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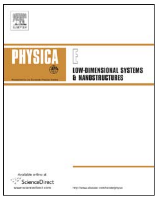

Quantitative determination of melamine in milk using Ag nanoparticle monolayer film as SERS substrate

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ABSTRACT

A Raman method employing silver nanoparticle (Ag NP) monolayer film as Surface-enhanced Raman Scattering (SERS) substrate was presented to rapidly detect melamine in milk. The Ag NPs with 80 nm diameter were modified by polyvinylpyrrolidone to improve their uniformity and chemical stability. The treatment procedure of liquid milk required only addition of acetic acid and centrifugation, and required time is less than 15 min. The Ag NP monolayer film significantly enhanced Raman signal from melamine and allowed experimentally reproducible determination of the melamine concentration. A good linear relationship (R^2=0.994) between the concentration and Raman peak intensity of melamine at 681 cm^{-1} was obtained for melamine concentrations between 0.10 mg L^{-1} and 5.00 mg L^{-1}. This implies that this method can detect melamine concentrations below 1.0 mg L^{-1}, the concentration currently considered unsafe.

1. Introduction

Melamine (2,4,6-triamino-1,3,5-triazine), as a cheap, water soluble compound and containing many nitrogen atoms, has been intentionally added to dairy products to fraudulently increase the apparent protein content [1,2]. In addition, because melamine is widely used in plastics and coatings industry, it may interfuse in food during food production, processing, packaging or transportation [3–5]. In many countries, melamine has been forbidden or restricted to add into food because it has been proven to cause renal failure [6–8]. For example, the Codex Alimentarius Commission (CAC) has restricted the maximum residue levels of melamine to 1.0 mg L^{-1} in liquid baby formula, and 2.5 mg L^{-1} in other milk-based foods and animal feeds (The Food Standards Programme of 23rd Session of the Joint FAO/WHO, 2008). Therefore, simple, highly efficient and reliable approaches are urgently needed to detect melamine quantitatively in the field of food safety. Melamine detection in milk is challenging because some milk ingredients are difficult to separate from melamine. Gas chromatography (GC) or high performance liquid chromatography (HPLC) coupled with other devices can meet the requirements of detecting sensitivity and quantification analysis. Unfortunately, they require time-consuming sample treatment processes, experienced operators, complex data analysis and expensive instruments [9–13]. These are all important factors which hinder the application of these methods in routine testing of foods for melamine.

Surface-enhanced Raman Scattering (SERS) technique is a powerful tool to detect trace analytes since it can provide “fingerprint information” of molecules at the single-molecule level [14–16]. SERS requires substrates containing “hot spots,” regions with high electromagnetic enhancement in the junctions between metallic nanostructures [17–19]. Although melamine detection by SERS has been reported [20–24], quantitative detection is rare and previously published methods are not adequate for routine testing of foods. In addition, most of the SERS substrates reported were Au nanostructure materials. Among all SERS substrates, Ag nanostructures have the best surface plasmon resonance properties in the visible region, which makes detection of trace melamine easier. Previously studied [25,26], Ag nanostructures possess low chemical stability. They also suffer from low reproducibility of signal enhancement level due to random and ununiform distribution of “hot spots” on Ag substrates. As a result, the practical application of the SERS technique with Ag nanostructures is limited. Here we show that appropriately synthesized, uniform Ag NP monolayer films result in Ag substrates with reproducible enhancement.

We studied Ag NPs modified by polyvinylpyrrolidone (PVP) and made into a monolayer film as SERS substrate. PVP modified Ag NPs are more stable and uniform than unmodified Ag NPs. A compact monolayer film was prepared using the modified Ag NPs, which improved the intensity and reproducibility of melamine signal, which is the prerequisite for quantitative detection with SERS. In addition, we also simplified the treatment process of milk samples, and only one substance, acetic acid was used. Compared with other methods or other

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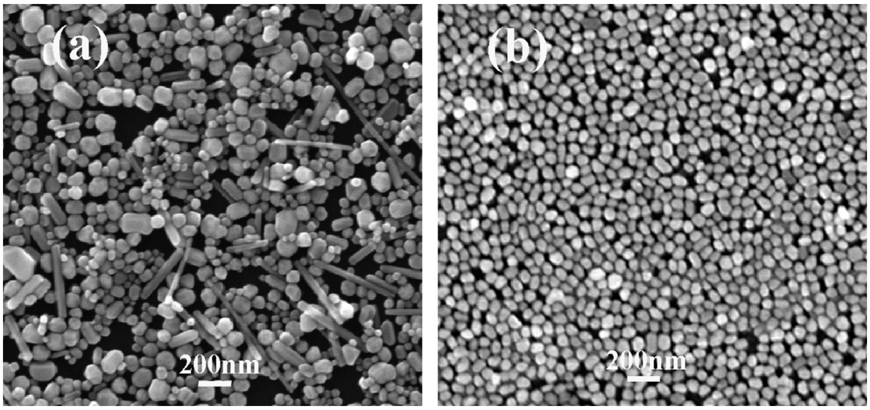

Fig. 1. The SEM images of Ag NP monolayer film. (a) unmodified Ag NPs; (b) PVP modified Ag NPs.

substances of treating milk [20,22,24], acetic acid is easily obtained and is eco-friendly. More importantly, the treatment time of milk samples was only less than 15 min, which is much shorter than other above methods.

2. Materials and methods

2.1. Reagents and materials

Silver nitrate ($AgNO_3$, 99.99%), sodium citrate (≥99.0%) and melamine (≥99%) were purchased from Aladdin. PVP (K30, GR) was obtained from Chinese Medicine Group Chemical Reagent Co., Ltd. Acetic acid (≥99.5%), cyclohexane (≥99.5%), and ethanol (≥99.7%) were purchased from Tianjin Fuyu Fine Chemical Co., Ltd in China. All reagents in our experiments are commercially available and were used without further purification. All solutions were prepared with deionized water (18 M Ω cm). Liquid milk was obtained from a local dairy farm.

2.2. Preparation of the Ag NP monolayer films

Ag NPs were synthesized using the method of Lee et al. [27], and PVP was introduced into the synthesis process to improve the stability and uniformity of Ag NPs. We prepared 80 nm Ag NPs. 4 ml sodium citrate solution (1 wt%) was rapidly injected into a round-bottom flask containing 200 ml boiling aqueous solution of $AgNO_3$ (0.017 wt%) and PVP (1.098 wt%). The mixture was stirred for 1 h while boiling. It was then cooled to room temperature, and centrifuged. In order to avoid signal interference from PVP in following SERS measurements, the sedimented Ag NPs were washed first in ethanol to remove the residual unbounded PVP molecules and then dissolved in deionized water to obtain the molar concentration before centrifugation.

The preparation of Ag NP monolayer film was based on Li et al. [28], and toluene was replaced by more eco-friendly cyclohexane in the preparation process. The above treated Ag NP colloid was poured into a beaker containing cyclohexane and gently stirred. Then ethanol was added into the above mixture and stirred gently for 30 s. The volume ratio of the Ag colloid, ethanol and cyclohexane was 3:2:1. The Ag NP monolayer film would occur between the interface of cyclohexane and water. After removing the cyclohexane supernatant with a syringe, Ag NP monolayer film was extracted on hydrophilic silicon wafer. After drying, the wafer was immersed in ethanol for at least 2 h to remove the residual cyclohexane, then dried again to obtain the clear monolayer film of Ag NPs.

2.3. The treatment of the milk samples

A concentrated aqueous solution of melamine, 500 mg L^{-1}, was prepared and added to liquid milk to obtain “spiked milk” samples with melamine concentrations of 5.00, 3.50, 2.50, and 0.50 mg L^{-1}, respectively. Dilute aqueous solutions with concentrations of 5.00, 3.50, 2.50, 1.00 and 0.10 mg L^{-1}, respectively were also prepared. The spiked milk was then treated to remove some components of the milk. Acetic acid (10 μL) was mixed with the 10 ml spiked milk samples. The resulting solution was stirred vigorously and allowed to stand for 2 min until the mixture stratified. The supernatant “whey” was extracted and centrifuged (~15000 r/min, ~10 min). The supernatant from the centrifugation was collected and additional milk solids “curds” were removed by filtration using 0.22 μm disposable pinhole filters. 10 μL of the filtrate was dropped on the Ag NP monolayer film. Samples used for standards, were also prepared, replacing the filtered supernatant with a dilute aqueous solutions of melamine. These films with added analyte were then dried for about 5 min at 60–80 °C.

3. Results and discussion

3.1. Characterization of as-prepared Ag NP monolayer film

In view of superior plasmon properties of Ag nanosturctures, Ag NPs were chosen as SERS substrate material in our work. Fig. 1(b) shows a scanning electron microscope (SEM) image of Ag NP film prepared using PVP. The average size of the PVP modified Ag NPs is about 80 nm. The closely-packed Ag NPs are distributed reasonably uniformly on the silicon wafer, and the average distance between neighbouring NPs was 2–3 nm. For comparison, a SEM image of Ag NPs without PVP modification is shown in Fig. 1(a). It is evident that the use of PVP increases the uniformity of Ag NPs. The Ag NPs in Fig. 1(a) have complex morphologies, which cause a large range of random distribution of “hot spots”, accordingly, quantitative measurement of analytes would not be realized using them as the SERS substrate.

3.2. Local electric field distribution between Ag NPs was simulated by finite-difference time-domain (FDTD) method

Light from a laser with appropriate wavelength can resonantly excite the collective oscillation of free electrons in metals. This phenomenon is called surface plasmon resonance of metals. The property of noble metals can dramatically enhance the local electric field on the surfaces of metal nanostructures. According to Raman theory [29], the SERS enhancement factor *EF* is proportional to the