Surface-Enhanced Raman Scattering-Active Substrate Prepared with New Plasmon-Activated Water

Chih-Ping Yang,§,∥ Sheng-Uei Fang,§,⊥ Kuang-Hsuan Yang,‡ Hsiao-Chien Chen,‡ Hui-Yen Tsai,‡ Fu-Der Mai,∥ and Yu-Chuan Liu‡,⊥

‡Department of Biochemistry and Molecular Cell Biology, and §Department of Internal Medicine, School of Medicine, College of Medicine, Taipei Medical University, No. 250, Wuxing Street, Taipei 11031, Taiwan
¶Division of Gastroenterology and Hepatology, Department of Internal Medicine, Taipei Medical University Hospital, No. 252, Wuxing Street, Taipei 11031, Taiwan
∥Department of Materials Science and Engineering, Vanung University, 1 Van-Nung Road, Taoyuan 32061, Taiwan

ABSTRACT: Conventionally, reactions in aqueous solutions are prepared using deionized (DI) water, the properties of which are related to inert “bulk water” comprising a tetrahedral hydrogen-bonded network. In this work, we demonstrate the distinguished benefits of using in situ plasmon-activated water (PAW) with reduced hydrogen bonds instead of DI water in electrochemical reactions, which generally are governed by diffusion and kinetic controls. Compared with DI water-based systems, the diffusion coefficient and the electron-transfer rate constant of K₃Fe(CN)₆ in PAW in situ can be increased by ca. 35 and 15%, respectively. These advantages are responsible for the improved performance of surface-enhanced Raman scattering (SERS). On the basis of PAW in situ, the SERS enhancement of twofold higher intensity of rhodamine 6G and the corresponding low relative standard deviation of 5%, which is comparable to and even better than those based on complicated processes shown in the literature, are encouraging.

INTRODUCTION

Water is the most commonly used environmentally friendly solvent for chemical reactions in solution. Compared with other solvents, water is able to form a flexible dynamic hydrogen-bonded network, in which hydrogen bonds (HBs) are dynamic in picoseconds, which makes investigating its local structure challenging. Thus, although most properties of liquid water are determined by its HBs, all of water’s commonly recognized properties are related to inert “bulk water” composed of tetrahedral hydrogen-bonded networks. Water as a solvent is conventionally considered a passive spectator in chemical reactions. Actually, liquid water has emerged as a promising active reactant using its characteristic property of donor-acceptor for proton transfer and electron donating. Moreover, liquid water is conventionally considered an independent reactant. However, as shown in the literature regarding the hydrogen evolution reaction (HER), the interaction energy of H₂O°−OH⁻ is 46.9 kJ mol⁻¹, but it increases approximately 2.5 times when H₂O° associates with an additional four water molecules by HBs. Meanwhile, gas-phase water is capable of catalyzing many chemical reactions through its ability to form HBs with other molecules because of more free water molecules being available in the gas phase, compared with liquid-phase water with a more perfect tetrahedral symmetry. These facts inspired us to create active liquid plasmon-activated water (PAW) with reduced HBs from deionized (DI) water at room temperature using hot electron transfer (HET) on resonantly illuminated gold nanoparticles (AuNPs). The created PAW can be innovatively employed as an environmentally friendly etching agent (vapor from hot electron-activated liquid water), in more efficient HERs and in increasing the efficiency and safety of hemodialysis. These innovative applications of PAW instead of conventional DI water in green chemistry, energy, and medicine open new aspects of the plasmonic efficiency of HET, novel hot dog-structured Au...
nanorod (NR)@Cu2O NPs were designed and synthesized.21 In this unique structure, the head-exposed Au NR provides a plasmonic enhanced local electric field at the surface of the photocatalyst to improve the transfer of electrons. A similar strategy was proposed for enhancing the plasmonic efficiency of HET based on Au-decorated ZnO corn silk.22 The formation of a Schottky barrier at the Au/ZnO interface can retard the electron−hole recombination, thus increasing the photoelectrochemical efficiency. With significant improvements in nanotechnology, the SERS enhancement is correspondingly increased, as expected, making the detection of single molecules possible.23,24 However, the reproducibility of SERS signals is also an important parameter of concern because of its reliable application. Unfortunately, high SERS enhancement generally corresponds to low reproducibility and to poor stability of SERS signals, which makes its application unreliable.25,26 Core@shell and array structures are two popularly employed strategies to improve SERS enhancement while maintaining both reproducibility and stability, but the fabrication procedures are correspondingly complicated.27,28

By using femtosecond transient absorption spectroscopy with visible pump and infrared probe to observe generation of injected electrons, plasmon-induced electron transfer from 10 nm Au nanodots to TiO2 nanocrystalline film was directly observed. It was revealed that the reaction time was within 240 fs and the yield was about 40%.29 On the other hand, reaction and diffusion rates in water cage are interesting. The much higher intramolecular kinetic isotope effects (KIEs) compared with the intermolecular KIEs of the same chemical reaction, R−H + "OH → R" + H2O, indicate a high degree of mobility of the two reaction partners inside of the solvent cage.30 As shown in our previous study,31 the activity of created PAW decays after a few days. Thus, we developed a multifunctional excited AuNP-decorated artificial kidney with efficient hemodialysis and therapeutic potential using PAW created in situ.32 In this work, we demonstrate the innovative advantages of in situ preparation of PAW used in electrochemical reactions with a higher diffusion coefficient and electron transfer rate, which generally govern electrochemical reactions. The effects of the in situ preparation of PAW on the correspondingly increased SERS signal and on improved signal reproducibility are also demonstrated in reference to traditional DI water.

RESULTS AND DISCUSSION

Electrochemical Reactions in PAW of Roughened Au Substrates Prepared Using PAW. As shown in the literature regarding SERS studies,33,34 controllable and reproducible surface roughness was readily produced by controlling electrochemical oxidation−reduction cycle (ORC) treatments using aqueous solutions containing chlorite-electrolytes. Figure 1a shows the image of in situ production of PAW. Figure 1b demonstrates typical triangular voltammetric curves in the fifth

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**Figure 1.** Reaction cell in the electrochemical experiment and the corresponding electrochemical reactions in PAW and DI water. (a) Equipment of the glass reaction cell under resonant illumination of green LEDs: 1, working electrode; 2, reference electrode; 3, counter electrode; and 4, green LEDs pasted onto a transparent plastic sheet the circled the glass cell. (b) CVs showing the fifth scan in ORC treatments at 500 mV s−1 for roughening the Au electrodes in 0.1 M KCl using the same Au substrate based on different waters with and without resonant illumination. (c) CVs showing the 20th scan in ORC treatments at 500 mV s−1 for roughening the Au electrodes in 0.1 M KCl using the same Au substrate based on different waters with and without resonant illumination. (d) CVs at 50 mV s−1 of 50 mM K3Fe(CN)6 in DI water with roughened Au substrates prepared using different waters with and without resonant illumination; the blank sample represents a mechanically polished Au electrode without further ORC treatment for reference.
The terms “roughened Au substrates (samples ‘DIW’) prepared using DI waters without resonant illumination. (a) DI water without resonant illumination. (b) PAW with resonant illumination. (c) Plots of anodic peak potential ($E_{pa}$) and cathodic peak potential ($E_{pc}$) vs $\ln \upsilon$ from plot (a) based on DIW. (d) Plots of anodic peak potential ($E_{pa}$) and cathodic peak potential ($E_{pc}$) vs $\ln \upsilon$ from plot (b) based on PAW in situ.

Figure 2. CVs at various scan rates ($50-600 \, mV \, s^{-1}$) of 50 mM K$_3$Fe(CN)$_6$ in different waters with and without resonant illumination with roughened Au substrates (samples “DIW”) prepared using DI waters without resonant illumination. (a) DI water without resonant illumination. (b) PAW with resonant illumination. (c) Plots of anodic peak potential ($E_{pa}$) and cathodic peak potential ($E_{pc}$) vs $\ln \upsilon$ from plot (a) based on DIW. (d) Plots of anodic peak potential ($E_{pa}$) and cathodic peak potential ($E_{pc}$) vs $\ln \upsilon$ from plot (b) based on PAW in situ.

Scan for anodic dissolution of Au and cathodic redeposition of Au on substrates in solutions of 0.1 M KCl based on PAW with reduced HBs and DI water with stronger HBs. In these plots, the terms “DIW” and “PAW”, respectively, represent ORC procedures performed in DI water and prepared PAW, without additional resonant illumination of green light emitting diodes (LEDs) in the experiments. The terms “DIW in situ” and “PAW in situ” represent ORC procedures, respectively, performed in DI water and prepared PAW under resonant irradiation of green LEDs in the experiments. Basically, the dissolution of Au and the redeposition of AuNPs onto substrates were easier in the PAW-based electrolytes, as indicated from the enhanced currents, than those in the DI water-based electrolytes. In the ORC treatment for roughening the Au substrate, AuNPs were deposited on the Au substrate. Therefore, water with stronger HBs at the AuNPs on the Au substrate under resonant illumination could be transferred into PAW with reduced HBs. Compared with the “DIW” sample, the cathodic redeposition currents at ca. 0.33 V versus Ag/AgCl, respectively, increased by 2.7 and 4.3% for the “DIW in situ” and “PAW” samples. These increases were more significant for the “PAW in situ” sample, that increased by 41.6%. This great increase of 41.6% compared with those of 9.5 and 10.0% indicated that the original PAW with reduced HBs at the AuNPs on the Au substrate under resonant illumination could be more easily transferred into in situ PAW with greater numbers of weaker HBs available. This also suggests that a synergistic effect on further reducing HBs of water indeed occurred for PAW with intrinsically reduced HBs on an AuNP-deposited Au electrode under resonant illumination. It is recognized that the obtained currents well depend on the kinetics- and diffusion-controls in electrochemical reactions. These results suggested that the employed KCl electrolytes could diffuse more efficiently in ORCs, and higher electron transfer rates could occur at the Au electrode in PAW-based solutions, especially in in situ-based PAW solutions, which contributed to the obtained higher currents at constant applied potentials. The different effects using PAW instead of conventional DI water on the corresponding ORC treatments for roughening Au substrates could result from the different respective kinetics- and diffusion-controlled reactions, which were shown in their significantly different cyclic voltamograms (CVs). These interesting phenomena are discussed below. After preparing roughened Au substrates as described in Figure 1c, the same substrates were further examined to obtain their specific surface areas, as shown in Figure 1d regarding the corresponding CVs at 50 mV $s^{-1}$ of 50 mM K$_3$Fe(CN)$_6$ in DI water-based solutions. An additional blank sample of a mechanically polished Au electrode without further ORC treatment was also used for reference. It can be observed that, as expected, both anodic and cathodic peak currents based on roughened Au substrates were higher than those of the blank flat Au electrode. Compared with the cathodic peak current of the blank Au electrode, those values, respectively, increased by
ca. 7.6, 10.9, 10.9, and 11.5% for the “DIW”, “DIW in situ”, “PAW”, and “PAW in situ” samples. Similarly, the increased currents for the “DIW in situ” and “PAW” samples based on waters with reduced HBs were at the same level, which were higher that that of the “DIW” sample based on water with stronger HBs. This increase was slightly enhanced for the “PAW in situ” sample based on water with greater numbers of reduced HBs. The specific surface areas of roughened Au electrodes can be calculated according to the Randles–Sevcik equation:

\[ I_p = 269,000AN^{3/2}D^{1/2}ν^{1/2} \]  

where \( I_p \) is the peak current, \( n \) is the number of electrons transferred (\( n = 1 \) for this system), \( A \) is the specific surface area of the electrode, \( D \) is the diffusion coefficient, \( C \) is the concentration of the electrolyte, and \( ν \) is the scan rate. In experiments using the same electrolyte, concentration, and DI water at the same scan rate, the specific surface area is proportional to the recorded current. Compared with the blank sample with a surface area of 0.238 cm\(^2\), the calculated specific surface areas were 0.256, 0.264, 0.264, and 0.265 cm\(^2\) for the illumination (calculated from Figure S2). This suggests that PAW in situ had a function of enhancing the diffusion of \( K_3Fe(CN)_6 \) in the experiment at 50 mV s\(^{-1}\). The calculated diffusion coefficient in DI water without resonant illumination at 50 mV s\(^{-1}\) was 1.54 \( × \) 10\(^{-6}\) cm\(^2\) s\(^{-1}\) with a relative standard deviation (RSD) of 6.1%. The calculated diffusion coefficient in DI water without resonant illumination was 1.14 \( × \) 10\(^{-6}\) cm\(^2\) s\(^{-1}\) with an RSD of 8.6%. The other two sets of reproducible experiments are demonstrated in Figure S1. This was a ca. 35% higher diffusion coefficient in PAW with resonant illumination in the experiment at 50 mV s\(^{-1}\) as compared with that in DI water without resonant illumination in the experiment at 50 mV s\(^{-1}\). Moreover, this increase in the diffusion coefficient (1.39 \( × \) 10\(^{-6}\) cm\(^2\) s\(^{-1}\), RSD = 8.3%) was ca. 22% of the magnitude for the experiment in PAW with resonant illumination on the roughened Au substrate (the “PAW in situ” sample) prepared using PAW with resonant illumination (calculated from Figure S2). This suggests that PAW in situ had a function of enhancing the diffusion ability of species in water. The reason might be attributed to the reduced size of hydrated \( Fe(CN)_6^{3−/4−} \) in the water. With a significantly reduced hydrogen-bonded structure, hydration is associated with large water clusters due to the strong HB network of water. Contrarily, breaking the HB structure can reduce the sizes of water clusters, resulting in higher mobility of \( Fe(CN)_6^{3−/4−} \). Similarly, the diffusion coefficients increased by ca. 36, 50, 40, and 46% in PAW with resonant illumination compared with experiments in the DI water system without resonant illumination in experiments at 100, 200, 400, and 600 mV s\(^{-1}\), respectively. Moreover, these diffusion coefficients increased by ca. 30, 43, 32, and 36% in PAW with resonant illumination on the roughened Au substrate (“PAW in situ” samples) prepared using PAW with resonant illumination (calculated from Figure S2) compared with those in the DI water system without resonant illumination in experiments at 100, 200, 400, and 600 mV s\(^{-1}\), respectively. In addition, as the scan rate (\( ν \)) increased from 50 to 600 mV s\(^{-1}\), the redox peak currents of \( Fe(CN)_6^{3−/4−} \) simultaneously increased. The low-conductivity solution, which cannot respond instantly at high \( ν \) because of the slow electron transfer rate in the absence of electrolytes, resulted in more positive and negative shifts in \( E_p \) and \( E_{pc} \), respectively, as \( ν \) increased. Furthermore, when the anodic peak-to-cathodic peak separation was >0.2 V and \( ν \) was >200 mV s\(^{-1}\), the peak potentials were proportional to the natural logarithm of \( ν \) (Figure 2c,d). Two linear equations (with most of \( R^2 \) values of >0.999) were obtained for the anodic and cathodic peak potentials. According to eqs 2 and 3:

\[ E_{pa} = E^0 - \frac{RT}{(1 − α)nF} \ln \frac{RTk_e}{(1 − α)nF} + \frac{RT}{(1 − α)nF} \ln ν \]  

\[ E_{pc} = E^0 + \frac{RT}{αnF} \ln \frac{RTk_e}{αnF} - \frac{RT}{αnF} \ln ν \]  

where \( E^0 \) is the formal potential (i.e., the average of \( E_p \) and \( E_{pc} \)), \( α \) is the electron-transfer coefficient, \( n \) is the number of electrons transferred, \( T \) is the absolute temperature, \( R \) is the gas constant, \( F \) is Faraday’s constant, \( k_e \) and \( k_c \) are the slopes of anodic and cathodic peaks potentials, respectively, and versus \( ν \). \( k_e \) is the apparent heterogeneous electron-transfer rate constant. The value of \( Fe(CN)_6^{3−/4−} \) was 1. Therefore, the \( k_e \) constant can be calculated to be 0.1083 s\(^{-1}\) (with an RSD of 3.2%) for PAW with resonant illuminations in the experiments, which was higher (ca. 15% of the magnitude) than that of 0.0945 s\(^{-1}\) (with an RSD of 0.3%) for DI water without resonant illumination in the experiments. Moreover, this increase in the electron-transfer rate constant was ca. 16% of the magnitude for the experiment in PAW with resonant illumination on the roughened Au substrate (the “PAW in situ” sample) prepared using PAW with resonant illumination (0.1097 s\(^{-1}\) with an RSD of 9.7%) calculated from Figure S2. This indicates that interactions of \( Fe(CN)_6^{3−/4−} \) with its surrounding water molecules could influence the ability of electron transfer. \( Fe(CN)_6^{3−/4−} \) being embedded within large water clusters hindered its electron transfer. Moreover, we performed a similar experiment in DI water as shown in Figure 2a, but the CV experiment of \( K_3Fe(CN)_6 \) was performed in a completely dark atmosphere, not in a normal condition with indoor lighting of fluorescent lamps, as shown in Figure S3. The calculated diffusion coefficient and electron-transfer rate constant were 1.06 \( × \) 10\(^{-6}\) cm\(^2\) s\(^{-1}\) and 0.0880 s\(^{-1}\), respectively, which were ca. 7 and 7% lower in magnitude compared with those corresponding values in experiments performed in DI water under normal indoor lights. These results suggest that available indoor lighting of fluorescent lamps with a full visible wavelength are also effective in creating in situ PAW from DI water on the roughened Au substrate. Certainly, this effectiveness was less than that with resonant illumination of green LEDs.

**Performances of SERS Signals on SERS-Active Au Substrates Prepared Using PAW and DI Water.** Figure 3 shows the Raman spectra of rhodamine 6G (R6G) adsorbed onto roughened Au substrates (similar to the “PAW in situ” sample shown in Figure 1c) prepared in 0.1 M KCl using PAW with resonant illumination in ORC treatments with different scans. They are characteristic of Raman spectra of R6G.37–39 The band at ca. 1184 cm\(^{-1}\) was assigned to the C−H in-plane bend mode; bands at ca. 1313 and 1576 cm\(^{-1}\) were assigned to N−H in-plane bend modes; and bands at ca. 1361, 1510, and
Au already deposited on the substrate if the number of cycles exceeds the optimum value. This results in a lower SERS effect demonstrated by increasing the number of cycles beyond 25 cycles. In this work, the ORC treatment was performed in PAW-based solutions with resonant illumination, in which electrolytes could diffuse and electrons could transfer to electrodes more efficiently. Thus, as shown in Figure 3, the observed optimum number of cycles to obtain the strongest SERS effect using the common ORC treatment for 20 cycles was sufficient. The obtained SERS intensity was comparable to that with 25 cycles, which is generally used in SERS studies. The SERS effect was decided based on the SERS intensity of R6G of the strongest bands (at ca. 1361 cm\(^{-1}\)). This suggests that the treatment time for obtaining the strongest SERS effect can be significantly reduced by ca. 20% in the commonly employed ORC method using in situ PAW instead of conventional DI water. In addition to enhanced SERS intensity, the reproducibility of the SERS intensities is also a concerned topic in its reliable application. As shown in Figure 3, the extremely low RSD of 4% for 20 scans compared with a little higher RSD of 14% for 25 scans indicated that the optimal SERS-active Au substrate could be prepared using the ORC treatment for 20 scans in PAW under resonant illumination. This developed SERS-active Au substrate with excellent reproducibility using the easy one-step electrochemical method was comparable to and even better than those of SERS-active metal arrays based on complicated procedures shown in the literature.\(^{41-44}\) The effect of the number of cycles used in the ORC treatment on the corresponding SERS performance of the roughened Au substrate (similar to the “PAW” sample shown in Figure 1c) was also examined using PAW without resonant illuminations in the ORC treatment, as shown in Figure S4. Similarly, 20 scans used in the ORC treatment were the optimal number of cycles from the viewpoints of the SERS effect and its reproducibility.

Figure 4 shows the SERS spectra of probed R6G adsorbed on SERS-active substrates produced employing different kinds of water with and without resonant illuminations in ORC treatments for 20 scans. The corresponding RSDs based on five measurements are also shown in these plots. The four prepared SERS-active Au substrates were similar to those shown in Figure 1c. Interestingly, the average relative SERS intensities of R6G observed on the Au substrates produced in DI water-based solutions with resonant illumination (the “DIW in situ” sample, Figure 4b) and in the PAW-based solution without resonant illumination (the “PAW” sample, Figure 4c) all increased, compared with that obtained on the Au substrate produced in the DI water-based solution without resonant illumination (the “DIW” sample, Figure 4a). The former and latter increased by ca. 67 and 52%, respectively. Moreover, this increase could be enhanced to ca. 2.1-fold (ca. 3-fold for other probe molecules of deposited polypyrrole, see Figure S5) for the Au substrate produced in the PAW-based solution with resonant illumination (the “PAW” sample, Figure 4d). Detailed calculations, compared with the DIW system, the increases in SERS intensities at 1184 (C–H in-plane bend mode), 1313 (N–H in-plane bend mode), 1361 (C–C stretching mode), and 1510 cm\(^{-1}\) (C–C stretching mode) could be enhanced to 2.7–1.8, 2.1, and 2.1-fold, respectively, for the PAW in situ system. In obtaining the relative intensities, the normalized Raman intensity was used. This was calculated from the ratio of the strongest signal intensity of R6G adsorbed on the SERS-active substrate produced in the PAW-based

![Figure 3. Raman spectra of 2 × 10^{-5} M R6G adsorbed onto roughened Au substrates (samples “PAW in situ”) prepared by using PAW with resonant illumination under different ORC cycles. Spectra were obtained from five selected sites on the same substrate. (a) With 15 cycles. (b) With 20 cycles. (c) With 25 cycles.](https://acsomega.acs.org/doi/10.1021/acsomega.8b00494)
solution to that of R6G adsorbed on the SERS-active substrate produced in the DI water-based solution without resonant illumination. Thus, it is not necessary for making a correction on the normal Raman scattering intensity to account for differences in sampling geometry and scattering phenomena, as shown in the literature. Moreover, the RSD of the SERS intensity of R6G adsorbed on the ORC treatment-prepared “DIW” sample prepared in DI water with strong HBs was acceptable at ca. 16%, as shown in Figure 4a. Encouragingly, this RSD was significantly reduced to 7% for the “DIW in situ” and “PAW” samples prepared in PAW-containing water with reduced HBs, as shown in Figure 4b,c. In particular, this reduction in RSD (5%) (RSD 6% for other probe molecules of deposited polypyrrole, see Figure S5) was more significant for the “PAW in situ” sample prepared in water with greater numbers of reduced HBs, as shown in Figure 4d. Examining the results shown in Figures 1, 2, and 4, it is found that a higher diffusion coefficient for electrolytes in a solution and a higher electron-transfer rate constant are responsible for the higher current recorded in the ORC treatment for obtaining a SERS-active Au substrate. Correspondingly, these higher diffusion coefficients and electron-transfer rate constants are responsible for the higher SERS activity and the better signal reproducibility. The electrochemical ORC procedure in a DI water-based system is a good method to prepare a SERS-active substrate with acceptable signal reproducibility with an RSD of ca. 16%. This RSD can be further reduced to 5% using an in situ PAW system (with a higher diffusion coefficient for electrolytes in a solution and a higher electron-transfer rate constant) in the ORC procedure.

In addition, the good reproducibility of the SERS signal of probed R6G obtained on SERS-active substrates produced in PAW-based solutions was further investigated using SERS mapping. Point-by-point maps recorded from an area of 20 × 20 μm² with 2 μm steps for SERS-active Au substrates produced in DI water- and PAW-based solutions with or without resonant illumination are demonstrated in Figure 5. In an individual plot, the variation in the color of the block is dependent on the highest and the lowest SERS intensities of R6G on the area for each sample. Thus, blocks with different colors represent different intensities of SERS signals of R6G. The maps were obtained using the band area of the baseline-corrected band at ca. 1361 cm⁻¹ (the strongest SERS band of R6G). The more uniform color of the blocks just means the more uniform measured SERS intensities on the area. As shown in Figure 5a, the SERS map on the Au substrate (the “DIW” sample) prepared in DI water-based solutions without resonant illumination demonstrated obviously large spatial variations in SERS intensities. In an individual plot, the variation in the color of the block is dependent on the highest and the lowest SERS intensities of R6G on the area for each sample.

Figure 4. Raman spectra of 2 × 10⁻⁵ M R6G adsorbed onto roughened Au substrates prepared by ORC treatments at 500 mV s⁻¹ for 20 scans in 0.1 M KCl by using different waters under resonant illumination or not (the same experimental conditions shown in Figure 1c). Spectra were obtained from five selected sites on the same substrate. (a) Using DI water without resonant illumination. (b) Using DI water with resonant illumination. (c) Using PAW without resonant illumination. (d) Using PAW with resonant illumination. The calculated RSDs based on SERS intensities of R6G of the strongest bands (at ca. 1361 cm⁻¹) at five selected sites on differently roughened Au substrates are shown in the individual plots.
and in PAW-based solutions without resonant illumination (the “PAW” sample, Figure 5c). Most importantly, the intensity map on the roughened Au substrate exhibited very uniform color for the substrate prepared in full-time PAW-based solutions with resonant illumination (the “PAW in situ” sample, Figure 5d). These phenomena were consistent with observations shown in Figure 4. This effect can be ascribed to the easier reduction of Au on the Au substrate in PAW-containing solutions with reduced HBs, in which electrolytes diffuse and electrons transfer at electrodes more efficiently. Thus, metal NPs with hot spots can be more uniformly deposited onto substrates. This available strategy for obtaining uniform SERS-active substrates is quite easy and convenient.

**Figure 5.** Area mapping (20 X 20 μm²) of point-by-point variations in the SERS signal of R6G of the strongest band (at ca. 1361 cm⁻¹) observed on roughened Au substrates prepared by ORC treatments at 500 mV s⁻¹ for 20 scans in 0.1 M KCl by using different waters under resonant illumination or not (the same experimental conditions shown in Figure 1c). (a) Using DI water without resonant illumination. (b) Using DI water with resonant illumination. (c) Using PAW without resonant illumination. (d) Using PAW with resonant illumination.

**Figure 6.** SEM images of roughened Au substrates prepared by ORC treatments at 500 mV s⁻¹ for 20 scans in 0.1 M KCl by using different waters under resonant illumination or not (the same experimental conditions shown in Figure 1c). (a) Using DI water without resonant illumination. (b) Using DI water with resonant illumination. (c) Using PAW without resonant illumination. (d) Using PAW with resonant illumination.

Therefore, more and more even hot spots should be observed on these PAW-based SERS-active Au substrates.

Surface morphologies of the roughened Au substrates prepared in different 0.1 M KCl-based waters were also examined by atomic force microscopy (AFM). Figure S6 shows 2D surface images of roughened Au substrates. As shown in all four of these images, structural features of the redeposited AuNPs demonstrated dimensions of ca. 100 nm, which is suitable for SERS studies.⁴⁸,⁴⁹ Calculated average values of the mean roughness (Rₐ) on roughened Au substrates prepared using PAW and DI water with and without resonant illuminations were 92.3 (RSD 15.8%), 66.9 (RSD 15.6%), 66.6 (RSD 9.9%), and 45.2 (RSD 5.0%) nm for the “DIW”, “DIW in situ”, “PAW”, and “PAW in situ” samples, respectively. In the calculations, we obtained five Rₐ values, which were automatically calculated from a program attached to the instrument, based on five randomly selected lines on the same sample (see Figure S7 of the “DIW” sample for an explanation). The five random lines were roughly equally spaced across the entire image and were similarly selected for different samples to the greatest extent. The average Rₐ of individual samples was determined from the three sets of medium Rₐ values, removing the largest and smallest ones. The higher value of Rₐ recorded for the DI water-based system without resonant illumination in preparation suggests that this AuNP-deposited substrate was rougher because of uneven sizes and a low density of deposited AuNPs on the substrate. Interestingly, Rₐ values were markedly reduced by ca. 28% in magnitude for the “DIW in situ” and “PAW” samples prepared in PAW-containing systems with reduced HBs because of more even surfaces with closely packed NPs. Moreover, this reduction was enhanced to ca. 51% in magnitude for the Au substrate produced in the PAW-based solution with resonant illumination (the “PAW in situ” sample). This phenomenon indicates that an even AuNP-deposited surface can be created employing the PAW-containing water instead of general DI water in the ORC treatment, especially when using the PAW-based solution with resonant illumination in preparation. This...
promises that the SERS technique can be reliably applied in analytical chemistry, which was discussed before in Figures 4 and 5. These encouraging results all suggest that excellent reproducible SERS signals from easy one-step electrochemical methods are practicable using in situ PAW developed in this work.

**CONCLUSIONS**

We successfully utilized the created in situ PAW with significantly reduced HBs to more efficiently perform electrochemical reactions. Compared with conventional DI water, the diffusion coefficient and electron-transfer rate constant on an Au substrate in this in situ PAW were, respectively, increased by ca. 35 and 15% in magnitude. These marked increases were responsible for more efficient chemical reactions, which generally are governed by diffusion and kinetic controls, in this newly developed water-based solvent. To prepare the SERS-active Au substrate using simple ORC treatment in this in situ PAW instead of DI water, the preparation time can be significantly reduced. Moreover, the SERS effect and signal reproducibility were all improved using the in situ PAW system. In particular, the extremely low RSD of 4% of SERS signals in experiments, the glass cell with DI water was illuminated with green LEDs to create PAW in situ at the AuNPs (see details in the Supporting Information).

**EXPERIMENTAL SECTION**

Preparations of PAW ex Situ and PAW in Situ. PAW (ex situ) was prepared using a previously described method.13 In particular, the extremely low RSD of 4% of SERS signals comparable to and even better than those of SERS-active metal substrates (PDF).

Preparation of the SERS-Active Au Substrate. The Au electrode was cycled in a deoxygenated 0.1 M KCl aqueous solution (40 mL based on PAW or DI water) from −0.28 to +1.22 V versus Ag/AgCl at 500 mV s⁻¹ with and without resonant illumination (green LEDs) for 20 scans. Respective durations at the cathodic and anodic vertices were 10 and 5 s, respectively. Finally, the potential was held at the cathodic vertex before the roughened Au electrode was taken from the solution and thoroughly rinsed with DI water (see details in the Supporting Information).

**ASSOCIATED CONTENT**

* Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b00494.

Detailed experimental methods, CVs of K₃[Fe(CN)]₆ in DI water and PAW, reproducible experiments, Raman spectra of R6G, and AFM images of roughened Au substrates (PDF).
