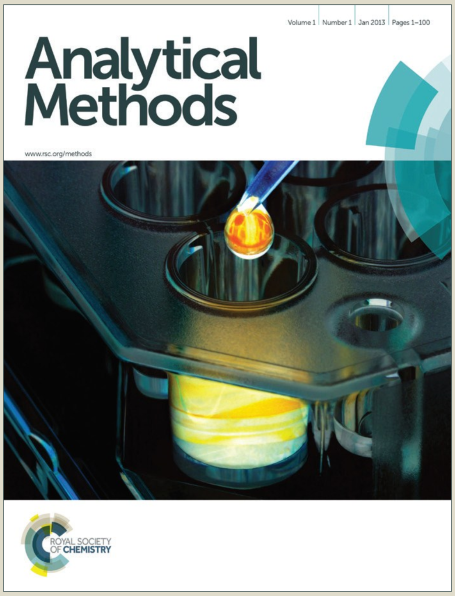
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Determination of Melamine and Malachite Green by Surface-Enhanced Raman Scattering Spectroscopy using Starch-Coated Silver Nanoparticles as Substrate

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Abstract

The silver nanoparticles (AgNPs) were synthesized by classic method. The surface-enhanced Raman scattering (SERS) activity of the as-synthesized substrates was evaluated by measuring the SERS signals of several different target analytes. The influence of starch concentration on AgNPs was studied and 1.00% (w/v) of starch was selected. The starch-coated AgNPs displayed a higher stability than the classic AgNPs. The practical application of the starch-coated SERS substrate was evaluated by determination of melamine and malachite green. Under the optimal conditions, melamine and malachite green were determined in the ranges of 2.00-50.0 μg L^{-1} and 0.500-35.0 μg L^{-1} with correlation coefficients 0.9992 and 0.9979, and the detection limits were 0.600 μg L^{-1} and 0.080 μg L^{-1}, respectively. The recoveries of melamine in spiked milk samples and malachite green in water samples were 94-104% and 96-107%, respectively. These results foresee promising application of starch-coated AgNPs as sensitive SERS substrates in both food and environmental water.

Key words: Surface-enhanced Raman scattering; Starch; Silver nanoparticles; Melamine.

1. Introduction

In recent years, surface enhanced Raman scattering (SERS) has induced significant interest on account of its molecular specificity and high sensitivity.[1,2] SERS is a kind of plasmonic effect and occurs when molecules are adsorbed onto rough metal surface. Thus Raman signals are dramatically enhanced, being 10^6-10^8 times stronger than conventional Raman signals.[3] This giant enhancement arises from two mechanisms, including electromagnetic field enhancement (EM) and chemical enhancement (CE).[4] For its easy operability, high sensitivity and non-destructive characteristic, SERS is widely applied in the studies of food, environmental and biological chemistry, health care, safety, terrorist threats and so on.[5,6]

In order to further apply SERS in real sample analysis, a large amount of metals have been used as substrates, including silver, gold, copper, platinum, iron, cobalt, etc.[7,8] Among these noble metals, silver nanoparticles (AgNPs) are commonly used as SERS-active substrates owing to its high sensitivity, low-cost and strong signals. In previous reports, a variety of AgNPs with different shapes, such as sphere, nanorod, nanoprism, and nanocube, have been studied.[9-11] Among these diverse shaped nanoparticles, AgNPs with close-to-spherical shapes are acknowledged as efficient and ultra-high sensitive SERS substrates.[12] However, it is not easy to get stable and reproducible SERS response when stored for a long time. Therefore, it is still a great challenge to synthesize stable, reproducible and sensitive SERS substrates.

In recent reports, modifying roughened SERS substrates is acknowledged as the most efficient way to improve its sensitivity and stability using metallic oxides, such as ZnO, TiO_2, Fe_3O_4, etc.[13-15] Most of these chemicals are highly reactive and can increase the risk of environmental damages. Utilization of nontoxic chemicals needs to be considered in a green synthesis orientation.[16] Biosynthesis of metal nanoparticles is a kind of chemical reduction. The method is very significant owing to its eco-friendly and quite simple nanoparticles synthesis procedures.[17] These green methods have been applied to the preparation of nanoparticles in short time period and large amounts.[18] Glucose, sucrose, maltose, starch, chitosan, and cyclodextrin are commonly used as reducing agents,[19-21] gaining different sizes and shapes of nanoparticles. Starch is universally acknowledged as the second most abundant biopolymer in nature and biodegradable, nontoxic, low-cost production, and used as raw material in different industries.[22] Silver nanoparticles are prepared using starch acting as stabilizing agents. Starch is a linear polymer formed by the α-(1→4) linkages between D-glucose units and behaves like a linear polymer, which can be used for the synthesis and stabilization of nanoparticles. Polyhydroxylated macromolecules present interesting dynamic supramolecular associations facilitated by inter- and intra-molecular hydrogen bonding resulting in molecular level capsules, which can act as templates for nanoparticle growth.[23] Starch encapsulate the AgNPs and itself plays a stabilizing role.[24] In this work, starch is used to modify the AgNPs to obtain stable and sensitive SERS substrate.

In order to improve the sensitivity and stability of AgNPs, the soluble starch was used as both the reducing agent and the stabilizer to synthesize SERS substrates. The procedure is simple and no extra reagents are used. The SERS substrate was very easy to be fabricated and surface modification was not required. Firstly, the as-synthesized starch-coated silver nanoparticles were characterized by means of UV-vis spectroscopy and TEM. The starch-coated AgNPs possessed both high sensitivity and satisfactory stability, which can be stored in room temperature for 3 months with little change in Raman signal. The aim of this work was secondly to evaluate the SERS efficiencies by detecting four analytes based on different concentrations of starch-coated AgNPs SERS substrates. Furthermore, the SERS determination of melamine in milk and malachite green in environmental water were performed and the results were satisfactory, which foreseed promising application of starch-coated AgNPs as SERS substrate.

2. Materials and methods

2.1 Chemicals and instruments

All reagents and chemicals used were at least of analytical grade. Silver nitrate ($AgNO_3$, 99.85%), sodium citrate (anhydrous, 99%), sodium hydroxide (NaOH), sodium chloride (NaCl, 99.5%), melamine (99.0%) and malachite green (98%) were purchased from J&K Chemical Company, hydrochloric acid (HCl, 37.5%) was purchased from Sinopharm Chemical Reagent Co. Ltd. 4,4'-bipyridine (99.0%) and Rhodamine 6G (R6G, 99.0%) were bought from Jinchun Reagent (Shanghai, China). Milk samples were bought from Wal-Mart and water samples were collected from local river of Changchun City, China. All aqueous solutions were prepared with deionized water purified with Milli-Q water purification system (18.0 MΩ cm).

0.010 g of melamine and 4,4'-bipyridine were dissolved in 100 mL of 50% methanol aqueous solution to prepare the standard stock solution, respectively. 0.010 g of malachite green and R6G were dissolved in 100 mL deionized water, respectively. To test the linear relationship for quantitative analysis, standard stock solutions of 100 mg L^{-1} melamine and malachite green were sequentially diluted with deionized water into series of concentrations, respectively. All stored solutions were kept in refrigerator at 4℃ and all experiments were carried out at room temperature.

Raman spectra were obtained using BTR111MiniRam (B&W Tek, Inc) equipped with 785 nm excitation laser and a 1 cm quartz cell. The laser power was chosen as 150 mW. The exposure time used for datum collection was 10 s. The surface morphologies of the AgNPs were measured on a Hitachi H800 transmission electron microscope (TEM, Hitachi Ltd, Japan), operating at 200 kV. Absorption spectra of the AgNPs colloids were recorded on a TU-1810C UV–vis spectrometer (Beijing Purkinje General Instrument Co., Ltd.).

2.2 Synthesis of starch-coated AgNPs

Lee and Meisel’s classic synthesis method of AgNPs has been improved as follows.[25] Starch was dissolved in 50 mL deionized water (starch concentration: 0.00, 0.10, 0.20, 0.50, 0.75, 1.00 and 1.50% (w/v)) and the resulting solution was heated to boil with constant agitation for 30 min to guarantee full gelatinization of starch.[26] For the synthesis of AgNPs, 9 mg $AgNO_3$ was added to the boiling solution under vigorous stirring for 1 min. Then 1.0 mL of 1.00% sodium citrate solution was quickly added into the mixing solution and boiled the solution for 1 h. After reaction, the solution was cooled down to room temperature gradually and a series of different colors of starch-coated AgNPs were obtained with Ag concentrations of about 0.95 $\times 10^{-3}$ mol L^{-1}. The solutions were stored in refrigerator at 4□.

3. Results and discussion

3.1 Characterization of classic and starch-coated AgNPs

UV-vis spectrum and TEM image were employed to characterize the dispersibility and morphology of the prepared AgNPs. From the UV-vis spectrum (Fig. 1), plasmon band at 402-418 nm indicates that nanometer-sized Ag particles were synthesized.[27] Visually, when the starch concentration is higher than 0.20%, the color of the solution become more and more transparent and deeper, until the starch concentration increase to 1.00%, the color do not show a remarkable change any more. The absorption peaks of the meantime also shift to short wavelength, until the starch concentration increases to 1.00%, which indicates that the synthetic particles become smaller. For a given metal system, the SERS intensity will depend on the size of nanostructure and the uniform distribution.[28] The TEM images of starch-coated AgNPs shown Fig. 2 reveal that AgNPs have a spherical shape with a narrow size distribution. When the concentrations of starch were 0.00, 0.10, 0.20, 0.50, 0.75, 1.00 and 1.50%, the diameters of particles were 40 nm, 35 nm, 33 nm, 30 nm, 25 nm, 20 nm and 26 nm, respectively. The size and size distribution of these AgNPs are ideal for using as SERS substrates because smaller particles (<10 nm) and larger particles (>100 nm) do not show a good SERS signal.[29]

3.2 SERS behavior of starch-coated AgNPs

To test the influence of starch concentrations on the performance of starch-coated AgNPs, SERS behaviors of several analytes, including R6G, 4,4'-bipyridine, melamine and malachite green were studied. For R6G, 200 μL of starch-coated AgNPs, 70 μL of 0.200 mol L^{-1} NaCl, 70 μL of 1.00 mol L^{-1} HCl and 200 μL of 5.00 μg L^{-1} R6G were added into 1 cm quartz cell in sequence. The resulting solution was kept at room temperature for 3 min. For 4,4'-bipyridine, 200 μL of starch-coated AgNPs, 100 μL of 0.200 mol L^{-1} NaCl, and 200 μL of 5.00 μg L^{-1} 4,4'-bipyridine were added into 1 cm quartz cell in sequence. For melamine, 200 μL of starch-coated AgNPs, 150 μL of 1.00 mol L^{-1} NaCl, 200 μL of 1.00 mol L^{-1} NaOH and 100 μL of 5.00 μg L^{-1} melamine were added into 1 cm quartz cell. 400 μL of starch-coated AgNPs, 70 μL of 0.200 mol L^{-1} NaCl, 70 μL of 1 mol L^{-1} HCl and 200 μL of 5.00 μg L^{-1} malachite green were added into 1 cm quartz cell in sequence for SERS testing of malachite green. Fig. 3 shows the schematic illustration of starch-coated AgNPs SERS measurement for determining the analytes.

Fig. 4(a) shows the Raman spectra of melamine, 4,4'-bipyridine, R6G and malachite green AgNPs using 1.00% starch-coated as SERS substrates. The characteristic SERS peaks of melamine at 621 cm^{-1} arises from NH_2 twisting vibration, 704 cm^{-1} is owing to ring breathing vibration, 1071 cm^{-1} is assigned to C-N-C or N-C-N bending vibration, respectively.[30] According to Fig. 4(b), the Raman signals obtained with the AgNPs coated using 1.00% starch were much higher than those obtained with the AgNPs at other concentrations. The results are in conformity with the UV-vis results. The UV-vis is a kind of localized surface plasmon resonance(LSPR), which can be used as a tool to estimate range of particle size and stability of the NP suspensions.[31] The AgNPs involved 1% starch exhibited the maximum SERS signals owing to uniform distribution of silver nanoparticles. In order to observe the enhancement factor (EF), the Raman EF of an analyte based on SERS substrate, is calculated as

$$EF = I_{SERS}C_{Raman}/I_{Raman}C_{SERS}$$ [32]

where I_{SERS} and I_{Raman} represent the SERS and normal Raman signal intensities, respectively; and C_{SERS} and C_{Raman} correspond to the concentrations of the analytes used for SERS and normal Raman measurements. In this study, the Raman *EF* was calculated based on the normal Raman peak at 679 cm^{-1} of 5000 mg L^{-1} melamine solution and the SERS peak at 704 cm^{-1} of 0.1 mg L^{-1} melamine solution. Finally, the Raman *EF* for melamine are 1.19×10^5, 2.20×10^5, 2.83×10^5, 3.95×10^5 , 5.56×10^5, and 4.23×10^5 in the presence of 0.10, 0.20, 0.50, 0.75, 1.00 and 1.50% (w/v) starch-coated AgNPs, respectively. It can be seen that the EF obtained with the AgNPs coated using 1.00% starch substrate is higher than those obtained with other concentration starch-coated AgNPs.

To further investigate the above conclusion, 4,4'-bipyridine was chosen as the probe molecule. The SERS spectrum of 4,4'-bipyridine agrees well with that from the literature: 1080 cm^{-1} and 1239 cm^{-1} are assigned to the inplane C-H deformation and ring stretch and 1290 cm^{-1} is assigned to C-C inter-ring stretch.[33] Peak intensities at 1290 cm^{-1} of 4,4'-bipyridine with different concentrations of starch-coated AgNPs were measured for Raman *EF*, with results of 8.76×10^4, 1.96×10^5, 3.85×10^5, 5.64×10^5 , 7.33×10^5, and 6.01×10^5 in the presence of 0.10, 0.20, 0.50, 0.75, 1.00 and 1.50% (w/v) starch-coated AgNPs, respectively.The results clealy show that the signal obtained with the AgNPs coated using 1.00% starch is stronger than those obtained with other concentrations starch-coated AgNPs, and the siginificant difference may arise from the difference of the activity of AgNPs and local concentration of the analyte adjacent to the surface of the SERS-active sites.

Similar to 4,4'-bipyridine, the AgNPs coated using 1.00% starch substrate exhibited a stronger SERS signal to R6G. Typical Raman band assignment for R6G are as followed: the peaks at 1649, 1511, and 1363 cm^{-1} are associated with the carbon skeleton stretching modes,[34] the peaks at 610 and 771 cm^{-1} are assigned to the C-C-C ring in-plane, out-plane bending, and C-C stretching vibrations, respectively,[35] the 1127 cm^{-1} should be noted to the C-O-C stretching mode. The peak intensities at 771 cm^{-1} were chose to calculate Raman *EF* and the results are 8.99×10^4, 9.58×10^4, 1.13×10^5, 2.25×10^5 , 3.15×10^5, and 2.77×10^5 in the presence of 0.10, 0.20, 0.50, 0.75, 1.00 and 1.50% (w/v) starch-coated AgNPs, respectively. As shown in Fig. 4(b), the Raman intensity increases with increasing the concentration of starch up to 1.00%, and then decreases, which may be attributed to the hot spots that can enhance the intensity of Raman signals of the substrates.

To confirm the above conclusion, malachite green was chosen as the forth probe to investigate the SERS activities of starch-coated AgNPs substrates. As shown in Fig. 4(a), the characteristic bands of SERS spectra of malachite green ranging from 850 to 1700 cm^{-1} are observed. According to previous studies,[36] the most prominent peaks for malachite green were at 1177, 1219, 1394, and 1616 cm^{-1}, which are attributed to the in-plane vibrations of ring C–H, rocking of C–H, stretching of N-phenyl and stretching of ring C–C, respectively. The Raman *EF* of SERS peak at 1177 cm^{-1} for malachite green are 1.07×10^5, 2.34×10^5, 3.46×10^5, 4.01×10^5 , 5.11×10^5, and 4.29×10^5 in the presence of 0.10, 0.20, 0.50, 0.75, 1.00 and 1.50% (w/v) starch-coated AgNPs, respectively. The SERS intensity of the the AgNPs coated using 1.00% starch at 1177 cm^{-1} is about 2 times stronger than the lowest. All the results show that the AgNPs coated using 1.00% starch exhibit good SERS activity and sensitivity.

3.3 Stability of starch-coated AgNPs

The limitation of using classic AgNPs as SERS substrate is the instability arisen from the storage, which would certainly influence the reproducibility for quantitative detection. The AgNPs may precipitate from solution, resulting in decrease of SERS signal. Macromolecular chains of starch posses a large number of hydroxyl groups that can complex well with the metal ion, which further enables good control of size, shape and dispersion of nanoparticles and increases the stability.[37] The UV-vis spectrum shows that the starch-coated AgNPs have a spherical shape with narrower size distribution than AgNPs, which reveals that starch-coated AgNPs are well distributed and have smaller diameters. This is important for improving the stability of SERS substrates.

To investigate the stability of the starch-coated AgNPs substrates, the AgNPs coated using 1.00% starch and the classic AgNPs were used to obtain the TEM micrograph. As shown in Fig. 5, 1.00% starch-coated silver nanoparticles have round shape morphology and fine dispersion, but some aggregates and irregularly shaped particles were found in the micrograph of classic AgNPs.

In order to further confirm the stability, the SERS performance of both the AgNPs coated using 1.00% starch and AgNPs in the presence of melamine were measured with aqueous solutions of melamine at the same conditions. The relative standard deviations (RSDs) of the intensities at 704 cm^{-1} were calculated, as shown in Fig. 6. It shows that the RSD (2.70%) obtained with the AgNPs coated using 1.00% starch is much lower than that 18.43% obtained with AgNPs. We only discuss the change within 30 days of the silver nanoparticles, although the SERS intensity of AgNPs became stable after 20 days, in fact AgNPs have already precipitated in the bottom. The SERS signal still reduced, in a relatively slow rate after 30 days. Unlike the classic AgNPs, the starch-coated AgNPs were stabilized and protected by the hydroxyl, which offered advantage of stable SERS signals by avoiding the AgNPs aggregation. Therefore, it can be indicated that the the AgNPs coated using 1.00% starch SERS substrate provides high stability.

3.4 Determination of melamine in milk

As we know, melamine (1,3,5-triazine-2,4,6-triamine, $C_3H_6N_6$) is an organic chemical and has been illegally mixed to milk products to show a trickly readout of protein content. When melamine content was 66% common test for total protein contents cannot distinguish melamine from other proteins in milk proteins.[38] It has also been reported that melamine in diary products induced serious kidney problems in a handful of Chinese infants.[39] Development of a simple, rapid and accurate method for monitoring melamine in milk is of great importance.

In this work, the AgNPs coated using 1.00% starch were used as SERS substrate for rapid determination of melamine in milk samples. The effect of experimental conditions was investigated. A stable and sensitive Raman signal was observed when 200 µL of as-synthesized AgNPs, 150 µL of 1.00 mol L^{-1} NaCl and 200 µL of 1.00 mol L^{-1} NaOH were used and incubation time was 4 min. Then, the SERS detection was performed by using a series of melamine standards under the optimum conditions. Fig. 7(a) displays the Raman signal of melamine. The figure shows that the Raman intensity increases with the increase of melamine concentration. To evaluate the precision of the present method, the intra-day and inter-day relative standard deviations (RSDs) were measured by analyzing 5 standard solutions over one day and over ten days, the results are listed in Table 1. The intensity of the band at 704 cm^{-1} versus concentration of the melamine in the range of 2.00-50.0 µg L^{-1} was plotted and correlation coefficient was 0.9992. The limit of detection (LOD) and quantification (LOQ) were computed based on the 3 and 10 times of standard deviation of the blank signal and the results are shown in Table 2.

In order to further validate the SERS substrate, the method was then applied to the analysis of milk samples. The RSDs of the results are less than 10%. The samples were then spiked with standard melamine solution of 0.500 and 1.00 mg kg^{-1} to evaluate the recoveries, which were in the range of 94%-104%, and the results are listed in Table 3.

3.5 Determination of malachite green

Malachite green is a kind of triphenylmethane dye and widely used in aquaculture industry owing to its effectiveness against fungal and parasite in fish, which has roused a public concern.[40] It has been prohibited in aquaculture industry because of its mutagenic and teratogenic effects to humans. However, malachite green is still illegally used by some people in many places due to its high efficiency, low cost and availability. Malachite green residues in water for fish culture may cause pollution to surface and ground water systems. Consequently, it is of great importance to study a sensitive method for the trace detection of malachite green in aquaculture.

In the study, we use SERS method to detect malachite green in environmental sample. The effects of experimental parameters on the SERS intensities were investigated. The 400 μL of as-synthesized AgNPs, 400 μL of 1.00 mol L^{-1} HCl, 70 μL of 0.200 mol L^{-1} NaCl and incubation time 5 min were considered as the optimum conditions. The precision of the present method are listed in Table 1. As we can see from the Fig. 7(b), the areas of SERS peaks increase with the increase of the malachite green concentration. The SERS peak areas at 1177 cm^{-1} were used for the quantification. The good linearity was obtained in the concentration, range of 0.500-35.0 μg L^{-1} with a correlation coefficient greater than 0.9979. LOD of malachite green was 0.0800 μg $L^{-1}$and LOQ was 0.230 μg L^{-1}. The results are listed in Table 2. Malachite green in water samples were determined. The results display that the recoveries are in the range of 96%-107%, as shown in Table 4. The starch-coated AgNPs can be used for determination of malachite green in environmental water.

Conclusions

In this work, the synthesis of AgNPs using starch was performed by a green and simple method. The starch-coated AgNPs were more stable and reproducible than AgNPs. R6G, melamine, malachite green and 4,4'-bipyridine were employed to test the SERS efficiencies of starch-coated AgNPs in the presence of different concentrations of starch. The results indicated that the the AgNPs coated using 1.00% starch possessed stronger signals than others. So the AgNPs coated using 1.00% starch was applied for quantitative analysis of melamine and malachite green. Under the optimum conditions, the melamine and malachite green in the samples were determined and the results were satisfactory. The present method based on the novel starch-coated AgNPs may accelerate the application of the analysis of food and environmental samples.

References

1. P. L. Stiles, J. A. Dieringer, N. C. Shah and R. P. Van Duyne, *Annu. Rev. Anal. Chem.,* 2008, 1, 601-626.
2. L.-X. Chen, D.-W. L, L.-L. Qu, Y.-T. Li and Y.-T. Long, *Anal. Methods*, 2013, 5, 6579-6582.
3. Y. Zhang, H. Hong, DV. Myklejord and W. Cai, *small*, 2011, 7, 3261-3269.
4. E. B. Santos, E. C. N. L. Lima, C. S. Oliveira, F. A. Sigoli and I. O. Mazali, *Anal. Methods*, 2014, 6, 3564-3568.
5. S. Nie and SR. Emory, *Science*, 1997, 275, 1102–1106.
6. K. Kneipp, Y. Wang, H. Kneipp, LT. Perelman, I. Itzkan, RR. Dasari and MS. Feld, *Phys Rev Lett*, 1997, 78, 1667–1670.
7. Z.-Q. Tian and B. Ren, *Annu. ReV. Phys. Chem.*, 2004, 55, 197-229.
8. Z.-Q. Tian, B. Ren and D. -Y. Wu, *J. Phys. Chem. B*, 2002, 106, 9463-9483.
9. Zh. H. Luo, K. Chen, D. L. Lu, H. Y. Han and M. Q. Zou, *Microchim Acta*, 2011, 173, 149-156.
10. B. Nikoobakht and M. A. El-Sayed, *Chem. Mater*., 2003, 15, 1957-1962.
11. A. Rai, A. Singh, A. Ahmad and M. Sastry, *Langmuir*, 2006, 22, 736-741.
12. EA. Vitol, Z. Orynbayeva, G. Friedman and Y. Gogotsi, *J Raman Spectrosc*, 2012, 43, 817-827.
13. H. Tang, G. Meng, Q. Huang, Z. Zhang, Z. Huang and C. Zhu, *Adv. Funct. Mater.*, 2012, 22, 218–224.
14. G. Shan, S. Zheng, S. Chen, Y. Chen and Y. Liu, *Colloids Surf. B*, 2012, 94, 157–162.
15. L. Sun, J. He, S. An, J. Zhang and D. Ren, *J Mo Struct*, 2013, 1046, 74-81.
16. M.A.EI-Sheikh, *The Scientific World Journal*, 2014, 2014, 514563-514574.
17. G. A. Valencia, L. C. O. Vercik and R. Ferrari, *Starch*, 2013, 65, 931-937.
18. P. Raveendran, J. Fu and S. L. Wallen, *J. Am. Chem. Soc.*, 2003, 125, 13940-13941.
19. J. Huang, Q. Li, D. Sun, Y. Lu, Y. Su, X. Yang, H, Wang, Y. Wang, W. Shao, N. He, J. Hong and C. Chen, *Nanotechnology*, 2007, 18, 105104–105115.
20. J. Y. Song and B. S. Kim, *Bioprocess Biosyst. Eng.,* 2009, 32, 79–84.

21. S. P. Dubey, M. Lahtinen and M. Sillanpää, *Process Biochem.*, 2010, 45, 1065–1071.

22. R. I. Mattos, A. Pawlickaa, J. F. Lima, C. E. Tambelli, C. J. Magon and J. P. Dnoso, *Electrochim. Acta,* 2010, 55, 1396–1400.

23. N. Vigneshwaran, R. P. Nachane, R. H. Balasubramanya and P .V. Varadarajan, *Carbohydr Res,* 2006, 341, 2012-2018.

24. A. Mandal, S. Sekar, N. Chandrasekaran, A. Mukherjee and T. P. Sastry, *RSC Adv.*, 2015, 5, 15763-15771.

25. P. C. Lee and D. Meisel, *J Phys Chem*, 1982, 86, 3391-3395.

26. C. -H. Chen, W. -S. Kuo and L.-S. Lai, *FOOD HYDROCOLLOID*, 2009, 23, 2132–2140.

27. P. Jiang, J.-J. Zhou, R. Li, Y. Gao, T.-L. Sun, X.-W. Zhao, Y.-J. Xiang and S. -S. Xie, *J. Nanopart. Res.*, 2006, 8, 927–934.

28. M. Moskovits, *J Raman Spectrosc*, 2005, 36, 485–496.

29. P. Pienpinijtham, X. Han, S. Ekgasit and Y. Ozaki, *Anal. Chem.*, 2011, 83, 3655-3662.

30. N. E. Mircescu, M. Oltean, V. Chis and N. Leopold, *Vib. Spectrosc.*, 2012, 62, 165 -171.

31. G. Hong, C. Li and L. Qi, *Adv. Funct. Mater.*, 2010, 20, 3774-3783.

32. J.-P. Su and J. S. Lin, *Res Chem Intermed*, 2014, 40, 2287–2302.

33. M. K. Singh, M. Singh, J. L. Verma, N. Kumar and R. K. Mandal, *Trans Indian Inst Met*, 2015, 68, 239-245.

34. R. Que, M. Shao, S. Zhuo, C. Wen, S. Wang and S.-T. Lee, *Adv. Funct. Mater.*, 2011, 21, 3337-3343.

35. X. Li, G. Chen, L. Yang, Z. Jin and J. Liu, *Adv. Funct. Mater.*, 2010, 20, 2815-2824.

36. L. He, N. Kim, H. Li, Z. Hu and M. Lin, *J. Agric. Food Chem.*, 2008, 56, 9843–9847.

37. S. Mohanty, S. Mishra, P. Jena, B. Jacob, B. Sarkar and A. Sonawane. *Nanomedicine*, 2012, 8, 916-924.

38. M. Thompson, L. Owen, K. Wilkinson, R. Wood and A. Damant, *Analyst*, 2002, 127, 1666-1668.

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

40. S. J. Culp and F. A. Beland, *Toxicol.*, 1996, 15, 219–238.

Figure Captions

Fig. 1 UV-vis absorption spectra of starch-coated AgNPs obtained with different concentrations of starch. Inset shows the color of different AgNPs solutions.

Fig. 2 TEM micrograph of AgNPs obtained with (a) 0.00%, (b) 0.10%, (c) 0.20%, (d) 0.50%, (e) 0.75%, (f) 1.00% and (g) 1.50% starch.

Fig. 3 Schematic illustration of starch-coated AgNPs SERS measurement for determining analytes.

Fig. 4 SERS spectra of melamine,4,4'-bipyridine, R6G and malachite green using 1.00% starch-coated AgNPs as substrates (a), SERS intensities of four analytes using starch-coated AgNPs as substrates at selected peak (b) (concentrations of starch: 0.10%, 0.20%, 0.50%, 0.75%, 1.00%, 1.50% (w/v)).

Fig. 5 TEM micrograph of classic AgNPs (a) and the AgNPs coated using 1.00% starch (b) 30 days later after synthesized.

Fig. 6 SERS intensities of melamine using both classic AgNPs (a) and the AgNPs coated using 1.00% starch (b) as substrates within 30 days.

Fig. 7 The relationships between SERS intensities and analytes concentrations: (a) melamine (band 704 cm^{-1}), (b) malachite green (band 1177 cm^{-1}).

Tables

Table 1 Precision of present method (n = 5)

Analyte	Spiked (μg L^{-1})	Intra-day precision		Inter-day precision(10 days)	
		Error (%)	RSD (%)	Error (%)	RSD (%)
Melamine	2.0	-4.1	3.16	-5.6	4.37
Malachite green	2.0	+3.2	2.87	+4.8	3.62

Table 2 Analytical performances of present method

Analyte	Linear equations	Linear range(μg L^{-1})	r	LOD （μg L^{-1}）	LOQ （μg L^{-1}）
melamine	I=2007.1C+24535	2.00-50.0	0.9992	0.600	1.80
malachite green	I=15537C+58843	0. 500-35.0	0.9979	0.0800	0. 230

Table 3 Determination of melamine in milk samples (n = 5)

Samples	Spiked (mg kg^{-1})	Found (mg kg^{-1})	RSD (%)	Recovery (%)
Sample1	0.50	0.48	4.53	96
	1.00	1.04	4.39	104

Sample2	0.50	0.51	3.40	102
	1.00	0.92	2.39	94

Table 4 Determination of malachite green in environmental water samples (n = 5)

Samples	Spiked (μg L^{-1})	Found (μg L^{-1})	RSD (%)	Recovery (%)
Sample1	2.0	2.15	2.25	107
	10.0	9.60	2.58	96
Sample2	2.0	1.97	4.09	98
	10.0	10.23	4.40	102

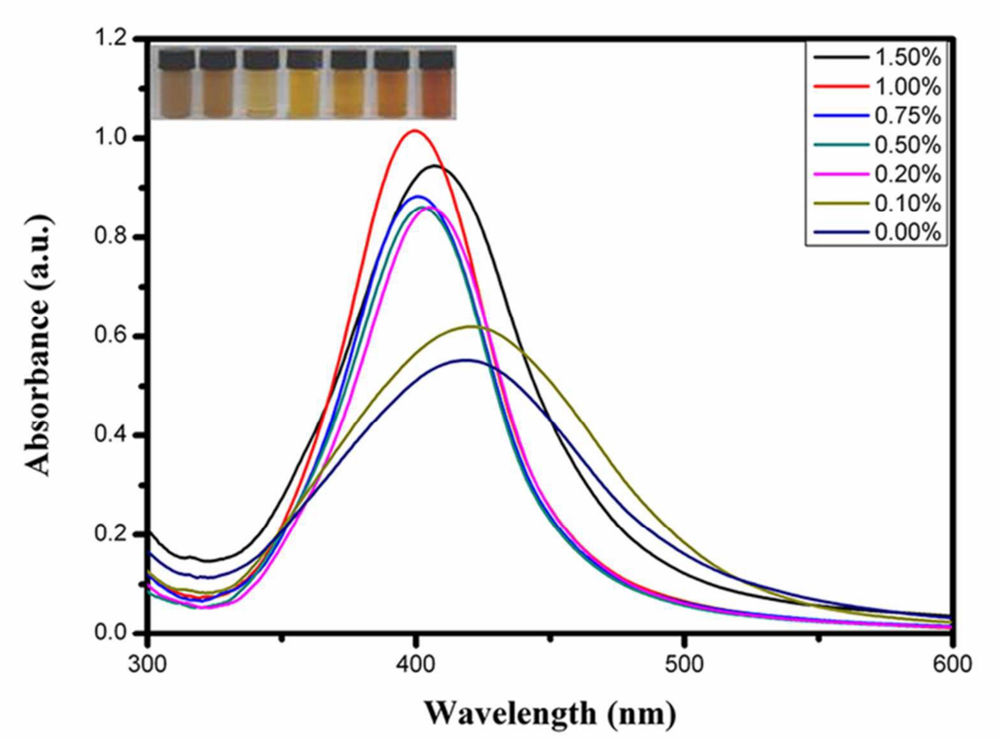

Fig. 1 UV-vis absorption spectra of starch-coated AgNPs obtained with different concentrations of starch. Inset shows the color of different AgNPs solutions.
59x43mm (300 x 300 DPI)

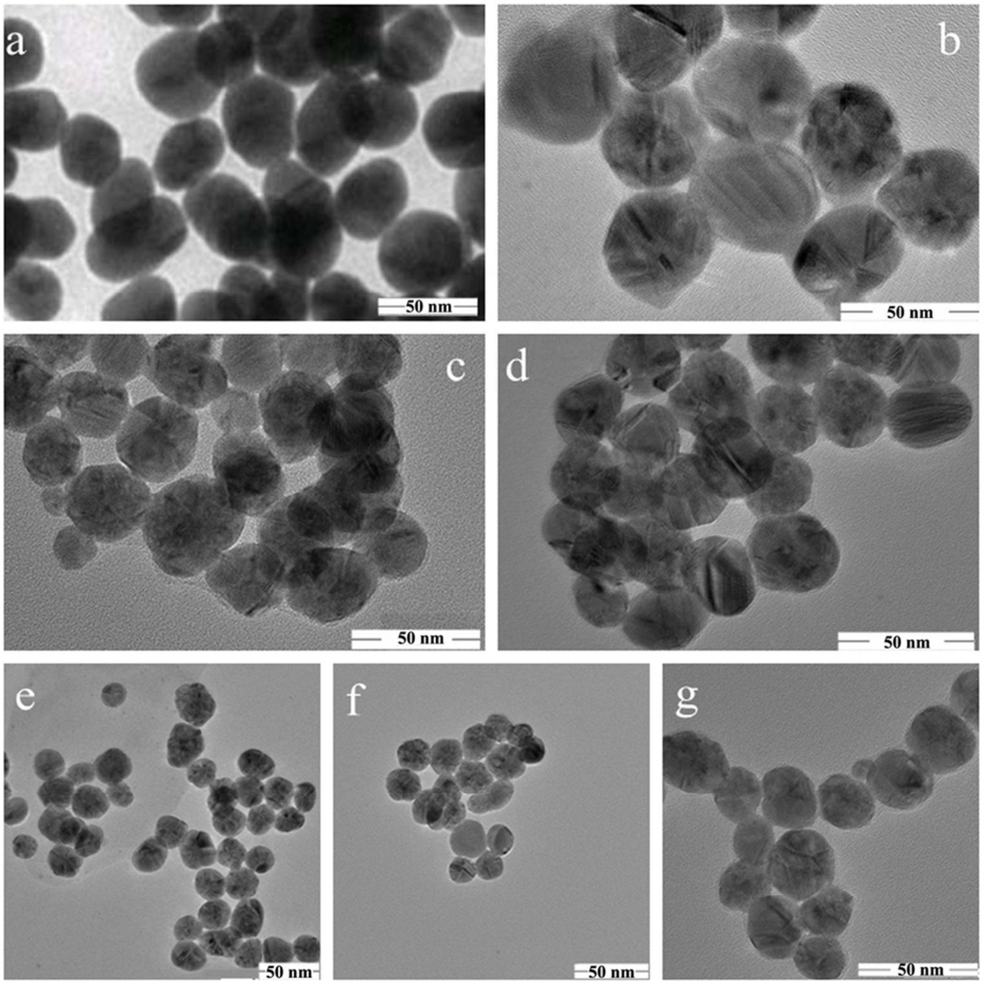

Fig. 2 TEM micrograph of AgNPs obtained with (a) 0.00%, (b) 0.10%, (c) 0.20%, (d) 0.50%, (e) 0.75%, (f) 1.00% and (g) 1.50% starch.
80x80mm (300 x 300 DPI)

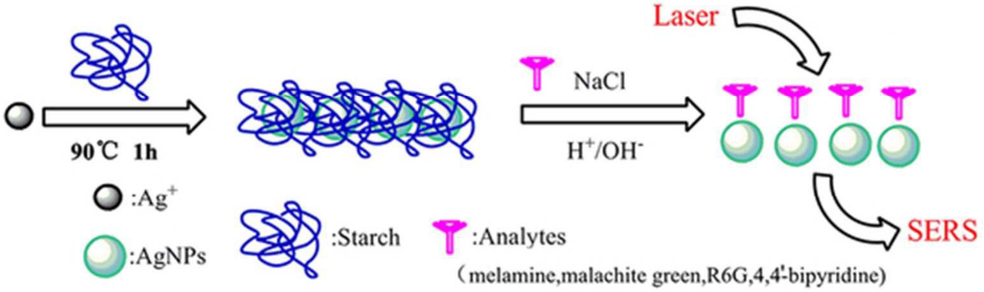

Fig. 3 Schematic illustration of starch-coated AgNPs SERS measurement for determining analytes.
48x14mm (300 x 300 DPI)

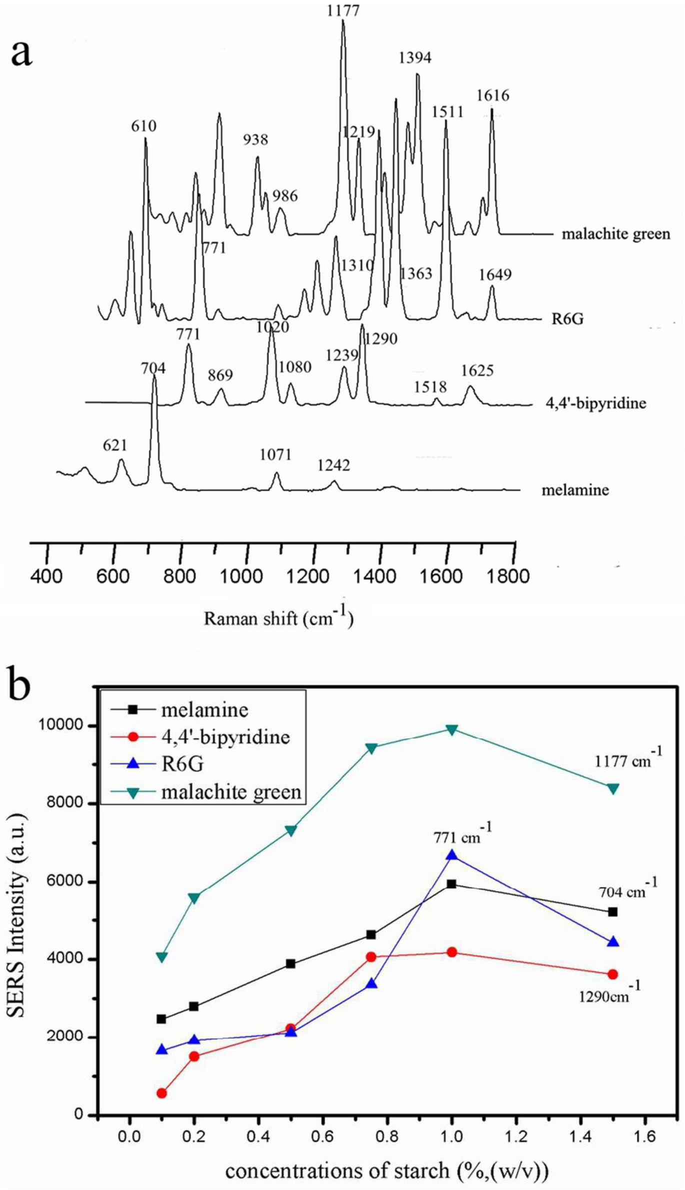

Fig. 4 SERS spectra of melamine,4,4'-bipyridine, R6G and malachite green using 1.00% starch-coated AgNPs as substrates (a), SERS intensities of four analytes using starch-coated AgNPs as substrates at selected peak (b) (concentrations of starch: 0.10%, 0.20%, 0.50%, 0.75%, 1.00%, 1.50% (w/v)).
136x232mm (300 x 300 DPI)

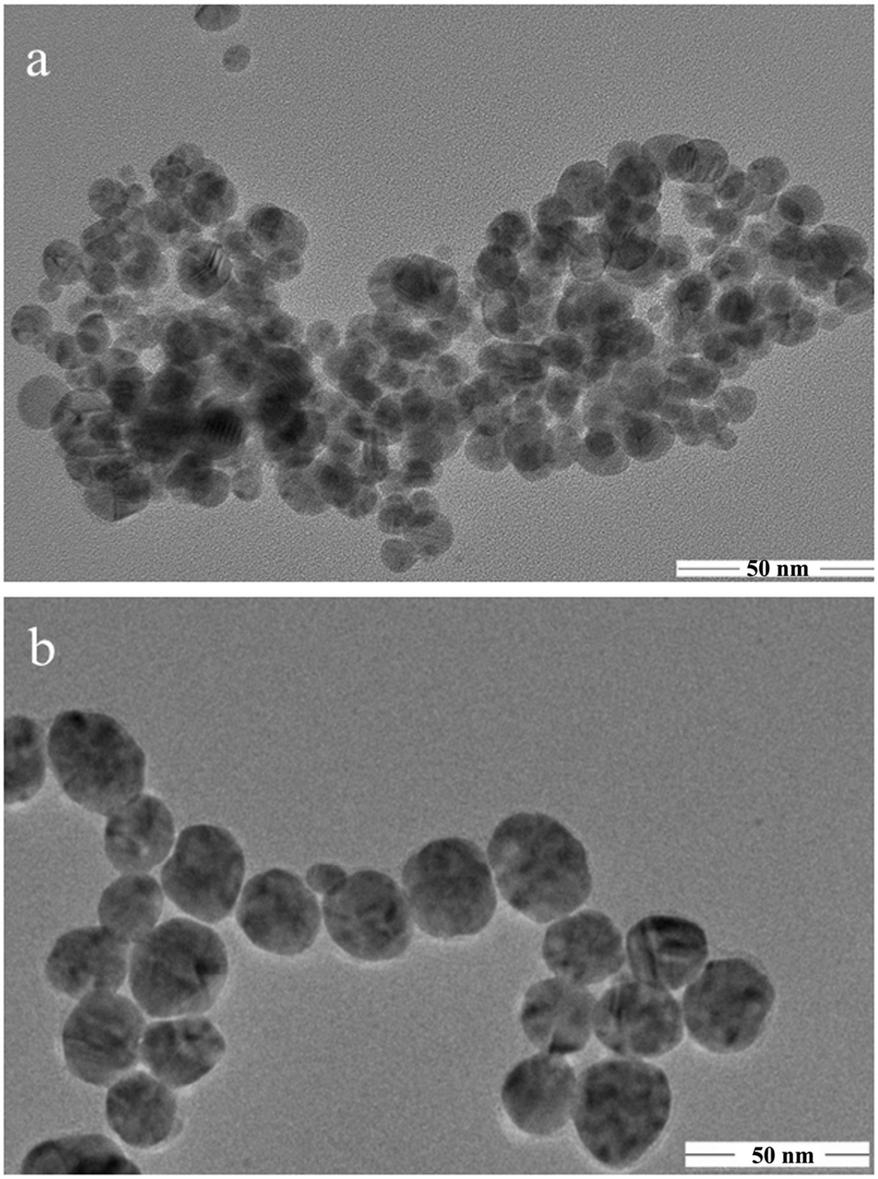

Fig. 5 TEM micrograph of classic AgNPs (a) and the AgNPs coated using 1.00% starch (b) 30 days later after synthesized.
106x142mm (300 x 300 DPI)

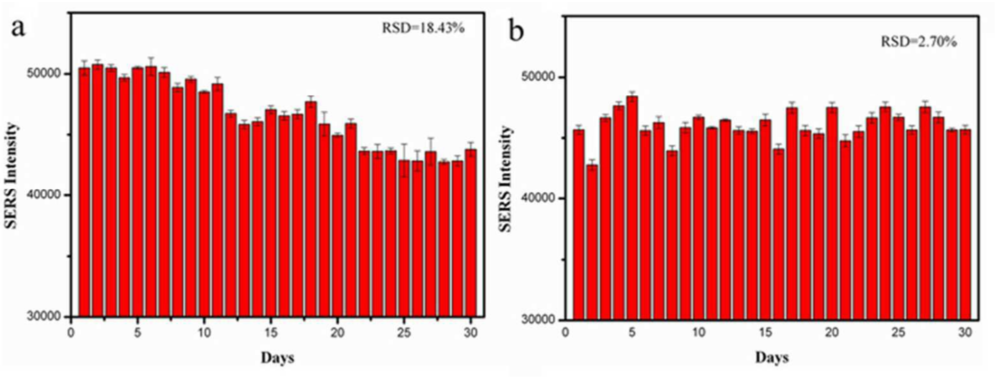

Fig. 6 SERS intensities of melamine using both classic AgNPs (a) and the AgNPs coated using 1.00% starch (b) as substrates within 30 days.
60x22mm (300 x 300 DPI)

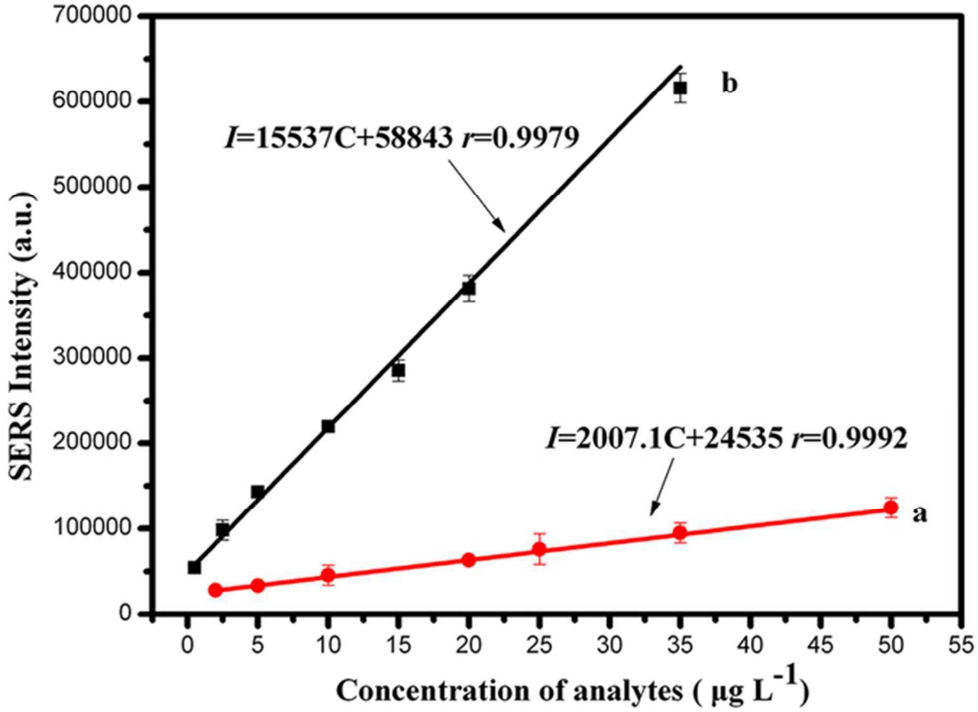

Fig. 7 The relationships between SERS intensities and analytes concentrations: (a) melamine (band 704 cm-1), (b) malachite green (band 1177 cm-1).
58x42mm (300 x 300 DPI)

Schematic illustration of starch-coated AgNPs SERS measurement for determining analytes.