Tunable Coffee Ring Formation on Polycarbonate Nanofiber Film for Sensitive SERS Detection of Phenylalanine in Urine

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ABSTRACT: A new method based on the coffee ring effect was developed for improving the sensitivity, simplicity, and robustness of surface-enhanced Raman scattering (SERS) in determining trace levels of analytes. In this method, a polyvinylpyrrolidone (PVP)-stabilized silver colloidal (AgC) solution was first prepared and mixed with a sample solution. Following deposition of the mixture solution on a solid substrate with a rough surface, a coffee ring was formed once the solvent had evaporated. The formation of a coffee ring not only concentrated the analyte but also reduced the space between silver nanoparticles (AgNPs) to strengthen the hotspot effect, thereby considerably improving SERS sensitivity. To strengthen the coffee ring effect further, the surface roughness of the solid support and PVP content of the AgC solution were investigated. The results indicated that an increase in surface roughness reduced the size of the coffee rings, whereas the addition of PVP not only stabilized the AgNPs but also improved the compactness of the coffee rings. When applying the proposed method to determine the phenylalanine (Phe) level in urine for rapid screening of the phenylketonuria disorder, strong chemical interference from uric acid (UA), which is a major component in urine, was observed. To minimize the interference from UA, ZnO powder was applied to the urine sample to adsorb UA prior to SERS detection. After cleaning by using ZnO, the SERS signals of Phe were revealed for quantitative purposes. Under the optimized conditions, both the sensitivity and reproducibility of SERS measurement considerably improved. Quantitative analyses revealed that the developed method is highly feasible for the rapid determination of Phe in real samples.

1. INTRODUCTION

Surface-enhanced Raman scattering (SERS) is the phenomenon in which the Raman scattering of molecules is enhanced by several orders of magnitude when these molecules are adsorbed on or nearby the rough metal surface. This enhancement of Raman scattering is usually explained using the electromagnetic field effect and chemical effect. Active SERS substrates are commonly prepared through the immobilization of suitable metal nanostructures on a common support and are used to detect compounds through either deposition or soaking of sample solution. For example, silver and gold nanostructures have been decorated on solid supports of polymer nanofibers, paper, glass, and other materials. Although the reported sensitivity for these types of substrates is promising, the short lifetime of the substrates, which is caused by the surface oxidation of metal nanostructures, and the tedious fabrication procedure involved have limited the practical application of this type of substrates. Colloidal solution-based metal nanostructures are easily fabricated using wet chemical methods and are available for mass production. With the assistance of stabilizing agents, the lifetime of colloidal solutions can be extended to several weeks or even months. However, the density of metal nanostructures in the colloidal solution must generally be low to prevent coagulation. This low density of metal nanostructures in the colloidal solution results in low sensitivity of SERS measurements, with the observed sensitivity usually 1 or 2 orders of magnitude lower than that observed for other substrate types.

To improve the sensitivity of using colloidal solutions for SERS detection, several methods have been developed, including heat-induced SERS sensing, solvent-induced hotspot, elasticity-induced stretchable membrane, coffee ring formation, and other methods. Among them, methods based on the coffee ring effect are simple in detection, while the sensitivity is acceptable caused by the strengthening of the hotspot effect. The formation of coffee rings involves a spontaneous process in which surface solvent evaporation leads to the concentration of the solute on the outer surface of the liquid droplet. The solute molecules and metal nanostructures can be packed closely during solvent evaporation, which causes strengthening of the hotspot effect in SERS measurements.

Coffee rings occur frequently when chemicals or biomolecules are deposited onto a suitable solid support. This phenomenon has been employed in SERS measurements through the deposition of a mixture of analytes and metal colloids on an appropriate solid support, with the mixture forming droplets. After solvent evaporation, suspended particles are deposited at the periphery to form a coffee ring because of the capillary action and weak Marangoni flow of the solvent. In SERS applications, the formed coffee ring pattern affects both detection sensitivity and reproducibility. For example, if a small and compact coffee ring is formed, the packing density of the metal colloids is high, which results in a
strong hotspot effect. Therefore, regulating the formation of the coffee ring is crucial for achieving high performance in SERS applications. The size of the coffee ring is affected by the contact angle of the droplet deposited on the solid support. The larger the contact angle, the smaller is the coffee ring. The contact angle is dependent on the roughness and hydrophobicity of the surface of the solid support. Moreover, the chemical composition of the droplet influences its surface tension and causes variation in the contact angle. Therefore, to control the formation of a coffee ring and enhance SERS intensity, the aforementioned parameters and their effects on SERS measurements were investigated in the present study. As illustrated in Figure 1, the substrate roughness and droplet chemical composition were the parameters of focus, with the objective of reducing the size of the formed coffee ring. Polycarbonate (PC) was selected as the substrate because of its hydrophobicity, which led to large contact angles and thus a small coffee ring. To alter the surface roughness of PC substrates, the PC nanofiber (PCNF) film was produced by electrospinning using different PC solutions. The prepared PCNF films were three-dimensional structures, and their surface roughness was controlled through the diameter of the nanofibers and air pockets within the structures, and hence, the contact angle of aqueous droplets could be varied. The effect of the droplet chemical composition on the formed coffee ring was also examined using various concentrations of the species in the droplets.

To investigate the performance of the formed coffee ring for SERS detection, phenylalanine (Phe) was selected because it is a key molecule in terms of phenylketonuria (PKU) disorder. Detecting this compound at a low level under strong interference in the urine matrix is challenging. The determination of Phe requires sensitive detection because its concentration in urine is 0.3–3.5 μM in healthy adults and can be as high as 100 μM in infants with the PKU disorder. Therefore, a coffee ring method was developed that detects Phe in urine and can be used to track the PKU disorder.

2. RESULTS AND DISCUSSION

2.1. Characteristics of the Prepared Silver Colloidal Solution and PCNF Film. When creating a coffee ring for SERS detection, the properties of the silver colloidal (AgC) solution used and solid support of the PCNF film are critical. Therefore, the morphology of silver nanoparticles (AgNPs) in AgC solutions prepared with the addition of different concentrations of polyvinylpyrrolidone (PVP) solutions was first examined using ultraviolet–visible (UV–vis) spectroscopy. A surface plasmon resonance (SPR) band located around 420 nm was observed for any of the prepared AgC solution, indicating that AgC solutions were successfully prepared (Figure 2A). SPR bands were marginally shifted within 10 nm for any examined PVP concentrations. This observation indicates that the prepared AgNPs using different PVP concentrations are similar in particle size.

For analytical considerations, the long-term stability of the AgNPs was investigated. As displayed in Figure 2B, the SPR band intensity for AgC0, AgC0.15, AgC0.3, and AgC0.45 changed marginally over the first two days and then remained relatively constant over 10 days. These results suggested that the PVP-stabilized AgC solutions can be used for long periods of time. Moreover, two AgNP solutions (AgC0 and AgC0.3) were characterized using transmission electron microscopy (TEM). The images of AgC0 (Figure 2C) reveal AgNPs with a particle size of approximately 60 nm, with a large variation in the particle size and the coagulation of some particles. By contrast, for the PVP-containing solution of AgC0.3, the particle size was narrowly distributed and some rod-like nanostructures were present (Figure 2D). According to the magnified TEM image (inset of Figure 2D), the formed particle size was approximately 50 nm. The distribution of the particle size of the formed AgNPs was narrower than that of the AgC0 solution not containing PVP. The aforementioned results indicate that the addition of PVP not only stabilized the AgNPs but also regulated the AgNPs to have narrow size distribution.

To determine the impact of the surface roughness of the solid support on the formation of the coffee ring, PCNF films with different morphologies were prepared according to our previous study by varying the concentration of the PC solution from 5 to 18%. The prepared PCNF films were characterized through scanning electron microscopy (SEM). The results indicated that bead-free PCNF films could be prepared when the concentration of the PC solution was higher than 13%. Figure 2E depicts an SEM image of a PCNF film prepared from 18% PC solution. Numerous beads with fine nanofibers can be observed in the image. When the PC concentration was higher than 13%, the prepared PCNF films were bead-free. Figure 2F displays the SEM image of the PCNF film prepared from 18% PC solution. This PCNF film contained bead-free nanofibers. The diameter of the fibers ranged from 200 to 500 nm, and the fibers were loosely packed.

2.2. Effect of Solid Support on Coffee Ring Formation. To determine the influence of the roughness and hydrophobicity of the solid support on the formation of a coffee ring, three types of solid support were examined: a glass plate, a PC plate, and PCNF films. PCNF films prepared using 18% PC solution were used in evaluations of the ring formation performance. To ensure that the AgC and PCNF films did not cause spectral interference, the Raman spectra of
the bare PCNF film and the coffee ring formed by depositing AgC0.3 on this PCNF film (AgC0.3@PCNF) were acquired (Figure 3A). The spectrum of the PCNF film itself does not have any spectral features; however, some weak spectral features are observable for AgC0.3@PCNF. After mixing 1 mM Phe with AgC0.3 in a volume ratio of 1:1, the formed solution (Phe−AgC0.3) was deposited on a PCNF film and dried (Phe−AgC0.3@PCNF). The SERS spectrum of Phe−AgC0.3@PCNF is displayed in Figure 3A along with the liquid spectrum of Phe−AgC0.3 and solid Phe. Considering the liquid spectra of Phe−AgC0.3, Phe could not be detected effectively if no coffee ring was formed. Once a coffee ring was formed on the PCNF film, a high-quality spectrum could be obtained and the spectral feature of Phe matched with that in the conventional Raman spectrum of solid Phe. Also, the spectral features are consistent with those in the literature,46,47 as shown in Figure 3A with band assignments. The intense symmetric ring stretching band at 997 cm−1 was selected for quantitative evaluation in later analyses.
To evaluate the effect of the surface roughness of the solid support on coffee ring formation, a series of PCNF films were produced using PC solutions with concentrations ranging from 5 to 18%. Phe–AgC0.3 (1 mM Phe, volume ratio 1:1) was used for characterization. Furthermore, a glass plate and PC plate were used as points of comparison with the PCNF films. After depositing and then drying 10 μL of Phe–AgC0.3 solution on the prepared PCNF films, SERS spectra were acquired from the outer band and center of the coffee rings. Photographs of the coffee rings formed on different solid supports are illustrated in Figure 3B. The coffee rings formed on PC and glass had a considerably larger diameter than those formed on the PCNF films. Also, when PCNF films produced with PC concentrations lower than 13% were used as the support, some dark areas could be observed inside the coffee rings. The SEM results indicated that PCNF films produced with a concentration of 11% had beads on their surface. These beads may hinder the movement of AgC solution during solvent evaporation and lead to dark spots inside the coffee rings. Raman spectra were acquired in the dark band and center areas of the coffee rings, and the variation in the Phe intensity at 997 cm⁻¹ for different solid supports is illustrated in Figure 3C. Considerably more intense SERS bands with smaller variation are observed in the spectra of the PCNF films produced from 13 to 18% PC solutions compared with those produced from 5 to 11% PC solutions. The SERS intensities are almost 15 times stronger in the PCNF film spectra than in the glass and PC plate spectra. On the other hand, the higher the surface roughness of the solid support, the higher is the intensity of the SERS signals.

2.3. Effect of PVP Concentration on Coffee Ring Formation. The size of a coffee ring is also affected by the surface tension of the liquid droplets. To examine this effect, AgC solutions were prepared with different amounts of PVP. PCNF films prepared with 18% PC solution were used as the solid support. After mixing an AgC solution with 1 mM Phe in equal volume, 10 μL of the mixture was deposited on a PCNF film. After drying, the formed coffee rings were subjected to SERS measurement. The coffee rings formed from Phe–AgC0.15, Phe–AgC0.3, and Phe–AgC0.45 were first cross-scanned from one end of the ring to the other. The intensity of Phe at 997 cm⁻¹ was plotted against the location, as displayed in Figure 4A. In addition, the Phe intensity at the dark ring and center regions was plotted against the PVP concentration, as illustrated in Figure 4B. Figure 4A reveals that the diameter of the formed coffee ring is approximately 2 mm, with small variation for all the examined solutions. Thus, the PVP concentration did not strongly affect the size of the coffee ring. However, the intensity of Phe varied significantly with different amounts of PVP, as illustrated in Figure 4B. The variation in the band intensity was caused by the formation of coffee rings of different widths of the dark band. To verify this finding, the coffee rings of Phe–AgC0, Phe–AgC0.15, Phe–AgC0.3, and Phe–AgC0.45 were examined through SEM. The resulting images for Phe–AgC0, Phe–AgC0.15, Phe–AgC0.3, and Phe–AgC0.45 are presented in Figure 4C–F, respectively. In these images, no clear band can be observed for the coffee ring formed when no PVP was added (Figure 4C, AgC0). The addition of PVP into the AgC solution resulted in clear bands, and the width of the band of the coffee ring increased as the PVP concentration was increased.

2.4. Effect of AgC Concentration on Coffee Ring Formation for SERS Detection. The formation of a coffee ring can increase the tightness in the packing of AgNPs to exert a strong hotspot effect. The amount of AgC required to form coffee rings that exert a sufficiently strong hotspot effect was investigated. AgC0.3 solution was diluted with different amounts of water. The diluted AgC solutions were then mixed with 5 mM Phe in a volume ratio of 9:1 to obtain a final Phe concentration of 0.5 mM, which is the same as the concentration used in above studies. To describe the findings clearly, the relative concentration of AgC (AgC %) was defined.

Figure 4. (A) Peak intensity of Phe at 997 cm⁻¹ obtained by scanning the SERS spectra across the formed coffee rings of Phe–AgC0.15 (●), Phe–AgC0.3 (■), and Phe–AgC0.45 (▲). (B) Peak intensity at 997 cm⁻¹ of Phe at the center (●) and ring (■) regions when AgC was stabilized using different PVP concentrations. (C–F) SEM images of the coffee rings of AgC0, AgC0.15, AgC0.3, and AgC0.45, respectively.
as \( V_{AgC}/(V_{AgC} + V_{water}) \times 100\%. As displayed in the plot of the observed SERS intensity of Phe at 997 cm\(^{-1}\) against AgC \(\%\) (Figure 5), the SERS intensity plateaued when AgC \(\%\) reached 55.6\%. Smaller concentrations of AgC result in considerably reduced SERS intensity. This result reveals that if AgC \(\%\) is lower than 55.6\%, the amount of AgC is insufficient to form dark bands and to exert a strong hotspot effect for detection. As illustrated in the photographs displayed in the inset of Figure 5, the darkness of the ring region of the coffee ring decreased as AgC \(\%\) was decreased, whereas the width of the dark band remained constant. If the AgC concentration is considerably diluted, it would be unable to form a coffee ring with a strong hotspot effect. When AgC \(\%\) is close to 55.6\%, the amount of AgNPs that form the coffee ring is close to the amount of undiluted AgC mixed with the sample solution in a volume ratio of 1:1.

2.5. Quantitative Aspects. The linearity of the SERS response of Phe was examined by mixing a 1:1 volume ratio of AgC\(^{0.3}\) with Phe solution. The original concentration of Phe (before mixing) in the sample solution was recorded and used to construct the calibration curve plotted in Figure 6. A linear relationship was obtained in the concentration region lower than 500 \(\mu\)M with a regression coefficient \((R^2)\) of 0.997. The estimated detection limit based on three times the noise level was approximately 2.35 \(\mu\)M. This detection limit indicates that our developed method improves the sensitivity in SERS detection by controlling the formation of coffee rings. Comparing with the literature reported methods, our developed method is competitive as the reported detection limits are in the range of 0.1–15 \(\mu\)M\(^{-1}\). For instance, separation techniques combined with mass detection showed a detection limit within 1–6 \(\mu\)M\(^{-1}\) and SERS methods gave a detection limit around 10 \(\mu\)M.\(^{-1}\)\(\mu\)M\(^2\)

In a real sample of urine, a strong matrix effect usually occurs. Therefore, the possible interfering species in a urine sample were first investigated. These species included amino acids and some metabolites. Selected interfering species were detected individually by using the optimized conditions determined previously in the present study. The spectra obtained for amino acids and metabolites are presented in Figure 7A,B, respectively. With the exception of tryptophan, amino acids could not be effectively detected (Figure 7A). Fortunately, the peak for the quantitative analysis of Phe is located at 997 cm\(^{-1}\), which is not spectrally interfered by tryptophan. In the analysis of the major metabolites in urine (Figure 7B), the spectral features of uric acid (UA), creatinine, and urea could be observed but not those of ammonia. In terms of spectral interference, only UA exhibits a weak broad band at the band position used for the quantitative analysis of Phe.

To further investigate the chemical interference caused by the aforementioned compounds, interfering species were added to the Phe solution. The results are presented in Table 1. Amino acids did not cause strong chemical interference with Phe detection. However, the compounds of UA, creatinine, and urea caused severe suppression of the Phe band intensity. This indicates that these species are capable of adsorbing onto the surface of AgC and occupying the active sites, thereby suppressing the SERS intensity of Phe. To overcome this problem, ZnO powder was employed to adsorb the major component of UA in the urine samples. According to the literature, ZnO strongly interacts with UA while remaining in the solid form. To examine its capability to remove UA, different amounts of ZnO powder were used to pretreat the sample solution for 10 min. Following centrifugation, the supernatant was employed as a sample for detection. Three aqueous solutions of 50, 100, and 200 \(\mu\)M UA were pretreated with different amounts of ZnO powder to examine the powder’s cleaning efficiency. The strong peak of UA at 1333 cm\(^{-1}\) (Figure 7B) was used to indicate the residual UA after treatment with ZnO powder. The SERS spectra obtained using the coffee ring method for nontreated UA and ZnO-treated solutions are displayed in Figure 8A. The spectra indicate that UA was completely removed, and the amount of ZnO required for complete cleaning varied with the UA concentration, as illustrated in Figure 8B. Approximately 2, 4, and 8 \(\%\) w/v of ZnO were required to completely remove 50, 100, and 200 \(\mu\)M UA, respectively (Figure 8B). Conversely, 1\% w/v ZnO could completely remove 25 \(\mu\)M of UA from the liquid solution.

To further study the influence of ZnO on the detection of Phe, 50 \(\mu\)M Phe was treated with different amounts of ZnO and the obtained SERS intensity of Phe at 997 cm\(^{-1}\) was plotted against the added amount of ZnO (Figure 8C). The addition of ZnO was discovered to reduce the Phe intensity; however, the Phe intensity remained at approximately 80% of...
the nontreated Phe intensity. These results indicate that the interaction between Phe and ZnO was weak. Therefore, the addition of ZnO effectively removed UA with a marginal decrease in the sensitivity of Phe detection. To further identify any cross-interaction between UA and Phe, a sample solution containing 50 μM Phe and 50 μM UA was examined. After treatment with 2% w/v ZnO, the SERS intensity of Phe remained at 80% of the original intensity, as illustrated in Figure 8D. This finding indicates that the cross-interaction between Phe and UA was also weak.

2.6. Application of the Proposed Approach for Detecting Phe in Urine Samples.

To evaluate the accuracy of the developed method for real-world samples, urine samples were collected from three healthy adult volunteers for 24 h and Phe was spiked into each of the collected sample with a concentration of 50 μM. According to the literature,55,56 the UA concentration in a urine sample ranges from 0.6 to 6 mM. The amount of ZnO powder required for removing such a high concentration of UA is too high to obtain a clear supernatant. Therefore, urine samples were diluted 10 times before analysis. Based on above studies, 1% w/v ZnO removes 25 μM UA. Considering that 6 mM is the highest concentration of UA found in urine samples, 24% ZnO powder can remove all UA after 10x dilution. Therefore, 30% w/v ZnO powder was used to clean up UA prior to Phe detection. The standard addition method was employed to eliminate the effect of ZnO powder on Phe detection. After the ZnO treatment, the concentration of Phe was determined using the one-point standard addition method. The estimated recovery was 99.2%(±6.8%), 102.4%(±1.6%), and 97.8%(±1.2%) for the three urine samples. These results indicate that our developed method is highly applicable for patients with PKU disorder.

3. CONCLUSIONS

In this study, coffee rings were successfully prepared on PCNF films by using PVP-stabilized AgC solutions. The coffee rings were used to determine the Phe concentration for addressing the PKU disorder. The preparation of an AgC solution is simple and the prepared AgC is highly stable. To improve the sensitivity of detection, the roughness of the solid support and composition of AgC were examined. The results indicated that a tight coffee ring formed on the hydrophobic PCNF film produced from a high-concentration PC solution. The composition of AgC did not affect the size of the coffee rings. The developed method was then employed to detect Phe in urine samples. The strong matrix effect from UA was
suppressed by employing a simple cleaning step. Successful results were obtained in the determination of Phe in urine samples. Thus, the developed method is sensitive for Phe detection and is readily applicable to the screening of the PKU disorder.

4. EXPERIMENTAL SECTION

4.1. Chemicals. Poly(bisphenol A carbonate) (PC, MW ≈ 45 000) was obtained from Sigma (St. Louis, MO, USA). Dimethylformamide (DMF) was purchased from J. T. Baker (Phillipsburg, NJ, USA). PVP (MW 1 300 000) and trisodium citrate were purchased from Janssen (Beerse, Belgium). L-Phenylalanine (Phe, 98.5%) was obtained from Acros Organics (Phillipsburg, NJ, USA). Silver nitrate was purchased from ProChem (Rockford, IL, USA). Tetrahydrofuran (THF) was obtained from Mallinckrodt Chemicals (St. Louis, MO, USA). Methanol was procured from Echo Chemical (Toufen, Taiwan). Deionized Milli-Q water was used to prepare all aqueous solutions. All the chemicals were used as received without further purification.

4.2. Apparatus. An electrospinning machine was purchased from MECC Co. (NANON-1A, Fukuoka, Japan). The machine comprised a 6 mL plastic syringe with an 18-gauge stainless-steel needle, an electrically controlled drum collector, and a high voltage supply for generating an electric field. The solution feed rate was maintained at 1.2 mL/h, and the distance between the collector and needle was maintained at 5 cm in all the productions. SERS spectra were obtained using a Triax 320 Raman system (Jobin Yvon Inc., Longjumeau, France) equipped with a 25 mW, 632.8 nm He/Ne laser (JDS Uniphase Co., Milpitas, CA, USA) and a liquid-nitrogen-cooled Ge charge-coupled device array detector (Jobin Yvon Inc.) having a spectral resolution of 0.06 nm. All the spectra were acquired using an exposure time of 1 s with 1 accumulation unless otherwise specified.

4.3. Preparation of AgC Solution. The method of preparing the AgC solution was modified from the methods in the literature. Citrate and PVP were used as the reducing and stabilizing agents, respectively. To prepare a 50 mL aqueous AgC solution, 1 mM silver nitrate and different concentrations of PVP (0−0.75% w/v) were first prepared in a conical flask. The solution was boiled for 10 min and 1 mL of 1% w/v trisodium citrate aqueous solution was then added dropwise, after which this solution was heated to a gentle boil for 15 min with mild stirring. The final AgC solution had a pale greenish brown color. The prepared AgC solution was stored in a refrigerator until use. To abridge the description, AgC solution prepared with x% w/v PVP is abbreviated as AgCx.

4.4. Preparation of PCNFs. PCNF films were prepared using our previously developed electrospinning method. Polycarbonate pellets of different weights were dissolved using 10 mL of a mixed solvent of THF and DMF (3:1). The PC solution with 1.8 g of PC pellets dissolved in 10 mL of 3:1 THF/DMF solvent was named as18% PC solution. After the PC had dissolved well, the solution was loaded into a syringe tube. A drum collector (1000 rpm) was used to collect the PCNF films. The electrospinning voltage, collector distance, and feed rate were fixed at 30 kV, 50 mm, and 1.2 mL/h, respectively. Within 3 h of spinning, self-standing PCNF films could be produced.

4.5. Detection Procedure. For detection, the sample and AgC solutions were mixed in a designed volume ratio. Then, 10 μL of this mixture was pipetted carefully onto the surface of a PCNF film. The liquid droplet that formed on the PCNF film was dried in a 70 °C oven for 10 min and subsequently subjected to Raman measurement.

Figure 8. (A) Detected SERS spectra of 50−200 μM UA with and without the ZnO treatment. (B) SERS intensity at 1133 cm−1 for 50 (▲), 100 (●), and 200 (■) μM UA after treatment with different amounts of ZnO powder. (C) SERS intensity of Phe at 997 cm−1 for the detection of 50 μM Phe after treatment with different amounts of ZnO powder. (D) SERS spectra of 50 μM UA, 50 μM Phe, and the solution of 50 μM Phe and 50 μM UA before and after the treatment with 2% w/v ZnO powder.
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