

STUDY OF DETECTION OF MELAMINE VIA SURFACE-ENHANCED RAMAN SCATTERING METHOD BASED ON SILVER NANOPARTICLES**Tran Thu Trang**

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ARTICLE INFO		ABSTRACT
Received:	01/4/2024	Silver nanoparticles (Ag NPs) were synthesized and characterized by transmission electron microscopy (TEM), X-ray powder diffraction (XRD), and absorption spectroscopy. Using Debye-Scherrer's formula and the result of the XRD spectrum, the size of the Ag NPs was estimated at approximately 8 nm. The fabricated silver nanoparticles were used to perform surface-enhanced Raman scattering (SERS) for detecting melamine. The SERS mechanism was discussed and indicated that electromagnetic enhancement played an important role; the contribution of chemical enhancement could be ignored due to the out-of-charge transfer resonance between Ag NPs surface and melamine molecules. The SERS activity of Ag NPs substrate in probing melamine was examined with a variety of concentrations ranging from 1.5×10^{-3} to 10^{-7} M. The SERS spectra of melamine molecules presented a good sensitivity with a limit of detection of 10^{-7} M, corresponding to an enhancement factor (EF) of 2×10^5 . The results show quite high sensitivity for probing melamine.
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NGHIÊN CỨU PHÁT HIỆN MELAMINE QUA PHƯƠNG PHÁP TĂNG CƯỜNG TÁN XẠ BỀ MẶT RAMAN DỰA TRÊN ĐỀ HẠT NANO BẠC**Trần Thu Trang**

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THÔNG TIN BÀI BÁO		TÓM TẮT
Ngày nhận bài:	01/4/2024	Hạt nano bạc (Ag NPs) được tổng hợp và phân tích các đặc trưng bằng phương pháp hiển vi điện tử truyền qua (TEM), nhiễu xạ tia X (XRD), và phổ hấp thụ. Sử dụng công thức Debye-Scherrer và phổ XRD, kích thước hạt nano bạc được đánh giá khoảng 8 nm. Hạt nano bạc được sử dụng làm đề tăng cường tán xạ Raman (SERS) để phát hiện melamine. Cơ chế SERS trên đề Ag NPs phát hiện melamine được thảo luận và chỉ ra cơ chế SERS là do cơ chế tăng cường điện từ trường. Sự đóng góp của cơ chế hóa học trong tăng cường tín hiệu SERS có thể được bỏ vì không trong dải cộng hưởng truyền điện từ giữa Ag NPs và melamine. Khả năng hoạt động của đề SERS trên cơ sở Ag NPs để phát hiện melamine được nghiên cứu trong dải nồng độ từ 1.5×10^{-3} đến 10^{-7} M. Phổ Raman trên đề SERS của melamine chỉ ra độ nhạy cao với giới hạn phát hiện đạt 10^{-7} M, ứng với hệ số tăng cường 2×10^5 . Kết quả nghiên cứu chỉ ra sử dụng đề SERS trên cơ sở Ag NPs để phát hiện melamine có độ nhạy tương đối cao.
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Ngày đăng:	24/5/2024	
TỪ KHÓA		
Hạt nano bạc		
Melamine		
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1. Introduction

Melamine is a heterocyclic organic compound containing a triazine ring and high nitrogen content. It is widely used to produce amino resins, plastic, and fertilizers in the industry [1], [2]. Due to its low cost and high nitrogen content, it is often illegally added to dairy products for infants and many other food products. This action can cause a great hidden danger to people's food safety [3], [4]. Some different methods have been developed and explored to detect melamine in milk, such as gas chromatography [5], and liquid chromatography [6]. Surface-enhanced Raman scattering (SERS) is a powerful tool for ultrasensitive surface chemical analysis [7], [8]. Using the SERS technique to detect melamine has been implemented based on various surfaces, such as ZnO [9], and Au nanoparticle-decorated ZnO/ZnFe₂O₄ composites [10].

Noble metal materials have been widely explored as conventional materials for SERS thanks to advantages such as high sensitivity, stability, and reproducibility [11]. Surface plasmon resonance (SPR) is known to play a primary role in the SERS mechanism in metal materials. On the other hand, the chemical enhancement, which is well-known with the charge transfer mechanism, is a minor contribution to SERS activities. For metal nanoparticles, the plasmon resonance is localized near the surface of the particles, and it results in considerably larger enhancement through aggregates of two or more nanoparticles oscillating collectively [12]. The range of localized surface plasmon effects of silver nanoparticles has been extended throughout the visible and near-infrared of the spectrum, implying that the vibrating molecule located near a metal nanoparticle would be better coupled with the exciting light. It is attributed to the high activity of SERS surfaces based on metal nanoparticles. Silver nanoparticles (Ag NPs) have been used as an effective SERS surface for detecting various analytes [13], [14].

In this report, the SERS based on Ag NPs for probing melamine molecules was investigated. The SERS mechanism was indicated as having a significant role in surface plasmon resonance under incident laser excitation. The contribution of the charge transfer mechanism could be ignored due to the out-of-charge transfer resonance between the energy levels of the melamine molecule and Ag NPs. Furthermore, the SERS sensitivity and repeatability activities based on Ag NPs were also studied and presented the good behaviors with a limit of detection (LOD) of 10⁻⁷ M, which corresponds to the highest enhancement factor (EF) of 2.10⁵.

2. Materials and methods

2.1. Materials

Silver nitrate (AgNO₃, 99.92%) was used as a silver precursor; sodium borohydride (NaBH₄, 99.9%) acted as both reducing and capping agents. Trisodium citrate dihydrate (TSC, 99%) was also used as a reducing chemical; and polyvinyl pyrrolidone (PVP) was used as a stabilizer.

2.2. Synthesis of silver nanoparticles.

The colloidal silver nanoparticles (Ag NPs) were synthesized through the reduction process of the of the silver precursor AgNO₃ in the presence of NaBH₄. The synthesis of colloidal AgNPs began with a simple aqueous phase mixture of AgNO₃ and TSC, PVP, and NaBH₄ solutions [15]. Briefly, a mixture of 4 mL of AgNO₃ concentration of 0.02 M, 400 μl TSC 0.6 M, 200 μL PVP 0.03 M, and 400 μL deionized water was mixed and stirred at room temperature for 15 minutes. After that, 1600 μL of NaBH₄ was injected into the mixture. Finally, 400 μL of NaOH 1 M was added to the mixture. Then, the total solution was stirred for 15 minutes. The obtained solution was used to prepare the SERS surface to detect melamine.

2.3. Characterizations

The morphology of the obtained Ag NPs was examined using transmission electron microscopy (TEM). The crystallinity of Ag NPs was investigated by X-ray powder diffraction (XRD) using monochromated Cu-K_α radiation (wavelength of 0.154056 nm) on the Bruker D8 Advances diffractometer (Germany). The absorption property of Ag NPs was recorded using a UV-Vis spectrometer (Jasco V-770). The investigation of normal Raman and SERS spectra was implemented on a Raman spectrometer (Raman Horiba Xplora plus microprobe, France). A laser with a wavelength of 532 nm was used for laser excitation. The acquisition time for each measurement was kept identical at 8 seconds. The calibration for the spectrometer was done using a silicon wafer with the characteristic band at 520 cm⁻¹. The various concentrations of melamine were prepared by diluting melamine powder in aqueous. The five SERS substrates based on Ag NPs with concentrations of melamine ranging from 1.5×10⁻³ M to 10⁻⁷ M were investigated. At such melamine concentrations, SERS intensities showed linear changes with concentration, and it was also the lowest concentration that could be clearly detected. In practice, each SERS surface was prepared with 100 μL of Ag NPs and 15 μL of melamine at each concentration.

3. Results and discussion

3.1. Morphological study of Ag NPs

The shape of synthesized Ag NPs was imaged using TEM spectroscopy. Figure 1a shows the TEM image of Ag NPs that are well-separated and spherical in shape. The absorption spectrum of Ag NPs indicates a characteristic band at 397 nm (Figure 1b). It is noted that the localized surface plasmon band reveals the figure's size and shape. The absorption property of Ag NPs at about 397 nm has contributed to the size of ~ 8 nm [16].

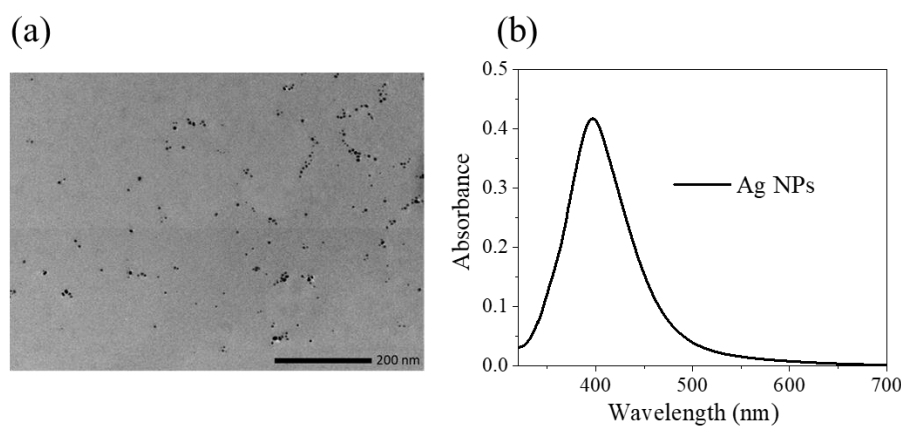


Figure 1. (a) TEM image and (b) absorption spectrum of Ag NPs

To gain a better understanding of the morphology of Ag NPs, the XRD spectrum of synthesized Ag NPs was measured. Figure 2a describes the XRD spectrum of Ag NPs. Five distinguishing peaks at 38.30^o, 44.35^o, 63.15^o, and 77.40^o correspond to the reflections of (111), (200), (220), and (311) Bragg crystal planes of the face-centered-cubic (FCC) Ag phase, respectively. It is consistent with the standard data file ICDD (International Centre for Diffraction Data) no. 01-071-3752. Furthermore, the diffraction peak positions can be used to determine the mean crystallite size of Ag NPs using Debye-Scherrer's formula:

$$D = k\lambda/(\beta\cos\theta) \quad (1)$$

In which, D is the size of Ag NPs, k is the shape factor, and for spherical NPs, it was taken of 0.89; λ is the X-ray wavelength for CuK_α; β is the line broadening; it is taken of the full width at half the maximum intensity (FWHM); and θ is the angle achieved from the 2θ value in the XRD pattern.

It has been found that peak broadening is inversely proportional to the size of the NPs [17]. In this study, the mean crystallite size of Ag NPs was estimated at approximately 8 nm. This is reasonable given the characteristic of the localized surface plasmon band at 397 nm of Ag NPs [18].

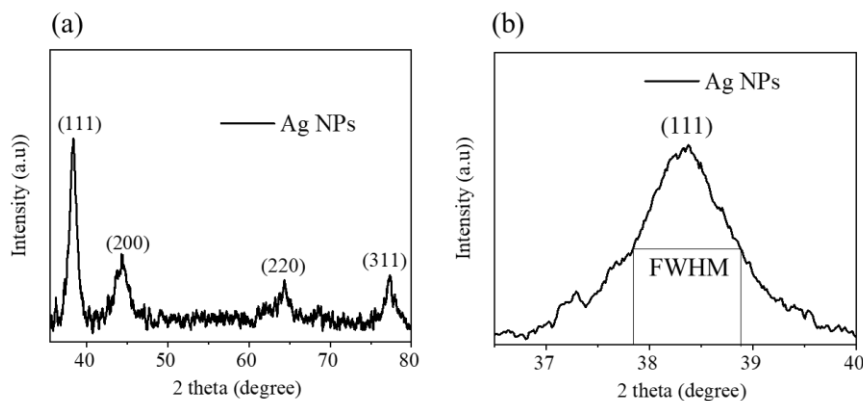


Figure 2. (a) XRD pattern of Ag NPs, and (b) zoom in diffraction peak (111) for estimation of crystalline size of Ag NPs

3.2. SERS study for the detection of melamine on Ag NPs surface

3.2.1. Study SERS mechanism based on Ag NPs surface in detection of melamine

Melamine molecules are classified into D_{3h} symmetry with a planar 1,3,5 s-triazine ring and three amino groups attached with each C atom. The normal vibration modes of melamine are as follows: $5A_1$ (Raman) + $4A_2$ (inactive) + $9E'$ (infrared/Raman) for the in-plane modes, and A_1'' (inactive) + $3A_2''$ (infrared) + $4E''$ (Raman) for the out-plane modes [19]. Due to the selection rules in the optical spectroscopies, the A_2 and A_1'' modes are absent. Figure 3 presents the SERS spectrum of melamine ($1.5 \times 10^{-3} M$) based on Ag NPs surface. The two most intense bands of melamine were observed at 678 cm^{-1} and 987 cm^{-1} , which have been assigned to the ring stretch and C-N_a stretch and are known as the totally symmetric line (A_1') [19, 20]. Two vibration modes at 630 and 1050 cm^{-1} could be attributed to the NH rocking and C-N_a stretch (E') [19]. For the Ag NPs-melamine system, it seems that s-triazine is attached to the surface of Ag NPs through a weak Ag – N bond and that the molecular plane is perpendicular to the Ag surface [12].

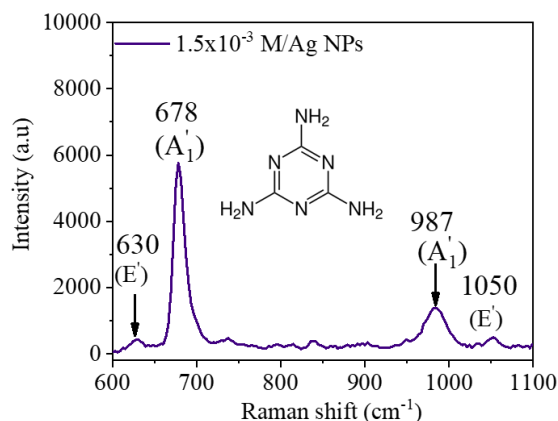


Figure 3. SERS spectrum of melamine $1.5 \times 10^{-3} M$ based on Ag NPs surface

Generally, the SERS mechanism is significantly contributed by electromagnetic enhancement causing the surface plasmon of the metal conduction band; and a lesser contribution stems from a chemical mechanism that was explained via the charge-transfer resonance mechanism [21]. To estimate the charge-transfer resonance mechanism, the relative energy level of Ag NPs and the

highest occupied molecular orbital (HOMO) – lowest unoccupied molecular orbital (LUMO) of melamine have to be involved. The HOMO and LUMO of melamine were located at 6.7 and 0.26 eV, respectively [22]. Likewise, the work function of Ag is 4.26 eV [23]; thus, the barrier for electron injection from the Fermi level of Ag to the LUMO of melamine is about 4 eV. It appears that under the excitation wavelength of 532 nm (2.3 eV), this energy is out of resonance with the charge transfer transition from the Ag Fermi level to the LUMO of melamine. Therefore, the charge-transfer resonance mechanism could be ignored for the contribution of the SERS mechanism to detect melamine based on Ag NPs. For Ag NPs, the plasmon resonance is localized near the surface of the particle. It is accepted that a vibrational molecule located near a metal nanoparticle will couple to the exciting light; and the polarizability tensor of the metal-molecule system will couple to the surface plasmon resonance field [12]. Thus, it should be deduced that SPR plays a significant role in the SERS mechanism based on the molecular-metal system Ag NPs and melamine.

3.2.2. Study SERS sensitivity based on Ag NPs in detecting melamine

To assess the sensitivity of the SERS activity of Ag NPs surface in detecting melamine, various concentrations of melamine (varying from 1.5×10^{-3} M to 10^{-7} M) were prepared. The SERS performance of melamine based on Ag NPs is presented in Figure 5. At a lower concentration of 10^{-7} M, the SERS spectra cannot be achieved in discrepancy peaks. Thus, the limit of detection (LOD) of melamine based on Ag NPs surface is as low as 10^{-7} M. This is a good LOD for detecting melamine in comparison with the previous studies [10], [24]. Figure 4b shows a linear relationship between the logarithm of the concentration of melamine and intensity.

To assess the sensitivity of detecting melamine based on the surface of Ag NPs surface, the enhancement factor (EF) was estimated using the peak at 678 cm^{-1} following the formula [25]:

$$EF = \left(\frac{I_{SERS}}{I_{nor}} \right) \times \left(\frac{C_{nor}}{C_{SERS}} \right) \quad (2)$$

in which I_{SERS} and I_{nor} are the SERS intensity of melamine adsorbed on Ag NPs and the normal Raman intensity of melamine on the glass substrates, respectively; C_{SERS} and C_{nor} are the concentrations of the melamine on the Ag NPs surface and that on a glass substrate, respectively. Melamine concentrations ranging from 1.5×10^{-3} to 10^{-7} M resulted in EF values of 2.2×10^3 to 2×10^5 . In comparison with the SERS activity in detecting melamine of other substrates, such as silver nanodecahedra [26], silver nanoparticles, and silver nanoparticle-coated HfO_2 [24], this study indicates an advantage in SERS activity for probing melamine.

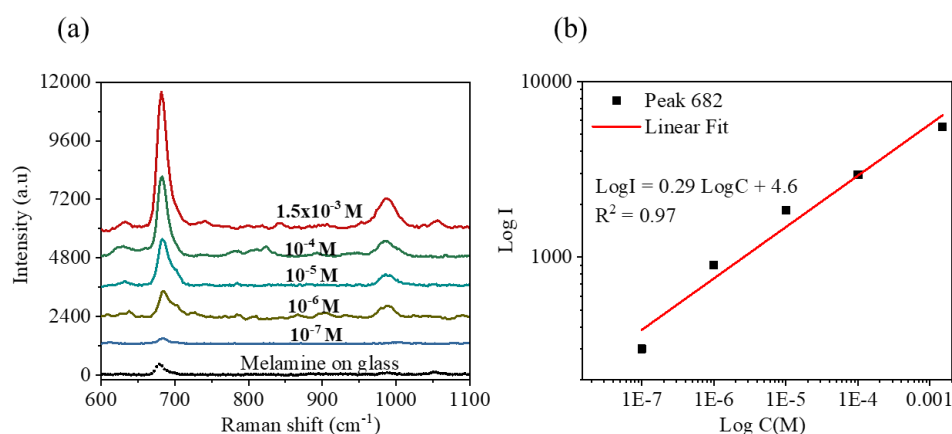


Figure 4. (a) SERS spectra of melamine adsorbed on Ag NPs surfaces with different concentrations ranging from 1.5×10^{-3} M to 10^{-7} M, using the excitation wavelength at 532 nm; and (b) the linear relationship between the log I of the 682 peak and the logarithm of melamine concentration

In addition to the good sensitivity, the repeatability of the SERS substrate based on Ag NPs was also studied by recording the SERS spectra of melamine at concentrations of 10^{-7} M from 5 random points on the surface. Figure 5 describes the SERS spectra of melamine (10^{-7} M), which were implemented at different positions on the substrate. The vibration modes of melamine indicate no shift, and their intensity is high, with the relative standard deviation of the signal at 678 cm^{-1} being about 14%. Thus, the results prove that the SERS activity based on Ag NPs surface could be a good candidate for detecting melamine.

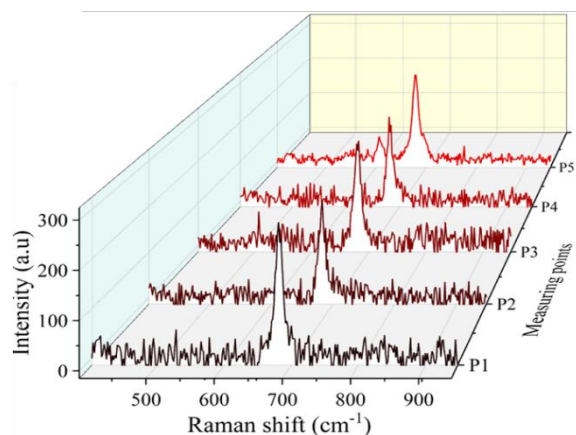


Figure 5. SERS spectra of melamine (10^{-7} M) based on Ag NPs surfaces from 5 random positions

4. Conclusion

In conclusion, Ag NPs surfaces has successfully been synthesized and used to study SERS activity in probing melamine. The SERS mechanism based on Ag NPs in detecting melamine was indicated due to electromagnetic enhancement through the plasmon resonance of metal conduction band of Ag NPs. The charge transfer resonance between Ag NPs and melamine molecule might be ignored. Furthermore, the sensitivity and repeatability of the SERS surfaces were investigated and presented a high performance of SERS behaviour with the LOD of 10^{-7} M corresponding EF of 2×10^5 .

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