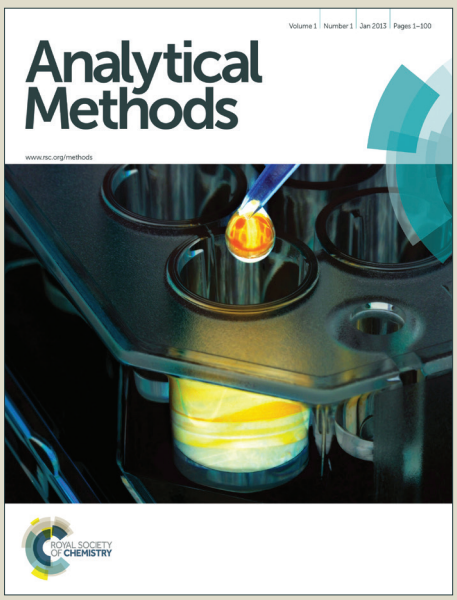
Analytical Methods

Accepted Manuscript



ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/methods

Application of silver nanoparticles decorated with β-cyclodextrin in determination of 6-mercaptopurine by Surface-enhanced Raman Spectroscopy

Lei Yang, Yanhua Chen, Hongli Li, Lan Luo, Ying Zhao, Hanqi Zhang, Yuan Tian *

College of Chemistry, Jilin University, Changchun, 130012, P. R. China

**To whom correspondence should be addressed.*

Phone: +86-431-85168399;

Fax: +86-431-85112355

E-mail: tianyuan@jlu.edu.cn

Abstract

Although classical silver nanospheres were widely used as the probes in surface-enhanced Raman spectroscopy (SERS), they lacked long-term stability. In the present work, the silver nanoparticles decorated with β-cyclodextrin (β-CD-AgNPs) were used as the probe and the stability was improved. The adsorption characteristics of 6-mercaptopurine (6-MP) on classical silver nanospheres and β-CD-AgNPs were studied cautiously using SERS, and chemical information of the molecular structure was obtained. The binding of 6-MP molecule to the surface of AgNPs is based on the stable Ag-S covalent bond. The electrostatic adsorption between protonated N^7 atom or N^1 atom and the silver surface results in a notable Raman enhancement. The intensity of the Raman band of 6-MP at 866 cm^{-1} was used to determine 6-MP. The experimental conditions, such as volume of CD-AgNPs solution, incubation time, pH value and concentration of sodium chloride were examined and optimized. Compared with classical silver nanospheres when β-CD-Ag nanoparticles were used, the sensitivity was higher, the analytical time was shorter, and the linear range was wider. A good linearity (r=0.996) in the range of (0.040-2.0) $\times 10^{-7}$ $mol \cdot L^{-1}$ was obtained for determining 6-MP. The limit of detection is 0.024×10^{-7} $mol \cdot L^{-1}$ and the limit of quantification is 0.080×10^{-7} $mol \cdot L^{-1}$. This method was successfully applied to the

determination of 6-MP in real samples and the results were satisfactory.

Keywords: β-cyclodextrin (β-CD); Silver nanoparticles (AgNPs); 6-mercaptopurine (6-MP); Surface-enhanced Raman spectroscopy (SERS)

1. Introduction

Surface-enhanced Raman spectroscopy (SERS) technique demonstrates unique advantages in material analysis and enjoys a huge potential as a high sensitive method of quantitative analysis. First of all, because of obvious and tangible molecular specificity, SERS is an extremely convenient method to get content information of the analyte in the sample and the structure information of the molecule simultaneously. The SERS can be applied to the simultaneous detection of multiple analytes because all molecules own their own characteristic Raman signals [1-3]. Secondly, SERS is superior to other methods in providing information of the surface properties, because its real-time signal will change with many factors such as the chemical environment (such as pH value) and the orientation or rearrangement of molecules adsorbed on the substrate surface [4-5].

Among a great variety of available SERS substrates, multifarious silver colloids are often used due to high activity and strong operability [6-12]. Silver colloids generally used for SERS active substrate were prepared by reducing silver nitrate with hydrazine, sodium borohydride [13] or sodium citrate [14]. The dispersion and stability of the silver colloids were achieved based on electrostatic repulsion between the nanoparticles.

However, there are numerous external irresistible factors that will lead the repulsive force somewhat to be weakened or even to disappear, so that silver nanoparticles in the solution are no longer stable and then coagulate. The uncontrolled coagulation eventually lead to a sharp decrease of the activity of SERS substrate and poor stability in the form of SERS spectra. In conclusion, the preparation of silver nanoparticles with excellent activity and stability is still difficult and a focus in current research.

Mercaptopurine (6-MP, Scheme 1) is a kind of chemotherapy drug and can be used as immunosuppressor [15]. 6-MP is mainly used in the treatment of lymphoblastic leukaemia, and used to treat variety of diseases, such as choriocarcinoma, chorioadenoma, polycythemia, psoriatic arthritis, and inflammatory bowel disease [16]. 6-MP dosage is an issue of crucial importance and underdosage will fail in the treatment of the disease. The relatively adverse effects can be caused by overdose. The slight symptoms may be diarrhoea, nausea, vomiting, etc. while severe symptoms can be hematuria, fatal liver toxicity, myelosuppression, etc. [17-18]. Therefore, the determination of 6-MP is of great significance for clinical applications. Some methods have been reported for the determination of 6-MP, including HPLC [19-21], GC/MS [22], voltammetric method [23], electrochemical reductive desorption method [24], electrochemical method [25], inhibition chemiluminescence [26], flow

injection chemiluminescence (FI-CL) [27], and capillary electrophoresis with laser-induced fluorescence detection [28] and fluorescence enhancement detection [29-30]. However, these methods show their limitations and demerits in practical application. The liquid chromatography is time-consuming. GC/MS is not suitable to distinguish between isomers. The electrochemical method requires frequently calibration. To develop a simple and reliable method for determination of 6-MP is necessary.

In this work, β- cyclodextrin (β-CD) was used as the protecting agent to stabilize silver nanoparticles (AgNPs). β-CD modified AgNPs were used as the substrate in SERS. 6-MP were covalently bonded on the surface of the silver nanoparticles. The present method was applied to the analysis of the real samples. The experimental results obtained using a portable miniature Raman spectrometer indicated that the present method had some advantages in sensitivity, simplicity, and rapidity.

Scheme 1

2. Experimental

2.1. Materials

Silver nitrate ($AgNO_3$, 99.85 %), sodium citrate (anhydrous, 99 %), β-cyclodextrin (β-CD, 98 %), ammonia ($NH_3 \cdot H_2O$), 6-mercaptopurine (99.0 %), glucose, and sodium chloride (NaCl) were purchased from Beijing Ding Guo Biotech. Co. Ltd, China. The Britton-Robinson (BR) buffer solution (ionic strength, 0.5) contained 0.04 $mol \cdot L^{-1}$ H_3PO_4, 0.04 $mol \cdot L^{-1}$ HAc, and 0.04 $mol \cdot L^{-1}$ H_3BO_3 and the pH value of the buffer solution was adjusted to appropriate pH using 0.2 $mol \cdot L^{-1}$ NaOH. BR buffer solution was used to control the acidity of the system. Aqua regia solution was used to clean the glassware and the glassware was then rinsed thoroughly with deionized water prior to use. Other chemicals used here were of analytical reagent grade and all the solutions used in this study were prepared with deionized water obtained with Milli-Q water purification system (18.2 MΩ·cm).

2.2. Equipment

Raman spectra were obtained using a BTR111MiniRam (B&W Tek, Inc.), equipped with 785 nm excitation laser and a fibre-optics probe. 1 cm quartz cell was used. The laser power was chosen as 105 mW and the integration time was 10s. Absorption spectra were recorded on an Australian GBC Cintra 10e UV-vis-NIR spectrometer in the wavelength range of 300 to 1000 nm. The TEM image was obtained with a

JEM-2100F transmission electron microscope operated at an accelerating voltage of 200 kV.

2.3.1. Synthesis of classical AgNPs

The AgNPs were synthesized based on the reduction of $AgNO_3$ by citrate following the procedure described by Lee and Meisel [14]. In a 250 mL round-bottom flask equipped with a condenser, 27 mg of $AgNO_3$ was dissolved in 150 mL of deionized water and heated to the boil with vigorous stirring. Rapid addition of 3 mL of 1 % sodium citrate in the boiling solution resulted in a green-gray and transparent colloid solution after boiling for 30 min. Then the heating was stopped and the stirring continued for an additional hour. Finally, the AgNPs were stored in a brown bottle at 4 °C

2.3.2. Synthesis of β-CD-AgNPs

β-CD-AgNPs were prepared based on the traditional silver mirror reaction in the presence of cyclodextrin according to literature [31] except that α-CD was placed with β-CD, which resulted in significant change in the nanoparticles size. $[Ag(NH_3)_2]^+$ was prepared by adding ammonia dropwise into 1 mL of 0.1 $mol \cdot L^{-1}$ $AgNO_3$ solution in a capped glass tube

and shaking vigorously till the precipitate was completely dissolved. The mixture was diluted to 10 mL with deionized water to obtain the solution of 10 mmol·L^{-1} $[Ag(NH_3)_2]^+$. In a 250 mL round-bottom flask, 1.16 g of β-CD and 92 mL of deionized water were heated slightly for about 5 min with vigorous stirring. Then 4 mL of 10 mmol·L^{-1} $[Ag(NH_3)_2]^+$ and 4 mL of 50 mmol·L^{-1} glucose were added sequentially. The color of the solution changed from colorless to light orange after boiling for 30 min with refluxing and magnetic stirring. The resulting solution was cooled down to room temperature in atmospheric environment and filtered through 0.22 μm filter membrane. The solution could be stored for up to 45 days in refrigerator at 4 °C without loss in activity.

2.4. Preparation of standard solution of 6-MP

The stock solution of 3 mmol·L^{-1} 6-MP was prepared by dissolving 6-MP in a certain amount of 0.1 mol·L^{-1} NaOH with the help of the ultrasound and diluting to fixed volume with deionized water. The working standard solution of 6-MP was prepared by diluting the stock solution with deionized water. The resulting working standard solution was filtered before use.

2.5. Preparation of sample

The commercial tablet was accurately weighed, ground, and transferred into a 50 mL flask. A certain amount of 0.1 $mol \cdot L^{-1}$ NaOH solution was added in the flask and the flask was ultrasonically shaken for about 15 min. The resulting solution was diluted to 250 mL with deionized water.

2.6. Determination of 6-MP

200 μL of β-CD-AgNPs solution and 800 μL of sample solution were added in the centrifuge tube and the resulting mixture was incubated for 2 min. 100 μL of buffer solution was added into the mixture. After gentle shaking, the appropriate volume of NaCl was added. The resulting solution was allowed to stand for 2 min at room temperature and then analyzed. The SERS intensity was expressed in the peak area. The peak area at 866 cm^{-1} was measured. All the experiments were performed in five replicates.

Similarly, 600 μL AgNPs solution, 600 μL of sample solution, 100 μL of buffer solution and appropriate volume of NaCl were mixted and the resulting solution was measured at 866 cm^{-1}.

3. Results and discussion

3.1. Characterization of AgNPs and β-CD-AgNPs

UV–vis spectrum and TEM are routinely used to assess the dispersibility and morphology of noble metal nanoparticles. Fig. 1 shows the UV–vis spectrum and TEM image of the prepared silver nanoparticles. The absorption maximum of the AgNPs is at 420 nm with a full width at half-maximum (FWHM) of 115 nm. These values correspond to the monodisperse colloidal AgNPs with the diameter in the range of 50-60 nm according to the literature [11]. The β-CD-AgNPs has a maximum absorption peak at 417 nm with FWHM of 71 nm. The size of the β-CD-AgNPs was obtained by measuring 106 nanoparticles from their TEM images and the average diameter of the nanoparticles is 34.1 nm. The prepared β-CD-AgNPs are monodispersed and homogeneous.

The absorption spectra of β-CD-AgNPs in the presence and absence of 6-MP are given in Fig. 1. The β-CD-AgNPs in the absence of 6-MP exhibit an absorption peak at 400 nm. With the increase of NaCl concentration, the intensity of the surface plasmon absorption band decreases and the absorption peaks of β-CD-AgNPs are broadened. The experimental result indicates that the β-CD-AgNPs congregate in the solution. A new peak located at around 769 nm can be observed in the presence of 6-MP. 6-MP was bound to the surface of silver nanoparticles strongly, which caused the aggregation of silver nanoparticles and the enhancement of the SERS intensity. The absorption peak also reveals that

the β-CD-AgNPs has a broad range of excitation wavelength and the laser excitation wavelength of 785 nm is a reasonable choice.

Figure 1

3.2. SERS bands of 6-MP

Shen et al. have studied the SERS and orientation of 6-MP self-assembled monolayers (SAMs) on a silver electrode [4] .The Raman and SERS spectra of 6-MP on silver colloid were reported [32], Fig. 2 shows the mechanism for determining 6-MP using β-CD-AgNPs as the SERS-active substrate. The chemical force of CD to interact with AgNPs should be hydrogen bond between the β-CD rim hydroxyl groups and the noble metal particles, and the wider end of β-CD may sitting on the surface of AgNPs [33] .The β-CD molecules have hydrophilic outer surface with hydroxyl groups, which can interact with AgNPs, and hydrophobic central cavity. 6-MP molecules can connect AgNPs with Ag-S bond when 6-MP in high concentration (6-MP molecules stand on the Ag surface); but maybe connect AgNPs with pi-Ag bond in low concentration (lie on the Ag surface). The β-CD can force 6-MP stand on the Ag surface either in high or low concentration.

As shown in Fig. 3, the dominating characteristic bands of the normal Raman and SERS spectra of 6-MP, ranging from 371 to 1499 cm^{-1}, are

all observed clearly. The difference of the spectra indicates that there is a very strong interaction between 6-MP and β-CD-AgNPs. There are many peaks in the SERS spectra of 6-MP. The major bands in these spectra are located at 435, 623, 681,866, 1003, 1145, 1207, 1288, and 1328 cm^{-1}. Fig. 3a shows that all these bands are characteristic of 6-MP molecules chemisorbed on the β-CD-AgNPs. The peak observed at 435 cm^{-1} corresponds to C^6-S^{10} stretching vibration and ring breathing vibration. The result suggests that 6MP should adsorb on the Ag surfaces through the S atom. The strongest peak observed at 866 cm^{-1} is mostly attributed to the C^8-H^8 out-of-plane bending vibration and N^7-C^8 bending. Subordinately, the peak observed at 1003 cm^{-1} corresponds to C^4-N^9 stretch, while the peak observed at 1145 cm^{-1} is thought to come from N^7-C^8 stretch, C-N^7-H^7 bending, and C^5-N^7 stretch. The overlapping peak observed at 1288-1328 cm^{-1} corresponds to the C^2-H^2 and C^8-H^8 deformation vibrations and the N^1-C^2-N^3 stretching vibration. Therefore, 6-MP was adsorbed on the silver surface with a tilted orientation via S, N^1, and N^7 atoms [4]. Weak peaks at 623, 681 and 1207 cm^{-1} correspond to ring C^5-C^6 bending. An increase of SERS signal could be observed when CD is decorated on AgNPs because of the change of surrounding environment of AgNPs. The stability of AgNPs was improved in the presence of β-CD.

Figure 2

Figure 3

3.4. Optimization of experimental conditions

3.4.1. Volume of β-CD-AgNPs solution and incubation time

The effect of the volume of β-CD-AgNPs solution in the range of 50 μL to 400 μL and the effect of incubation time on the SERS intensity were investigated. The experimental results are shown in Fig. 4. The results show the importance of properly controlling of the volume of β-CD-AgNPs and incubation time for obtaining an optimized Raman enhancement. For 50 and 100 μL, the SERS intensities increase in the first 10 minutes. For 200 μL, the SERS intensity reaches a clear-cut plateau in 2 min at room temperature and slightly changes after 8 min. For 300 and 400 μL, the SERS intensities firstly increase and then decrease with the increase of incubation time. The SERS intensity increases with the increase of the amount of β-CD-AgNPs and go to a maximum at 300 μL, and then the SERS intensity decreases with the increase of the amount of β-CD-AgNPs. 300 μL should be the best choice. However, the accuracy of experimental results obtained with 300 μL was lower than that obtained with 200 μL. Therefore, 200μL and 2 min were

selected in this method.

For the AgNPs, as shown in Fig. 4, the reaction between silver nanospheres and 6-MP occurs rapidly and silver colloids can achieve the best coagulation at 4 min. After 4 min the SERS intensities decrease slightly. So, the incubation time 4 min was selected in this experiment.

Figure 4

3.4.2. Effect of pH

The pH plays an important role in the experiment. BR buffer solution in the pH range of 2.7 to 6.7 was used to adjust the pH values of the analytical solution. Fig. 5 shows the effect of the pH value on the SERS intensity of the system. With the increase of pH value, the SERS intensity for the 6-MP increases, reaches maximum at pH 5.9, and then decreases significantly. So pH 5.9 of BR buffer solution was selected for the determination of 6-MP.

For the AgNPs, the silver colloids are negatively charged due to the adsorption of citrate groups, which is responsible for the stability of colloidal silver. It can be seen from Fig. 5 that the SERS intensity for the 6-MP reaches a maximum at the pH value of 3.5. When the pH value is higher than 3.5, there is a general trend of decreasing intensity. So pH 3.5

was selected for the determination of 6-MP when AgNPs was used.

Figure 5

3.4.3. Effect of concentration of sodium chloride

An aggregating agent is routinely added into SERS-active colloids to achieve a degree of enhancement of SERS signals [34]. NaCl is frequently used as the aggregating agent for AgNPs in the enhancement process and the concentration of NaCl is an important factor for the aggregation degree of AgNPs [31]. The effect of sodium chloride concentration on the SERS intensity of 6-MP was investigated and the experimental results are shown in Fig. 6. The SERS intensity of 6-MP in the presence of β-CD-AgNPs is enhanced gradually with the increase of NaCl concentration in the range of 0.12 $mol \cdot L^{-1}$ to 0.31 $mol \cdot L^{-1}$. The peak area at 866 cm^{-1} obtained with 0.22 $mol \cdot L^{-1}$ NaCl solution is largest. Therefore, 0.22 $mol \cdot L^{-1}$ NaCl solution was selected for β-CD-AgNPs.

Similarly, 0.016 $mol \cdot L^{-1}$ NaCl solution in the concentration range of 0.012 $mol \cdot L^{-1}$ to 0.020 $mol \cdot L^{-1}$ was selected for AgNPs. The result indicates the Ag colloids have gotten to the optimum aggregation.

Figure 6

3.5. Effect of coexisting substances

The coexisting substances with positive or negative charges will affect on the SERS intensity. The effect of the substances on the SERS intensity was investigated. The experimental results are summarized in Table 1. It can be seen that most of the foreign substances have little influence on the SERS intensity and can be allowed at high concentration levels. Compared with silver nanospheres, when the β-CD-AgNPs was used, selectivity was higher.

Table 1

3.6. Calibration curves and Stability of β-CD-AgNPs

As shown in Fig. 7, the Raman peak at 866 cm^{-1} is the strongest peak in the SERS spectra. Under the optimum conditions, the calibration curves were constructed by plotting the peak areas of the vibrational band at 866 cm^{-1} versus the concentrations of analyte. For the β-CD-AgNPs, the SERS intensity is directly proportional to the 6-MP concentrations in the range of (0.040-2.0) $\times 10^{-7}$ $mol \cdot L^{-1}$. The regression equation is I_{SERS} =108931+808173C (C: $mol \cdot L^{-1}$). The correlation coefficient (r) is 0.996 which indicates that there are good linear relationships between the SERS

intensity and 6-MP concentration. The limit of detection (LOD), corresponds to signal-to-noise ratio 3, is 0.024×10^{-7} mol·L^{-1}. The limit of quantification (LOQ) is 0.080×10^{-7} mol·L^{-1}. For the AgNPs, the regression equation is I_{SERS} =-335166+372791C. There is a linear relationship in the range of (0.80-4.8) $\times10^{-7}$ mol·L^{-1} and the corresponding correlation coefficient (r) is 0.995. The LOD is 0.14×10^{-7} mol·L^{-1} and LOQ is 0.47×10^{-7} mol·L^{-1}. It can be concluded that compared with silver nanospheres, when β-CD-Ag were used the sensitivity for the determination of 6-MP was higher and the linear range was wider. Compared with methods reported previously, the SERS method using β-CD-AgNPs as substrates also has some advantages for 6-MP determination, as shown in Table 2. Fig. 8 shows that the β-CD-AgNPs as SERS substrates is stable within 45 days.

Figure 7

Table 2

Figure 8

3.7. Analytical application

The analytical applicability of the present method was validated by

analyzing two commercial tablets of 6-MP. The results are shown in Table 4. For the β-CD-AgNPs, the contents of the 6-MP per tablet in the two samples are 51.5 and 51.0 mg. For the AgNPs, the contents of the 6-MP per tablet in the two samples are 43.0 and 38.2 mg, respectively. Table 3 shows the recoveries and RSDs of 6-MP in the commercial tablet. The results indicate that the two methods for determining 6-MP are reliable and satisfactory. Compared with AgNPs, when the β-CD-AgNPs were used, the recoveries are higher and RSD is lower.

Table 3

4. Conclusions

In this work, we report the preparation of β-CD-AgNPs nanoparticles and describe a high sensitive and selective method for the determination of 6-MP. As the SERS substrate, the β-CD-AgNPs were more sensitive than the AgNPs for determination 6-MP. The effects of some experimental conditions are discussed and optimized in detail. A good linearity (r=0.996) in the range of (0.040-2.0) $\times 10^{-7}$ mol·L^{-1} was obtained. In addition, present method is suitable for the routine determination of 6-MP in different dosage forms and also can be applied to drug control.

References:

1. N. Guarrotxena and G. C. Bazan, *Advanced Materials*, 2014, 26, 1941-1946.
2. H. Kang, S. Jeong, Y. Park, J. Yim, B. H. Jun, S. Kyeong, J. K. Yang, G. Kim, S. Hong and L. P. Lee, *Advanced Functional Materials*, 2013, 23, 3719-3727.
3. C. L. Wong, U. Dinish, M. S. Schmidt and M. Olivo, *Analytica chimica acta*, 2014, 844, 54-60.
4. H. Chu, H. Yang, S. Huan, G. Shen and R. Yu, *The Journal of Physical Chemistry B*, 2006, 110, 5490-5497.
5. K. V. Kong, U. Dinish, W. K. O. Lau and M. Olivo, *Biosensors and Bioelectronics*, 2014, 54, 135-140.
6. R. A. Alvarez-Puebla and R. F. Aroca, *Analytical chemistry*, 2009, 81, 2280-2285.
7. L.-X. Chen, D.-W. Li, L.-L. Qu, Y.-T. Li and Y.-T. Long, *Analytical Methods*, 2013, 5, 6579-6582.
8. N. Leopold and B. Lendl, *The Journal of Physical Chemistry B*, 2003, 107, 5723-5727.
9. A. Raza and B. Saha, *Forensic science international*, 2014, 237, e42-e46.

10. J. Wu, F. Zhang and H. Zhang, *Carbohydrate polymers*, 2012, 90, 261-269.

11. Q. Yang, F. Liang, D. Wang, P. Ma, D. Gao, J. Han, Y. Li, A. Yu, D. Song and X. Wang, *Analytical Methods*, 2014, 6, 8388-8395.

12. J. P. Camden, J. A. Dieringer, Y. Wang, D. J. Masiello, L. D. Marks, G. C. Schatz and R. P. Van Duyne, *Journal of the American Chemical Society*, 2008, 130, 12616-12617.

13. R. Keir, D. Sadler and W. Smith, *Applied spectroscopy*, 2002, 56, 551-559.

14. P. Lee and D. Meisel, *The Journal of Physical Chemistry*, 1982, 86, 3391-3395.

15. S. Sahasranaman, D. Howard and S. Roy, *European journal of clinical pharmacology*, 2008, 64, 753-767.

16. O. Nielsen, B. Vainer and J. Rask‐Madsen, *Alimentary pharmacology & therapeutics*, 2001, 15, 1699-1708.

17. B. Nørgård, L. Pedersen, K. Fonager, S. Rasmussen and H. T. Sørensen, *Alimentary pharmacology & therapeutics*, 2003, 17, 827-834.

18. A. R. Davis, L. Miller, H. Tamimi and A. Gown, *Obstetrics & Gynecology*, 1999, 93, 904-909.

19. T. L. Ding and L. Z. Benet, *Journal of Chromatography B: Biomedical Sciences and Applications*, 1979, 163, 281-288.

20. Y. Su, Y. Y. Hon, Y. Chu, M. E. Van de Poll and M. V. Relling, *Journal of Chromatography B: Biomedical Sciences and Applications*, 1999, 732, 459-468.

21. K. Tsutsumi, Y. Otsuki and T. Kinoshita, *Journal of Chromatography B: Biomedical Sciences and Applications*, 1982, 231, 393-399.

22. S. Floberg, P. Hartvig, B. Lindström, G. Lönnerholm and B. Odlind, *Journal of Chromatography B: Biomedical Sciences and Applications*, 1981, 225, 73-81.

23. M. Keyvanfard, V. Khosravi, H. Karimi-Maleh, K. Alizad and B. Rezaei, *Journal of Molecular Liquids*, 2013, 177, 182-189.

24. Y. Sato, Y. Ishikawa, H. Matsuura, K. Uosaki, F. Mizutani and O. Niwa, *Electroanalysis*, 2005, 17, 965-968.

25. P. Zhou, L. He, G. Gan, S. Ni, H. Li and W. Li, *Journal of Electroanalytical Chemistry*, 2012, 665, 63-69.

26. H. Sun, T. Wang, X. Liu and P. Chen, *Journal of Luminescence*, 2013, 134, 154-159.

27. L. Wang, B. Ling, H. Chen, A. Liang, B. Qian and J. Fu, *Luminescence*, 2010, 25, 431-435.

28. S. R. Rabel, J. F. Stobaugh and R. Trueworthy, *Analytical biochemistry*, 1995, 224, 315-322.

29. X.-C. Shen, L.-F. Jiang, H. Liang, X. Lu, L.-J. Zhang and X.-Y. Liu,

Talanta, 2006, 69, 456-462.

30. Z. Chen, G. Zhang, X. Chen, J. Chen, J. Liu and H. Yuan, *Biosensors and Bioelectronics*, 2013, 41, 844-847.

31. P. Ma, F. Liang, Y. Sun, Y. Jin, Y. Chen, X. Wang, H. Zhang, D. Gao and D. Song, *Microchimica Acta*, 2013, 180, 1173-1180.

32. A. V. Szeghalmi, L. Leopold, S. Pînzaru, V. Chis, I. Silaghi-Dumitrescu, M. Schmitt, J. Popp and W. Kiefer, *Journal of Molecular Structure*, 2005, 735-736, 103-113.

33. S. Jaiswal, B. Duffy, A. K. Jaiswal, N. Stobie and P. McHale, International journal of antimicrobial agents, 2010, 36, 280-283.

34. N. R. Yaffe, A. Ingram, D. Graham and E. W. Blanch, *Journal of Raman Spectroscopy*, 2009, 41, 618-623.

Figure Captions:

Scheme1： Structure of 6-Mercaptopurine in thiol form (a) and its tautomer (b) .

Fig. 1： (A) UV–Vis spectrum of AgNPs (a), β-CD-AgNPs (b), β-CD-AgNPs in the presence of NaCl (0.22 $mol \cdot L^{-1}$) and trace 6-MP (c) and β-CD-AgNPs in the presence of NaCl (0.22 $mol \cdot L^{-1}$) (d). (B) Statistical analysis of the size distribution of β-CD-AgNPs. (C) The TEM image of β-CD-AgNPs. (D) The TEM image of β-CD-AgNPs in the presence of NaCl (0.22 $mol \cdot L^{-1}$)

Fig. 2： The sensing mechanism for determining 6-MP using β-CD-AgNPs as the SERS-active substrate

Fig. 3： Raman and SERS spectra of 6-MP. SERS spectrum of 6-MP based on β-CD-AgNPs, the concentration of 6-MP is 1.0×10^{-7} $mol \cdot L^{-1}$ (a), SERS spectrum of 6-MP based on AgNPs, the concentration of 6-MP is 1.0×10^{-7} $mol \cdot L^{-1}$ (b), Raman spectrum of solid 6-MP (c), Raman spectrum of 6-MP (10 $mmol \cdot L^{-1}$) (d). 785 nm laser wavelength, 10 s exposure time, 150 mW laser power.

Fig. 4： Effects of volume of β-CD-AgNPs solution and incubation time on SERS intensity of 6-MP. For β-CD-AgNPs, the concentration of 6-MP is 1.0×10^{-7} $mol \cdot L^{-1}$, the concentration of

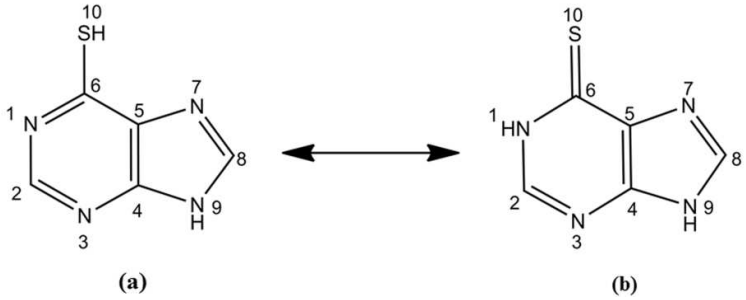
NaCl is 0.22 mol·L^{-1}, pH is 5.9. For AgNPs, the concentration of 6-MP is 2.4×10^{-7} mol·L^{-1}, the concentration of NaCl is 0.016 mol·L^{-1}, pH is 3.5.

Fig. 5： Effect of pH on SERS intensity of 6-MP. For AgNPs, the concentration of 6-MP is 2.4×10^{-7} mol·L^{-1}, the concentration of NaCl is 0.016 mol·L^{-1} (a). For β-CD-AgNPs, the concentration of 6-MP is 1.0×10^{-7} mol·L^{-1}, the concentration of NaCl is 0.22 mol·L^{-1} (b).

Fig. 6： Effect of concentration of NaCl on SERS intensity of 6-MP. For AgNPs, the concentration of 6-MP is 2.4×10^{-7} mol·L^{-1}, pH is 3.5 (a). For β-CD-AgNPs, the concentration of 6-MP is 1.0×10^{-7} mol·L^{-1}, pH is 5.9 (b).

Fig. 7： Calibration curves of 6-MP and photographic images of β-CD-AgNPs and AgNPs. Error bars represent the standard deviation of five measurements. For β-CD-AgNPs, the concentration of NaCl is 0.22 mol·L^{-1}, pH is 5.9. For AgNPs, the concentration of NaCl is 0.016 mol·L^{-1}, pH is 3.5.

Fig. 8： Effects of storage time of β-CD-AgNPs at 4 °C on SERS intensity of 6-MP, Inset shows the SERS intensity distribution of the 866cm^{-1} band. Each datum point represents the average value of the experimental results obtained in five replicates. Error bar represents the standard deviation.



Scheme1

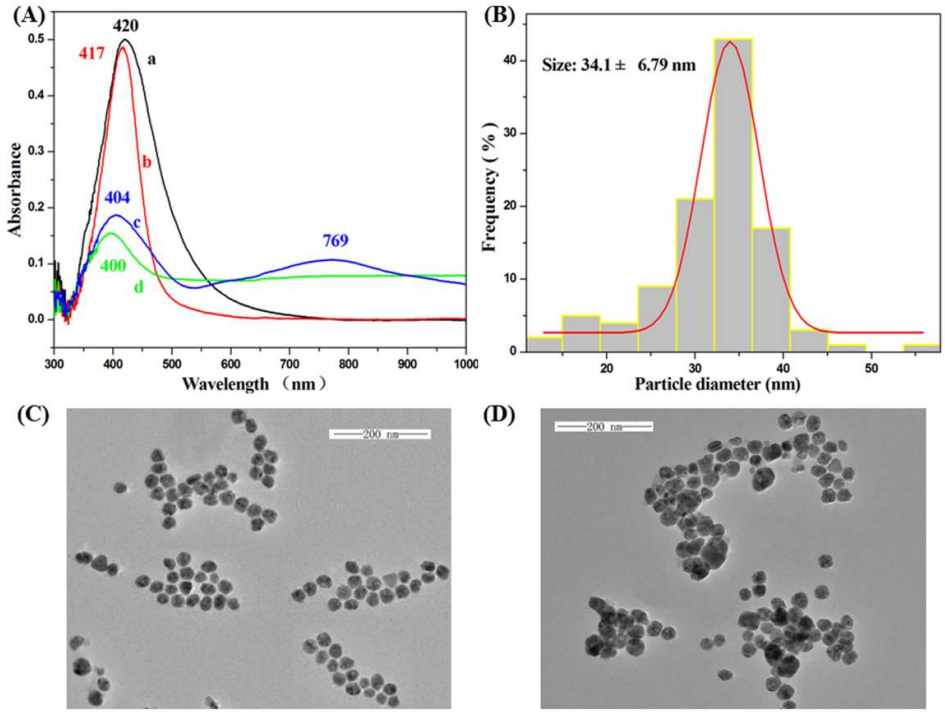


Fig. 1

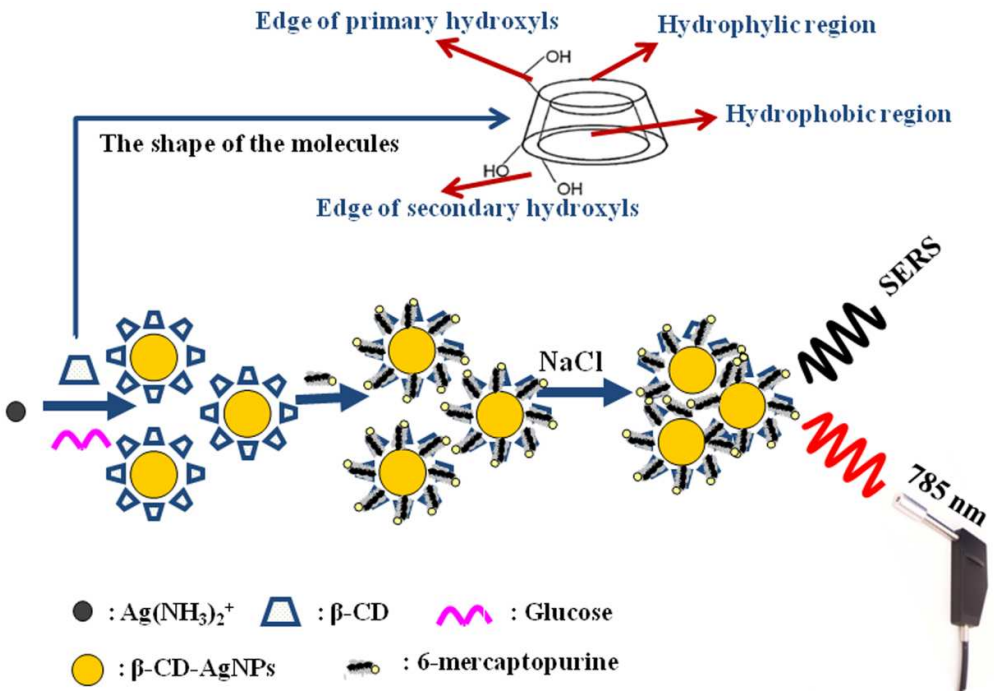


Fig. 2

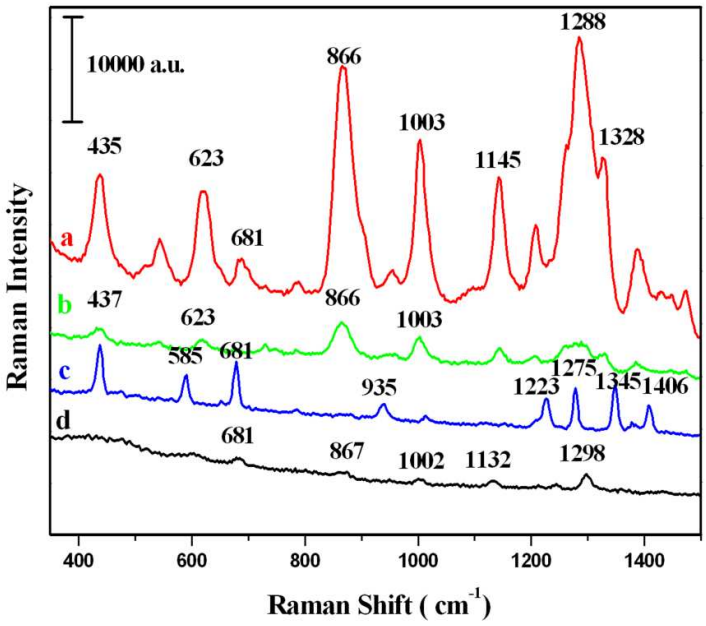


Fig. 3

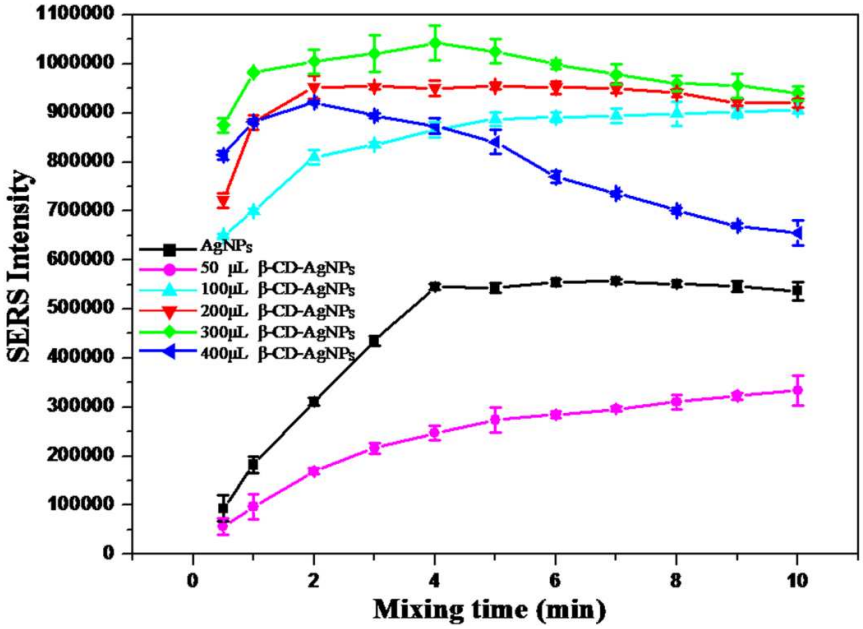


Fig. 4

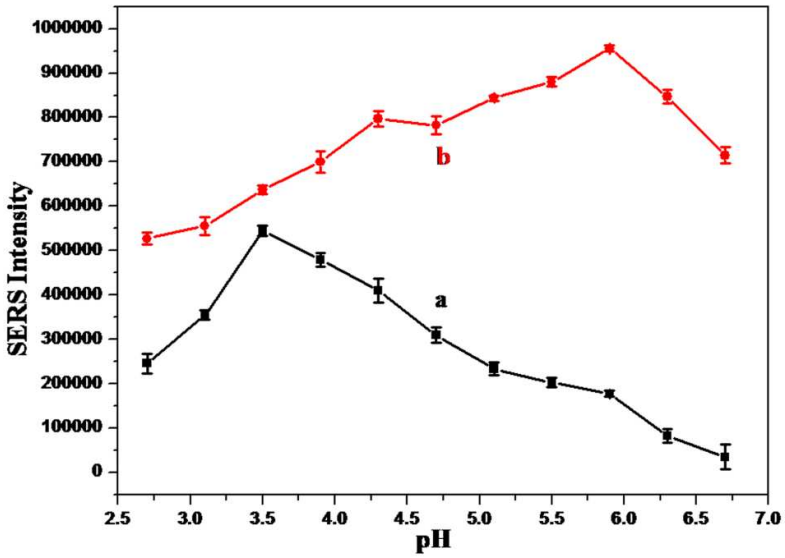


Fig. 5

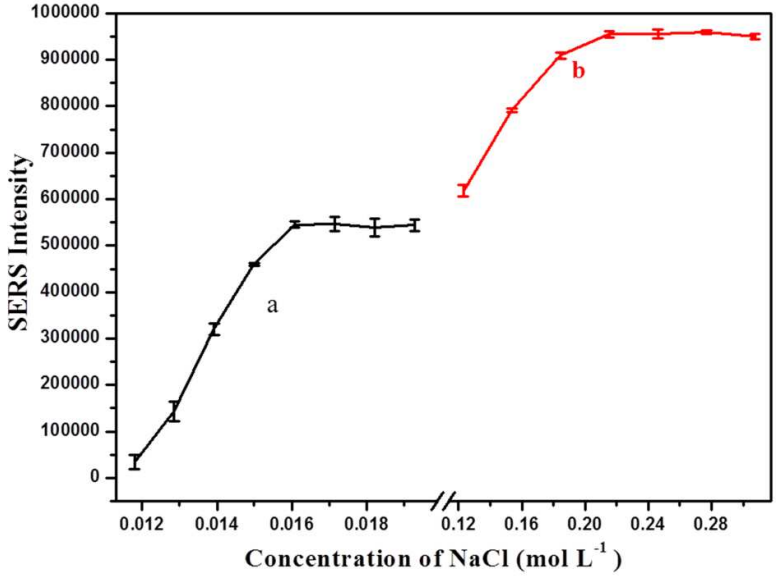


Fig. 6

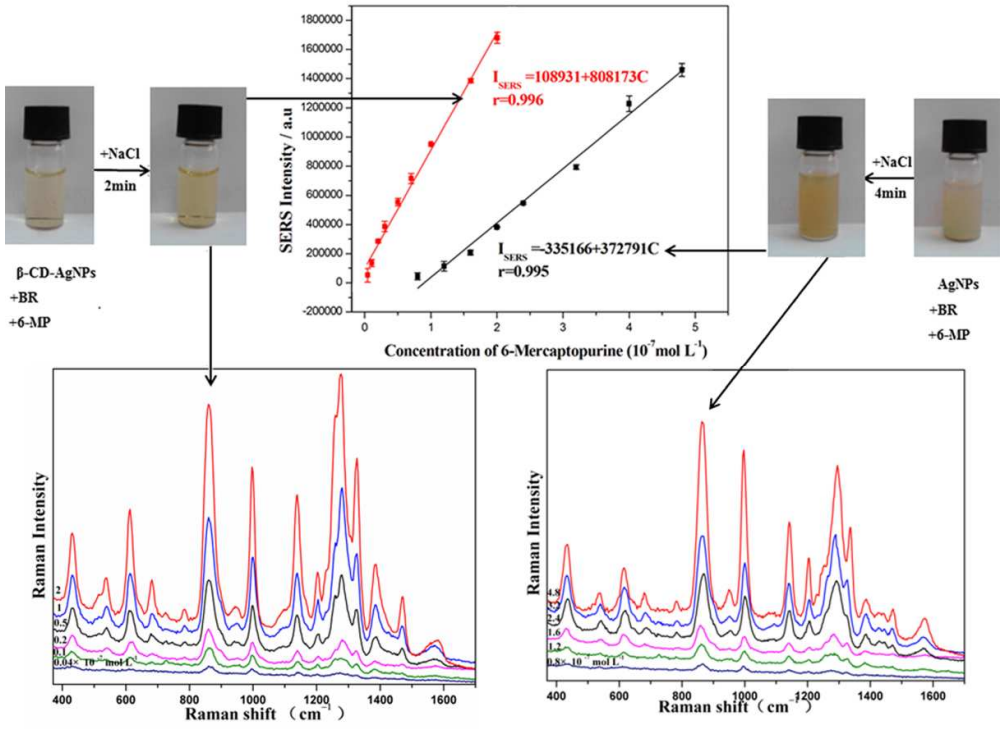


Fig. 7

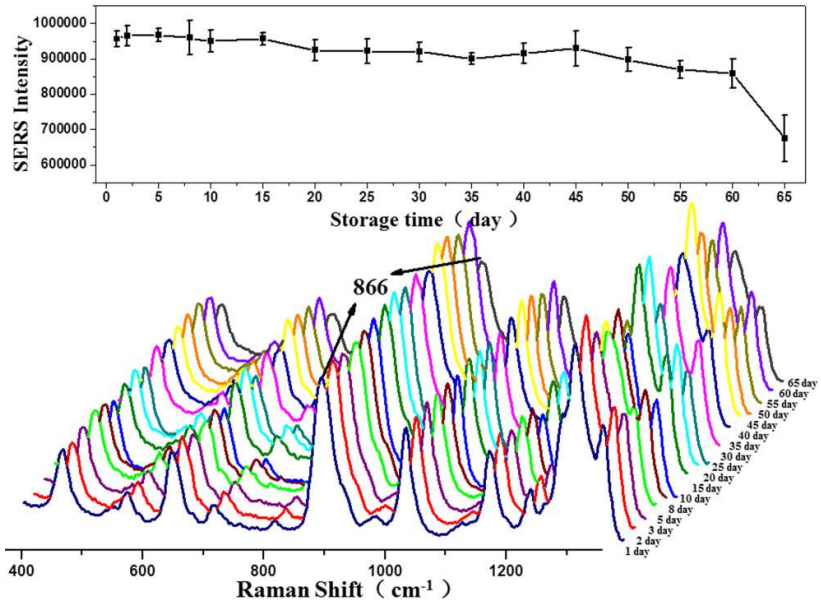


Fig. 8

Table 1 Effect of coexisting substances

Coexisting substances	Concentration ($\mu mol \cdot L^{-1}$)	Relative error (%)	
		β-CD-AgNPs	AgNPs
K^+, Cl^-	200.0	-3.1	-6.5
Na^+, Br^-	200.0	-5.4	-8.7
Co^{2+},Cl^-	200.0	-3.7	6.5
Fe^{3+}, Cl^-	200.0	-1.5	3.1
Na^+, HPO_4^{2-}	100.0	-2.3	-4.4
Mg^{2+}, Cl^-	100.0	-1.7	2.6
Ca^{2+},Cl^-	100.0	1.2	-7.8
Na^+, SO_4^{2-}	100.0	3.2	-4.3
Na^+, CO_3^{2-}	100.0	-2.3	-8.6
Fe^{2+}, Cl^-	100.0	-2.4	6.3
Cr^{3+}, SO_4^{2-}	2.0	-0.7	-2.6
Ag^+, NO_3^-	2.0	-1.5	-3.2
Ba^{2+}, Cl^-	1.0	0.8	4.4
Hg^{2+},Cl^-	1.0	1.7	6.3
Ni^+, Cl^-	1.0	-5.4	-13.3
Cu^{2+}, Cl^-	1.0	3.1	9.4

Table 2 Comparison of reported methods with the present method for the determination of 6-MP

Method	LOD (10^{-7} mol·L^{-1})	Linear range (10^{-7} mol·L^{-1})	References
SERS	0.024	0.040-2.0	This work
HPLC	0.20	0.10–6.4	19
Voltammetry	1.0	5.0–9000	23
The Desorption of Electrochemical Reduction	0.10	0.10-1.0	24
Electrochemistry	0.050	2.0–2000	25
Fluorescence Enhancement Method	0.0050	0.64–3.0	29

Table 3 Recovery of 6MP detection in pharmaceutical tablets.

Sample	Silver nanoparticles	Added (10^{-7} mol·L^{-1})	Found (10^{-7} mol·L^{-1})	Recovery (%)	RSD (n=5, %)
Tablet 1	β-CD-AgNPs	1.00	1.03	103.0	2.45
	AgNPs	2.00	1.72	86.0	3.81
Tablet 2	β-CD-AgNPs	1.00	1.02	102.0	3.72
	AgNPs	2.00	1.53	76.5	4.19

A high sensitive and selective method using silver nanoparticles decorated with β-cyclodextrinas SERS-active substrate was developed for detecting 6-mercaptopurine.