A Binary Functional Substrate for Enrichment and Ultrasensitive SERS Spectroscopic Detection of Folic Acid Using Graphene Oxide/Ag Nanoparticle Hybrids

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ABSTRACT Herein graphene oxide/Ag nanoparticle hybrids (GO/PDDA/AgNPs) were fabricated according to a self-assembly procedure. Using the obtained GO/PDDA/AgNPs as SERS substrates, an ultrasensitive and label-free detection of folic acid in water and serum was demonstrated based on the inherent SERS spectra of folic acid. The modified graphene oxide exhibited strong enrichment of folic acid due to the electrostatic interaction, and the self-assembled Ag nanoparticles greatly enhanced the SERS spectra of folic acid, both of which led to an ultrahigh sensitivity. Therefore, although the SERS enhancement of *p*-ATP on GO/PDDA/AgNPs was weaker than that on Ag nanoparticles, the SERS signals of folic acid on GO/PDDA/AgNPs were much stronger than that on Ag nanoparticles. To improve the detection, the concentration of GO/PDDA/AgNPs was optimized to reduce background of the graphene oxide. The SERS spectra of the folic acid showed that the minimum detected concentration of folic acid in water was as low as 9 nM with a linear response range from 9 to 180 nM. To estimate the feasibility of the detection method based on GO/PDDA/AgNPs for the practical applications, diluted serum containing different concentrations of folic acid was taken as real samples. It was established that the sensitivity and the linear range for the folic acid in serum were comparable to that in water. This ultrasensitive and label-free SERS detection of folic acid based on GO/PDDA/AgNPs offers great potential for practical applications of medicine and biotechnology.

KEYWORDS: graphene oxide/Ag nanoparticle hybrids · folic acid · SERS detection · serum

In recent decades, surface-enhanced Raman spectroscopy as a very important analytical technique for biomedical detection has received increasing attention.[1−3] Until now, based on surface-enhanced Raman scattering (SERS), various applications have been reported, such as the detection of DNA/RNA, vesicles, proteins, pathogens and so on.[4−8] As a widely used method, SERS shows its unique and excellent properties for the biological system. Although Raman spectroscopy is limited by low sensitivity, SERS could provide signal intensity of the molecules enhanced by orders of magnitudes on the proper substrates. Nie's group and Kneipp's group have reported the single molecule detection by SERS, indicating the high sensitivity of SERS for ultrasensitive biological detection.[9,10] Hot spots formed in the nanoscale junctions and interstices of the SERS substrates could greatly increase the Raman cross section of biomolecules, leading to a low detection limit. Because of the limited influence of water, SERS, compared with infrared spectroscopy, is a better fit for the main biological systems that reside in water. Benefiting from the very narrow bandwidth of a typical Raman peak, SERS is suited for the detection of multicomponent samples. Although SERS tags with organic Raman reporter molecules with strong characteristic SERS signature could indirectly identify the trace biomolecules, it is also possible to recognize the analytes according to their inherent vibrations of SERS spectra. SERS substrates with large enhancement factor and good reproducibility are essential for the biomedical applications of SERS. Silver is often selected to fabricate SERS substrates for the detection of biomolecules because of its high SERS activity. During the reported SERS applications, Ag-based multifunctional substrate composites of Ag and other materials exhibit improved properties for bioanalysis compared with the SERS substrates of silver, such as roughed Ag electrodes, Ag nanoparticles (AgNPs), colloid and assembly AgNP films. For instance, the encapsulation of SiO_2 and polymer improves the stability and biocompatibility of the SERS substrates, and the combination with magnetic nanostructures adds the SERS substrates with the capability of controllability and enrichment

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of the analytes.[11−15] Consequently, the Ag-based hybrid multifunctional SERS substrates have become a hot research topic for the further development of SERS applications in biomedical systems.

Graphene, which is a monolayer of carbon atoms packed closely into a two-dimensional honeycomb lattice, has drawn significant attention because of its fascinating properties and potential applications since the experimental discovery of single layers by Novoselov and Geim in 2004.[16] In the previous biomedical applications, graphene and graphene oxide were used as nanocarriers for drug loading and delivery,[17,18] indicating the enrichment of drug molecules on graphene and graphene oxide. It was reported that the loading ratio (weight ratio) of graphene oxide is much higher than that of other loading nanostructures,[19] suggesting a more efficient extraction for target molecules. Because of the large surface area in low manufacturing cost of the graphene oxide and its interaction with the target molecules, this enrichment is also utilized to improve the sensitivity of the biomedical detection.[20,21] However, due to the strong van der Waals interactions, the aggregation of graphene oxide in solutions, especially in salt or other biological solutions, limits their biological applications. To resolve this problem, it has been demonstrated that the graphene oxide/nanoparticles hybrid can avoid the aggregation of graphene oxide.[22] Meanwhile, the hybridization of graphene oxide and nanoparticles as an efficient strategy enhances the electronic, catalytic, and optical properties of the graphene oxide/nanoparticle hybrids.[22−25] Until now, hybrid materials have been reported to consist of graphene oxide and various nanoparticles, such as gold, platinum, palladium, and so on.[23,24,26,27] Nonetheless, there are only a few works about the composites of Ag and graphene, and most of them are produced by reducing silver salt on graphene oxide.[28,29] Self-assembly is an ordinary method to fabricate the graphene oxide/nanoparticle hybrids in which the loading ratio and the morphology of the nanoparticles are tunable, though this strategy is rarely used to fabricate the graphene oxide/AgNPs. It is anticipated that the nanostructures of graphene oxide/Ag nanoparticles fabricated by the self-assembly method can be used as SERS substrates, while AgNPs supply strong SERS activity and the graphene oxide can concentrate the target molecules.

Herein, graphene oxide/Ag nanoparticle hybrids (GO/PDDA/AgNPs) were obtained based on a self-assembly strategy, in which poly(diallyldimethyl ammonium chloride) (PDDA) was used as the functional macromolecules to prepare the stable cationic polyelectrolyte-functionalized graphene oxide and anchor the AgNPs, and the modified GO with positive potential can concentrate the negatively charged target molecules. The obtained GO/PDDA/AgNPs exhibited strong SERS activity resulting from the AgNPs and the enrichment of the folic acid molecule on the graphene oxide due to the electrostatic interaction. On the basis of the GO/PDDA/AgNPs, ultrasensitive and label-free detection of folic acid by SERS spectra was demonstrated. Folic acid, known as a widely distributed water-soluble vitamin, is reported to be a very significant component for human health which relates to a series of diseases such as gigantocytic anemia, mental devolution, heart attack, and congenital malformation, and

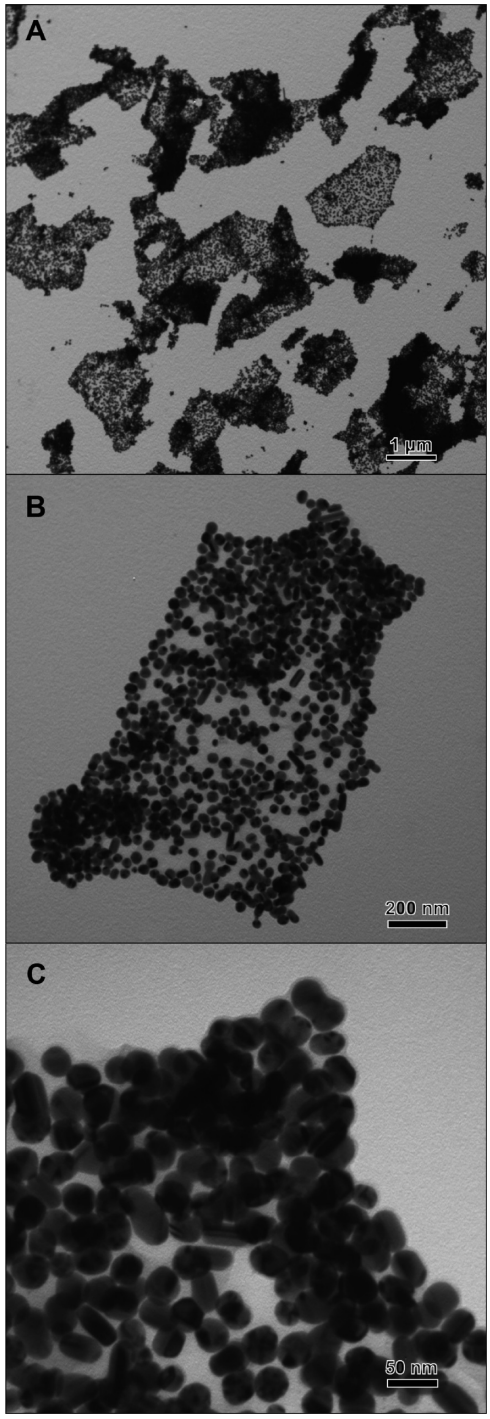


Figure 1. TEM images of the GO/PDDA/AgNPs at different magnifications.

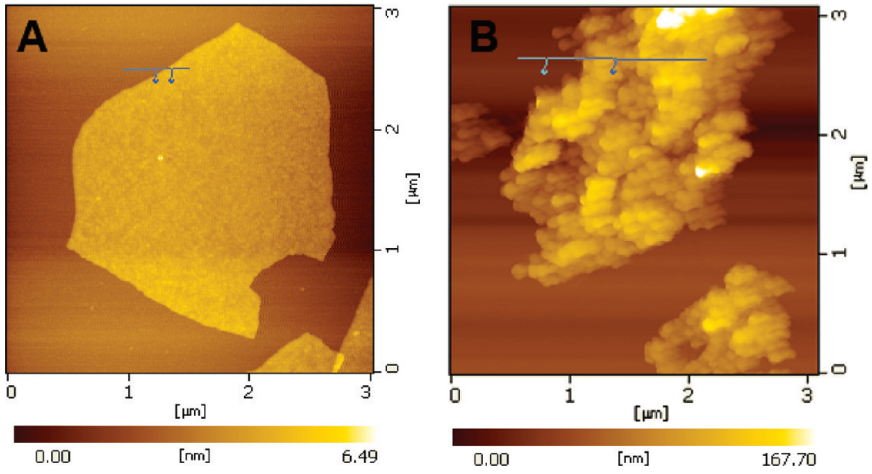


Figure 2. AFM images of PDDA/GO (A) and GO/PDDA/AgNPs (B).

folic acid also has drawn attention as a possible targeting agent of cancer cells because the receptors of folic acid are found on the surfaces of various human tumor cells.[30−33] Therefore, it is interesting to develop an ultrasensitive detection method for the practical applications of medicine and biotechnology. On the basis of the GO/PDDA/AgNPs with strong SERS enhancement and enrichment of folic acid, low limit of detection with wide linear response range was achieved by SERS.

RESULTS AND DISCUSSION

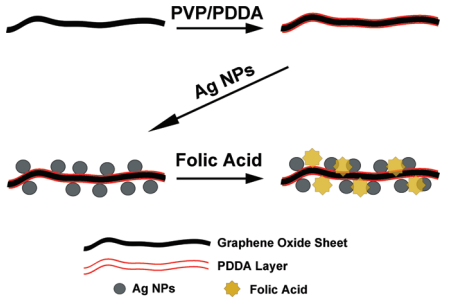
The loading of the AgNPs onto graphene oxide was characterized by TEM, and the typical images of GO/PDDA/AgNPs are shown in Figure 1 at different magnifications. It was observed (Figure 1A) that all of the graphene oxide nanosheets were modified by AgNPs, and the amount of AgNPs on a single graphene oxide was large. Some areas of the GO/PDDA/AgNPs with dark color were also found, which were presumably attributed to the overlapping of the loaded AgNPs. Figure 1B shows that all of the AgNPs were confined in the range of graphene oxide, indicating that the AgNPs were loaded on the graphene oxide. The average size of AgNPs adsorbed on the graphene oxide was about 35 nm, while there were a few short rods and smaller nanoparticles that exhibited strong SERS activity. Meanwhile, the arrangement of the AgNPs on the graphene oxide was observed to be not very close, therefore, there were still some blanks between the AgNPs for the enrichment of the folic acid molecules in the procedure of SERS detection. In a zoomed image of the graphene oxide shown in Figure 1C, the superposition of AgNPs was noticed, suggesting that the self-assembly of AgNPs could occur on both sides of the graphene oxide. The TEM images show the formation of GO/PDDA/AgNPs and proved that the loading of AgNPs was efficient on the platform of graphene oxide.

To further study the linkage between the AgNPs and graphene oxide modified with PDDA, atomic force microscope (AFM) measurements were carried out and the results are shown in Figure 2. On the basis of the AFM images, the changing of the thickness and the roughness was monitored. Unlike our previous work,[23] the AFM image in Figure 2A shows that the surface of the typical graphene oxide modified with PDDA was smooth. The thickness of graphene oxide modified with the PDDA was about 2.1 nm, which was also thinner than that of the modified graphene in ref 23. The differences were attributed to the smaller amount of PDDA modified on the graphene oxide. After the self-assembly of AgNPs, it was found that the roughness of the graphene oxide increased and a lot of nanoparticles were observed. The thickness of the hybrids was demonstrated to be 79.3 nm, about 2 times the average diameters of the AgNPs, indicating that the nanoparticles were assembled on both sides of the graphene oxide corresponding to the TEM results.

The self-assembly of nanoparticles onto graphene oxide can be performed by directly adsorbing the as-prepared nanoparticles onto the graphene oxide because the graphene oxide was negatively charged due to residual defects such as carboxyl, hydroxyl, and epoxy.[34] However, the negative charges of the graphene oxide were quite weak for anchoring nanoparticles, and for silver, most of the AgNPs synthesized with negatively charged capping agents are difficult to be adsorbed directly onto graphene oxide, which might be a primary reason for the lack of papers about the assembly of AgNPs onto the graphene. The modification of the graphene oxide with positively charged polymer has been proven to be a good strategy for the assembly of metal nanoparticles because the polymer as a linker can anchor the negatively charged nanoparticles onto graphene oxide. The electrostatic interaction between the graphene modified with PDDA and the AgNPs was characterized by zeta-potential measurement, and the results are shown in Table 1. The mean zeta-potential of PDDA/graphene oxide was positive (33.9 mV) due to the modification of PDDA.

TABLE 1. Measured Average Zeta-Potential of the Modified Graphene Oxide at 20 °C

	PDDA/GO	AgNPs	AgNPs (control)	GO/PDDA/AgNPs
zeta-potential (mV)	33.9	−31.3	−20.3	14.9



Scheme 1. Illustration of the fabrication of GO/PDDA/AgNPs and the procedure of SERS detection of folic acid using GO/PDDA/AgNPs as substrates.

Then the added negatively charged AgNPs (−31.3 mV) were adsorbed on the PDDA/graphene oxide because of the electrostatic interaction. AgNPs synthesized by another method[35] were used as the control group; however, these AgNPs cannot be assembled on PDDA/graphene oxide. Zeta-potential of the control group AgNPs was −20.3 mV, much lower than that of the AgNPs used in the fabrication of GO/PDDA/AgNPs, while the average diameter of the control group AgNPs, 55 nm, was bigger. Therefore, it is believed that the size and the amount of negative charges of AgNPs are important for the formation of GO/PDDA/AgNPs. Finally, zeta-potential measurement demonstrated that the obtained GO/PDDA/AgNPs were still positively charged (14.9 mV), indicating that the assembly of the AgNPs was not complete corresponding to the low intensity of AgNP adsorption shown in TEM images. The GO/PDDA/AgNPs with positive surface charge were suited for the enrichment of the folic acid molecules due to the electrostatic effect.

The whole procedure of fabrication of GO/PDDA/AgNPs and its application in detection of folic acid are illustrated in Scheme 1. First, the graphene oxide was modified by poly(*N*-vinyl-2-pyrrolidone) (PVP) and PDDA, and a stable solution of cationic functional graphene oxide was obtained. The adsorption of the PDDA on GO was mainly attributed to the electrostatic effect.[23] Then the as-prepared graphene oxide/PDDA solution was mixed with AgNPs, which were negatively charged due to the capping reagent, sodium citrate. The AgNPs were self-assembled onto the graphene oxide/PDDA because of the electrostatic effect. These AgNPs were used to enhance the SERS spectra of the folic acid molecules. The zeta-potential of the obtained GO/PDDA/AgNPs was shown to be positive. Therefore, folic acid molecules reported to be negatively charge in solution were extracted to the GO/PDDA/AgNPs due

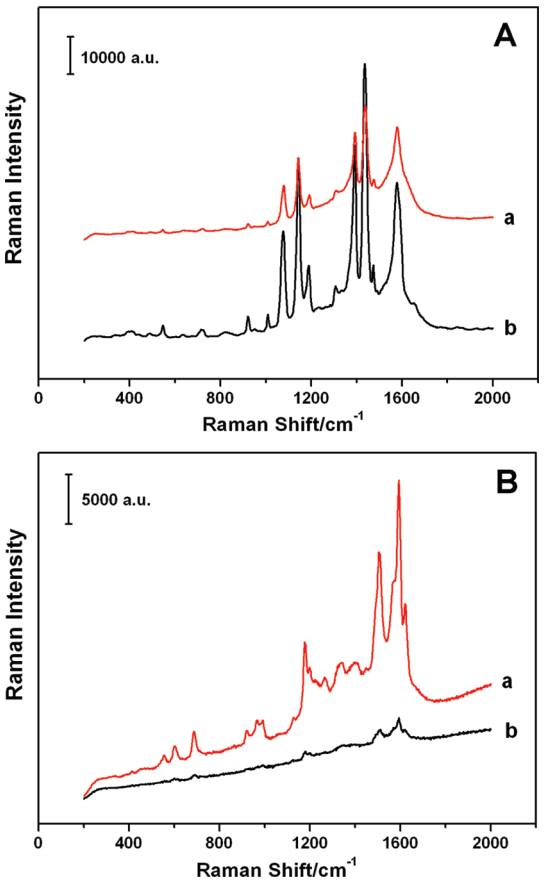


Figure 3. SERS spectra of 10^{-5} M *p*-ATP (A) on GO/PDDA/AgNPs (a) and AgNP colloid (b), and SERS spectra of 10^{-4} M folic acid (B) on GO/PDDA/AgNPs (a) and AgNP colloid (b).

to the electrostatic interaction when added to the solution of GO/PDDA/AgNPs.[36] Benefiting from the enrichment from the modified graphene oxide, the SERS signals of the folic acid were further amplified.

To estimate the SERS activity of the GO/PDDA/AgNPs, 10^{-5} M aqueous solution of *p*-aminothiophenol (*p*-ATP) was chosen as the probe molecule (obtained SERS spectrum is shown in Figure 3Aa). The SERS spectra of *p*-ATP on the AgNPs used in the fabrication of GO/PDDA/AgNPs were also collected as the contrast (shown in Figure 3Ab). The primary vibrations of *p*-ATP were confirmed according to the reported work.[37] Two sets of bands were observed on the SERS spectra of *p*-ATP on two kinds of SERS substrates: 1577, 1184, and 1075 cm^{-1} assigned to a_1 vibration modes and 1434, 1389, and 1139 cm^{-1} assigned to the b_2 vibration modes. The relative intensity of the peaks in the spectra on AgNPs and GO/PDDA/AgNPs indicated that the *p*-ATP adsorbed on AgNPs according to the cleavage of the Ag−S bond in the same orientation,[38] and it is known that, in water (pH = 7), the amino group of *p*-ATP is almost deprotonated.[39] Therefore, it was believed that the modified graphene oxide did not assist the adsorption of *p*-ATP on AgNPs of GO/PDDA/AgNPs. It was distinct that the intensity of

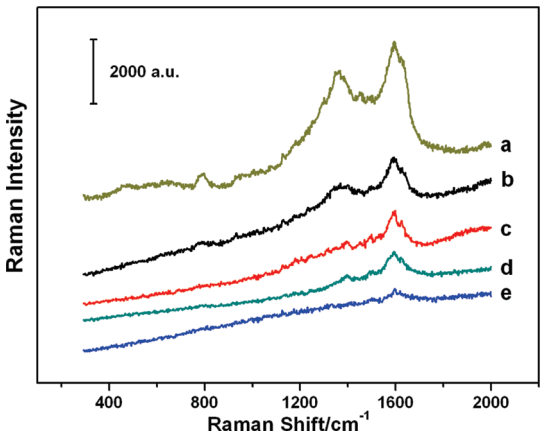


Figure 4. SERS spectra of 9 nM folic acid obtained in the GO/PDDA/AgNP solutions with 0.02 mg/mL (a), 0.01 mg/mL (b), 0.004 mg/mL (c), 0.0025 mg/mL (d), 0.002 mg/mL (e) graphitic carbon.

SERS spectra on GO/PDDA/AgNPs was weaker than that of AgNPs, though the vibrations of *p*-ATP on GO/PDDA/AgNPs and the AgNPs were at the same positions. A possible reason was proposed to explain the difference of the intensity: the adsorption of *p*-ATP on AgNPs in the colloid would induce the aggregation of AgNPs, and the hot spots formed in the nanoscale junctions and interstices due to the aggregation would supply strong SERS enhancement; however, there was no further aggregation observed of the AgNPs assembled on the graphene oxide after the addition of *p*-ATP, and TEM images showed that the arrangement of AgNPs was not very close; thus the amount of hot spots in GO/PDDA/AgNPs was fewer, leading to the weaker SERS intensity of *p*-ATP. According to the results of *p*-ATP SERS spectra, it was evident that, without the enrichment from the modified graphene oxide, the SERS intensity of the probe molecules on GO/PDDA/AgNPs was weaker than that on AgNPs.

It has been reported that graphene and graphene oxide are used for drug delivery, indicating the enrichment of the target molecules. On the basis of the same concept, herein the graphene oxide was used to concentrate the folic acid molecules, and the AgNPs adsorbed on the graphene oxide enhanced the Raman signal of folic acid. Therefore, a strong SERS spectrum of folic acid was achieved with the GO/PDDA/AgNPs, leading to an ultrasensitive detection of folic acid. As shown in Figure 3Ba, the SERS spectra of 10^{-4} M folic acid with a strong signal on GO/PDDA/AgNPs were recorded. Main vibrations of folic acid shown in the SERS spectrum were confirmed according to the reported work.[40] These characteristic peaks providing the information of the molecules could be used to identify folic acid in the samples. The SERS spectrum of the folic acid on AgNPs as the contrast is shown in Figure 3Bb, in which the primary vibrations were at the same positions as that on GO/PDDA/AgNPs. Although the intensity of the *p*-ATP on GO/PDDA/AgNPs was weaker than that on AgNPs, it was distinct that the SERS signal of folic acid on GO/PDDA/AgNPs was much stronger than that on AgNPs in the colloid. The additional enhancement of SERS signal of folic acid on GO/PDDA/AgNPs was attributed to the enrichment of folic acid molecules from the PDDA-modified graphene oxide in the solution. The electrostatic interaction between the negatively charged folic acid molecules and the modified graphene oxide with positive surface potential can increase the concentration of folic acid in the region near the GO/PDDA/AgNPs. With the large surface area of graphene oxide, the enrichment of folic acid from the GO/PDDA/AgNPs greatly enhanced the SERS signal of folic acid compared with that on AgNPs in the colloid.

To achieve a lower limit of detection, the strongest peak located at 1595 cm^{-1} was chosen as the signature to determine the concentration of folic acid in the samples. However, the G bands of graphene and graphene oxide at about 1600 cm^{-1} may overlap the signature band of folic acid and disturb the detection of folic acid in low concentration.[41] Therefore, the amount of the GO/PDDA/AgNPs was optimized to get stronger folic acid SERS spectra with a lower intensity of the G band of graphene oxide. As shown in Figure 4, SERS spectra of 9 nM folic acid were obtained in a series of concentration of GO/PDDA/AgNPs. Figure 4a shows the SERS spectrum of folic acid recorded in the GO/PDDA/AgNP solution with 0.02 mg/mL graphitic carbon. The peak of 1595 cm^{-1} attributed to the folic acid was observed, while the G band was at about 1600 cm^{-1}. Meanwhile, the D band at about 1355 cm^{-1} was distinct in the spectrum. According to the intensity of the D band, the background was estimated in the respect that the intensity ratio of the D band and G band is constant for the graphene oxide used in GO/PDDA/AgNPs. The GO/PDDA/AgNP solution with 0.004 mg/mL graphitic carbon was selected to determine the folic acid because in the corresponding SERS spectrum the peak at 1595 cm^{-1} was clear enough with the ignorable D band of graphene oxide at 1355 cm^{-1}.

In the GO/PDDA/AgNP solution with 0.004 mg/mL graphitic carbon, SERS spectra of the folic acid in a series of concentrations are shown in Figure 5A. One can see that the intensity of the SERS spectra rose with the increasing concentrations of folic acid, suggesting that the intensity was proportional to the amount of folic acid molecules adsorbed on the graphene oxide. The minimum concentration of folic acid detected was 9 nM, shown as Figure 5Ab, which was lower than the calculated limit of detection (LOD) reported by Graham (18 nM),[40] indicating that ultrasensitive detection of folic acid was achieved based on the GO/PDDA/AgNPs. The SERS intensity of the vibration located at 1595 cm^{-1} *versus* the concentration of folic acid is also

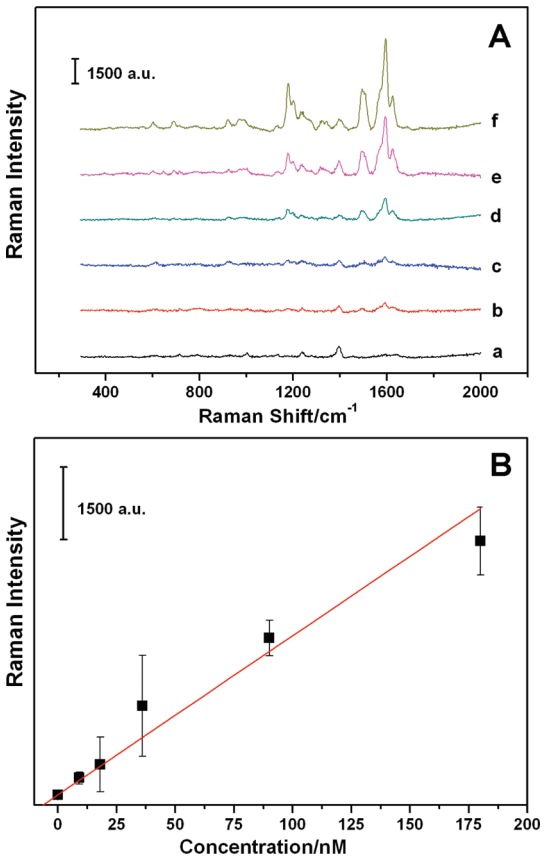


Figure 5. SERS spectra of different concentrations of folic acid in water (A): blank (a), 9 nM (b), 18 nM (c), 36 nM (d), 90 nM (e), and 180 nM (f); and SERS dilution series of folic acid in water based on the peak located at 1595 cm^{-1} (B).

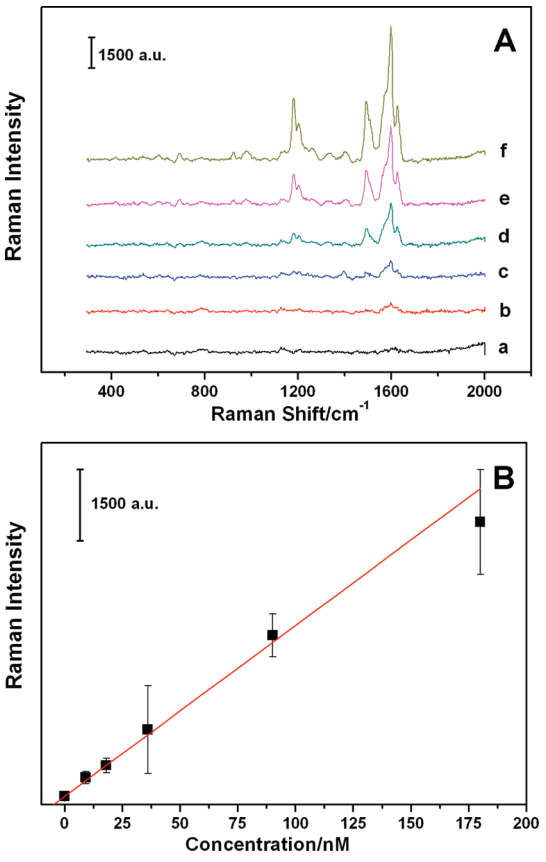


Figure 6. SERS spectra of different concentrations of folic acid in diluted serum (A): blank (a), 9 nM (b), 18 nM (c), 36 nM (d), 90 nM (e), and 180 nM (f); and SERS dilution series of folic acid in diluted serum based on the peak located at 1595 cm^{-1} (B).

plotted in Figure 5B, which revealed a linear SERS response from 9 to 180 nM of folic acid (R^2 = 0.985). The distribution of AgNPs on graphene oxide modified by PDDA was not very even, leading to a non-uniformity of hot spots in the substrate, which should be the reason for the unsatisfactory standard deviations of the intensity for some concentrations.

To investigate the feasibility of the detection of folic acid in real biological samples, the known amount of folic acid was added to the diluted human serum. Then the diluted serum containing folic acid was mixed with GO/PDDA/AgNP solution. Additional procedure to remove the protein in serum was unnecessary. The SERS spectra of folic acid in different concentrations in diluted serum are illustrated in Figure 6A. As the folic acid dissolved in water, the intensity of the SERS spectra of folic acid in serum was proportional to the concentration of the folic acid in diluted serum. Besides, it was evident that the SERS spectra of folic acid in serum were quite similar to that in water with comparable intensity. There were only a few additional peaks in the spectrum of blank serum shown in Figure 6Aa attributed to the components in serum which did not disturb the recognition of folic acid. On the basis of the above results, it can be deduced that the influence of the remaining protein in serum was almost ignorable in the procedure of folic acid detection. The lowest detected concentration of folic acid in serum was 9 nM, corresponding to the spectrum shown in Figure 6Ab, as low as that in water. To represent the capability of the quantitative detection of folic acid in serum and its reproducibility, the linear fit calibration curve (R^2 = 0.992) with error bars is illustrated in Figure 6B. The linear response of SERS was observed from 9 to 180 nM. The concentration gradient experiments of folic acid proved that obtained GO/PDDA/AgNPs were good SERS substrates for the detection of folic acid in serum, and the ignorable protein background indicated a potential application to detect folic acid in other practical biological systems.

CONCLUSION

Herein graphene oxide/AgNP hybrids (GO/PDDA/AgNPs) were prepared as SERS substrates according to a self-assembly method. On the basis of the obtained GO/PDDA/AgNPs, an ultrasensitive and label-free SERS strategy was developed for the detection of folic acid in water and serum according to the inherent SERS spectra of folic acid. The modified graphene oxide was used to

enrich the folic acid molecules due to the electrostatic interaction, on which the self-assembled AgNPs supplied strong SERS enhancement to obtain the SERS signal of folic acid. The contrast of SERS spectra of *p*-ATP and folic acid on AgNP colloids and GO/PDDA/AgNPs showed that the enrichment due to the graphene oxide was very important for the ultrasensitivity of the SERS detection of folic acid based on GO/PDDA/AgNPs. To improve the detection, the concentration of GO/PDDA/AgNPs was optimized, and it was established that, in the GO/PDDA/AgNP solution with 0.004 mg/mL graphitic carbon, clear SERS spectra of folic acid were obtained with the weakest background of graphene oxide. It was demonstrated that the minimum detected concentration of the folic acid in water was as low as 9 nM, and the calibration curve showed a good linear relation with a linear response range from 9 to 180 nM. The capability of SERS detection of folic acid in real samples based on GO/PDDA/AgNPs was investigated with serum containing a known amount of folic acid, and the results showed that the sensitivity and the linear response range were comparable to that in water. In this procedure, the influence from the proteins remaining in serum was almost ignorable. On the basis of the substrates of GO/PDDA/AgNPs, the versatility of this ultrasensitive SERS detection of folic acid in varied matrices was expected for the practical applications of medicine and biotechnology.

MATERIALS AND METHODS

Chemicals and Reagents. Graphite was obtained from Alfa Aesar. Folic acid, *p*-aminothiophenol (*p*-ATP), and poly(diallyldimethyl ammonium chloride) (PDDA) (M_W = 40 000−50 000, 20 wt % in water) were purchased from Aldrich. $AgNO_3$, sodium citrate dihydrate, KCl, and poly(*N*-vinyl-2-pyrrolidone) (PVP) (K30, M_W = 30 000−40 000) were obtained from Shanghai Chemical Reagent Co. (Shanghai, China). All of the reagents were used as received. Fresh human serum was purchased from a local hospital. Water used throughout all experiments was purified with a Millipore system. All glassware used in the following procedures was cleaned in a bath of freshly prepared 3:1 HCl/HNO_3 (aqua regia) and rinsed thoroughly in Millipore water prior to use.

Apparatus. Transmission electron microscope (TEM) measurements were performed by using a HITACHI H-800 EM with an accelerating voltage of 200 kV. The samples for TEM measurement were prepared by dropping the product solution on carbon-coated copper grids and drying in ambient conditions. Zeta-potential measurements were conducted with a Zetasizer NanoZS (Malvern Instruments). The atomic force microscope (AFM) images were obtained by using a SPI3800N microscope (Seiko Instruments, Inc.) operating in the tapping mode with standard silicon nitride tips. Surface-enhanced Raman scattering (SERS) spectra were obtained by using a Renishaw 2000 (Renishaw Co., United Kingdom) equipped with an Ar^+ ion laser giving the excitation line of 514.5 nm with a laser spot diameter of 1.6 μm and air cooling charge-coupled device (CCD) as the detector. The Raman band of a silicon wafer at 520 cm^{-1} was used to calibrate the spectrometer.

Synthesis of AgNPs. AgNPs were synthesized based on a previous method.[42] Briefly, 0.53 mL of 0.1 M $AgNO_3$ was added to 50 mL of water at 45 °C and heated rapidly to boiling. Then 1 mL of a 1% sodium citrate dihydrate was injected under vigorous stirring, and the resulting solution was held at boiling for 45 min. The resulting solution was cooled in the ambient conditions.

Synthesis of the control group AgNPs was performed following the reported method.[35] First, 0.25 mL of $AgNO_3$ (0.1 mol/L) aqueous solution was added to 50 mL of water under strong stirring. When the solution was boiling, 3 mL of citrate sodium (1 wt %) was quickly added into the solution, followed by injecting ascorbic acid (2 mL, 0.1 mol/L) aqueous solution. Then the solution was boiled for 5 min and cooled in ambient conditions.

Synthesis of PDDA-Functionalized Graphene Oxide. The PDDA-functionalized graphene oxide was synthesized based on a previous work of our group.[23] First, the graphene oxide was prepared from natural graphite powder by the modified Hummers method.[43,44] Then the graphene oxides obtained were exfoliated by ultrasonication in water for more than 1 h. At last, water solution of homogeneous graphene oxide (1.0 mg/mL) was obtained. PVP-capped graphene oxide was prepared according to the as-reported work.[45] In a typical procedure, 80 mg of PVP was added to 20 mL of 0.25 mg/mL graphene oxide solution, followed by stirring for 30 min. The resulting dispersion was washed and centrifuged three times and dissolved in 5 mL of water. To obtain PDDA-functionalized graphene oxide (PDDA/GO), 0.1 mL of 20 wt % PDDA was mixed well with 16.8 mL of 0.625 M KCl, followed by injecting 4.2 mL of PVP-capped graphene oxide, and the resulting solution was sonicated for 1.5 h. The products were washed and centrifuged three times. Finally, the PDDA/GO was redispersed in 4 mL of water.

Fabrication of Graphene Oxide/Ag Nanoparticle Hybrids. Forty microliters of 1 mg/mL of PDDA/GO was added to 4 mL of as-prepared AgNPs under stirring. Then the solution was sonicated for 3 min before standing overnight. The precipitate was washed several times and dissolved in 2 mL of water.

Detection of Folic Acid. The samples were prepared by adding a known concentration of folic acid aqueous solution to the solution of GO/PDDA/AgNPs, and the obtained mixtures were incubated in a 25 °C water bath for 3 h, followed by slight sonication to redisperse the GO/PDDA/AgNPs which have adsorbed the folic acid molecules. Then the samples were measured by Raman spectrometry using an accumulation time of 30 s. One percent serum (in water) with known concentration of folic acid was used to estimate the detection method in real samples. The rest of the procedure for the detection in diluted serum was the same as that in water.

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Supporting Information Available: SERS spectra of the GO/PDDA/AgNP solutions with 0.02 mg/mL (a), 0.01 mg/mL (b), 0.004 mg/mL (c), 0.0025 mg/mL (d), 0.002 mg/mL (e) graphitic carbon. This material is available free of charge *via* the Internet at http://pubs.acs.org.

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