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Surface-enhanced Raman spectroscopy Detection of Organic Molecules and *in situ* Monitoring of Organic Reactions by Ion-induced Silver Nanoparticle Clusters†

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Abstract

Surface-enhanced Raman spectroscopy (SERS) finds wide applications in the field of organic molecule detection. However, reliable SERS detection of organic molecules and in-situ monitoring of organic reactions under natural conditions by metal colloids are still challenging due to unstable formation of nanoparticle clusters in solution as well as low solubility of the organic molecules. Here, we approach the problems by introducing calcium ions to aggregate silver nanoparticles to form stable hot spots and acetone to promote uniform distribution of organic molecules on the nanoparticle surface. Significantly, our method exhibits stable SERS detection of up to 6 types of organic molecules in liquid. With acetone signals as an internal standard, we are able to determine molecule concentrations as well as monitor 3 kinds of organic reactions in situ. Our method shows potential for biomedical analysis, environmental analysis, and organic catalysis research.

Introduction

Hydrocarbons and their derivatives are often referred to as organic compounds, which are known to play an important role in pharmaceutical [1] and medical research [2], as well as the development of solar cells and polymer materials [3]. Researchers have developed various label-free methods of organic molecule detection, such as nuclear magnetic resonance spectroscopy (NMR) [4,5], UV-visible absorption spectroscopy (UV-Vis) [6,7], mass spectrometry (MS) [8], molecularly imprinted polymers (MIPs) [9], electrochemically modified electrodes [10,11], and infrared spectroscopy (IR) 12. However, most of these methods are complex and have several limitations. For example, In IR, water molecules and other organic solvents exhibit strong infrared adsorption that presents a high noise background. Therefore, IR is not the technique of choice to detect the organic molecules in the solution [12]. Therefore, it is necessary to develop an efficient, highly sensitive, economical, and simple method for the detection of organic molecules.

Compared with the traditional methods mentioned above, surface enhanced Raman spectroscopy (SERS) offers high sensitivity and accuracy, which can provide fingerprint without the interference of water molecules. SERS detection of molecules relies on plasmonic metal nanostructures that, upon resonant laser excitation, exhibit intensively enhanced electromagnetic field on their surface, termed “hot spot”. When the molecules of interest are adsorbed in the hot spot, they would be excited to emit strong SERS signals to provide vibration fingerprint of the unique molecular structure [13-18].

While analyzing organic molecules, SERS technology exhibits the following characteristics: First, SERS technology can perform non-destructive rapid detection of samples [19]. It has a simple process of sample preparation, and organic molecules can exist either in solution or in a solid state. Second, SERS detection has extremely high sensitivity up to single-molecule level [20,21,22]. For different molecules and enhancement materials, enhancement factor of SERS lies in the range of 10^2 to 10^{14}. At the same time, since the enhancing substrate is generally a rough metal surface [23], it provides large surface to absorb and enrich the molecules of interest to allow study of behavior of organic molecules on the interface. Third, as a complementary method of IR, Raman spectroscopy possesses similar spectral library [24]. Each organic functional group has its own characteristic Raman peak position, which is beneficial for the analysis of organic molecules and the monitoring of organic reactions.

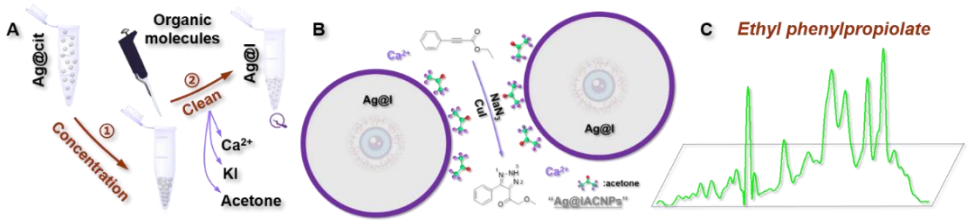
Although the SERS method has several advantages, it is extremely difficult to apply SERS to the detection of organic molecules in solution. The main challenge involves efficient molecular manipulation that captures the molecules in solution and place them into the hot spots for SERS detection. SERS system of metal colloids mostly rely on plasmonic metal nanoparticles in solution that can adsorb molecules in solution on their surface and form different clusters stochastically and instantly in light cone of an excitation laser [25]. The unpredictable nanogaps in the clusters lead to non-uniform distribution of hot spots, which result in irreproducible SERS signals of the adsorbed molecules and hamper reliable SERS analysis. On the other hand, solid-state SERS substrates have uniform distribution of hot spots. But the hot spots are usually small nanogaps that prevent molecules in solution from diffusing into them [26].

Additional methods of molecular manipulation are needed to integrate with the SERS substrates, complicating the fabrication and leading to extra background noise. For example, Shoji et al. used a poly-N-isopropylacrylamide (PNIPAM) micro-assembly to optically trap organic molecules in aqueous solutions [27]. Therefore, obtaining stable SERS signals of organic molecules and monitoring organic reactions in situ under natural conditions in solution are still challenging.

Recently, we have developed an ion-induced aggregation method to construct silver nanoparticle clusters (Ag@I) with controllable hot spots in solution for reproducible SERS detection of biomolecules [28,29]. The method was to coat a layer of iodide ion on the surface of the chemically synthesized silver nanoparticles, which not only cleaned the surface of the nanoparticles and changed the interface structure of the nanoparticles, but also avoided chemical interaction between the metal surface and the organic molecules.

In this study, we extended this method to detecting organic molecules in solution by using calcium ions, Ca^{2+}, as the aggregation agent and acetone as modifier and internal standard, termed Ag@IACNPs, to analyze different kinds of organic molecules as well as monitor the progress of organic reactions, as shown in Schematic 1. The working principle consists of 3 elements: (1) Iodine ions can replace citrate ions on the surface of silver nanoparticles, that is Ag@I, ensuring that the obtained sample signal is not interfered by citrate ions[30]; (2) Acetone not only increases the solubility of organic molecules in the enhanced substrate, but also promotes the uniform distribution of organic molecules in the system, which becomes Ag@IA. (3) Finally, Ca^{2+} ions are introduced to induce the aggregation of silver nanoparticles to form "hot spots", leading to Ag@IAC nanoparticles (Ag@IACNPs). The organic molecules to be tested are squeezed into the "hot spots" of modified silver nanoparticles, which can generate good SERS signals.

Compared with the common metal cationic aggregators (Al^{3+}, Mg^{2+}, etc.), the addition of Ca^{2+}



Schematic 1. A. The preparation flow chart of Ag@IACNPs: 1) silver nanoparticles modified by citrate (Ag@cit) was incubated with iodide ions, and acetone was added after centrifugation. 2) After silver nanoparticles modified by iodide ions (Ag@I) became stable, the organic sample to be tested was added, followed by the addition of Ca^{2+} ions as aggregation agent. Finally, a capillary glass tube was used to draw the sample for measurement by a Raman spectrometer. B. The schematic diagram of the hot spots in the formation of the enhanced substrate Ag@IACNPs; C. The SERS spectrum of ethyl phenylpropiolate obtained on Ag@IACNPs.

reduced the strong electrostatic interaction between the cation and the enhanced substrate (Figure S1), which was beneficial for the uncharged organic molecules to enter the hot spot and obtain enhanced Raman signals[31,32]. Due to the surface activity of acetone, the addition of acetone into the system increased the solubility of organic molecules in the enhanced substrate, promoted the uniform distribution of the organic molecules in the system, resulting in the generation of the SERS signals with good reproducibility. We used this method to perform SERS analysis on a variety of typical organic molecules (aliphatic, aromatic, alcohols, carboxylic acid, aldehyde, esters), and all molecules showed unique fingerprint peaks. Additionally, we also used the acetone signal as an internal standard to monitor the progress of three classical organic reactions using this method. This method offers broad application prospects in the fields of medical diagnosis and organic reaction mechanism research.

Results and discussion

Some organic molecules are known to have low solubility in water, and their Raman signals are very weak at low concentrations. Generally, silver nanoparticles synthesized in the water phase are used as the enhanced substrates. It is difficult to form hot spots in the organic phase, which inhibits the enhancement of the Raman signals of the organic molecules. Figure 1A (red line) shows a Raman spectrum of 5 mM ethyl phenylpropiolate in acetone where no Raman signal were observed due to low concentration. Next, silver nanoparticles modified with iodide ions were added to the system, which resulted in the appearance of some weak solvent signals (Figure 1A, blue line); however, the signal of ethyl phenylpropiolate was still not visible. When acetone and Ca^{2+} ions were introduced (Figure 1A, green line), we observed an incredibly enhanced Raman signal of ethyl phenylpropiolate, which was almost the same as the Raman signal of its pure sample (liquid) and had extremely high signal-to-noise ratio. The stability of the method was also high that 15 sets of random SERS spectra of ethyl phenylpropiolate were reproducible (Figure S2). It is worth noting that acetone was introduced as a solvent to dissolve ethyl phenylpropiolate in the system. Moreover, the amount of acetone in the system was much higher than that of the test sample, but the signal of the solvent acetone did not interfere with the signal of the test sample. Furthermore, the enhanced substrate effectively suppressed the peak position of the acetone and selectively enhanced the SERS signal of the organic molecule. To further verify this feature of the new enhanced substrate, we tested a variety of typical organic molecules (toluene, acetic acid,

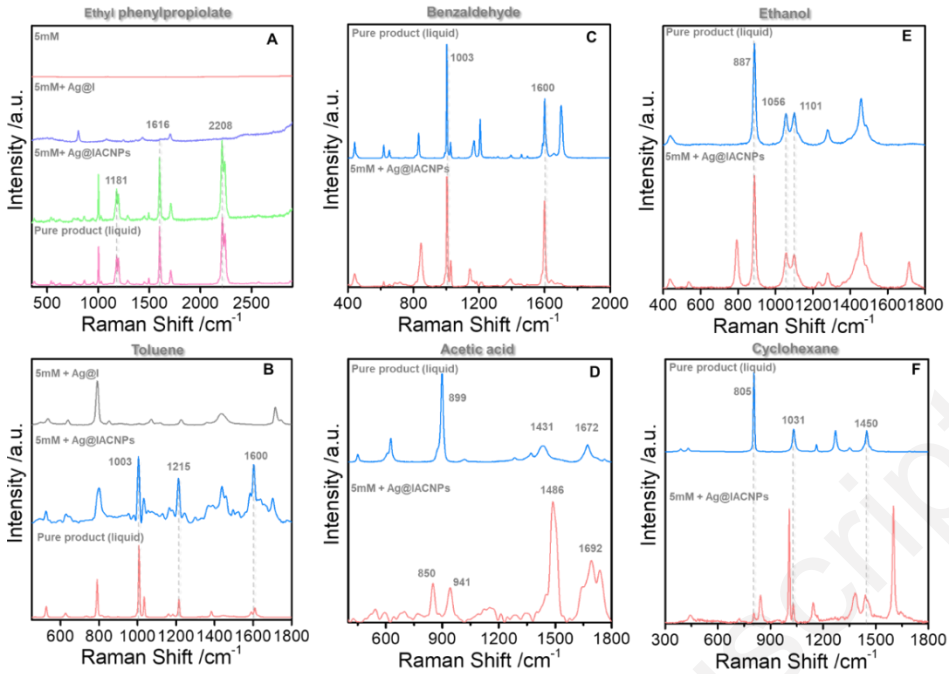


Figure 1. A: Raman spectra of ethyl phenylpropiolate under various conditions; B: Raman spectra of toluene under various conditions: Raman spectrum (red line), SERS spectra (blue line and gray line). SERS spectra of benzaldehyde (C), acetic acid (D), ethanol (E), and cyclohexane (F). The blue line represents the Raman spectra of the above elements, and the red line is the SERS spectra obtained by the current method.

cyclohexane, and benzaldehyde). Figure 1B-F show the SERS spectra of these organic molecules and the Raman spectra of their pure products (liquid). In toluene, we found that the Raman signal of the mixture was mainly acetone signal in 5mM acetone solution (Figure 1B, gray line), but when the above-mentioned enhanced substrate was added, the SERS signal of toluene itself was observed (Figure 1B, blue line), and its peak position was consistent with the Raman spectrum of the elemental sample (Figure 1B, red line). Almost all organic molecules showed similar experimental results (see SI for detailed spectra and peak assignments), which proved that our method did selectively enhance the Raman signal of organic molecules.

For quantitative SERS by our method, we selected acetone as the internal standard to analyze the organic molecules at different concentrations. Figure 2A shows the SERS spectra obtained for

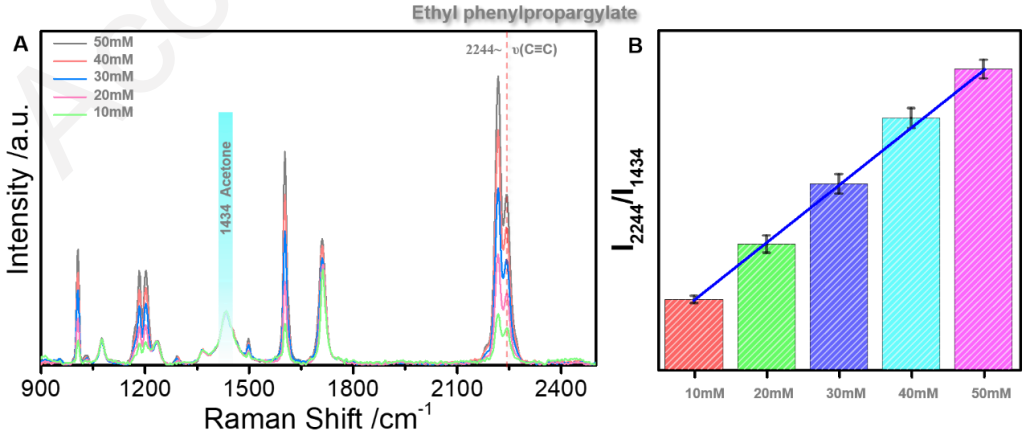


Figure 2. A: SERS spectra obtained for ethyl phenylpropiolate at different concentrations (10-50 mM; concentration difference is 10 mM) normalized to the aceton peak at 1434 cm^{-1}. B: The bar graph of I_{2244}/I_{1434} against the change in the concentration of ethyl phenylpropiolate.

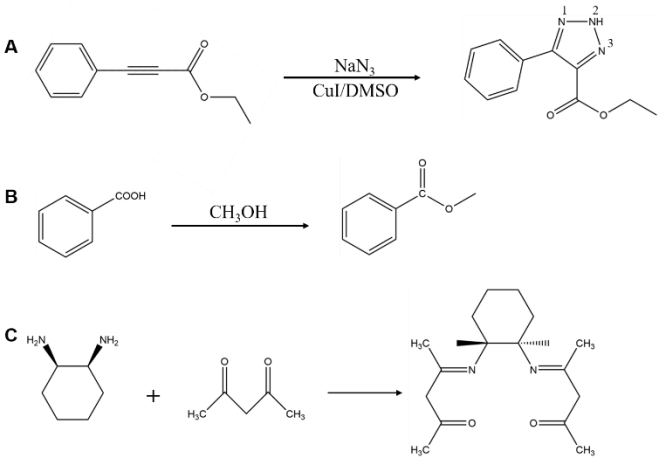


Figure 3. Organic reactions being detected by the method. A: Click reaction; B: Esterification reaction; C: Schiff base reaction.

five functional groups of ethyl phenylpropiolate (2244, 2218 cm^{-1} υ(C≡C), 1709 cm^{-1} υ(C=O), 1602, 1497, 1433 cm^{-1} δbk(ring), 1201, 1182cm^{-1} υ(C-O-C)) using the current method. The spectra were obtained by normalizing the acetone signal at 1434 cm^{-1}. With an increase in the sample concentration, an increase in the relative intensity of the sample signal was observed. We used the ratio of 2244 cm^{-1} in the signal of ethyl phenylpropiolate to the signal at 1434 cm^{-1} of acetone to plot the concentration of ethyl phenylpropiolate in the mixture, which resulted in a linear relationship (Figure 2B). The threshold of each error bar was much smaller than the threshold needed to distinguish the difference in concentration. Thus, this method could be used to detect the concentration of organic molecules in the solution, which would facilitate the application of SERS spectroscopy in the fields of food safety, pesticide residues, and organic reaction monitoring.

With high sensitivity and stability, we used this method to monitor the progress of several classical organic reactions: Click reaction, Esterification reaction and Schiff base reaction (Figure 3). Figure 4A shows the SERS monitoring chart of the click reaction. The gray line represents the SERS spectrum of a mixture of ethyl phenylpropiolate and dimethyl sulfoxide (DMSO) at the beginning of the reaction. The characteristic peak positions of ethyl phenylpropiolate (2208 cm^{-1} υ(C≡C), 1709 cm^{-1} υ(C=O), 1616 cm^{-1} δ(ring)) were clearly observed. The red line shows the SERS spectrum of the

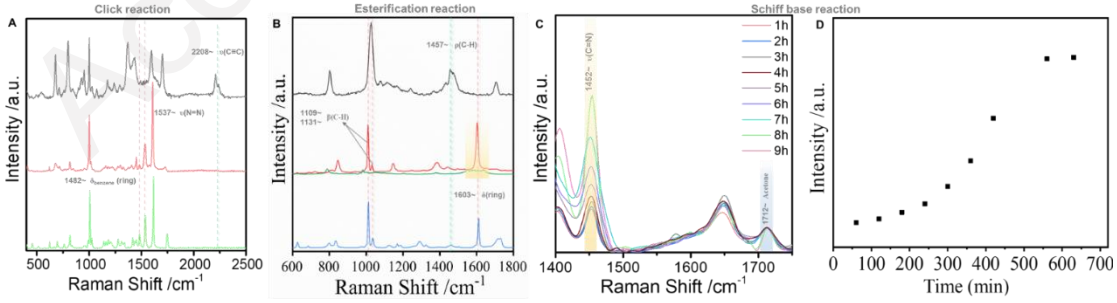


Figure 4. A: SERS spectrum for monitoring the click reaction: the black line shows the SERS spectrum of the sample taken from the system before the addition of sodium azide; the red line shows the SERS spectrum of the sample taken from the system two minutes after the addition of sodium azide; The green line shows the SERS spectrum of the pure triazole product. B: SERS spectrum for monitoring the esterification reaction: the black line shows the SERS spectrum of the sample taken from the system before the addition of benzoic acid; the red line shows the SERS spectrum of the sample taken from the system two minutes after the addition of sodium benzoate; the blue line shows the SERS spectrum of pure methyl benzoate. C: SERS spectrum for monitoring the Schiff base reaction; D: Time-dependent Raman intensity at 1452 cm^{-1} of the Schiff base reaction.

mixture 2 min after the addition of sodium azide. The 1,2,3 -triazole peak (1482 cm^{-1},1537 cm^{-1}) was clearly visible. The green line shows the SERS spectrum of the pure triazole sample. Notably, the signal of the mixture was almost dominated by triazole. This could be due to the nature of the click reaction: the reaction proceeded relatively quickly at the beginning. Therefore, this method could objectively reflect the progress of organic reactions. Figure 4B shows the SERS monitoring chart of the esterification reaction. The gray line represents the SERS spectrum of the mixture of potassium bisulfate and methanol at the beginning of the reaction. The characteristic peak positions of methyl (1457 cm^{-1}) were clearly visible. The red line represents the SERS spectrum of the mixture 1 h after the start of the reaction, and after the addition of benzoic acid, the characteristic peaks of methyl benzoate (1603 cm^{-1} δ(ring), 1131,1109 cm^{-1} β(C-H)) were clearly visible (the blue line represents the SERS spectrum of the pure methyl benzoate sample). Using esterification reaction as an example, we compared the SERS signal to decide whether to add enhanced substrate or not. The signal-to-noise ratio of the red line and green lines were very different. Unlike the click reaction, the rate of the esterification reaction was relatively slow. We monitored the SERS spectra of the system 1 h and 2 h after the reaction started (Figure S3). Using the acetone peak intensity for normalization, we found that the peak intensity of methyl benzoate was still very weak after 1 h of the reaction, and a significant increase in peak intensity was observed after 2 h of reaction.

SERS monitoring spectrum of another classic organic reaction: Schiff base reaction (Figure 4C). We monitored the reaction throughout the entire process (Figure 4D). From the beginning of the reaction, the mixture samples were detected by SERS every hour, and the obtained spectrum was normalized using the acetone peak (1712cm^{-1} υ(C=O)). With an increase in the reaction time, we observed a significant increase in the SERS signal of the product Schiff base (1452 cm^{-1} assign to υ(C=N)); it is worth noting that the increase in the peak intensity of the product was not uniform. As a demonstration of real-time monitoring of the chemical reaction, the time dependent Raman peak intensity at 1452 cm^{-1} is displayed in Figure 4D. The peak intensity of the product increased slowly within 1-4 h from the beginning of the reaction, and then the signal intensity increased significantly (4-8 h). At the end of the reaction (8-9 h), there was almost no change in the SERS signal of the product, which was consistent with the objective law of organic reactions (see SI for the SERS spectra of the reactants). This method used the characteristic fingerprint information of organic molecules to comprehensively analyze the organic reaction process, and thus, could be used for obtaining the information regarding the intermediates involved in the organic reaction, resulting in an improved understanding of the mechanism of the organic reaction process.

Conclusions

To summarize, we developed a new label-free method for the detection of organic molecules to monitor organic reactions in situ, using Ca^{2+} ions as the aggregating agent to obtain the stable hot spot, the SERS signal of low concentration organic molecules could be sensitively captured. The introduction of acetone enhanced the dispersion of the organic molecule in the substrate system and increased the solubility of the organic molecules while obtaining a reproducible SERS signal. This method detected various types of classical organic molecules indiscriminately under identical conditions and obtained the characteristic peaks of the basic functional groups. Using acetone as

an internal standard, we were able to identify the content of organic molecules with a low-concentration difference and monitor organic reactions in situ under natural conditions. This method would promote the application of SERS in the field of detection of organic molecules and help to analyze the life processes involved in organic molecules. These above works are being carried out in our laboratory.

Experimental

Schematic 1. shows the specific experimental process of using Ca^{2+} ions and acetone to improve SERS hot spots. Silver sol (0.45 mL) was incubated with iodide ions after centrifugation, and 100 μL of acetone was added. After the system became stable, the organic sample (1 μL) to be tested was added, followed by the addition of 2 μL of Ca^{2+} ions. Next, use a capillary glass tube to draw the sample and test the Raman spectroscopy. The silver nanoparticles (AgNPs) were synthesized following the Lee [33] method, then I- ions, acetone and Ca^{2+} ions were added to form Ag@IACNPs. The silver sol of the system before adding Ca^{2+} ions is green, and the system turns red after adding Ca^{2+} ions. Transmission electron microscopy (TEM, Figure S1) and dynamic light scattering (DLS, Figure S2) were used to characterize the silver sol before and after adding Ca^{2+} ions and organic molecule, which proved the aggregation of silver nanoparticles. The TEM test instrument was manufactured made by JEM-2100(UHR) (Japan) the DLS test instrument was manufactured made by Beckman Coulter DelsaNanoC (America) and the SERS test instrument was manufactured by WITec alpha 300R (Germany). The specific experimental procedure is detailed in ESI.

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Conflicts of interest

There are no conflicts to declare.

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