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# Free-Labeled Nanogold Catalytic Detection of Trace $UO_2^{2+}$ Based on the Aptamer Reaction and Gold Particle Resonance Scattering Effect

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**Abstract** In pH 5.5 2-(*N*-morpholino)-ethanosulfonic acid buffer solution containing 0.0125 M NaCl at 80 °C, the single-stranded substrate DNA hybrid with enzyme DNA to form double-stranded DNA (dDNA). The substrate chain of dDNA could be cracked catalytically by $UO_2^{2+}$ to produce a short single-stranded DNA (ssDNA) that adsorbed on the nanogold (NG) surface to form stable NGssDNA conjugate, and the unadsorbed NG take place aggregation to produce the NG aggregations in blue color. Both NG and NGssDNA exhibited strong catalytic activity on the gold particle reaction between $HAuCl_4$ and ascorbic acid that can be monitored by resonance scattering (RS) spectral technique at 620 nm. However, the catalytic effect of NG aggregation was very weak and it cannot be separated from the cracked reaction solution. When the $UO_2^{2+}$ concentration increased, the ssDNA increased, the NGssDNA increased, the formed gold particles increased, and the RS intensity at 620 nm increased. The increased RS intensity $\Delta I_{620\ nm}$ was linear to $UO_2^{2+}$ concentration in the range of 3.35–23.45 pM, with a regression equation of $\Delta I_{620\ nm}=27.6C+29.1$, and detection limit of 0.1 pM. This new RS assay was applied to analysis of $UO_2^{2+}$ in water sample with satisfactory results.

**Keywords** $UO_2^{2+}$ · Catalytic aptamer cracking · Nanogold catalysis · Resonance scattering spectral assay

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## Introduction

Uranium is a radioactive metal element that exists in the environment [1]. It is one of the main materials in nuclear energy generation, and enriched uranium is a major material in nuclear weapon. If human beings were exposed to uranium-polluted environment, that could threaten the human health [2, 3]. Thus, highly selective and sensitive detection of uranium is very important for environmental protection and human health. At present, some analytical techniques such as inductively coupled plasma, atomic absorption spectrometry and phosphorimetry have been used to detect uranium [4–7], with high sensitivity. However, those methods require expensive and complicated instruments. In recent years, some new methods have been developed for metal ions, including fluorescence [8, 9], surface plasmon resonance (SPR) [10], electrochemistry [11, 12], and colorimetry [13, 14]. However, there are only a few reported methods for trace uranium [15–19], and most of them lack high sensitivity and selectivity, and low cost.

Aptamer is a short single-stranded oligonucleotide containing 20–100 bases. It can bind with high affinity and specificity to a wide range of target molecules including protein, polypeptide or organic compound and metal ion, with very small dissociation constant. Due to the high affinity, easy and quick preparation in vitro, and easy functionalizing, aptamers are used as research tools in specific protein function and interaction study, disease treatment, medical diagnosis, and bioanalysis [20]. At present, spectrophotometry, fluorescence, electrochemistry, SPR, and surface-enhanced Raman scattering have been utilized in aptamer analysis [21–23]. Resonance scattering (RS) spectroscopy is simple, rapid, and sensitive and has been applied to analysis of trace proteins, nucleic acids, and inorganic ions [24–26]. Nanogold (NG), being of easy preparation, high electron density and good

biocompatibility, has been applied to protein, nucleic acid, and metal ion analysis [27]. Recently, NG has been also utilized in RS technique to develop immunonanogold RS, immunonanogold catalytic RS, aptamer–nanogold RS, and aptamer–nanogold catalytic RS assays, with high selectivity and sensitivity [28]. Based on our knowledge, there is no report about the catalytic active difference of nanogold and its aggregation, and aptamer RS assay for uranium. In this article, based on $UO_2^{2+}$-dDNA catalytic cutting reaction and the NG aggregation reaction, the catalytic active differences both NG and the NG aggregation on the $HAuCl_4$–ascorbic acid (AA) reaction, and RS effect of the gold particles, a new nanogold catalytic RS assay was established for the determination of trace uranium ion in water sample.

## Experimental

### Apparatus

A model Cary Eclipse fluorescence spectrophotometer (Varian Company, USA) was used to record RS spectra, with the synchronous scanning technique ($\lambda_{ex}-\lambda_{em}=\Delta\lambda=0$), a voltage of 500 V, both excited and emission slit width of 5.0 nm, emission filter of 1%T attenuator. A model TU-1901 double beams UV–visible spectrophotometer (Beijing Purkinje General Instrument Limited Co., China), a model 79-1 magnetic heat agitator (Zhongda Instrumental Plant, Jiangsu, China), a model nanoparticle and zeta potential analyzer (Malvern Company, England), and a model FEI Quanta 200 FEG scanning electron microscopy (SEM, Netherlands) were used.

### Reagents

$HAuCl_4$ $2.4\times10^{-2}$ mol/L (National Pharmaceutical Group Chemical Reagents Company, China), single-strand DNA (substrate chain 1, SS1) probe (Biological Technology Co., Ltd., Shanghai, China) with sequence of CTCACTATAG GAAGAGATGGACGTG, enzyme chain1 (ES1) of CACGGTCCATCTCTGCAGTCGGGTAGGCTTTC TACTGTT AACCGACC, substrate chain 2 (SS2) of ACTCACTATAGGAAGA ATGGACGG, enzyme chain 2 (ES2) of CACGTCCATCTCTGCAGTCGGGT GTTAAACCGAC TTCAGACAA TGAGT, substrate chain 3 (SS3) of ACTCACTATAGGTCCCGGATA GAGATG GACGTG, and enzyme chain 3 (ES3) of CACGTC CATCTCCGGGTTAAAACCCATAGTGAGT were used. A pH 5.5 2-(*N*-morpholino)-ethanosulfonic acid buffer solution (MES) with 0.025, 2.0 mol/L NaCl solution, 2.0 mol/L NaOH solution, 0.05 mol/L Tris solution, $1.00\times10^{-6}$ and $1.00\times10^{-7}$ mol/L $UO_2^{2+}$ standard solution, 0.5 mg/mL $NaBH_4$ solution, $9.6\times10^{-3}$ mol/L $HAuCl_4$ solution, 0.1 mol/L HCl solution, 0.1 mol/L cetyltrimethyl ammonium bromide (CTAMB) solution, and 0.057 mol/L AA solution were prepared. Nanogold particles were prepared by sodium borohydride reduction procedure: A 35 mL doubly distilled water, 0.50 mL $2.4\times10^{-2}$ M $HAuCl_4$ solution, and 3.5 mL 1% sodium citrate solution were added into a 50-mL conical bottle under magnetic stirring. Then, 3.0 mL 0.5 mg/mL $NaBH_4$ solution was dripped slowly, and the color immediately changed from dark red to black red, and to red. After 10 min, the mixture was diluted to 50.0 mL. The nanogold particle concentration was 58.0 μg/mL Au. The NG aggregation was prepared as follows: A 25-μL 2.0 mol/L NaCl solution and 300-μL 58.0 μg/mL NG solution were added into a tube and diluted to 1.0 mL and mixed well. The concentration of NG aggregation was 17.4 μg/mL Au. All reagents were of analytical grade, and the water was doubly distilled.

### Hybridization of DNA

A 0.50 mL 0.17 μmol/L of both SS1 and ES1 were added to a 15-mL colorimetric tube. Then 1.0 mL 2.0 mol/L NaCl solution, 2.8 mL of MES buffer solution, and 3.2 mL water were added. After mixed well, the mixture was heated at 80 °C for 15 min and cooled down to room temperature for 1.5 h [29]. The double DNA (dDNA) concentration was 21.5 nm/L.

### Procedure

A 75-μL 21.5 nmol/L dDNA solution and a certain amount of $UO_2^{2+}$ solution were added into a 5-mL marked test tube and mixed well. After 8 min, 25 μL 0.05 mol/L Tris solution was added immediately to stop the cracking reaction. Then 0.20 mL 58.0 μg/mL gold nanoparticle solution was added and diluted to 1.5 mL. The mixture is the cracking reaction solution.

Into a 5-mL tube, 10 μL $9.6\times10^{-3}$ mol/L $HAuCl_4$, 100 μL 0.1 mol/L HCl solution, 30 μL of the cracking reaction solution diluted 20 times, and 150 μL 0.1 mol/L CTMAB solution were added and diluted to 2.0 mL. Then 8 μL 0.057 mol/L AA solutions were added and diluted to 3.0 mL and heated at 60 °C for 15 min. The nanocatalytic reaction was stopped by tap water cooling. The RS spectrum, the $I_{620\ nm}$, and the blank value $(I_{620\ nm})_0$ without $UO_2^{2+}$ were recorded. The value of $\Delta I=I_{620\ nm}-(I_{620\ nm})_0$ was calculated.

## Results and Discussions

In pH 5.5 MES buffer solution containing 0.0125 mol/L NaCl, the substrate chain of dDNA could cracked by $UO_2^{2+}$ to release a short single-stranded DNA (ssDNA), also called as cleaved strand. The ssDNA can adsorb on the NG surface to form NGssDNA to prevent its aggregation.

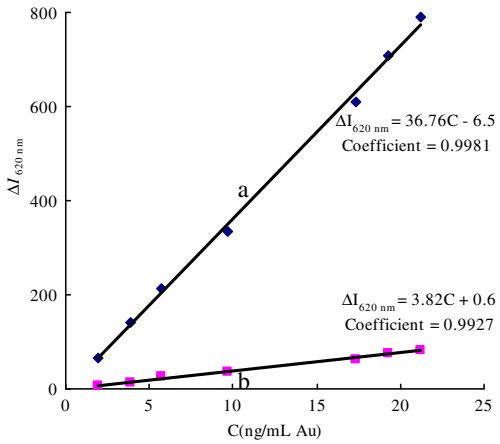


**Fig. 1** Relationship of gold concentration and $\Delta I_{620\ nm}$. *a* Nanogold: 32 μmol/L $HAuCl_4$, 3.3 mmol/L HCl, 5 mmol/L CTMAB, 34 mmol/L AA; *b* Nanogold aggregation: 32 μmol/L $HAuCl_4$, 3.3 mmol/L HCl, 5 mmol/L CTMAB, 34 mmol/L AA

When concentration of $UO_2^{2+}$ increased, the formed ssDNA and NGssDNA increased, the NG aggregation decreased, and the color changed from blue to red gradually. Thus, the RS peak decreased at 610 nm. If no $UO_2^{2+}$ was added, all NG aggregate, the solution is blue. We observed that the NG and NG aggregation exhibited great catalytic active difference on the gold particle reaction of $HAuCl_4$–CTMAB–AA. As Fig. 1 showed, NG particles had strong catalytic effect with a slope of 36.76, and the NG aggregation was very weak, with a slope of 3.82. If the NGssDNA was used as the nanocatalyst, the catalyzed products had a strong RS peak at 620 nm. Under the chosen conditions, when the concentration of $UO_2^{2+}$ increased, the content of NGssDNA in the cracking reaction solution

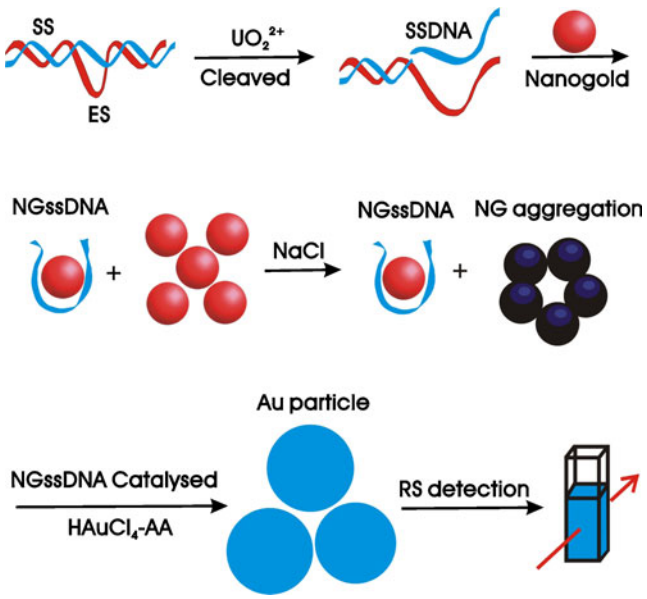


**Fig. 2** Principle of dDNA cleaved NGssDNA catalytic RS assay for $UO_2^{2+}$

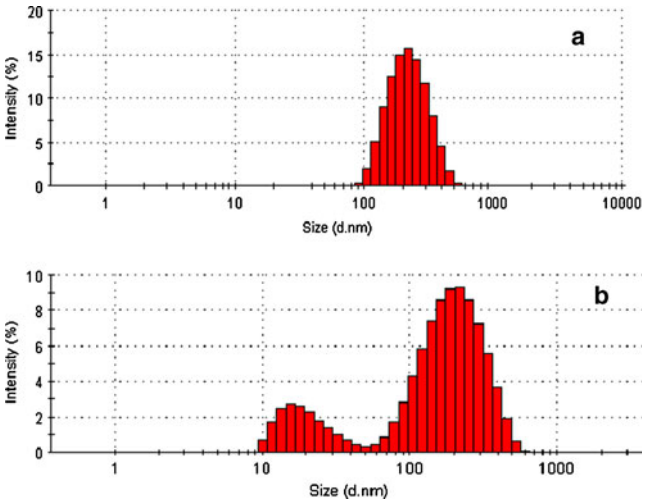


**Fig. 3** Laser scattering **a** 1.07 nmol/L dDNA, 7.74 μg/mL NG, 0.0125 mol/L NaCl; **b** 1.07 nmol/L dDNA, 7.74 μg/mL NG, 0.0125 mol/L NaCl, 26.8 nmol/L $UO_2^{2+}$

increased, the formed gold particles increased, and RS peak at 620 nm increased linearly. On those grounds, a new nanogold catalytic RS assay would be proposed for trace $UO_2^{2+}$ as in Fig. 2.

## Laser Scattering

In the absence of $UO_2^{2+}$, the dDNA could not protect the NG particles, which was gathered to form big gold particles by salt, with an average size of 200 nm

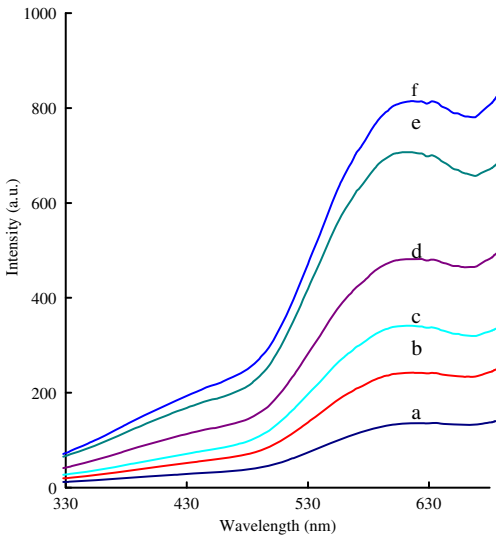


**Fig. 4** RS spectra of the NGssDNA catalytic system. *a* 32 μmol/L $HAuCl_4$, 3.3 mmol/L HCl, 5 mmol/L CTMAB, 34 mmol/L AA; *b* to *a* 7.74 μg/mL NG, 1.07 nmol/L dDNA, 3.35 pmol/L $UO_2^{2+}$; *c* to *a* 7.74 μg/mL NG, 1.07 nmol/L dDNA, 6.7 pmol/L $UO_2^{2+}$; *d* to *a* 7.74 μg/mL NG, 1.07 nmol/L dDNA, 10.05 pmol/L $UO_2^{2+}$; *e* to *a* 7.74 μg/mL NG, 1.07 nmol/L dDNA, 20.1 pmol/L $UO_2^{2+}$; *f* to *a* 7.74 μg/mL NG, 1.07 nmol/L dDNA, 23.45 pmol/L $UO_2^{2+}$

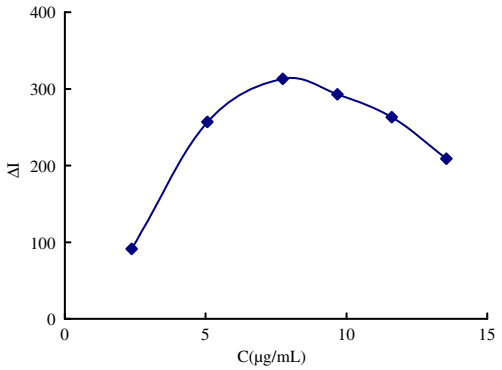


**Fig. 5** Effect of AuNPs concentration 32 μmol/L $HAuCl_4$, 3.3 mmol/L HCl, 5 mmol/L CTMAB, 34 mmol/L AA, 7.74 μg/mL NG, 9.50 pmol/L $UO_2^{2+}$

(Fig. 3a). When $UO_2^{2+}$ was added to the solution, the dDNA was cleaved to form a short ssDNA that could protect NG particles without aggregation. That is, the NGssDNA and the NG aggregation coexist in the system as in Fig. 3b. The average size of these particles was 135 nm. This was consistent with the analytical principle.

## Scanning Electron Microscopy

The SEM of the cracking reaction solution cannot be observed by scanning electron microscope because it contained high concentration of salt. To remove large amount of salt, the cracking reaction solution was centrifuged for 15 min with 15,000 rpm, and the particles were dispersed in water by ultrasonic wave. Then a 20-μL solution was taken into a silicon slice and dried by air. Using a scanning electron microscope, the graph was obtained. The results indicated that the small NG particles in size of 8 nm were aggregated to form large aggregations.

## Resonance Scattering Spectra

In pH 5.5 MES buffer solution containing 0.0125 M NaCl, dDNA do not protect NG, and NG formed large NG aggregations that had an RS peak at 610 nm. Upon addition of $UO_2^{2+}$, the substrate chain of dDNA could be cracked to a short ssDNA that prevent NG to be aggregated. Thus, the RS peak decreased linearly with the concentration of $UO_2^{2+}$ increasing and can be used to detect $UO_2^{2+}$. But, the sensitivity was not high.

To overcome the above problem and enhance the sensitivity, the nanocatalytic reaction was utilized. When there is no $UO_2^{2+}$, the NG aggregations in the cracking reaction solution showed weak catalytic effect on the $HAuCl_4$–CTMAB–AA reaction, and the system exhibited a weak RS peak at 620 nm as in Fig. 4. With the concentration of $UO_2^{2+}$ increasing, the concentration of NGssDNA increased in the cracking reaction solution, the gold particles increased, and the RS intensity at 620 nm enhanced linearly. Thus, a wavelength of 620 nm was chosen for the catalytic detection of $UO_2^{2+}$.

## Selection of Assay Conditions

The NG aggregation reaction need a high concentration of NG, and the NG catalytic reaction takes place at trace NG. Thus, the diluted cracking reaction solution was necessary for the nanocatalytic RS assay. For the cracking reaction, the effect of EMS pH buffer solution on the $\Delta I_{620\ nm}$ was tested. When the pH was 5.5, the $\Delta I_{620\ nm}$ reached the maximum, so pH 5.5 EMS buffer solution was chosen. The cracking reaction of $UO_2^{2+}$ is a dynamic process, and the reaction time should be controlled accurately, and a stopping reaction reagent should be used. Tris is a good reagent to stop the $UO_2^{2+}$ cracking reaction. The effect of Tris concentration on the $\Delta I_{620\ nm}$ was considered. A 0.83 mM Tris, giving maximum $\Delta I_{620\ nm}$, was chosen for use. The results showed that when the reaction time was 8 min, the $\Delta I_{620\ nm}$ was maximal. Thus, 8 min reaction time was chosen. Figure 5 showed that when NG concentration was 7.74 μg/mL, the $\Delta I_{620\ nm}$ had the maximum. Therefore 7.74 μg/mL NG was selected. When the dDNA concentration was 1.07 nmol/L, the $\Delta I_{620\ nm}$ of the system was maximum. Thus, 1.07 nmol/L dDNA was selected for use.

Nanocatalytic reaction is an important way to amplify analytical signal. We found that NG and NGssNDA have strong catalytic effect on $HAuCl_4$–CTMAB–AA reaction. We studied the effects of concentration of HCl, $HAuCl_4$, AA, and CTMAB concentrations, reaction time, and temperature on the $\Delta I_{620\ nm}$. The results showed that a 10-μL $9.69\times10^{-3}$ mol/L $HAuCl_4$ solution, 100 μL 0.1 mol/L HCl solution, 150 μL 0.10 mol/L CTMAB solution, 18 μL 0.057 mol/L AA solution, and reaction temperature of 60 °C for 15 min, giving maximum $\Delta I_{620\ nm}$, were

**Table 1** Analytical result for $UO_2^{2+}$ in water sample

| Sample | This assay (nmol/L) | RSD (%) | LF method (nmol/L) | Sample | This assay (nmol/L) | RSD (%) | LF method (nmol/L) |
|---|---|---|---|---|---|---|---|
| 1 | 10.1±4.1 | 4.1 | 9.3 | 4 | 0.092±0.0041 | 4.4 | 0.010 |
| 2 | 12.0±4.6 | 3.8 | 12.6 | 5 | 0.210±0.012 | 5.7 | 0.245 |
| 3 | 2.00±0.12 | 6.0 | 1.95 | 6 | 0.043±0.0035 | 8.1 | 0.048 |

chosen for use. We also examined the effect of the volume for cracking reaction solution diluted 20 times on the $\Delta I_{620\ nm}$. The results showed that when the diluted solution was 30 μL, $\Delta I_{620\ nm}$ reached maximum. So 30 μL of the diluted solution was chosen for use.

### Influence of Coexistent Substances

According to the procedure, the influence of coexistent ions on the determination of $UO_2^{2+}$ was tested, with a relative error of ±10%. Results indicated that a 1.5-μmol/L $Pd^{2+}$, 2.1 μmol/L $Fe^{3+}$, 2.4 μmol/L $Ag^{+}$, 1.5 μmol/L $Co^{2+}$, 1.8 μmol/L $Ca^{2+}$, 2.8 μmol/L $Pb^{2+}$, 1.6 μmol/L $Mn^{2+}$, 5.5 μmol/L $Mo^{6+}$, 2.0 μmol/L $Zn^{2+}$, 7.5 μmol/L $Al^{3+}$, 2.03 μmol/L $Mg^{2+}$, 3.5 μmol/L $Cr^{3+}$, 251 μmol/L HSA, 25 μmol/L glucose, 150 μmol/L albumin, 512 μmol/L lysine, 145 μmol/L valine, 156 μmol/L phenylalanine, and 148 μmol/L tyrosine do not interfere with the 20 pmol/L $UO_2^{2+}$ determination. This showed the method had good selectivity.

### Linear Relationship

Under the optimal conditions, the analytical features for the three different chain lengths of DNA hybridization systems (SS1-ES1, SS2-ES2, and SS3-ES3) were studied. The SS1-ES1 system was best. For the RS assay, the decreased intensity $\Delta I_{620\ nm}$ was linear to $UO_2^{2+}$ concentration in the range of 0.67–60.3 nmol/L, with a regression equation of $\Delta I_{620\ nm}=10.5C+61.2$, coefficient of 0.9972, and detection limit of 0.05 nmol/L. In comparison to the reported assays [15–19, 26], this method was simple, rapid, low cost, sensitive and selective, and the linear range was wider.

Selecting the SS1-ES1 system, an NG catalytic RS assay was proposed, according to the procedure. The working curve was obtained to plot the $UO_2^{2+}$ concentration C (in picomoles per liter) vs $\Delta I_{620\ nm}$. The increased intensity $\Delta I_{620\ nm}$ was linear to the $UO_2^{2+}$ concentration in the range of 3.35–23.45 pmol/L, with a regression equation of $\Delta I_{620\ nm}=27.6C+29.1$, coefficient of 0.9911, and detection limit of 0.1 pmol/L. This NG catalytic RS assay was one of most sensitive, and the signal is linear to $UO_2^{2+}$ concentration.

### Analysis of $UO_2^{2+}$ in Water Sample

Six water samples, including river, lake, reservoir, fountain, well, and pond, were pretreated according to the reference [30]. The $UO_2^{2+}$ was determined five times according to the procedure. The results of Table 1 showed that the RS assay was in agreement with that of the laser fluorescence (LF) method. The relative standard deviation was in the range of 3.8%–8.1%. The known 0.10 and 5.00 nmol/L $UO_2^{2+}$ were added into the sample to measure the recovery. A recovery range of 92.2%–107.5% was obtained.

## Conclusions

The catalytic cracking reaction of dDNA-$UO_2^{2+}$ was studied firstly by nanogold RS spectral method. Results show that the nanogold catalytic activity was stronger than its aggregations on $HAuCl_4$–vitamin C particle reaction that can be also monitored by the RS technique at 620 nm. A novel and highly sensitive and selective nanocatalytic RS assay was proposed for detection of trace $UO_2^{2+}$ in water, based on the catalytic cracking and nanocatalytic reactions.

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