#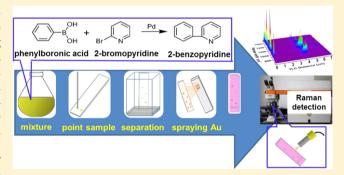 Thin Layer Chromatography Coupled with Surface-Enhanced Raman Scattering as a Facile Method for On-Site Quantitative Monitoring of Chemical Reactions

Zong-Mian Zhang, Jing-Fu Liu,* Rui Liu, Jie-Fang Sun, and Guo-Hua Wei

State Key Laboratory of Environmental Chemistry and Ecotoxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

Ⓢ Supporting Information

ABSTRACT: By coupling surface-enhanced Raman spectroscopy (SERS) with thin layer chromatography (TLC), a facile and powerful method was developed for on-site monitoring the process of chemical reactions. Samples were preseparated on a TLC plate following a common TLC procedure, and then determined by SERS after fabricating a large-area, uniform SERS substrate on the TLC plate by spraying gold nanoparticles (AuNPs). Reproducible and strong SERS signals were obtained with substrates prepared by spraying 42-nm AuNPs at a density of 5.54×10^{10} N/cm^2 on the TLC plate. The capacity of this TLC-SERS method was evaluated by monitoring a typical Suzuki coupling reaction of phenyl-

boronic acid and 2-bromopyridine as a model. Results showed that this proposed method is able to identify reaction product that is invisible to the naked eye, and distinguish the reactant 2-bromopyridine and product 2-phenylpyridine, which showed almost the same retention factors (R_f). Under the optimized conditions, the peak area of the characteristic Raman band (755 cm^{-1}) of the product 2-phenylpyridine showed a good linear correlation with concentration in the range of 2−200 mg/L ($R^2 = 0.9741$), the estimated detection limit (1 mg/L 2-phenylpyridine) is much lower than the concentration of the chemicals in the common organic synthesis reaction system, and the product yield determined by the proposed TLC-SERS method agreed very well with that by UPLC-MS/MS. In addition, a new byproduct in the reaction system was found and identified through continuous Raman detection from the point of sample to the solvent front. This facile TLC-SERS method is quick, easy to handle, low-cost, sensitive, and can be exploited in on-site monitoring the processes of chemical reactions, as well as environmental and biological processes.

For their advantages like simple operation and fast separation, thin layer chromatography (TLC)-based separation technologies endow a lasting popularity in various research fields, and they play a major role in monitoring synthetic organic reactions.[1,2] Generally, after spotted on the plate and developed in the mobile phase via capillary action, the separated sample spots are visualized by the absorbance or fluorescence, either native or enhanced with visualizing agents, and the relevant products are identified by their characteristic colors or fluorescence combined with the retention factor (R_f) values. However, the following challenges remain:
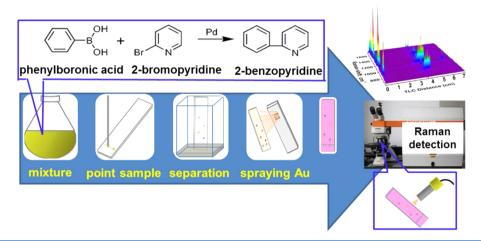
(i) because of the limited specificity and sensitivity provision capacity of TLC, spectroscopic routines like nuclear magnetic resonance (NMR) and mass spectroscopy (MS) are needed to further characterize reaction products after the extraction of the chemicals from the TLC plate,[3,4] which is time-consuming and labor-intensive;

(ii) some chemicals like alkanes are fluorescence-insensitive, and cannot be detected under an ultraviolet (UV) lamp, thus prohibiting their monitoring; and

(iii) since the separation on TLC is mainly based on the chemicals' different affinity with the stationary and mobile phase, chemicals with similar structure or polarity are difficult to separate completely.

To overcome these limits, various technologies, such as infrared (IR), Raman, and mass spectrometry have been explored with TLC for compound specific information. Among these technologies, special attention has been paid to surface-enhanced Raman scattering (SERS), partly for its high-sensitivity with detection limit down to the single-molecule level under optimized conditions,[5] fingerprint-based high specificity and capacity in simultaneous detection of multi-analytes,[6] and high tolerance to various analyte configurations including liquid, powder, gas and solid samples like foods[7,8] and artworks.[9] Furthermore, the development in instrument automation enables the fulfillment of large area mapping with

Scheme 1. Schematic Representation of the TLC-SERS Study of Continuous Raman Scanning

Raman spectrometer in subminute time scale. Therefore, the combination of SERS and TLC enables the conduction of separation and detection on the same plate, and distinguishment of the incompletely separated compounds from their characteristic Raman bands, without loss of chemicals through the imaging-mode of SERS.

Since first reported by Hezel,[10] TLC-SERS had been applied to the analysis of various analytes,[11−13] including the natural dyes on artwork,[9] pesticide residue from crops,[14] some biomarkers in biological samples,[15−18] and on-site detection of aromatic pollutants in environmental water samples.[19] In most of the above methods, silver nanoparticles (AgNPs) was dropped on the spots of preseparated TLC plate, and then the SERS spectrum was collected from each spot for the information on the surface analytes. Therefore, these methods are only valid for the analysis of visible analytes on the plate in the millimeter-sized area (mm^2), but not valid for automatic and continuous analysis of all the chemicals, including invisible analytes on the plate. Freye et al.[20] realized the analysis in larger area and distinguished the compounds with similar R_f values through establishing a conformal blotting process and transferring analytes from TLC plate to nanocomposite films. However, the preparation of silver-polymer nanocomposites and a transferring procedure are required, which is very complicated and time-consuming.

Herein, we report a new TLC-SERS method for continuous and automatic on-site monitoring of chemical reaction processes on the TLC plate. After separation of the products on a commercially available TLC plate, SERS substrate with high uniformity of gold nanoparticles (AuNPs) was fabricated on the plate by spraying Au colloid on it, and then the SERS scanning was conducted along a line from the point of sample to the solvent front at submicrometer-scale intervals. The SERS spectra along the line provide all the information on the separated components, including fingerprint spectra for identification of chemicals that are incompletely separated or invisible to the eyes on the plate.

EXPERIMENTAL SECTION

Chemicals and Materials. Chlorauric acid ($HAuCl_4$), sodium citrate, potassium phosphate, petroleum ether, and ethyl acetate were purchased from Sinopharm Chemical Reagent Co. (Beijing, China). Palladium acetate, palladium nitrate, and palladium chloride were supplied by Aladdin

Reagent Database, Inc. (Shanghai, China). Phenylboronic acid, 2-bromopyridine, 2-phenylpyridine, biphenyl, and 2,2-bipyridine were purchased from Sigma−Aldrich (St. Louis, MO). Silica gel TLC plates (G and GF_{254}) were acquired from Anhui LiangChen Silicon Material Co. (Anhui, China). The sprayer was purchased from Ningbo Danlong Electric Manufacturer Co. (Zhejiang, China). All the chemicals are at least analytic grade, and pure water produced with a Millipore Milli-Q Gradient system (Billerica, MA) was used throughout this study.

Organic Synthesis Procedure. The Suzuki coupling reaction of phenylboronic acid and 2-bromopyridine was chosen as the model for evaluating the developed TLC-SERS method. The synthesis reaction was carried out in a three-necked bottle under constant stirring. The 10 mL of ethanol/water (1:1) solvent containing 2.5 mmol potassium phosphate was poured into the three-necked bottle, followed by adding 1.3 mmol 2-bromopyridine, 1.9 mmol phenylboronic acid, and 17.8 μmol palladium acetate. The reaction solution was performed at room temperature to slow the reaction rate, which can fulfill the requirement of the monitoring.

Synthesize and Characterization of AuNPs. AuNPs were prepared using sodium citrate as the reducing and capping agent according to the literature.[21] Briefly, 150 mL of 1 mmol/L chlorauric acid solutions was heated to boil. After adding appropriate amounts of sodium citrate and continued boiling and stirring of the mixture for ∼30 min, different sizes of AuNPs were obtained in the solutions. The produced AuNPs were characterized by transmission electron microscopy (TEM), and results are shown in Figure S1 in the Supporting Information (SI).

TLC Separation. After being activated by heating at 110 °C for 30 min, the silica gel TLC plate was micropipetted with 1-μL samples. After drying in the air, the plate was placed in a 125-mL chamber and developed using petroleum ether and ethyl acetate (v/v = 5:1) as the mobile phase solvent. After the separation of the samples, Au dispersions were sprayed onto the plate with a sprayer. The sprayer allowed aqueous AuNP dispersions to form fine drops of colloid (in nanometer range) to uniformly coat the TLC plate. In this way, the plate surface was entirely covered with uniform AuNPs without diffusion of the chemical spots. The size and amount of AuNPs sprayed on the TLC plate was optimized to obtain the best SERS signal.
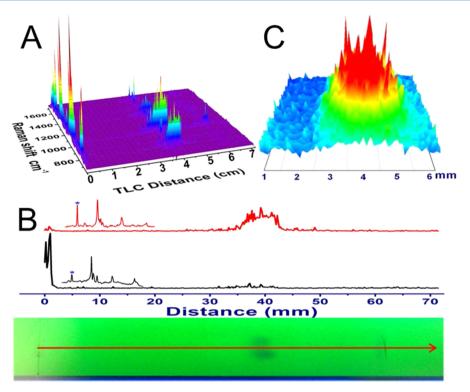
Figure 1. (A) Raman image from the point of the sample to the solvent front along a line; (B) comparison of Raman signal distribution of 2-phenylpyridine (red, 755 cm^{-1}) and phenylboronic acid (black, 737 cm^{-1}) on the plate with the fluorescence image of the TLC plate; (C) Raman image of 2-phenylpyridine in the product area.

SERS Detection. For the Raman spectra collection, a Renishaw Invia Raman microscope equipped with a 785-nm laser was used, and a 20× long working-distance objective was used with an output power of 1 mW and a 20-μm beam diameter on the plate. The TLC plate was placed on the XYZ stage of the Raman spectrometer and scanned from the sample point to the solvent front at 300 μm interval along a line, and all the spectra were scanned from 618 cm^{-1} to 1719 cm^{-1}. The SERS signals were obtained and mapped with Raman shift as the *X*-axis, movement distance as the *Y*-axis, and the Raman signal intensity as the *Z*-axis. In this way, the SERS spectra of all the chemicals along the line were recorded in several minutes and the concentration of the chemicals were obtained at the same time. The detection can be finished in several minutes (Scheme 1).

UPLC-MS/MS Determination of Product. For ultra-performance liquid chromatography (UPLC) monitoring the reaction, 10 μL of reaction solution was sampled at different reaction time and terminated by adding 40 μL of 0.4 mol/L acetic acid solutions, and then diluting to suitable concentrations with the 10 mmol/L acetic acid. The reaction product (2-phenylpyridine) was determined by a TSQ Quantum Access triple quadruple mass spectrometer (Thermo Scientific, Waltham, MA) coupled with a Waters Acquity UPLC (Waters, Milford, MA). Separation of the products was accomplished using the UPLC system equipped with a Waters BEH C$_{18}$ column (2.1 mm × 50 mm, 1.7 μm). The injection volume was 10 μL, and the column temperature was 45 °C. The mobile phase was a mixture of 0.1% formic acid and methanol (v/v = 1:1) with a flow rate of 0.2 mL/min. The retention time of the product was 1.37 min. For the mass spectrometer detection, the electrospray ionization (ESI) was operated in the positive-ion mode, and the best conditions were set as follows: spray voltage

= 4000 V and capillary temperature = 300 °C. Nitrogen was used as the sheath gas (35 units) and auxillary gas (20 units).

RESULTS AND DISCUSSION

Method Development and Analytical Performance. Figure 1A shows the SERS spectra of reaction solution of the Suzuki coupling of phenylboronic acid and 2-bromopyridine. To avoid the interference from each other, the Raman bands of 737 and 755 cm^{-1} were used for the quantification of phenylboronic acid and 2-phenylpyridine, respectively, and the distribution of the two chemicals on the plate was found to match well with the spots on the GF$_{254}$ TLC plate (Figure 1B). To test the applicability of this method for detection in larger areas, the spot of the 2-phenylpyridine (6 mm in diameter, visualized under a 254-nm UV lamp) was detected in a spectral acquisition density of 0.1 mm × 0.1 mm. The over 3000 SERS spectra obtained were mapped based on the characteristic Raman band of 755 cm^{-1}, which corresponds to the totally symmetric in-plane vibration of phenyl ring.[22,23] As shown in Figure 1C, the Raman signal intensity of 2-phenylpyridine distributed like a torch light in the measuring area, which represents its concentration distribution. The above results suggested that this method can be used for monitoring of a process by continuous detection in large area, and the missing of analytes can be avoided by using the image mode at the interval of 300 μm.

The size and concentration of AuNPs for fabricating the SERS substrates were optimized to improve the method sensitivity. Environmental scanning electron microscopy (ESEM) surface images showed the presence of a difference in structure of the SERS substrates fabricated with different sizes of AuNPs (18, 33, 42, 71 nm) on the TLC plate (Figure S2 in the Supporting Information). The SERS spectra of series
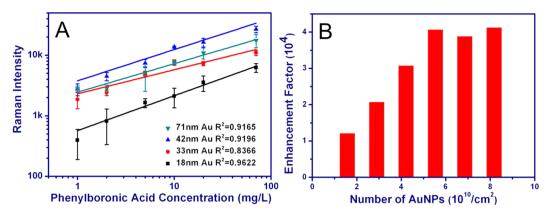
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Figure 2. Impacts of AuNP size and concentration on the SERS response. (A) SERS intensity detected with SERS substrates fabricated from different-sized AuNPs; the concentrations of phenylboronic acid were 1, 2, 5, 10, 20, and 70 mg/L. (B) The Raman-enhanced factor for phenylboronic acid detected by spraying with 1.58×10^{10}, 2.9×10^{10}, 4.22×10^{10}, 5.54×10^{10}, 6.86×10^{10}, and 8.18×10^{10} N/cm^2 AuNPs (42 nm) on the TLC plate. A Raman spectrometer was used with a laser intensity of 1 mW, an acquisition time of 1 s, and two accumulate times.
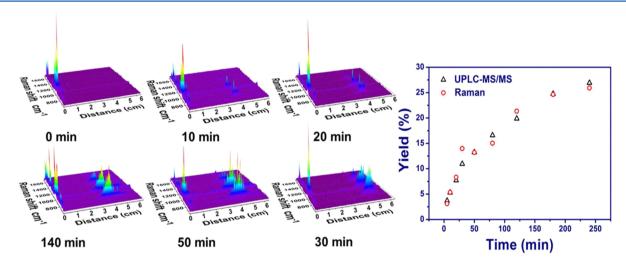

Figure 3. (Left) Monitoring the Suzuki coupling reaction of phenylboronic acid and 2-bromopyridine at different reaction times. (Right) Product yield determined by Raman and UPLC-MS/MS at different reaction times.

concentrations of phenylboronic acid (1−70 mg/L) were detected with SERS substrates formed by spraying different-sized AuNPs on the preseparated TLC plate pointed with phenylboronic acid samples. Considering the roughness of the TLC plate surface, 11 spectra of each sample spot were collected and the average value was adopted to calculate the SERS signal intensity. Figure 2A shows the SERS intensity of 999 cm^{-1} Raman band, the strongest breathing mode (ν_{12}) of phenyl ring,[24] at a series of phenylboronic acid concentrations, indicating that 42-nm AuNPs showed the best performance.

The concentration of 42-nm AuNPs for fabricating the SERS substrates was optimized by spraying different concentrations of AuNPs onto the preseparated TLC plates pointed with 10 mg/L phenylboronic acid as the probe, and calculating the enhanced factors (EFs) by comparing the Raman intensity at the 999 cm^{-1} band to the bulk Raman signal obtained from the TLC plate with 2 g/L phenylboronic acid. The AuNP concentration was calculated based on the equation given by eq 1:[25,26]

$$C_{AuNPs} = \frac{C_{chlorauric\ acid}}{4\pi(R-\delta)^3/3V_g}$$ (1)

where $C_{chlorauric\ acid}$ is the concentration of HAuCl$_4$, R the radius of AuNPs, δ the length of the protecting ligand ($\delta \ll R$), and V_g the volume of one Au atom (17 Å^3). The EF was calculated according to eq 2:

$$R_{EF} = \frac{I_{SERS}}{I_{bulk}}\left(\frac{N_{bulk}}{N_{SERS}}\right)\left(\frac{P_{bulk}}{P_{SERS}}\right)\left(\frac{t_{bulk}}{t_{SERS}}\right)$$ (2)

in which I represents the Raman intensity, N is the number of probe molecules, P is the laser power, and t represents the acquisition time; and subscripts "SERS" and "bulk" represent surface-enhanced Raman spectroscopy and normal Raman scattering of bulk solution, respectively. Results shown in Figure 2B indicated that the EFs increased with AuNP concentration until reaching a value of 5.5×10^{10} N/cm^2 and then leveled off, suggesting that AuNPs at this concentration on the TLC plate provided suitable spacing between the particles and the target molecules. In the following experiments, 42-nm AuNPs were sprayed on the TLC plate to provide concentration of 5.5×10^{10} N/cm^2.

Under the above optimized conditions, the SERS images of 2-phenylpyridine (755 cm^{-1}) at various concentrations were recorded, and the peak area of the characteristic Raman band (755 cm^{-1}) showed a good linear correlation with 2-

phenylpyridine concentrations in the range of 2−200 mg/L (R^2 = 0.9741) (see Figure S3 in the Supporting Information). The estimated detection limit is 1 mg/L 2-phenylpyridine, which is much lower than the concentration of the chemicals in the common organic synthesis reaction system, verifying the great applicability of this method in on-site monitoring of organic synthesis process.

Application in Monitoring the Reaction Process. The proposed TLC-SERS method was employed to monitor the Suzuki reaction process through the SERS detection of phenylboronic acid and 2-bromopyridine using the GF$_{254}$ TLC plate as the separation and detection substrate. Figure 3 (left) shows the SERS images of the preseparated mixture recorded at different reaction times. The Raman signal intensity of the product (2-phenylpyridine) increased constantly with time up to 50 min and then remained constant, suggesting that either the reaction has reached equilibrium or the Raman signal intensity of the product had reached saturation under the given instrument conditions. To further verify the reason, the reaction solution can be diluted and analyzed again under the same conditions.

The applicability of the proposed method was further verified by monitoring the yields of the product, with UPLC-MS/MS detection as a reference method. Results (Figure 3 (right)) showed that the product yield increased quickly with the time up to 200 min and then continued to experience slow growth to the end. The profiles of product yield to reaction time obtained by these two methods agreed very well, indicating the reliability of TLC-SERS in quantification of the analytes. Given that the TLC-SERS method is much simpler and quicker than the UPLC-MS/MS approach, the TLC-SERS is a promising alternative for monitoring the process, both qualitatively and quantitatively.

Isolation and Identification of Analytes in Mixture. It is interesting to note that after the development of the Suzuki coupling reaction mixture of phenylboronic acid and 2-bromopyridine on the GF$_{254}$ TLC plate, no Raman signal of 2-bromopyridine was detected, even at the initial stage of the reaction. We ascribed it to the swamping of the 2-bromopyridine signals in the green fluorescence background of the GF$_{254}$ TLC plate. This speculation was verified by the distinct Raman signal of 2-bromopyridine detected by using the G TLC plate under the same detection conditions as that with GF$_{254}$ TLC plate (see Figure S4 in the Supporting Information).

When G TLC plate was used, however, the Raman intensity of the product was found to remain almost constant during the reaction process (see Figure S5 in the Supporting Information). We conjectured that 2-bromopyridine and 2-phenylpyridine have similar R_f values and overlapped Raman spectra after TLC separation, thus the product signal balanced due to the increase of product 2-phenylpyridine and the decrease of reactant 2-bromopyridine with the reaction time. To verify this speculation, 2-bromopyridine, 2-phenylpyridine, and their mixture were respectively pointed on the same G TLC plate, while 2-bromopyridine and 2-phenylpyridine were respectively pointed on the same GF$_{254}$ TLC plate. After development under the same conditions, nothing can be seen on the G TLC plate under the 254-nm UV light (Figure 4A). However, with the characteristic Raman band of 703 cm^{-1} (ν_{C-Br}) for 2-bromopyridine[27] and 755 cm^{-1} (ν_{Ph}) for 2-phenylpyridine, Raman mapping can be made continuously on the G TLC plate from the point of sample to the solvent front. Results showed
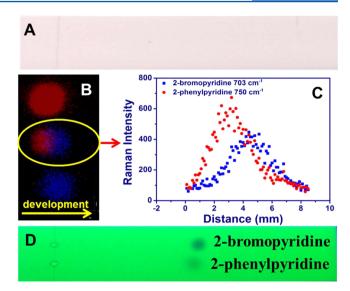

Figure 4. TLC separation and SERS detection of 2-bromopyridine and 2-phenylpyridine: (A) image of the G TLC plate after the development of 2-bromopyridine, 2-phenylpyridine, and the mixture; (B) SERS image of 2-bromopyridine, 2-phenylpyridine, and their mixture quantified by their characteristic Raman bands after the development with a G TLC plate; (C) Raman intensity distribution of 2-bromopyridine and 2-phenylpyridine along the line across the middle of mixture spot; and (D) fluorescence image of the GF$_{254}$ TLC plate after the development of 2-bromopyridine and 2-phenylpyridine.

that the two compounds, represent by different colors, have similar developing distances and overlapped in the mixture spot (Figure 4B). This was confirmed by the distribution profiles of the Raman intensity against distance acquired from their characteristic Raman bands along a line across the middle of the mixture spot on the G TLC plate (Figure 4C), as well as by the two black spots with similar R_f values observed under the 254-nm UV light when the two chemicals were developed on the same GF$_{254}$ TLC plate (Figure 4D). Notably, the above results demonstrated that, for products and reactants that are invisible for TCL only, and even have similar R_f values and cannot be separated completely on the TLC plate, the reaction process can be estimated through the relative intensity of their characteristic SERS bands. Thus, the TLC-SERS method has great potential in monitoring the reaction process, especially for the reactions with chemicals that are hardly separated or invisible to the eye for TLC only.

Identification of Unknown Compounds. The above success of TLC-SERS in identification of analytes in reacting mixtures encouraged us to test its applicability in identification of unknown compounds, e.g., byproducts or new complexes formed by catalysts and substrates. Thus, TLC-SERS detection was performed continuously from the point of sample to the solvent front, to determine if byproducts formed in the Suzuki coupling reaction of phenylboronic acid and 2-bromopyridine. To our delight, a strong SERS signal, which cannot be assigned to the reactants or products (see Figure S6 in the Supporting Information), was found at the point of the sample (Figure 5 (left)), indicating that a byproduct did form. Considering that, under alkaline conditions, the self-coupling between the reactants may occur in the Suzuki coupling reaction,[28,29] the unknown Raman signal might come from biphenyl or 2,2-bipyridine. The high similarity between the unknown Raman signal and that of 2,2-bipyridine (Figure 5 (right)) suggests that the unknown compound is relevant to 2,2-bipyridine. There-
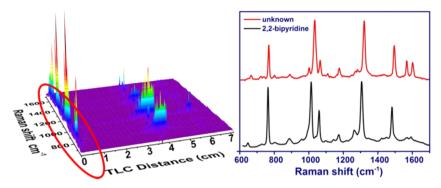
Figure 5. (Left) Full Raman mapping of the product mixture after the separation on the plate. (Right) Comparison of the Raman spectra of the unknown compound with 2,2-bipyridine.

fore, a series of experiments were conducted to identify the unknown compound.

Three pairwise combinations of the two reactants (phenylboronic acid, 2-bromopyridine) and catalyst (palladium acetate) were added into 3 three-necked bottles, respectively, to conduct the reaction under the same conditions. TLC-SERS detection results showed that the unknown SERS signal was detected only in the reaction mixture of 2-bromopyridine and palladium acetate, demonstrating that the unknown compound is related to the 2-bromopyridine. Because of the high similarity of the Raman signal between the unknown compound and 2,2-bipyridine, further experiments were carried out to ascertain whether the unknown signal came from 2-bromopyridine or 2,2-bipyridine. For this purpose, 2-bromopyridine and 2,2-bipyridine were mixed, respectively, with palladium acetate to conduct the reaction under the same conditions. TLC-SERS detection results showed that the unknown SERS signal in the reaction solution of 2,2-bipyridine with palladium acetate was much stronger than that of 2-bromopyridine with palladium acetate (see Figure S7A in the Supporting Information), indicating that the unknown compound was more relevant to 2,2-bipyridine and the self-coupling of 2-bromopyridine occurred during the reaction process.

To further test if the acetate in the catalyst contributed to the unknown compound, three kinds of catalysts—including palladium acetate, palladium nitrate, and palladium chloride—were compared. The quite similar TLC-SERS detection results (see Figure S7B in the Supporting Information) suggested that the acetate was not contained in the constitutional formula of the unknown compound.

We also tested if the buffer contributed to the unknown compound by conducting the reaction in potassium phosphate (pH 13.4), sodium carbonate (pH 12.0), and water (pH 6.7), respectively. Results of TLC-SERS detection (see Figure S7C in the Supporting Information) suggested that the production of unknown compound was enhanced in alkalescent buffers, but the components of the buffer were not contained in the constitutional formula of the unknown compound.

Based on the above TLC-SERS results, it can be concluded that, most likely, the unknown SERS signal stemmed from the complex of 2,2-bipyridine and palladium. However, the exact formula of the unknown product remains a topic for further study, because of the lack of standard SERS spectra and standards of the complexes of 2,2-bipyridine and palladium for validation.

CONCLUSIONS

By spraying gold nanoparticles (AuNPs) on a thin layer chromatography (TLC) plate with preseparated analytes to form surface-enhanced Raman scattering (SERS) substrates, we have developed a facile TLC-SERS method for on-site quantitative monitoring of chemical reactions. This method enables continuous detection in a line and a large area on TLC plate, as well as separation and detection on the same TLC plate to provide the concentration information on the analyte. Optimization of the AuNP size and concentration led to the evenly distributed and highly efficient SERS response on the surface of the plate, which enhanced the method sensitivity and precision. By continuous detecting along a line, this method provides full and accurate information about the process in specific chemical reaction, without missing substances that even are invisible to the naked eyes. Importantly, using the characteristic band of the Raman spectra, this proposed TLC-SERS can also distinguish analytes that cannot be separated completely by TLC. Furthermore, through the full scan of a large area on the plate, new byproducts can be found and their primary structures can be identified by the Raman spectra. Thus, this facile TLC-SERS method can be exploited in monitoring the progresses of reaction, as well as environmental and biological process.

ASSOCIATED CONTENT

Ⓢ Supporting Information
Experimental details and additional results are provided as Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author
*Tel./Fax: +86 10 62849192. E-mail address: jfliu@rcees.ac.cn.
Notes
The authors declare no competing financial interest.

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