Controlled Citrate Oxidation on Gold Nanoparticle Surfaces for Improved Surface-enhanced Raman Spectroscopic Analysis of Low-affinity Organic Micropollutants

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ABSTRACT

Surface-enhanced Raman spectroscopy (SERS) provides an ultrasensitive, fast, and inexpensive method for organic micropollutant analysis, but its applications are limited by the low affinity of most organic micropollutants towards plasmonic nanoparticle surfaces. Particularly, the citrate layer on gold nanoparticle (AuNP) surfaces exerts strong resistance to ligand exchange and prevents carboxylic and phenolic pollutants from entering SERS "hot spots". In this study, we aim to extend the application of SERS to low-affinity organic micropollutants by oxidative decomposition of citrate layer on AuNP surfaces. The kinetics of citrate oxidation were carefully controlled using sulfate radicals that were slowly released from peroxydisulfate photolysis, which guarantees both the stability of AuNP colloid and generation of a high density of SERS "hot spots" for pollutant analysis. *In situ* Raman spectroscopic monitoring demonstrates that citrate is first oxidized to di- and monocarboxylate acids and subsequently displaced by guest ligands. This oxidation-induced ligand exchange has been applied for SERS analysis of various low-affinity organic micropollutants, including monochloro-substituted carboxylates and phenols, as well as a widely used herbicide – 2.4-dichlorophenoxyacetic acid. This study substantially broadens the library of organic micropollutants for label-free SERS analysis and advances SERS towards a holistic analytical tool for water quality monitoring.

KEYWORDS

Surface-enhanced Raman spectroscopy, Citrate, Chlorobenzoic acid, Chlorophenol, Sulfate radical

INTRODUCTION

As a tremendous amount of organic chemicals are manufactured and discharged into aquatic environments at an increasing rate, an emerging concern has been raised that people might be chronically exposed to these trace-level compounds (i.e., organic micropollutants) via drinking water.[1-3] It has been reported that many recalcitrant organic micropollutants can survive multiple wastewater treatment processes and eventually make their way into drinking water supplies.[4, 5] Therefore, there is an urgent demand to identify and quantify different groups of organic micropollutants in drinking water on a large scale and at a high frequency, and thus minimize human exposure. However, the most frequently used methods for organic micropollutant quantification, e.g., liquid chromatography – tandem mass spectrometry (LC-MS/MS), require tedious sample pretreatment, addition of isotopic internal standards, as well as expensive instrumentation operated by well-trained personnel. Because of the high cost and long turnover time, these methods are unlikely to meet the growing demand for rapid and inexpensive monitoring of organic micropollutants in natural and engineered water systems.

Raman spectroscopy provides vibrational and rotational fingerprints of chemicals and thus a powerful tool for pollutant identification.[6, 7] Each Raman spectrum can be collected within one second, which can potentially be developed into a real-time water monitoring tool. Moreover, Raman spectroscopy relies on light for chemical information collection and transmission, so water samples do not contact the optical and electronic elements inside the Raman spectrometer and thus do not require extensive sample purification. However, Raman spectroscopy exhibits low sensitivity when applied for pollutant detection in water because of the low production efficiency of Raman scattering (about one out of one million scattering events). Surface-enhanced Raman spectroscopy (SERS) can substantially amplify the inherently weak Raman signals (up to 10^{11}

times) and improve the sensitivity for chemical analysis in water.[8-10] The strong interactions between incident light at specific wavelengths and plasmonic nanoparticles result in coherent oscillation of conduction electrons, which is known as localized surface plasmon resonance (LSPR).[11, 12] LSPR gives rise to a significantly enhanced electromagnetic field within nanoscale proximity of nanoparticle surfaces, which enhances the Raman cross-sections of the chemicals that are located within this zone.[13-15] Although SERS has regularly demonstrated single-molecular sensitivity in organic chemical analysis,[16-18] it remains a great challenge to extend this extraordinary sensitivity to a broader spectrum of organic micropollutants in water.[19, 20]

One of the biggest limitations for SERS applications in water pollutant analysis is the low affinity of most organic micropollutants towards plasmonic nanoparticle surfaces. Citrate-coated gold nanoparticle (AuNP) colloid is one of the most commonly used SERS substrates because the synthetic method is simple and easy to be scaled up.[21, 22] Citrate serves as both the reducing agent and the stabilization layer of AuNPs by providing electrostatic repulsion forces between nanoparticles.[23, 24] Ligand exchange or interaction between citrate and target molecules is an essential process during AuNP functionalization.[25, 26] Because of the presence of the negatively charged citrate layer on AuNP surfaces, only chemicals that can displace or adsorb on the citrate layer (e.g., thiolates and positively charged compounds) can be readily detected using SERS.[19, 27, 28] For most organic micropollutants that are either neutral or negatively charged, citrate acts as a barrier that prevents these organic micropollutants from approaching AuNP surfaces. Therefore, it is highly desirable to experimentally evaluate the role of citrate in SERS analysis of low-affinity organic micropollutants and to devise strategies to overcome this potential barrier.

How citrate binds onto AuNP surfaces recently garners intensive research interest because of its crucial role in AuNP stabilization and subsequent functionalization.[29-32] Basset et al. reported that at a high surface coverage, citrate can coordinate with AuNP surfaces via a monocarboxylate monodentate mode with one oxygen atom in one of its terminal carboxylates bound to one gold atom.[19] As surface coverage decreases, citrate transforms its conformation on AuNP surfaces to a monocarboxylate bridging mode with both oxygen atoms of a terminal carboxylate bound to two adjacent gold atoms or even a bi-carboxylate bridging mode with all four oxygen atoms of the two terminal carboxylates bound to four gold atoms.[19] The bridging mode exhibits much higher binding energy than the monodentate mode (up to 55 kcal/mol vs. 2 kcal/mol),[30, 32] making citrate at a low coverage very difficult to be displaced by guest ligands. Other studies also identified the key role of the central carboxylate and carboxylate protonation state in citrate coordination with AuNP surfaces.[30, 31] Gold adatoms resulting from AuNP aging can enhance citrate binding affinity by coordinating with additional terminal carboxylate.[31] In addition, the intermolecular forces (e.g., hydrogen bonding) between citrate molecules further stabilize the layer on AuNP surfaces.[30] As a result, excess thiolates co-exist with citrate molecules on AuNP surfaces rather than completely remove them.[29, 30] This high resistance of citrate to displacement by guest ligands further highlights the challenges for SERS analysis of low-affinity organic micropollutants and the necessities to devise an innovative way to facilitate ligand exchange on AuNP surfaces.

In this study, we will examine a hypothesis that an external oxidation stress can disrupt the citrate layer and subsequently improve the SERS analysis of low-affinity organic micropollutants. Chlorobenzoic acids (CBAs), chlorophenols (CPs), and 2,4-dichlorophenoxyacetic acid (2,4-D) that have only one carboxylate or hydroxyl group, are selected as typical representatives for low-affinity organic micropollutants. Citrate on AuNP surfaces is oxidized by a strong oxidant, i.e., sulfate radicals, that are generated from controlled photolysis of peroxydisulfate (PDS). The oxidant concentration and ionic strength in the suspension are carefully controlled to maximize

SERS 'hot spot" density and avoid the collapse of the AuNP colloid. UV-Vis spectroscopy and Raman spectroscopy are used for *in-situ* monitoring the kinetics of citrate oxidation with and without the presence of organic micropollutants. Time windows for efficient ligand exchange, i.e., SERS analysis, are identified during controlled citrate oxidation. To our knowledge, this is the first study that employs oxidants to facilitate the exchange of citrate and organic micropollutants on AuNP surfaces. It not only provides *in-situ* and real-time insights on citrate transformation under an oxidation stress, but also extends SERS applications to the analysis of low-affinity organic micropollutants in water.

MATERIALS AND METHODS

Chemicals. Gold(III) chloride trihydrate ($HAuCl_4$, ≥99.9%), potassium persulfate ($K_2S_2O_8$, ≥99.0%), 2-chlorobenzoic acid (2-CBA, 98%), 3-chlorobenzoic acid (3-CBA, ≥99%), 4-chlorobenzoic acid (4-CBA, 99%), and 2-chlorophenol (2-CP, ≥99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). 2,4-dichlorophenoxyacetic acid (2,4-D, 98%), sodium citrate dihydrate (≥99.0%), and potassium sulfate (≥99.0%) were purchased from Fisher Scientific (Waltham, MA, USA). All solutions were prepared with ultrapure water (18.2 MΩ•cm) generated from a Milli-Q Advantage Water Purification System (Millipore, Billerica, MA, USA).

AuNP synthesis. AuNPs were synthesized by citrate reduction of the gold precursor (i.e., $HAuCl_4$) at 100 °C.[33] One hundred milliliter of $HAuCl_4$ solution (1 mM) was boiled in a 250-mL round-bottom flask with magnetic stirring. A refluxing condensation equipment was turned on as $HAuCl_4$ solution was heated to avoid volume reduction due to water evaporation. After $HAuCl_4$ solution started to boil, an aliquot of sodium citrate stock solution (150 mM) was pipetted into the flask to reach the total concentration of 3.88 mM. The endpoint of the reaction was indicated as the colloid turned into a wine-red color. The flask was then removed from the heating mantle, followed by turning off the condensation equipment until the colloid was cooled down to room temperature. The as-synthesized AuNPs (1.88×10^{15} particles per liter) are uniform spheres with a diameter of 13±2 nm based on transmission electron microscopy (TEM) image analysis.[34]

***In-situ* monitoring the kinetics of citrate oxidation.** All experiments were conducted in 4-mL quartz cuvettes with airtight stoppers. An aliquot of 3-mL AuNP suspension and 1-mL PDS solution were mixed in a cuvette. Addition of PDS to the uniformly pre-synthesized AuNPs is expected to provide better sensing reproducibility and stability than adding it during AuNP synthesis. The concentration of the added PDS solution varied in order to achieve a desired final concentration, i.e., 5, 10, 15, and 20 mM. To generate sulfate radicals ($SO_4^{-\bullet}$) via PDS photolysis, the cuvette was placed under the irradiation of simulated sunlight generated by a solar simulator (Abet Technologies, Milford, CT, USA). The light output of solar simulator was calibrated using a Si reference cell provided by the same manufacturer. Light intensity at the cuvette was maintained at 2 Sun (2000 W/m^2) by adjusting the distance between the beam turner and the cuvette. To investigate the role of the UV portion in the simulated sunlight on $SO_4^{-\bullet}$ production, a longpass filter (ThorLabs, Newton, NJ, USA) was stacked in the beam turner to block light with a wavelength <400 nm.

To monitor the kinetics of citrate oxidation on AuNP surfaces, AuNP suspensions containing 0-20 mM PDS were characterized using a UV-Vis spectrometer and a bench-top Raman spectrometer after the irradiation of simulated sunlight for 0, 15, 30, 45, 60, 75, 90, 120, 180, 240, 300, and 360 min. As the dark control, a parallel mixture was also characterized by UV-Vis and Raman spectroscopy after being placed under dark conditions for the same periods of time. Raman spectra provide the kinetics of citrate transformation on AuNP surfaces in the presence of $SO_4^{-\bullet}$, while the UV-Vis spectra provide the kinetics of AuNP aggregation – an indirect evidence of citrate oxidation on AuNP surfaces.[35] Before each spectroscopic measurement, the cuvette was gently shaken three times to make sure the thorough mixing of all the components inside.

SERS analysis of low-affinity organic micropollutants. For organic micropollutant detection, the experimental settings were kept the same as the citrate oxidation experiments with slight modifications to accommodate the cuvette capacity. The volume of AuNP colloid was kept at 3 mL, to which 0.5-mL PDS stock solution and 0.5-mL analyte stock solution were added successively. The concentration of the PDS stock solution was thereby increased to 80 mM to obtain a final concentration of 10 mM. The concentrations of analyte stock solutions depended on the water solubilities of the organic micropollutants, which resulted in the final concentrations of 2-CBA, 3-CBA, 4-CBA, 2,4-D, and 2-CP at 0.64, 0.32, 0.057, 0.11, and 0.78 mM, respectively.

The cuvette containing the mixture of AuNP colloid, PDS, and analyte was placed under the irradiation of simulated sunlight. Meanwhile, another suspension with the identical composition was placed in dark for the same period of time as a control group. Kinetics of the ligand exchange between citrate and organic micropollutants were acquired by UV-Vis spectroscopic and Raman spectroscopic analysis. Spectra were taken every 15 min in 2 hours or every 1 hour in 6 hours under both sunlit and dark conditions. To examine the potential impact of ionic strength on AuNP aggregation, an extra control experiment was conducted by replacing the PDS solution with a K_2SO_4 solution of the same concentration.

Instrumentation. All Raman spectra were acquired from aqueous suspensions/solutions using a bench-top Raman spectrometer (Horiba MacroRAM, NJ, USA). The excitation source is a 785-nm diode-pumped solid-state laser with the maximum power intensity of 450 mW. Raman scatterings were recorded by a multichannel charge-coupled device detector. The spectrometer is based on 120 mm focal length spectrograph, with a holograph grating of 685 gr/mm. A single

Raman spectrum was collected with an integration time of 1 s and 3 accumulations. All Raman spectra were smoothed and baseline corrected using Origin (OriginLab, Northampton, MA, USA). To determine the intensity ratios between Raman bands, overlapped bands were deconvoluted using the Gauss function in Origin. The extinction spectra of the AuNP suspensions were acquired using an UV-Vis spectrofluorometer (Horiba Aqualog, NJ, USA). A 150-W Xenon arc-lamp was used as the continuous light source. The wavelength of incident light ranged from 300 to 1000 nm with an increment of 1 nm, and the integration time was set as 0.1 second for each wavelength.

RESULTS AND DISCUSSION

Sulfate radical generation for controlled citrate oxidation. The overall objective of this study is to control the kinetics of citrate oxidation on AuNP surfaces in order to create a time window for subsequent adsorption and SERS analysis of low-affinity organic micropollutants. During this time window, the citrate layer on AuNP surfaces should be substantially oxidized and reduced while the AuNPs should aggregate into an appropriate size range for efficient SERS analysis. Simulated sunlight was employed to photolyze PDS and produce sulfate radicals ($SO_4^{\bullet-}$) for citrate oxidation.[36-38] The small portion of UV light in solar irradiance and the relatively high stability of PDS enable the slow release of $SO_4^{\bullet-}$ to the suspension and great controllability of citrate oxidation rate. To test this hypothesis, an aliquot of PDS solutions with different concentrations was added into the AuNP suspensions under the illumination of the simulated sunlight at 2 Sun. A low PDS concentration (5 mM) was not enough to break the citrate layer on AuNP surfaces and induce AuNP aggregation, which is manifested by the almost unchanged UV-Vis spectra during 3 hours under both dark and sunlit conditions (Fig. 1A&B). When PDS concentration was increased to 10 mM, slight aggregation of AuNPs was observed in 2 hours under the dark condition, as evidenced by the slight shift of LSPR band from 520 to 525 nm and the slightly increasing extinction within

600-800 nm (Fig. 1C). Under simulated sunlight irradiation, intensive AuNP aggregation was observed (Fig. 1D), indicating that 10 mM PDS was sufficient to break the citrate layer on AuNP surfaces. As PDS concentration was raised higher than 15 mM, aggregation occurred immediately after adding PDS to AuNP colloid under both dark and sunlit conditions (Fig. 1E&F), which is attributed to the electric double layer compression induced by the high ionic strength.[39] At high PDS concentrations (i.e., 15 and 20 mM), AuNPs aggregated so fast that big black flocs appeared in 30-45 min, indicating the collapse of AuNP colloid (Fig. 1E-H).

Efficient SERS analysis requires a high density of "hot spots", i.e., the nanogaps within small AuNP aggregates.[40-42] Low-concentration PDS (5 mM) was not able to induce AuNP aggregation, thus resulting in no visible SERS signals of citrate (Fig. S1). While 15 and 20 mM PDS induced very fast AuNP aggregation and flocculation under sunlit conditions, which resulted in rapid decrease and even disappearance of citrate SERS bands after 15 min (Fig. S2). This leaves a very short time window for efficient SERS analysis of organic micropollutants, which makes the 15-20 mM PDS unsuitable for the purpose of this study. In addition, it is challenging to differentiate the contributions of high ionic strength and citrate oxidation to AuNP aggregation at such high PDS concentrations. On the contrary, 10 mM PDS produced the right amount of sulfate radicals to oxidize the citrate layer, but kept the ionic strength lower than the threshold to induce severe AuNP aggregation (Fig. 1C-D). Collectively, AuNPs kept aggregating during 120 min without flocculation (formation of large black flocs) and settling, leaving a wide time window for efficient SERS analysis. Therefore, 10 mM PDS was selected for controlled citrate oxidation in the following experiments.

Kinetics and pathways of citrate oxidation on AuNP surfaces. To investigate how citrate was transformed by sulfate radicals, SERS spectra of the citrate layer on AuNP surfaces were collected for an extended time, i.e., 6 hours. After PDS stock solution was added to reach a final concentration of 10 mM, two Raman bands at 835 and 1075 cm^{-1} appeared immediately and lasted for 6 hours under the dark condition (Fig. 2A), which were attributed to S-O stretching and symmetric S=O stretching of PDS (Fig. S3).[43] Sulfate radicals are short-lived reactive species and cannot be present, i.e., generate interferent Raman bands, during SERS measurements. The absence of citrate SERS bands was attributed to the low "hot spot" density in the AuNP suspension without irradiation (Fig. 1C), which also indicated that the citrate layer could not be oxidized by PDS itself (E°=2.05 V vs NHE).[44] In contrast, under the sunlit condition, citrate SERS bands were readily observed after 1-hour illumination and kept varying throughout the 6-h period (Fig. 2B). Raman bands from citrate were barely observed during 0-45 min due to the lack of SERS "hot spots" (Fig. 2D). At 1 h, clear Raman bands at 1020 and 1385 cm^{-1} started to appear, which are assigned to the symmetric stretching of terminal carboxylates (-COO) and C-C stretching of the central carboxylate of citrate, respectively (Fig. 2B).[31] Meanwhile, the intensity of the PDS Raman band at 1075 cm^{-1} decreased by 57% after 1 h and almost disappeared after 2 h (Fig. 2B). Compared to the dark control, the PDS consumption under sunlight illumination is attributed to PDS photolysis by the UV light in solar irradiance, which was subsequently validated by another control experiment conducted under UV-blocked sunlight illumination (Fig. S4).

The two citrate Raman bands at 1385 and 1020 cm^{-1} kept decreasing during 2-6 h (Fig. 2B), which can be attributed to the decrease of either the citrate coverage on AuNP surfaces or the SERS enhancement factor. To rule out the latter possibility, variation of the ratio between the intensities of Au-Cl band at 255 cm^{-1} and citrate carboxylate band at 1385 cm^{-1} as a function of time was plotted in Fig. 2C. The gradually increasing ratio from 2-6 h confirmed that the citrate layer was progressively removed from AuNP surfaces. This is also supported by the UV-Vis spectra showing that the AuNPs kept aggregating during 2-6 h (Fig. S5). As the intensities of citrate Raman bands decreased, a new Raman band appeared at around 950 cm^{-1} after 1-h illumination (Fig. 2B). Compared with the C-C Raman band at 1020 cm^{-1}, the intensity of this new broad band first increased during 1-2 h and gradually decreased afterwards until almost disappeared at 6 h (Fig. 2C), suggesting that it is originated from the intermediate products of citrate oxidation. During the process of oxidation, citrate first lost its central carboxylate transforming into 1,3-acetone-dicarboxylate (ADC), then lost one of the terminal carboxylates transforming into acetoacetate (AA), and eventually transformed into carbon dioxide.[31, 45] According to the literature, the most prominent SERS bands of ADC and AA are all located within 900-1000 cm^{-1}, so the broad band at around 950 cm^{-1} observed in this study is assigned to ADC and AA that were generated during citrate oxidation. At a higher temporal resolution, the ratio between intensities of the intermediate band at 950 cm^{-1} and C-C band at 1020 cm^{-1} gradually approached towards a plateau during the second hour of illumination (Fig. 2C&D). This is consistent with the results shown in Fig. 2B, which suggests that the generation and consumption rates of such intermediates were roughly equal at 2 h. After that, the consumption rate exceeded the generation rate (Fig. 2B&C), leading to a rapid decrease and eventual disappearance of the intermediate band because of the further oxidation and mineralization of ADC and AA. Although AuNP aggregation is a dynamic process, similar kinetics and pathways of citrate oxidation were repetitively observed (Fig. S6). All these results collectively demonstrate that the oxidation kinetics of citrate on AuNP surfaces can be well controlled by simulated sunlight-photolyzed PDS.

In situ monitoring the ligand exchange induced by citrate oxidation. SERS has demonstrated excellent efficiency for the detection of amine-containing organic micropollutants, e.g., atrazine, melamine, carbamazepine, and various aromatic amines.[19, 27, 28] However, many organic micropollutants in water lack amine groups and thus exhibit low affinity towards AuNP surfaces, making it challenging for label-free SERS analysis. Taking 2-CBA as an example, it has only one carboxylate group that can effectively bind with AuNP surfaces in contrast to citrate that has three carboxylate groups.[29] Therefore, it is difficult for 2-CBA to displace the dense citrate layer and enter SERS "hot spots". Via controlled $SO_4^{\bullet -}$ oxidation, the citrate coverage on AuNP surfaces can be substantially reduced, which is expected to facilitate its displacement by 2-CBA. In addition, citrate layer oxidation reduces the electrostatic repulsive force between AuNPs and creates a high density of SERS "hot spots" for 2-CBA analysis.

To test our hypothesis, 2-CBA and PDS were added to AuNP colloid followed by the collection of UV-Vis and SERS spectra every 1 h during a 6-h period. Under the dark condition, the AuNP suspension containing 0.64 mM 2-CBA and 10 mM PDS did not exhibit apparent aggregation in 6 h (Fig. 3A). As a result, no SERS bands from both 2-CBA and citrate were observed (Fig. 3C). Under solar irradiation, the UV-Vis extinction within 600-1000 nm increased substantially indicating the aggregation of AuNPs (Fig. 3B). Compared with the suspension without 2-CBA (Fig. 1D), AuNPs aggregated slower in the presence of 2-CBA (Fig. 3B), indicating that 2-CBA adsorbed onto AuNP surfaces and thereby stabilized the AuNP colloid.

Under sunlit condition, a new set of SERS bands at 600, 800, 912, 974, 1306, and 1574 cm^{-1} appeared from 1 hour and slightly increased during 1-6 h (Fig. 3D). These bands, either from 2-CBA itself or its stable oxidation product, were highly reproducible and thus can be used for SERS analysis of 2-CBA, which cannot be achieved without controlled citrate oxidation. A series of control experiments were conducted to validate this finding. First, UV-Vis and SERS spectra were collected with PDS replaced by K_2SO_4 at the same concentration (Fig. S7). Neither the apparent change of UV-Vis spectra and the appearance of SERS bands of citrate and 2-CBA were observed in 2 h under both sunlit and dark conditions because of the lack of SERS "hot spots" (Fig. S7). This further confirms that the PDS-induced AuNP aggregation is caused by citrate oxidation instead of double layer compression. Furthermore, 15 mM K_2SO_4 was added to induce the aggregation of AuNPs, i.e., to generate sufficient "hot spots". In this case, SERS bands of citrate dominated the spectra, indicating barely any ligand exchange occurred on AuNP surfaces (Fig. S8). These control experiments show that the ionic strength threshold to induce severe AuNP aggregation is 90 mM and highlight the indispensable role of citrate oxidation in SERS analysis of 2-CBA.

Similar to citrate oxidation by PDS (Fig. 2B), PDS was primarily consumed during the first hour when 2-CBA was present (Fig. 3D). However, in the presence of 2-CBA, the SERS bands of citrate at 1380 cm^{-1} and its intermediate products at around 950 cm^{-1} reduced significantly after 1 h and almost disappeared after 2 h, which was drastically different from that without 2-CBA (Fig. 2B & 3D). These results suggested that the ligand exchange between citrate and 2-CBA almost completed during the initial 2 h when a large amount of citrate was degraded into smaller intermediate products. To acquire a higher temporal resolution of ligand exchange, SERS spectra were collected at a 15-min interval, which showed that the ligand exchange started at least from 45 min and kept proceeding as evidenced by the increasing intensity ratio of the Raman bands at 1306 and 1385 cm^{-1} (Fig. 3E&F). As the oxidation power was reduced (5 mM PDS + 5 mM K_2SO_4), the kinetics of ligand exchange became slower because more citrate was left on AuNP surfaces (Fig. 3E & S9). The set of Raman bands from the guest ligand remained highly consistent during the oxidation process and under different oxidation powers (Fig. 3D, 3F & S9), further confirming that they can be used as a stable and reproducible SERS signature for 2-CBA analysis. Since the ligand exchange predominantly occurred within 2 h, we will only focus on the first 2 h to investigate SERS analysis of other organic micropollutants.

Improved analysis of other CBA isomers and CBA-based herbicide. To further validate our findings, two other isomers of chlorobenzoic acid, i.e., 3-CBA and 4-CBA, were also used to investigate the kinetics of oxidation-induced ligand exchange (Fig. 4). Lower concentrations of 3-CBA and 4-CBA were selected due to their water solubility restrictions. As shown in Fig. S10, AuNPs aggregated much faster under the sunlit condition when 4-CBA was added probably because of its higher pK_a (3.98) and lower concentration (0.057 mM) than 2-CBA (2.89, 0.64 mM) and 3-CBA (3.82, 0.32 mM). Less negative charges and lower guest ligand coverage following citrate displacement may act collectively to reduce the stability of AuNP colloid. As shown in Fig. 4A&C, no new Raman bands (except those from PDS and citrate) were observed in the presence of 3-CBA and 4-CBA under the dark condition because of the high resistance of citrate towards ligand exchange. Under sunlight illumination, a set of new and reproducible Raman bands appeared, indicating that both 3-CBA and 4-CBA can be analyzed by SERS with the assistance of controlled citrate oxidation (Fig. 4B&D). It is worth mentioning that the citrate layers on AuNP surfaces were completely and partially displaced following 2-h illumination in the presence of 3-CBA and 4-CBA, respectively (Fig. 4B&D). This can be explained by the lower concentration of 4-CBA than 3-CBA, which further highlights the detrimental role of citrate in SERS analysis of CBAs and the importance of controlled citrate oxidation to broaden the SERS applicability for low-affinity organic micropollutant analysis.

In addition to the simple monochloro-substituted benzoic acids, the controlled citrate oxidation was further employed for the detection of 2,4-D, a herbicide that is widely used for efficiently and selectively removing most broadleaf weeds. The World Health Organization lists

2,4-D as a moderately toxic compound (Class II) and recommends a maximum concentration of 100 ppb (0.45 μM) in drinking water.[46] For freshwaters, only 2,4-D with concentrations lower than 10 ppb can be mineralized by microorganisms.[47] Furthermore, the overspray of pesticides and herbicides has been recognized as the main emission source of halogenated organic compounds.[48] Therefore, regular monitoring and advanced oxidation processes in drinking water treatment are still required to prevent 2,4-D from entering tap water.

Similar to the model CBAs, there is also a chlorinated benzene ring and a carboxylate group in the chemical structure of 2,4-D (Fig. S11A), so it is expected to be analyzed by SERS with the assistance of controlled citrate oxidation. Under the dark condition, the aggregation of AuNPs was mild in 2 hours in the presence of 2,4-D, as indicated by the almost unchanged UV-Vis spectra (Fig. S11A). In addition, only PDS and weak citrate bands were observed in the SERS spectra (Fig. 4E) due to the high resistance of citrate towards 2,4-D displacement. On the contrary, the UV-Vis extinction of AuNPs within 600-1000 nm significantly increased in 2 hours under the irradiation of simulated sunlight, as a result of the aggregation of AuNPs following citrate layer oxidation (Fig. S11B). Meanwhile, a new set of Raman bands were observed in the presence of 2,4-D (Fig. 4F). The Raman spectra of 2,4-D and the model CBAs shared some similarities because of their similar chemical structures, but also exhibited unique features (e.g., around 400 cm^{-1}) that can be used for the identification of individual species. These results demonstrate that controlled citrate oxidation is essential for the improved SERS analysis of CBA-based pollutants.

Chlorophenol analysis enabled by controlled citrate oxidation. Controlled citrate oxidation-enabled SERS was further applied for the analysis of chlorophenols, another type of organic micropollutants with low affinity towards AuNP surfaces. Phenolic pollutants are commonly found in natural waters as a result of pesticide application and industrial wastewater discharge.[49,

[50] Halogenation of phenols can greatly increase their recalcitrancy and toxicity,[49, 51] which entails vital importance of accurate and fast detection of these contaminants. However, the hydroxyl groups of phenols do not have sufficient affinity to AuNP surfaces for efficient citrate layer displacement and SERS analysis. Taking 4-chlorophenol (4-CP) as a representative halogenated phenolic pollutant, we herein demonstrated that this bottleneck can be overcome by controlled citrate oxidation.

In the presence of 4-CP, the UV-Vis and Raman spectra collected from AuNP suspensions barely changed in 2 hours under the dark condition (Fig. 5A&C). While severe aggregation of AuNPs was observed under sunlight irradiation, as manifested by a new UV-Vis extinction band at around 650 nm (Fig. 5B).[52] The UV-Vis extinction of AuNPs in the presence of 4-CP did not extend toward the near-infrared region as far as that in the presence of CBAs or in the absence of any pollutants (Fig. 1, 5B, & S10). This demonstrates the unique surface chemistry of AuNPs coated by 4-CP or its stable oxidation product compared with those coated by CBA-based pollutants or oxidized citrate. On the other hand, a new set of SERS bands at 700, 1167, 1256, and 1558 cm^{-1} appeared in the presence of 4-CP (Fig. 5D), which were distinctively different from those collected in the presence of CBA-based pollutants (Fig. 3&4). This not only demonstrates the applicability of controlled citrate oxidation to a variety of low-affinity organic micropollutants, but also highlights a huge potential of this method for differentiating micropollutants with different functional groups.

CONCLUSIONS

Interferent Raman bands from citrate are usually unavoidable when citrate-capped AuNPs are used as SERS substrates due to its resistance to ligand exchange with organic micropollutants. As a result, it is impossible to observe SERS signals of low-affinity organic micropollutants using conventional methods to generate SERS hot spots, e.g., inducing AuNP aggregation by adding electrolytes. This study reports a novel method to minimize the citrate interference and improve SERS analysis of a variety of low-affinity organic micropollutants, including 2-, 3-, 4-chlorobenzoic acids, 2,4-D, and 4-chlophenol. This was achieved by carefully controlling the oxidation kinetics of citrate using sulfate radicals produced from PDS photolysis.

Despite its low-cost and high sensitivity, SERS has been restricted to the analysis of amine-containing and thiol-containing organic pollutants that exhibit high affinity towards AuNP surfaces. With this new method, the applicability of SERS was extended to carboxylic and phenolic compounds and potentially much more pollutants with low affinity towards AuNP surfaces. The "fingerprint" SERS spectra provide abundant information on the bonding vibration and rotation of chemicals and can be used to differentiate organic micropollutants with different functional groups. Although sulfate radicals can possibly react with target analytes, the stable and unique SERS signatures acquired in this study guarantee the reproducible and selective detection of individual organic micropollutants. For quantitative analysis, it is suggested that the remaining citrate layer on AuNP surfaces could be used as an internal standard to calibrate the SERS intensity variations caused by the changing SERS hot spot density. To tackle a mixture of organic micropollutants, we propose a hierarchical process where the high-affinity pollutants are detected first under dark conditions while the low-affinity ones are subsequently detected with the assistance of controlled citrate oxidation. Natural organic matter (NOM) can affect the performance of this method depending on its concentration and composition in environmental water matrices. Since NOM is generally not halogenated, it is possible to use the unique carbon-halogen vibrational modes to differentiate the organic micropollutants from NOM. After further development and optimization according to the challenges associated with the practical scenarios, this innovative analytical method will enable the development of a compact and modular device for rapid, onsite, and online detection of organic micropollutants in water.

In addition to micropollutant analysis, this study also provides an inexpensive method for *in situ* and real-time characterization of reactive aqueous media. Understanding how organic pollutants transform in the presence of reactive species in water is important for the development and optimization of water treatment technologies, e.g., advanced oxidation processes. Previous studies usually rely on LC-MS/MS to identify key reaction intermediates and delineate reaction pathways. However, LC-MS/MS analysis is *ex situ*, i.e., it cannot be used to monitor ongoing reactions. In this study, SERS was used to monitor the degradation of citrate in the presence of sulfate radicals, which identified key reaction intermediates and delineated the reaction pathways. Upon proper design, this method can be readily extended to monitor the degradation of organic micropollutants during water treatment processes *in situ* and in real time.

ASSOCIATED CONTENT

Supporting Information:

Variation of SERS spectra as a function of time collected in the presence of 5, 15, and 20 mM PDS (Fig. S1-2); Raman spectra of ultrapure water, AuNP colloid, and 40 mM PDS solution (Fig. S3); Variation of UV-Vis and SERS spectra as a function of time collected when UV light was blocked from the simulated sunlight (Fig. S4); Variation of UV-Vis spectra after 6-h simulate sunlight irradiation (Fig. S5); Repetitive SERS spectra showing the pathways of citrate degradations (Fig. S6); Control experiments when 10 mM PDS was replaced by 10 and 15 mM K_2SO_4 (Fig. S7-8); SERS spectra showing citrate oxidation and subsequent ligand exchange under a lower oxidation power (Fig. S9); Variation of UV-Vis spectra as a function of time in the presence of 3-CBA, 4-CBA, and 2,4-D (Fig. S10-11).

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Author Contributions

This study was designed and conducted by both authors. The manuscript was also written by both authors.

Notes

The authors declare no competing financial interest.

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REFERENCES

1. Luo, Y. L.; Guo, W. S.; Ngo, H. H.; Nghiem, L. D.; Hai, F. I.; Zhang, J.; Liang, S.; Wang, X. C. C., A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. *Sci. Total Environ.* **2014,** *473*, 619-641.

2. Petrie, B.; Barden, R.; Kasprzyk-Hordern, B., A review on emerging contaminants in wastewaters and the environment: current knowledge, understudied areas and recommendations for future monitoring. *Water Res.* **2015,** *72*, 3-27.

3. Li, X.; Tian, T.; Shang, X.; Zhang, R.; Xie, H.; Wang, X.; Wang, H.; Xie, Q.; Chen, J.; Kadokami, K., Occurrence and health risks of organic micro-pollutants and metals in groundwater of Chinese rural areas. *Environ. Health Perspect.* **2020,** *128*, (10), 107010.

4. Falas, P.; Wick, A.; Castronovo, S.; Habermacher, J.; Ternes, T. A.; Joss, A., Tracing the limits of organic micropollutant removal in biological wastewater treatment. *Water Res.* **2016,** *95*, 240-249.

5. Clara, M.; Strenn, B.; Gans, O.; Martinez, E.; Kreuzinger, N.; Kroiss, H., Removal of selected pharmaceuticals, fragrances and endocrine disrupting compounds in a membrane bioreactor and conventional wastewater treatment plants. *Water Res.* **2005,** *39*, (19), 4797-4807.

6. Kneipp, K.; Kneipp, H.; Itzkan, I.; Dasari, R. R.; Feld, M. S., Ultrasensitive chemical analysis by Raman spectroscopy. *Chem. Rev.* **1999,** *99*, (10), 2957-2975.

7. Stockle, R. M.; Suh, Y. D.; Deckert, V.; Zenobi, R., Nanoscale chemical analysis by tip-enhanced Raman spectroscopy. *Chem. Phys. Lett.* **2000,** *318*, (1-3), 131-136.

8. Campion, A.; Kambhampati, P., Surface-enhanced Raman scattering. *Chem. Soc. Rev.* **1998,** *27*, (4), 241-250.

9. Halvorson, R. A.; Vikesland, P. J., Surface-enhanced Raman spectroscopy (SERS) for environmental analyses. *Environ. Sci. Technol.* **2010,** *44*, (20), 7749-7755.

10. Li, D.; Qu, L.; Zhai, W.; Xue, J.; Fossey, J. S.; Long, Y., Facile on-site detection of substituted aromatic pollutants in water using thin layer chromatography combined with surface-enhanced Raman spectroscopy. *Environ. Sci. Technol.* **2011,** *45*, (9), 4046-4052.

11. Willets, K. A.; Van Duyne, R. P., Localized surface plasmon resonance spectroscopy and sensing. *Annu. Rev. Phys. Chem.* **2007,** *58*, 267-297.

12. Mayer, K. M.; Hafner, J. H., Localized surface plasmon resonance sensors. *Chem. Rev.* **2011,** *111*, (6), 3828-3857.

13. Michaels, A. M.; Jiang; Brus, L., Ag nanocrystal junctions as the site for surface-enhanced Raman scattering of single Rhodamine 6G molecules. *J. Phys. Chem. B* **2000,** *104*, (50), 11965-11971.

14. Bosnick, K. A.; Jiang; Brus, L. E., Fluctuations and local symmetry in single-molecule Rhodamine 6G Raman scattering on silver nanocrystal aggregates. *J. Phys. Chem. B* **2002,** *106*, (33), 8096-8099.

15. Stiles, P. L.; Dieringer, J. A.; Shah, N. C.; Van Duyne, R. R., Surface-enhanced Raman spectroscopy. *Annu. Rev. Anal. Chem.* **2008,** *1*, 601-626.

16. Xu, H. X.; Bjerneld, E. J.; Kall, M.; Borjesson, L., Spectroscopy of single hemoglobin molecules by surface enhanced Raman scattering. *Phys. Rev. Lett.* **1999,** *83*, (21), 4357-4360.

17. Nie, S. M.; Emery, S. R., Probing single molecules and single nanoparticles by surface-enhanced Raman scattering. *Science* **1997,** *275*, (5303), 1102-1106.

18. Kneipp, K.; Wang, Y.; Kneipp, H.; Perelman, L. T.; Itzkan, I.; Dasari, R.; Feld, M. S., Single molecule detection using surface-enhanced Raman scattering (SERS). *Phys. Rev. Lett.* **1997,** *78*, (9), 1667-1670.

19. Wei, H. R.; Huang, Q. S.; Vikesland, P. J., The aromatic amine pK_a determines the affinity for citrate-coated gold nanoparticles: in situ observation via hot spot-normalized surface-enhanced Raman spectroscopy. *Environ. Sci. Technol. Lett.* **2019,** *6*, (4), 199-204.

20. Chen, J. M.; Huang, Y. J.; Kannan, P.; Zhang, L.; Lin, Z. Y.; Zhang, J. W.; Chen, T.; Guo, L. H., Flexible and adhesive surface enhance Raman scattering active tape for rapid detection of pesticide residues in fruits and vegetables. *Anal. Chem.* **2016,** *88*, (4), 2149-2155.

21. Turkevich, J.; Stevenson, P. C.; Hillier, J., A study of the nucleation and growth processes in the synthesis of colloidal gold. *Discuss. Faraday Soc.* **1951**, (11), 55-75.

22. Ji, X. H.; Song, X. N.; Li, J.; Bai, Y. B.; Yang, W. S.; Peng, X. G., Size control of gold nanocrystals in citrate reduction: the third role of citrate. *J. Am. Chem. Soc.* **2007,** *129*, (45), 13939-13948.

23. Munro, C. H.; Smith, W. E.; Garner, M.; Clarkson, J.; White, P. C., Characterization of the surface of a citrate-reduced colloid optimized for use as a substrate for surface-enhanced resonance Raman scattering. *Langmuir* **1995,** *11*, (10), 3712-3720.

24. Mabuchi, M.; Takenaka, T.; Fujiyoshi, Y.; Uyeda, N., Surface enhanced Raman scattering of citrate ions adsorbed on gold sol particles. *Surf. Sci.* **1982,** *119*, (2-3), 150-158.

25. Bucci, R.; Maggioni, D.; Locarno, S.; Ferretti, A. M.; Gelmi, M. L.; Pellegrino, S., Exploiting ultrashort α, β-peptides in the colloidal stabilization of gold nanoparticles. *Langmuir* **2021,** *37*, (38), 11365-11373.

26. Okyem, S.; Awotunde, O.; Ogunlusi, T.; Riley, M. B.; Driskell, J. D., Probing the mechanism of antibody-triggered aggregation of gold nanoparticles. *Langmuir* **2021,** *37*, (9), 2993-3000.

27. Wei, H. R.; Vikesland, P. J., pH-triggered molecular alignment for reproducible SERS detection via an AuNP/nanocellulose platform. *Sci. Rep.* **2015,** *5*, 18131.

28. Wei, H.; Leng, W.; Song, J.; Liu, C.; Willner, M. R.; Huang, Q.; Zhou, W.; Vikesland, P. J., Real-time monitoring of ligand exchange kinetics on gold nanoparticle surfaces enabled by hot spot-normalized surface-enhanced Raman scattering. *Environ. Sci. Technol.* **2019,** *53*, (2), 575-585.

29. Park, J. W.; Shumaker-Parry, J. S., Strong resistance of citrate anions on metal nanoparticles to desorption under thiol functionalization. *ACS Nano* **2015,** *9*, (2), 1665-1682.

30. Park, J. W.; Shumaker-Parry, J. S., Structural study of citrate layers on gold nanoparticles: role of intermolecular interactions in stabilizing nanoparticles. *J. Am. Chem. Soc.* **2014,** *136*, (5), 1907-1921.

31. Grys, D. B.; De Nijs, B.; Salmon, A. R.; Huang, J. Y.; Wang, W. T.; Chen, W. H.; Scherman, O. A.; Baumberg, J. J., Citrate coordination and bridging of gold nanoparticles: the role of gold adatoms in AuNP aging. *ACS Nano* **2020,** *14*, (7), 8689-8696.

32. Al-Johani, H.; Abou-Hamad, E.; Jedidi, A.; Widdifield, C. M.; Viger-Gravel, J.; Sangaru, S. S.; Gajan, D.; Anjum, D. H.; Ould-Chikh, S.; Hedhili, M. N.; Gurinov, A.; Kelly, M. J.; El Eter, M.; Cavallo, L.; Emsley, L.; Basset, J. M., The structure and binding mode of citrate in the stabilization of gold nanoparticles. *Nat. Chem.* **2017,** *9*, (9), 890-895.

33. Frens, G., Controlled nucleation for the regulation of the particle size in monodisperse gold suspensions. *Nat. Phys. Sci.* **1973,** *241*, (105), 20-22.

34. Wei, H.; Loeb, S. K.; Halas, N. J.; Kim, J. H., Plasmon-enabled degradation of organic micropollutants in water by visible-light illumination of Janus gold nanorods. *Proc. Natl. Acad. Sci. U.S.A.* **2020,** *117*, (27), 15473-15481.

35. Vaid, K.; Dhiman, J.; Sarawagi, N.; Kumar, V., Experimental and computational study on the selective interaction of functionalized gold nanoparticles with metal ions: sensing prospects. *Langmuir* **2020,** *36*, (41), 12319-12326.

36. Hori, H.; Yamamoto, A.; Hayakawa, E.; Taniyasu, S.; Yamashita, N.; Kutsuna, S., Efficient decomposition of environmentally persistent perfluorocarboxylic acids by use of persulfate as a photochemical oxidant. *Environ. Sci. Technol.* **2005,** *39*, (7), 2383-2388.

37. Lee, J.; Von Gunten, U.; Kim, J. H., Persulfate-based advanced oxidation: critical assessment of opportunities and roadblocks. *Environ. Sci. Technol.* **2020,** *54*, (6), 3064-3081.

38. Zhang, W.; Zhou, S.; Sun, J.; Meng, X.; Luo, J.; Zhou, D.; Crittenden, J., Impact of chloride ions on UV/H_2O_2 and UV/persulfate advanced oxidation processes. *Environ. Sci. Technol.* **2018,** *52*, (13), 7380-7389.

39. Wei, X.; Popov, A.; Hernandez, R., Electric potential of citrate-capped gold nanoparticles is affected by poly(allylamine hydrochloride) and salt concentration. *ACS Appl. Mater. Interfaces* **2022,** *14*, (10), 12538-12550.

40. Camden, J. P.; Dieringer, J. A.; Wang, Y. M.; Masiello, D. J.; Marks, L. D.; Schatz, G. C.; Van Duyne, R. P., Probing the structure of single-molecule surface-enhanced Raman scattering hot spots. *J. Am. Chem. Soc.* **2008,** *130*, (38), 12616-12617.

41. Li, W. Y.; Camargo, P. H. C.; Lu, X. M.; Xia, Y. N., Dimers of silver nanospheres: facile synthesis and their use as hot spots for surface-enhanced Raman scattering. *Nano Lett.* **2009,** *9*, (1), 485-490.

42. Kleinman, S. L.; Frontiera, R. R.; Henry, A. I.; Dieringer, J. A.; Van Duyne, R. P., Creating, characterizing, and controlling chemistry with SERS hot spots. *Phys. Chem. Chem. Phys.* **2013,** *15*, (1), 21-36.

43. Wang, J.; Zheng, W.; Zhang, Y.; Song, S.; Chou, I. M.; Hu, M.; Pan, Z., Raman spectroscopic technique towards understanding the degradation of phenol by sodium persulfate in hot compressed water. *Chemosphere* **2020,** *257*, 127264.

44. Lau, T. K.; Chu, W.; Graham, N. J. D., The aqueous degradation of butylated hydroxyanisole by UV/$S_2O_8^{2-}$: study of reaction mechanisms via dimerization and mineralization. *Environ. Sci. Technol.* **2007,** *41*, (2), 613-619.

45. Redmond, P. L.; Wu, X. M.; Brus, L., Photovoltage and photocatalyzed growth in citrate-stabilized colloidal silver nanocrystals. *J. Phys. Chem. C* **2007,** *111*, (25), 8942-8947.

46. Hameed, B. H.; Salman, J. M.; Ahmad, A. L., Adsorption isotherm and kinetic modeling of 2,4-D pesticide on activated carbon derived from date stones. *J. Hazard. Mater.* **2009,** *163*, (1), 121-126.

47. Brillas, E.; Calpe, J. C.; Casado, J., Mineralization of 2,4-D by advanced electrochemical oxidation processes. *Water Res.* **2000,** *34*, (8), 2253-2262.

48. Muñoz, M.; Gullett, B. K.; Touati, A.; Font, R., Effect of 2,4-dichlorophenoxyacetic acid (2,4-D) on PCDD/F emissions from open burning of biomass. *Environ. Sci. Technol.* **2012,** *46*, (17), 9308-9314.

49. Davì, M. L.; Gnudi, F., Phenolic compounds in surface water. *Water Res.* **1999,** *33*, (14), 3213-3219.

50. Hu, Y.; Li, D.; Sun, F.; Weng, Y.; You, S.; Shao, Y., Temperature-induced phase changes in bismuth oxides and efficient photodegradation of phenol and p-chlorophenol. *J. Hazard. Mater.* **2016,** *301*, 362-370.

51. Dragstedt, C. A., The halogenated hydrocarbons: their toxicity and potential dangers. *AMA Arch. Intern. Med.* **1956,** *97*, (2), 261-262.

52. Zhou, J.; Li, Y.; Wang, W.; Lu, Z.; Han, H.; Liu, J., Kanamycin adsorption on gold nanoparticles dominates its label-free colorimetric sensing with its aptamer. *Langmuir* **2020,** *36*, (39), 11490-11498.