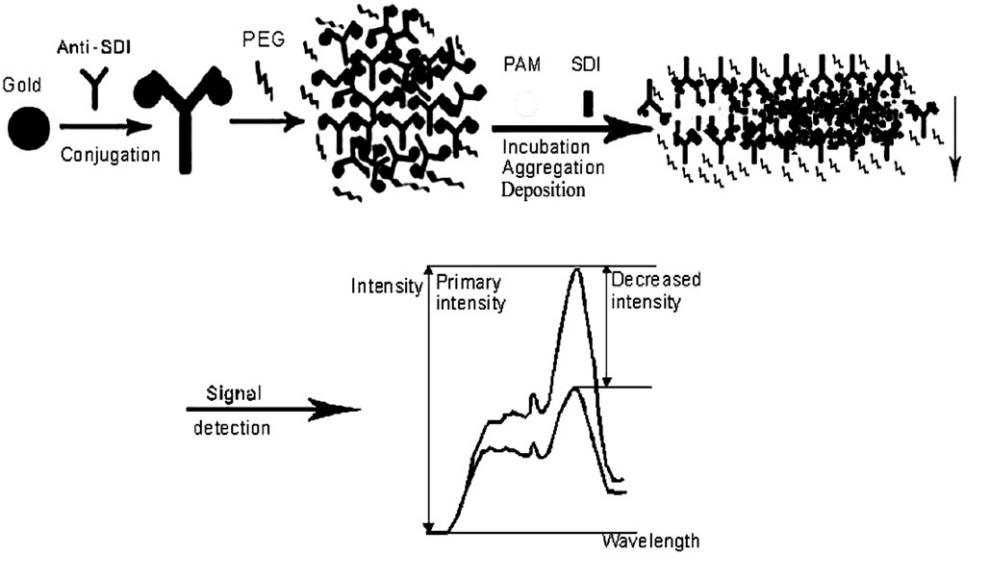

Figure 2. Immunonanogold reaction principle.
Notes: Step 1: anti-SDI Ab was labelled by nanogold; step 2: the gold-labelled anti-SDI Ab aggregated by PEG; step 3: SDI reacted with gold-labelled anti-SDI Ab in presence of PAM; step 4: recording RS intensity.

and deposited. The aggregates of gold-labelled anti-SDI Ab in the solution reduced, so the RS intensity reduced, SDI in the certain range was linear to the decreasing RS intensity. Thus, an immuno-RS spectral assay was developed for SDI without phase separation and washing steps.

3.1 *Transmission electron microscopy*

We prepared 10 nm sized spherical gold nanoparticles using the improved trisodium citrate-reduced procedure. When the gold particles were coated by the anti-SDI Ab, they kept their original diameter. Some particles displayed an insignificant aggregation. Most particles were dispersed (Figure 3a). In suitable pH condition, after ultrasonic irradiation, immuno-gold complex formed and the gold nanoparticles were aggregated. We study the effect of PEG on the aggregation of gold nanoparticles in liquid phase system. In the absence of PEG, the gold-labelled anti-SDI Ab were dispersed as shown in Figure 3b. The immunogold complex formed after immunoreaction, the gold nanoparticles were aggregated (Figure 3c). In the presence of high concentration PEG, the gold-labelled anti-SDI Ab was aggregated loosely (Figure 3d). Furthermore, the gold nanoparticles presented obvious aggregation after immuno-reaction (Figure 3e). This indicated that in certain conditions, whether in the presence of PEG or not, the reaction of the gold-labelled anti-SDI Ab with SDI happened and the gold nanoparticles aggregated after immuno-reaction in aqueous solutions. In the presence of PEG, the gold nanoparticles aggregated obviously and deposited, so PEG could enhance the detecting signals, which is also proved by Schneider's group [19].

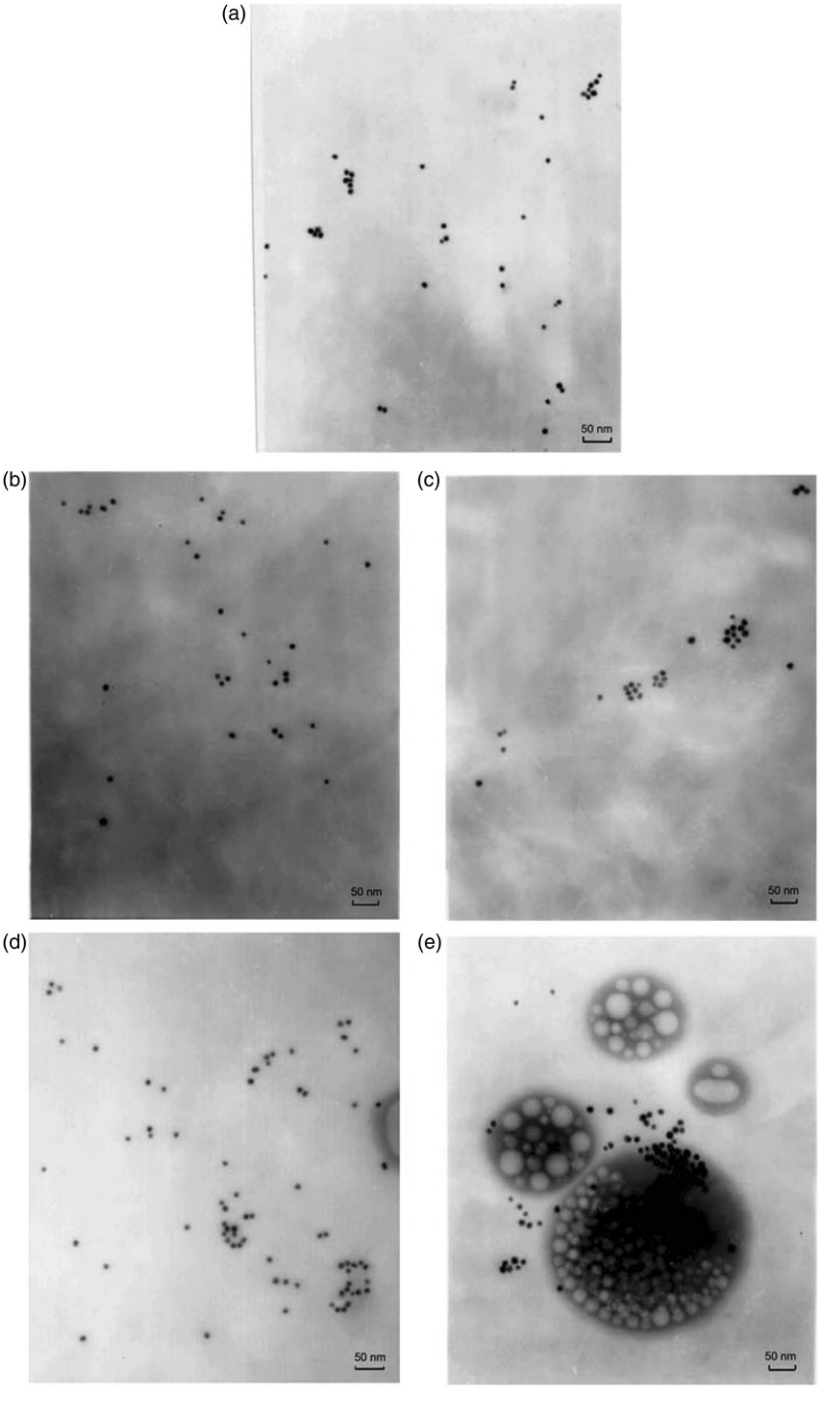

Figure 3. Transmission electron microscopy.
Notes: (a) 18.47 μg mL^{-1} gold-labelled anti-SDI Ab; (b) pH 4.9–21.85 μg mL^{-1}gold-labelled anti-SDI Ab; (c) pH4.92–22.5 ng mL^{-1} SDI –18.47 μg mL^{-1}gold-labelled SDI Ab; (d) pH 4.92–18.47 μg mL^{-1}gold-labelled anti-SDI Ab-52.5 mg ml^{-1} PEG-6000; (e) pH 4.92–18.47 μg mL^{-1} gold-labelled anti-SDI Ab-22.5 ng mL^{-1} SDI-52.5 mg mL^{-1} PEG-6000.

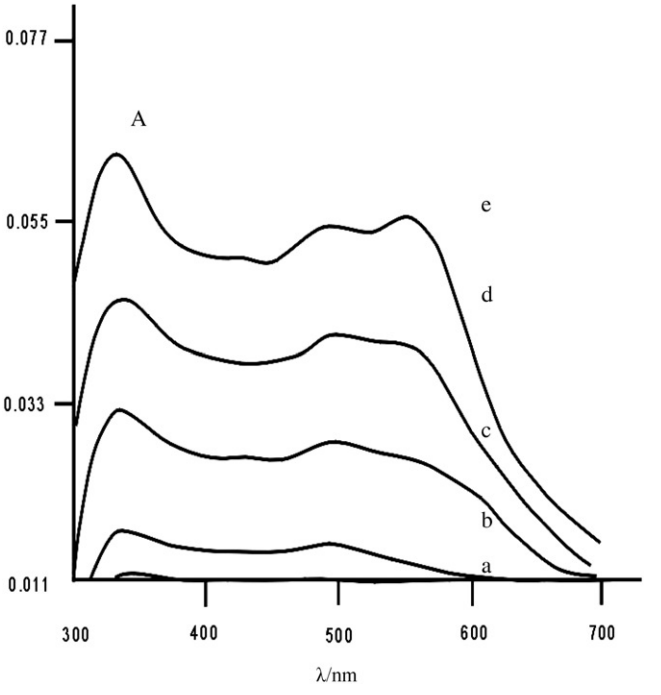

Figure 4. Absorption spectra.
Notes: (a) pH 4.92–0.20 mg mL^{-1} PAM; (b) a-0.5 μg mL^{-1} SDI; (c) a-1.0 μg mL^{-1} SDI; (d) a-1.5 μg mL^{-1} SDI; (e) a-2.0 μg mL^{-1} SDI.

3.2 *Absorption spectrum*

A weak absorption peak at 336 nm appears for more than 0.25 μg mL^{-1} SDI. The same concentration of SDI solution was prepared by 0.20 mg mL^{-1} PAM solution, it also displayed a weak peak at 336 nm. In the range of 300–700 nm, the absorption values for the both solutions are weak. Figure 4 shows the absorption spectrum of the SDI solution containing 0.20 mg mL^{-1} PAM.

In the gold-labelled system of SDI, the strongest absorption wavelength moves to the blue wavelength with SDI increasing (Figure 5). The strongest absorption peak at 536 nm is of the blank, while the strongest peaks are 530, 528, and 526 nm for 15.0, 30.0 and 45.0 μg mL^{-1} SDI, respectively. According to references [18 and 20], the absorption peaks always shift towards blue when the size of the gold nanoparticles decrease. It proves the size of aggregates for the gold nanoparticles decreased after immuno-reaction because the gold nanoparticles were aggregated, deposited, and the gold-labelled anti-SDI Ab reduced in the solution after the immuno-gold complex formed.

3.3 *Resonance scattering spectrum*

The SDI solution (more than 0.25 μg mL^{-1}) is orange red, shows two obvious synchronous (Rayleigh) scattering peaks at 470 and 570 nm. The study indicates that three factors, including lamp source, free molecular absorption and the RS effect of nanoparticles,

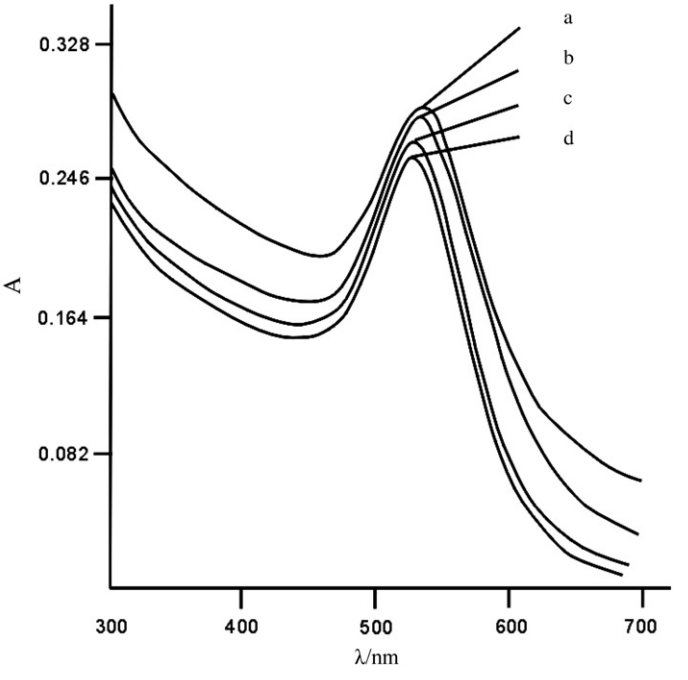

Figure 5. Absorption spectra of gold-labelled rabbit anti-SDI Ab and SDI.
Notes: (a) pH 4.92–18.47 μg mL^{-1} gold-labelled SDI Ab-52.5 mg mL^{-1} PEG-6000; (b) a-15.0 ng mL^{-1} SDI; (c) a-30.0 ng mL^{-1} SDI; (d) a-45.0 ng mL^{-1} SDI.

produce the synchronous scattering peaks [21]. The SDI solution has no strong absorption peaks and shows weak absorption in the range of 300–700 nm (Figure 4). The strongest emission peak appears at 470 nm for the spectrofluorometer and produced a synchronous scattering peak at 470 nm [21]. So the peak at 570 nm is the RS peak of SDI. And the light near 570 nm is orange red. It is the significant reason that the SDI solution displays the same colour. PAM promoted the dissolution of SDI and made SDI stabilise. The system of SDI-0.20 mg mL^{-1} PAM shows two obvious synchronous scattering peaks at 470 and 570 nm. The RS peak at 570 nm is also strong, the RS intensity increased linearly in the range of 0.10–2.00 μg mL^{-1} SDI (Figure 6). We developed a direct detecting method for SDI by RS spectrum, but the sensitivity was poor. We compared the SDI solution by water, but because PAM has great viscosity it alleviates the aggregation for the molecule of SDI [22], SDI diluting by PAM stabilised in the solution for a longer time, so the RS intensity at 570 nm decreased.

The synchronous scattering signals of anti-SDI Ab and SDI were weaker. For the small diameter gold nanoparticles, the strongest peak is at about 580 nm, and moves to the red wavelengths slowly with an increase in the concentration [23]. When gold particles were coated by SDI antiserum, the signals enhanced faintly and the spectrum shape seemed the same as the gold nanopartiles. Under the suitable solution, immunogold complex formed, there were six synchronous scattering peaks at 360, 400, 420, 440, 470 and 580 nm, the peaks at 360, 400, 420, 440 and 580 nm were RS peaks, the peak at 580 nm is strongest (Figure 7). Their intensities decreased greatly and changed linearly with added SDI concentration, indicating that gold particles were aggregated and deposited after immunoreaction. A wavelength of 580 nm was chosen for assay.

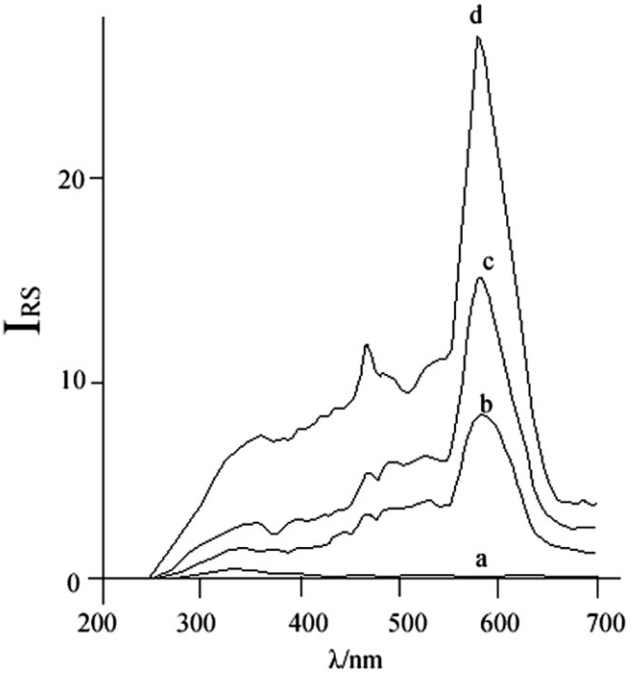

Figure 6. Resonance scattering spectra of SDI in PAM.
Notes: pH4.92–0.20 mg mL^{-1} PAM solution. (a) Blank without SDI; (b) 0.50 μg mL^{-1} SDI; (c) 1.00 μg mL^{-1} SDI; (d) 2.00 μg mL^{-1} SDI.

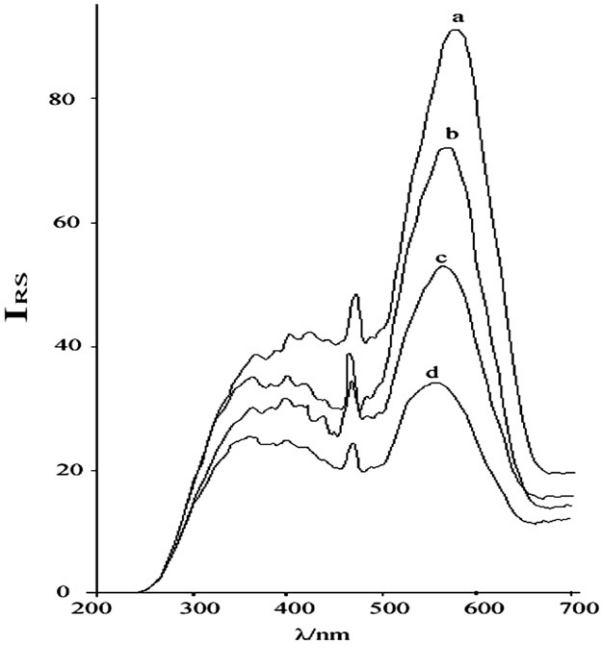

Figure 7. Resonance scattering spectra of SDI-immunonanogold complex.
Notes: pH 4.92–18.47 μg mL^{-1} gold-labelled SDI Ab-52.5 mg mL^{-1} PEG-6000. (a) Blank; (b) 15.0 ng mL^{-1} SDI; (c) 30.0 ng mL^{-1} SDI; (d) 45.0 ng mL^{-1} SDI.

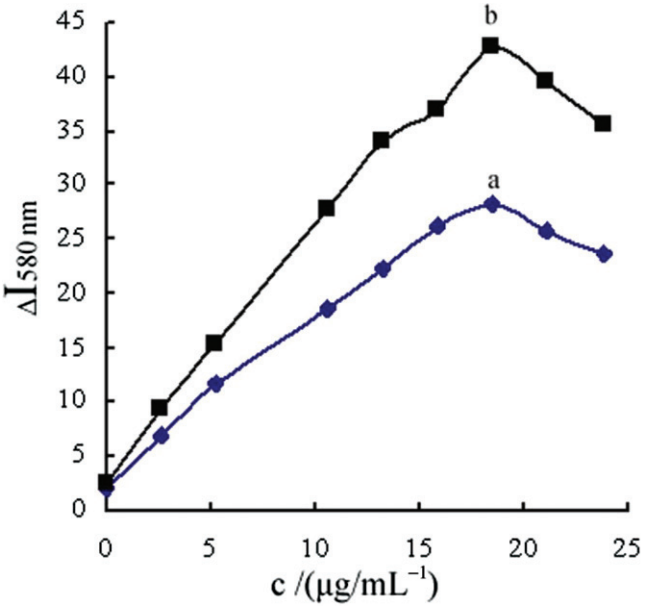

Figure 8. The effect of gold-labelled anti-SDI Ab on ΔI_{580nm}.
Notes: pH 4.92–18.47 $\mu g\,mL^{-1}$ gold-labelled SDI Ab-52.5 $mg\,mL^{-1}$ PEG-6000. (a) 22.5 $ng\,mL^{-1}$ SDI; (b) 30.0 $ng\,mL^{-1}$ SDI.

3.4 *Selection of pH, type, and volume of the buffer*

The effect of KH_2PO_4–Na_2HPO_4 (pH = 4.49–9.18) and citric acid-Na_2HPO_4 (pH = 2.6–8.0) buffer on $\Delta I_{580\,nm}$ was tested. The blank signal of citric acid–Na_2HPO_4 buffer was strong. KH_2PO_4–Na_2HPO_4 buffer had better effect on $\Delta I_{580\,nm}$. The optimum $\Delta I_{580\,nm}$ occurred in pH 4.92 KH_2PO_4–Na_2HPO_4 buffer. $\Delta I_{580\,nm}$ was high in range of 0.30–0.70 mL of buffer, 0.40 mL of pH 4.92 buffer was chosen.

3.5 *Effect of gold-labelled anti-SDI Ab concentration*

The concentration effects of gold-labelled SDI Ab on $\Delta I_{580\,nm}$ are shown in Figure 8. In a certain range of concentration, with the increasing amount of gold-labelled SDI Ab, $\Delta I_{580\,nm}$ increased. The maximum $I_{580\,nm}$ was obtained at 18.47 $\mu g\,mL^{-1}$ gold-labelled SDI Ab.

3.6 *Effect of different PEG concentration*

PEG not only strengthens the immunoreaction, reduces un-special absorption and reaction time, but also enhances the detecting signals [19]. We, therefore, choose PEG to make $\Delta I_{580\,nm}$ enhance clearly. We study the effect of PEG-4000, PEG-6000, PEG-10000 and PEG-20000 on $\Delta I_{580\,nm}$, as shown in Figure 9. Maximum $\Delta I_{580\,nm}$ appeared at $5.25 \times 10^4\,\mu g\,mL^{-1}$ PEG-6000.

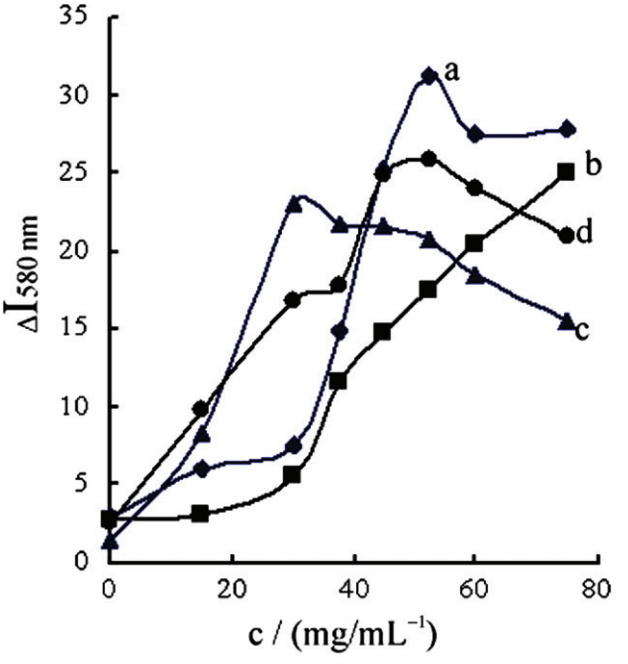

Figure 9. The effect of PEG concentration on ΔI_{580nm}.
Notes: pH 4.92–18.47 μg mL^{-1} gold-labelled SDI Ab-22.5 ng mL^{-1} SDI. (a) PEG-6000; (b) PEG-4000; (c) PEG-10000; (d) PEG-20000.

3.7 *Effect of incubation time*

The effect of incubation time on $\Delta I_{580\,nm}$ was tested. Under ultrasonic irradiation, the incubation was relatively quick and the results kept stable. We obtained the maximum ΔI_{580nm} after 20 min. The intensity was stabilised in 25 to 45 min, and decreased after 45 min. The incubation at 37°C completed and the results kept stable after 40 min, while it was stabilised at room temperature of 20°C after 50 min. Therefore, we choose the ultrasonic irradiation at 20°C for 25 min for the experiment.

3.8 *Analytical feature*

According to the procedure, with SDI concentrations 'c' increasing, their corresponding intensities $\Delta I_{580\,nm}$ weres obtained. The linear range was 0.23 to 45.0 ng mL^{-1} SDI. The linear regression equation was $\Delta I_{580\,nm} = 1.20c + 2.01$ ($R = 0.9975$, $n = 6$). Using 3δ, the LOD was determined to be 0.13 ng mL^{-1}. Compared with the pressed assay [2–7], it is evident that the sensitivity was improved. In the whole course, the steps for separation and washing were omitted, so the method was simple and fast. And the detecting signals were linear to the concentration of SDI.

3.9 *Effect of coexistent substance*

According to the procedure, using 22.5 ng mL^{-1} SDI, we studied the interferences of coexistent foreign substance such as proteins, amino acids, HSA, BSA, lecithin, sudan III

Table 1. Effect of foreign substances (22.5 ng ml^{-1} SDI).

Coexistent substance	Tolerance ($\mu g\,mL^{-1}$)	Relative error (%)	Coexistent substance	Tolerance ($\mu g\,mL^{-1}$)	Relative error (%)
Glucose	2500	−3.6	DL-tryptophan	40	−6.3
Glycine	50	8.8	cane sugar	2000	8.3
HSA	17	7.9	aspartate	30	−4.9
IgG	20	−2.8	BSA	5	8.5
Vitamin B_2	10	−1.4	L-tyrosine	30	6.5
Folic acid	4	−5.6	DL-methionine	15	4.6
Quinine sulphate	40	−8.6	polyacrylamide	12.5	−6.9
Vitamin K	40	6.1	tetradecyl dimethyl benzyl ammonium chloride	4	−7.3
Vitamin C	250	−9.7	lecithin	3	9.5
Vitamin B_6	75	4.4	nicotine	38	−5.2
Complement C_4	0.8	−3.0	sodium dodecyl benzene sulphonate	12	−9.8
L-glutamate	75	9.3	nicotinic acid	75	−1.8
L-lysine	15	4.2	Mg^{2+}	38	−9.1
IgA	7.2	−2.8	Ca^{2+}	50	1.0
Complement C_3	3.8	−2.8	Zn^{2+}	48	−8.5
Vitamin B_1	400	−1.3	L-cystine	20	−4.9
Sudan III	0.7	−9.7	IgM	3.4	−3.0

Table 2. Results of SDI in egg samples.

Samples	Single value ($\mu g\,g^{-1}$)							Mean ($\mu g\,g^{-1}$)	HPLC ($\mu g\,g^{-1}$)
1	2.707,	3.240,	2.594,	2.681,	2.624,	2.990,	2.850	2.812 ± 0.234	2.790
2	4.176,	4.232,	4.093,	3.893,	4.093,	4.036,	4.149	4.096 ± 0.109	4.006
3	5.304,	4.372,	5.108,	4.456,	4.572,	5.335,	4.402	4.793 ± 0.437	4.986
4	4.456,	4.346,	4.629,	4.712,	5.078,	5.048,	4.402	4.667 ± 0.299	4.892
5	5.644,	5.475,	4.938,	5.758,	5.531,	4.682,	5.361	5.341 ± 0.391	5.567
6	6.067,	5.811,	5.897,	6.234,	5.614,	5.221,	5.504	5.764 ± 0.346	5.990
7	5.727,	5.784,	5.701,	5.841,	5.305,	5.022,	6.094	5.639 ± 0.359	5.584
8	8.242,	8.242,	7.562,	8.099,	7.336,	7.500,	8.408	7.913 ± 0.433	8.012

and so on, the tolerance limit was taken as the maximum concentration of the foreign substance that resulted in approximately ±10% relative error in the determination. As shown in Table 1, the coexistent foreign substance did not interfered the analysis, thus the method had good selectivity.

3.10 *Analysis of samples*

Eight samples of eggs polluted by SDI were cooked and peeled. A 1.0 g of egg yolk was added into a weighing bottle and baked to constant weight in bake oven at 80°C, respectively. 0.40 g of egg yolk was weighed and added into beaker, dissolved with 10 mL water, and left overnight. Next day, the sample solutions were oscillated by ultrasonic

vibration for 1 h, stood for 10 min, the mixed solution was centrifuged at 12000 rpm for 30 min, and then the upper solution was taken out and stored. According to the optimum procedure, 150 μL of the upper solution was added and determined, the results indicated as in the Table 2, and were agreement with that of the HPLC [1].

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