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Portable SERS-Based POCT Kit for Ultrafast and Sensitive Determining Paraquat in Human Gastric Juice and Urine

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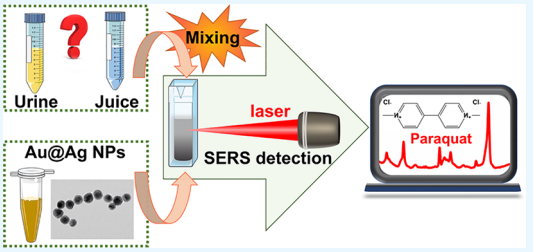
ABSTRACT: Paraquat (PQ) poisoning poses a significant public health concern. Unfortunately, point-of-care testing (POCT) of PQ in biofluids remains challenging. This study developed a portable kit that enables swift and reliable identification and quantification of PQ in human urine and gastric juice. The approach employed the surface-enhanced Raman scattering (SERS) technique, leveraging gold−silver core−shell nanoparticles (Au@Ag NPs) as the substrate. The kit comprised a portable Raman spectrometer and three sealed tubes containing Au@Ag NPs colloid, KI solution, and $MgSO_4$ solution. A discernible correlation was observed between signal intensity and the logarithmic concentration, spanning from 5 to 500 μg/L in urine and 10 μg/L to 1 mg/L in gastric juice. The detection limits, calculated from the characteristic peak at 1648 cm $^{-1}$, were 1.36 and 4.05 μg/L in human urine and gastric juice, respectively. Notably, this POCT kit obviated the need for pretreatment procedures, and the detection process was accomplished within 1 min, yielding satisfactory recoveries. This expeditious time frame is crucial for clinical diagnosis and rescue operations. Compared to conventional methods, this kit demonstrated real-time determinations in nonlaboratory settings. The simplicity and practicality of this POCT assay suggest its significant potential as an innovative alternative for poisoning detection applications.



1. INTRODUCTION

Paraquat (1,1′-dimethyl-4,4′-bipyridinium dichloride, PQ) is a nonselective quaternary ammonium herbicide known for its heightened toxicity to humans.[1,2] Recent reports highlight a significant surge in PQ poisoning cases, prompting heightened public concern.[3] The deleterious impact of PQ is prominently manifested in local renal dysfunction, severe pulmonary fibrosis, and other potentially fatal consequences.[2] Particularly, the well-documented toxic effects of PQ on the lungs underscore its status as an exceedingly hazardous substance.[4] Given the serious consequences of PQ poisoning, expeditious identification of PQ in patient biofluids assumes paramount importance, serving as a crucial step for prompt diagnosis and timely intervention in patient rescue efforts.[5]

Contemporary techniques employed for PQ detection in biosamples predominantly encompass high-performance liquid chromatography (HPLC),[6] gas chromatography−mass spectrometry (GC−MS),[7] and liquid chromatography−mass spectrometry (LC−MS).[8] While these methodologies serve as robust foundations for PQ poisoning diagnosis, their rapid clinical application is impeded by protracted procedures involving sample purification, separation, and analysis, compounded by the requisite expertise.[7−9] The exigency arises from the pressing need for an optimized approach to facilitate efficient on-site detection of PQ in human biological fluids. This imperative stems from the inherent limitations of current methods, accentuated by their time-consuming nature and the demand for specialized skills, which collectively restrict their practical utility within clinical settings.

Surface-enhanced Raman spectroscopy (SERS) emerges as a compelling analytical technique, offering numerous advantages such as the ultrasensitive acquisition of distinctive molecular fingerprints, straightforward operation, rapid readout, and minimal susceptibility to interference from water.[10,11] The signal is greatly amplified when molecules are adsorbed onto the SERS substrate, owing to either electromagnetic or chemical enhancement.[12] By dramatically boosting the electromagnetic fields and dense plasmonic hotspots that are constantly produced by the surrounding metal nanostructures, SERS platforms offer exceptional sensitivity and specificity.[13] Given these attributes, SERS technology has been widely used in biomedicine[14,15] and stands out as a promising alternative for the analysis of paraquat (PQ).[16] While recent approaches

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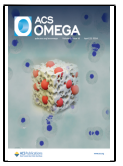

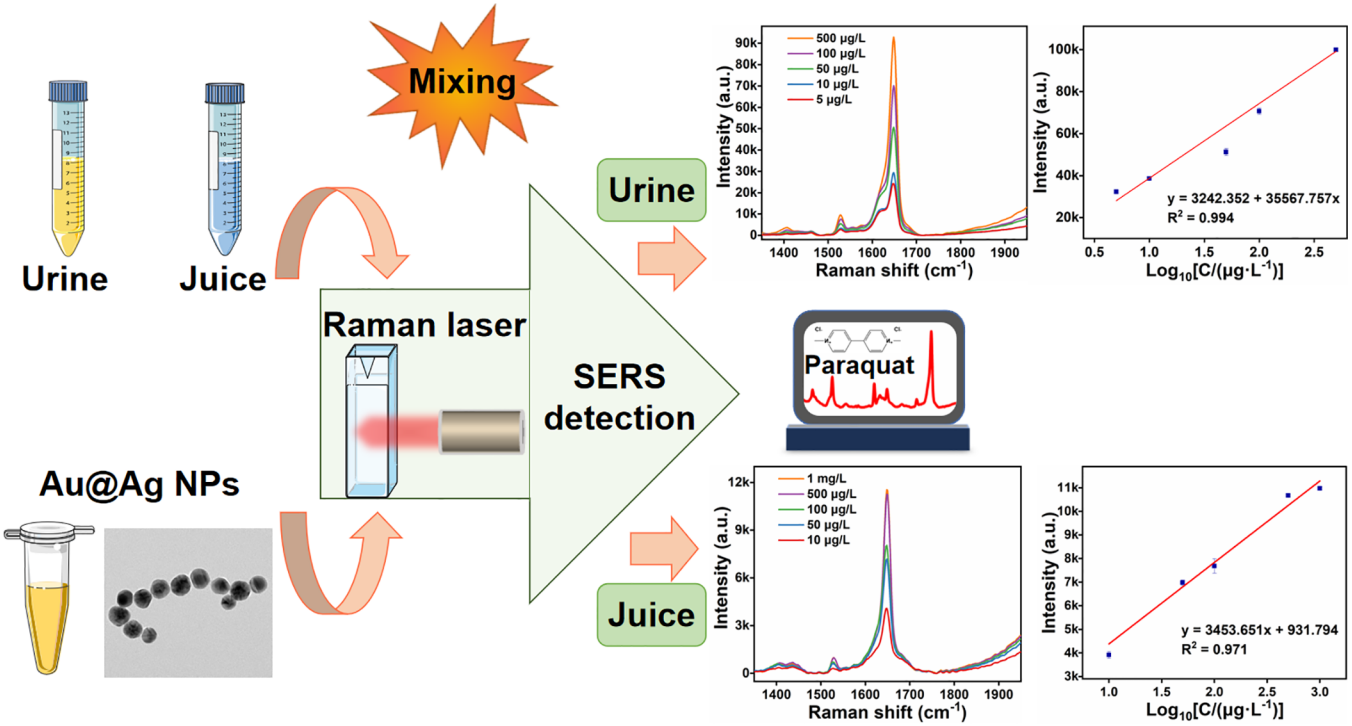

Figure 1. Scheme of the detection process using the POCT kit.

have explored SERS detection of PQ, the predominant focus has been on environmental samples[17] and food, such as apple juice,[18] adzuki beans,[19] and oily matrix.[20] However, there are few studies on the SERS detection of PQ in body fluids. A notable gap still persists between the need for clinical emergencies and the available methods for rapid testing, necessitating the development of SERS methods explicitly tailored for detecting PQ in the biological fluids of poisoned individuals. The rising popularity of point-of-care diagnostic testing platforms in clinical settings can be attributed to their ability to deliver rapid results, user-friendly interfaces, and cost-effectiveness.[21] Leveraging the advantages of high sensitivity and the distinctive fingerprint effect, the SERS technique holds significant promise in the realm of point-of-care testing (POCT).[22] The adoption of a portable Raman spectrometer approach may thus represent a novel alternative for POCT applications, markedly enhancing detection efficiency and furnishing crucial information for the treatment of PQ poisoning.

This study introduces a portable SERS-based POCT kit for rapidly and sensitively determining the PQ in human gastric juice and urine. The kit mainly consists of three sealed tubes containing gold/silver core−shell nanoparticles (Au@Ag NPs), KI solution, and agglutinating agent. The KI solution, gastric juice or urine, and agglutinating agent are sequentially added to the Au@Ag NPs and mixed thoroughly, followed by a rapid SERS test with a portable Raman spectrometer. Remarkably, the proposed SERS-based POCT kit facilitates direct PQ quantification in complex biofluids, eliminating the need for any pretreatment procedures. Notably, the entire analytical process can be accomplished within a mere 1 min time frame, demonstrating superior efficiency compared to conventional methods. The expeditious, user-friendly, and cost-effective attributes of this methodology render it well-suited for timely POCT of PQ, even in nonlaboratory settings. The commendable detection outcomes underscore the significant potential of this SERS-based POCT strategy in diverse clinical applications.

2. MATERIALS AND METHODS

2.1. Reagents and Materials. All of the reagents were analytical grade and used without further purification. PQ (98%) was purchased from Guangzhou Sopo Biological Technology Co., Ltd. (Guangzhou, China). Silver nitrate ($AgNO_3$) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Chloroauric acid tetrahydrate ($HAuCl_4{\cdot}4H_2O$), potassium iodide (KI), magnesium sulfate ($MgSO_4$), aluminum nitrate ($Al(NO_3)_3$), aluminum sulfate ($Al_2(SO_4)_3$), and sodium chloride (NaCl) were purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). All solutions were prepared using ultrapure water (≥18.2 MΩ·cm).

2.2. Instruments. Transmission electron microscopy (TEM) was used to examine the uniformity and morphology of the nanoparticles with an accelerating voltage of 100 kV (JEOL, JEM-1011, Japan). Absorbance measurements were conducted by using a Shimadzu 2600 ultraviolet−visible (UV−vis) spectrometer. SERS analyses were performed by using a portable Raman system (QE Pro, Ocean Optics, USA). The laser wave was set 785 nm and the spot diameter of the laser was 158 μm. The laser had an incident intensity of 250 mW, and the integration time was set to 5 s.

2.3. Preparation of Au@Ag NPs. The synthesis of Au@Ag nanoparticles (NPs) was conducted by reducing $HAuCl_4$ or $AgNO_3$ with sodium citrate, following our established methodology outlined in ref 23. In brief, 100 mL of 0.1% $HAuCl_4$ aqueous solution was boiled for 15 min at 130 °C. Subsequently, 1.5 mL of 1% sodium citrate was introduced, and the mixture was allowed to boil for an additional 15 min under continuous stirring at 600 rpm until the formation of Au NPs. A colloidal solution comprising 25 mL of Au NPs was

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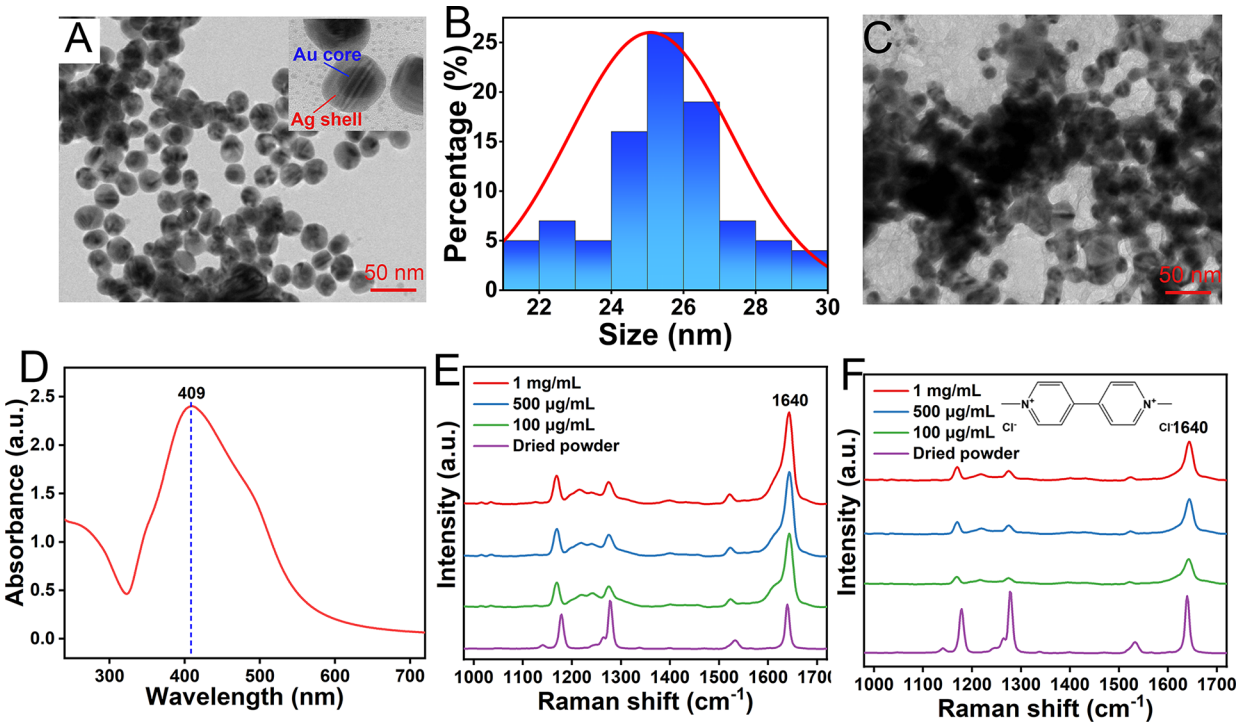

Figure 2. TEM images of (A) and size distribution (B) of Au@Ag NPs. (C) TEM image of 25 nm Au@Ag NPs before and after aggregation. (D) UV−vis spectra of colloidal Au@Ag NPs. Reference Raman spectrum of solid PQ (purple line) and SERS spectrum of PQ in urine (E) and gastric juice (F). The upper part of (F) displays the structural formula of PQ.

blended with 75 mL of water and brought to the boiling point. Consecutively, 2.2 mL of an $AgNO_3$ solution (10 mM) and 2 mL of 1% sodium citrate solution were incorporated into the mixture, followed by an additional heating period of 40 min, inducing the deposition of Ag NPs onto the Au NPs' surface.

2.4. Preparation of Solutions and Samples. Urine samples, generously contributed by 12 volunteers from Shandong First Medical University, were derived from surplus volumes following routine analyses. To mitigate interindividual variability, pooled urine was employed. Hydrochloric acid and pepsin were utilized to prepare simulated gastric juice (pH = 1.2).[24] A stock solution of PQ at a concentration of 1 mg/mL was meticulously prepared in water. Urine and simulated gastric juice solutions containing PQ were subsequently produced by diluting the stock solution with the respective biological matrices. Notably, the absence of extraction procedures ensured direct testing of all samples. The study adhered to the ethical principles outlined in the WMA Declaration of Helsinki and received approval from the Research Ethical Committee under reference number SDZFY-EC-H-2023-02.

2.5. SERS Detection of PQ. The detection of PQ was performed by using the proposed POCT strategy. Simulated samples from poisoned patients were prepared by adding a specific quantity of PQ to pooled urine or gastric juice. The experimental conditions were optimized using a urine solution spiked with 100 μg/L of PQ. The aggregation effect was compared using solutions of $MgSO_4$, $Al(NO_3)_3$, $Al_2(SO_4)_3$, and NaCl.

The portable kit comprised a portable Raman spectrometer, a quartz cuvette, and three reagent tubes containing a nanocolloid solution, KI solution, and agglomerating agent. The procedural sequence involved adding 10 μL of 10 mM KI solution to 930 μL of a nanocolloid solution and introducing 50 μL of the sample. Subsequently, 10 μL of the agglomerating agent was added and mixed. The mixture was then transferred to a quartz cuvette. The laser beam was directly focused on the quartz cuvette containing the analyte to be measured for SERS measurements, and the SERS spectrum was collected under the same conditions. Method detection limits (MDLs) were determined using the calculation method prescribed by the U.S. Environmental Protection Agency.[25] The standard deviation of the samples analyzed for repeated spiked samples was calculated, and then the MDL was calculated by the formula $MDL_S = t_{(n-1,1-\alpha=0.99)}S_S$. The SERS detection of PQ in human urine and gastric juice is illustrated in Figure 1.

3. RESULTS AND DISCUSSION

3.1. Characterization of the Substrate. Figure 2A shows a representative TEM image of the bare Au@Ag NPs prepared in this study along with a histogram showing the size dispersion of the nanoparticles. With an average diameter of 25.1 ± 2.23 nm, the nanoparticles are fairly monodisperse (Figure 2B). Additionally, Figure 2C shows TEM images of Au@Ag NPs after aggregation. After adding salt, the particle gaps became smaller and fused, and the dispersed nanoparticles in the colloidal solution agglomerated. Figure 2D displays that the UV−vis absorption band for the Au@Ag NP solution was detected at 409 nm, which is consistent with a previous study.[23] The observed bandwidth was relatively narrow, further suggesting that the nanoparticles exhibit a high degree of uniformity. The uniformity of the NPs provides the basis for efficient SERS enhancement through local surface plasmon resonance.

3.2. Raman Characterization of PQ. The specific fingerprint of PQ can be obtained directly. The prominent vibrational bands of PQ primarily consist of C−N stretching (841 cm^{-1}), C=C bending (1173 cm^{-1}), C−C bending (1281

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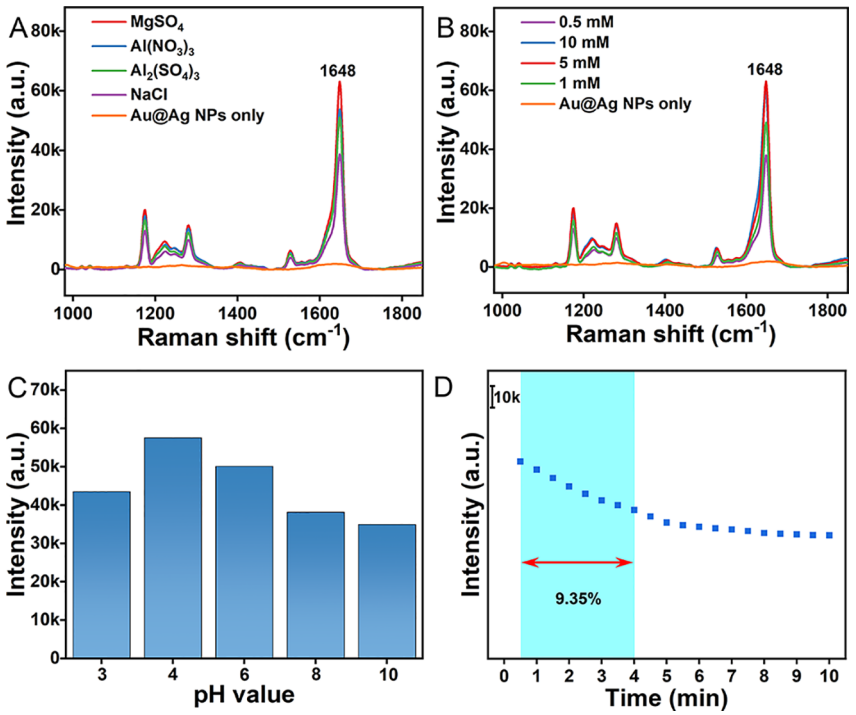

Figure 3. Different types (A) and concentrations (B) of agglomerating agents on the SERS sensitivity toward the characteristic 1648 cm^{-1} peak of 100 μg/L PQ. (C) Impact of pH values ranging from 3 to 10 on the SERS signal of PQ in urine. (D) Temporal evolution of the SERS intensity of 1648 cm^{-1} peaks in the urine.

cm^{-1}), and C=N stretching (1648 cm^{-1}) (Figure 2E). In this study, the band at 1648 cm^{-1} was chosen for the quantitative analysis of PQ due to its distinct separation and high intensity. No significant alteration in the SERS spectrum of PQ was observed when compared to the Raman spectrum of solid PQ, as demonstrated in urine (Figure 2E) and gastric juice (Figure 2F). PQ can be quickly and qualitatively identified by directly comparing the SERS spectrum of biological samples with reference fingerprints of solid standards. This simple yet practical comparison is crucial in diagnosing poisoned patients and holds great potential for facilitating rapid qualitative analysis in POCT scenarios.

3.3. Optimization of SERS Detection of PQ. The potency of the SERS signal is markedly contingent on the interparticle distance. In isolation, a singular nanoparticle yields a SERS effect of modest strength, insufficient for substantial signal amplification.[26] Introducing salt into the colloidal solution is a common practice to overcome this limitation, aiming to foster nanoparticle aggregation and induce localized electromagnetic (EM) field enhancement.[27] The addition of KI expels the anionic citrate molecules from the NP surface, allowing the analyte molecules to attach to the gold surface, where I^- and positively charged PQ interact electrostatically. The most pronounced EM interactions materialize when the nanogap between particles diminishes to less than 1 nm.[28] Consequently, an optimization step to facilitate aggregation becomes imperative.

This part aims to determine the concentration at which the aggregation of nanoparticles occurs and at which cations interact with the nanoparticles to yield optimal SERS enhancement. The optimization of the SERS effect was pursued using the signal derived from 100 μg/L PQ. Diverse aggregating conditions were optimized, including the type and concentration of salts. Four salts—$MgSO_4$, $Al(NO_3)_3$, $Al_2(SO_4)_3$, and NaCl—were selected as aggregating agents at concentrations of 0.5, 1, 5, and 10 mM to discern their impact on signal amplification. As depicted in Figure 3A,B, the most robust SERS signal was manifested with 5 mM $MgSO_4$, consequently establishing its selection for subsequent steps. The addition of salt induces nanoparticle destabilization, culminating in the formation of "hot spots" that amplify the signal through localized enhancement of the electric field.[29] During the salt-induced aggregation process, the cation assists in the destabilization of the nanoparticles, the divalent and trivalent cations produce a lower critical coagulation concentration and exhibit a more pronounced effect in promoting nanoparticle agglomeration when compared to monovalent cations.[30] Nevertheless, at a certain salt concentration, the signal begins to decline, indicative of achieving a state of "full aggregation" and diminishing efficacy of SERS enhancement. Leveraging the insights from the optimized aggregation-aided SERS, ultrasensitive detection of PQ can be effectively realized in this study.

Subsequently, an examination of the impact of pH values spanning from 3 to 10 on the SERS intensity was conducted (Figure 3C). It was observed that the solution with a pH of 4 demonstrated the highest enhancement in the SERS signal. Additionally, pH values of 3 and 6 were also found to be satisfactory in terms of signal strength. Given that the pH levels of urine and gastric juice are typically neutral or acidic, and that PQ remains stable under such conditions.[31] Therefore, the pH values adopted in this study were 4.0−6.0 in urine and 0.9−1.5 in gastric fluid. This study omitted the pH adjustment step to enhance the clinical detection efficiency. The effect of aggregation time on SERS intensity in urine (Figure 3D) was also examined by recording continuous SERS signals. The results showed that the signal remained relatively stable over 4 min with an RSD of 9.35%. Here, considering the detection

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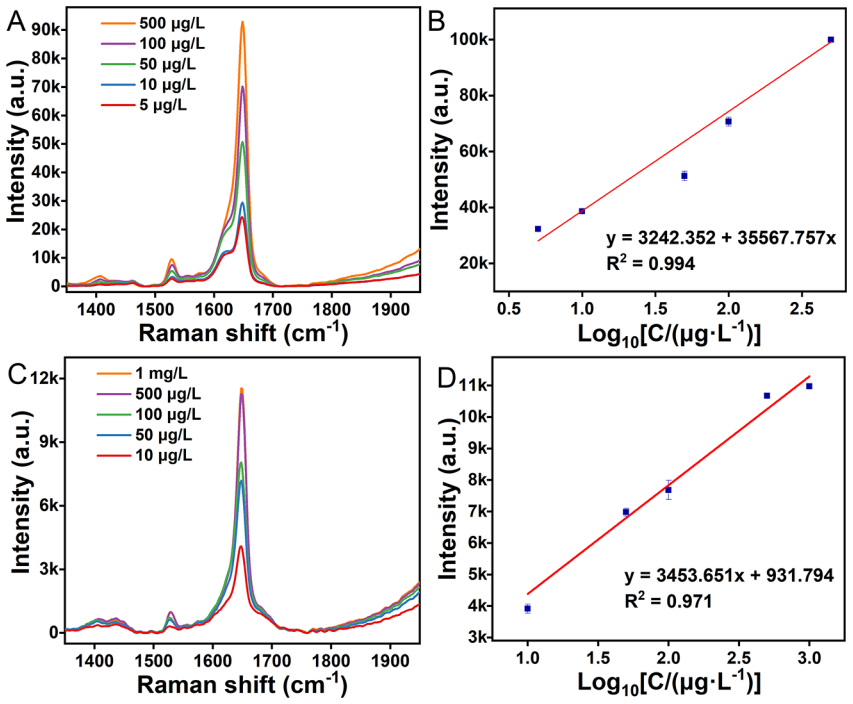

Figure 4. SERS spectra of PQ in urine measured using the proposed POCT kit at 1648 cm^{-1}, and (C) was simulated gastric juice. The linear relationship between the signal intensity and the logarithm of the PQ concentrations in (B) human urine and (D) simulated gastric juice, with three measurements of each concentration.

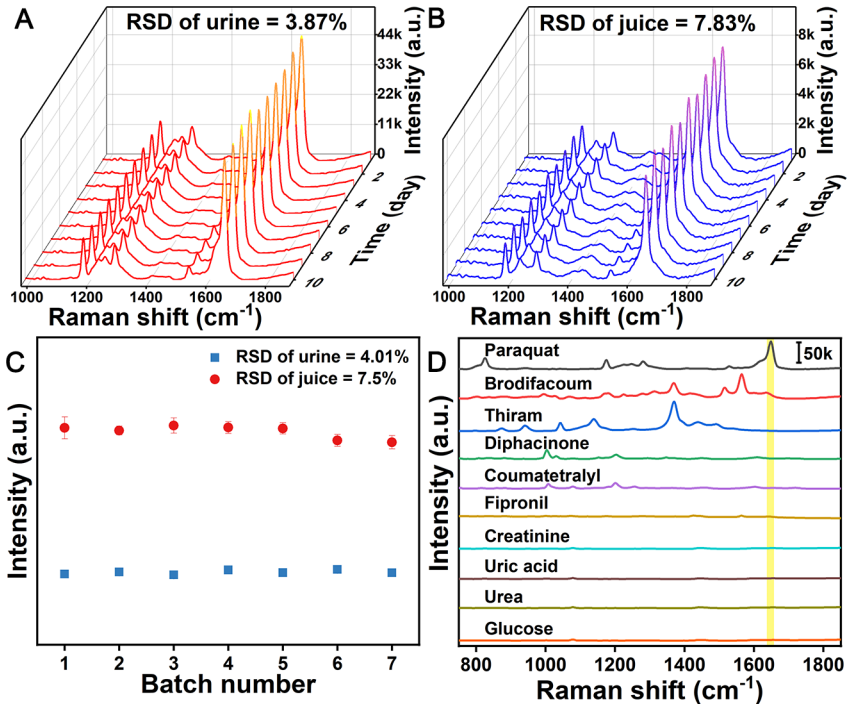

Figure 5. Signal intensity variations of 1648 cm^{-1} of 100 μg/L PQ in urine (A) and gastric juice (B) via 10 repetitive detections, (C) between 7 batches of SERS substrate, and (D) spectra of other 100 μg/L pesticides and potential components of the body fluids.

efficiency and the standardization of operation, 1 min was chosen as the aggregation time. The analytical enhancement factor (EF) for PQ was calculated using the equation EF = $(I_{SERS}/C_{SERS})/(I_{RS}/C_{RS})$, where I_{SERS} and I_{RS} indicate the SERS signal intensity of PQ at 1648 cm^{-1} under SERS and non-SERS substrate conditions, respectively, and C_{SERS} and C_{RS} are the corresponding concentrations. In this study, the EF is 6.72 × 10^5, the values of C_{SERS} and C_{RS} are 0.1 and 2000 mg/L, respectively.

3.4. SERS Quantitative Detection of PQ in Urine and Gastric Juice. In cases of oral misadministration and self-administration, patients often undergo gastric lavage upon admission, as documented in previous studies,[32] making testing of gastric fluid of great significance. Furthermore, as PQ is

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primarily excreted in its prototype form through urine,[33] both gastric juice and urine serve as ideal samples for analyzing PQ poisoning. In this study, we employed the standard addition method by adding known quantities of PQ to the samples. The main steps include adding unequal amounts of standard solution to several portions of the sample solution and measuring the SERS signal according to plotting the standard curve followed by plotting the absorbance−addition concentration curve. The characteristic peak intensities were plotted against the logarithmic concentration of the PQ. A good linear relationship was observed in urine between SERS intensity and the logarithm of PQ concentration, ranging from 5 to 500 μg/L (Figure 4A). The regression equation (I_{SERS} = 35567.757 log $C_{PQ(\mu g/L)}$ + 3242.352) achieved a high R^2 value of 0.994 (Figure 4B). A similar trend was observed in gastric juice, with the PQ concentration ranging from 10 μg/L to 1 mg/L (Figure 4C). The regression equation (I_{SERS} = 3453.651 log $C_{PQ(\mu g/L)}$ + 931.794) obtained a R^2 value of 0.971 (Figure 4D). The calculated MDLs for PQ-spiked urine and gastric juice samples were 1.36 and 4.05 μg/L, respectively, meeting the poisoning diagnosis requirements. The lower SERS intensity of PQ in gastric juice than that in urine is due to the instability of the nanocolloid solution caused by gastric acid. These results provided strong evidence for the rapid quantitative analysis of PQ in biofluids.

3.5. Analysis Performance. The reproducibility and batch-to-batch variation of the signal are critical discussion parameters for convincing SERS response.[13] Urine (Figure 5A) and gastric juice samples (Figure 5B) spiked with 100 μg/L PQ were tested 10 times. The corresponding RSDs were 3.87 and 7.83% for urine and gastric juice samples, respectively, demonstrating good reproducibility. The signal variation of seven batches synthesized on different days was also addressed by determining the peak intensity of 1648 cm^{-1}. The RSDs of the substrate were 4.01 and 7.50% for urine and gastric juice samples, respectively (Figure 5C). These results showed good analysis performance, reinforcing its great potential for POCT applications. This study synthesized the Au@Ag NPs with a particle size of approximately 25 nm. This size ensures excellent colloidal solution stability and provides an ideal signal enhancement effect.[13] With excellent RSDs and minimal induced SERS signal loss, the stability was deemed good. Practical clinical applications rely on the substrate's long-term stability, which guarantees a long enough duration for practical use. Additionally, the recoveries were investigated by using the standard-addition method. Table 1 summarizes the recoveries of PQ in urine and juice were 79.21−103.99 and 73.55−94.59%, ensuring good practical applications. SERS measurements of several pesticides in urine, including paraquat ($C_{10}H_{11}C_{l2}N_5O_3$), brodifacoum ($C_{31}H_{23}BrO_3$), thiram ($C_6H_{12}N_2S_4$), diphacinone ($C_{23}H_{16}O_3$), coumatetralyl ($C_{19}H_{16}O_3$), and fipronil ($C_{12}H_4Cl_2F_6N_4OS$) with the same concentration of 1 mg/L, and other potential components of the body fluids such as 1 mg/dL creatinine, 1 mg/dL uric acid, 20 mg/dL urea, and 100 mg/dL glucose,[13] were used to assess the selectivity of the proposed POCT kit. Figure 5D shows the spectra of other pesticides obtained by using the proposed SERS approach. The SERS spectra of brodifacoum, diphacinone, coumatetralyl, thiram, and fipronil were observed. Each of the pesticides displayed a distinct fingerprint, and no significant signals interfering with PQ. As the signals of intrinsic urinary substances such as creatinine and uric acid were not be observed, the coexistence of the above pesticides and intrinsic urinary substances with PQ will not cause significant signal interference. These findings substantiate the exceptional selectivity of the POCT kit for paraquat identification. This proposed method may broaden the application of SERS technology in clinical and is expected to be transformed into a practical method, contributing to effective patient resuscitation.

Table 2 compares various methods used to detect PQ and other pesticides in biological samples. Most chromatographic methods for detecting PQ require complex equipment and time-consuming preparation procedures.[6−8,36] Although the methods are very sensitive, they are not suitable for POCT. The SERS detection method for PQ in plasma and urine established by our research group previously utilized electro-enhanced solid phase adsorption, which significantly improved the preprocessing efficiency but still could not realize on-site detection.[34] In contrast, the SERS-based portable kit proposed in this study realized POCT with the advantages of easy use, no need for complex sample preparation, and cost-effectiveness. A comparison of the present SERS method with the LC-MS assay for PQ in urine has been conducted. We detected

Table 1. Recoveries PQ in Spiked Biofluids Using the POCT Kit Based on SERS[a]

sample	spiked (μg/L)	found ± SD (μg/L)	recovery (%)	RSD (%, n = 3)
urine	10	9.8 ± 0.9	98.9%	0.9%
	100	79.2 ± 8.1	79.2%	10.3%
	1000	1039.9 ± 16.3	103.9%	15.7%
gastric juice	10	7.3 ± 7.1	73.5%	9.7%
	100	94.5 ± 13.7	94.5%	14.5%
	1000	811.7 ± 3.2	81.1%	3.9%

[a]SD, standard deviation; RSD, relative standard deviation.

Table 2. Comparison of Different Methods for the Detection of PQ in a Biological Matrix[a]

analyte	method	media	preprocessing operation	detection limit	ref
PQ	SERS	urine	not needed	1.36 μg/L	this study
PQ	SERS	gastric juice	not needed	4.05 μg/L	this study
PQ	SERS	urine	solid−phase extraction	0.036 μg/L	34
PQ	SERS	plasma	solid−phase extraction	0.35 μg/L	34
PQ	LC/MS	urine	magnetic-phase extraction	0.12 μg/L	6
PQ	LC/MS	blood	solvent extraction	100 μg/L	8
PQ	LC/MS	urine	solvent extraction	100 μg/L	8
PQ	GC/MS	urine	monolithic spin column extraction	100 μg/L	7
PQ	GC/MS	serum	monolithic spin column extraction	100 μg/L	7
PQ	GC/MS	urine	solid-phase extraction ction	50 μg/L	36
DQ	SERS	urine	not needed	17.5 μg/L	13
CMTT	SERS	urine	solvent extraction	13.71 μg/L	35

[a]CMTT, coumatetralyl.

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PQ in urine at 150 and 200 ppb by two methods, and the results were comparable, but the SERS method has the advantage of convenience and efficiency.

The above results proved the selectivity, reproducibility, and sensitivity of this portable kit. The corresponding SERS signal can be obtained within 1 min. These advantageous features of the SERS approach hold remarkable significance in the realm of clinical applications, underscoring the considerable promise of this straightforward yet effective kit for expedited and accurate POCT in clinical settings.

4. CONCLUSIONS

This study successfully presents the development of a portable POCT kit, leveraging SERS, for the rapid and sensitive detection of PQ in human urine and gastric juice. This innovative POCT strategy capitalizes on using Au@Ag NPs as the substrate, obviating the necessity for preliminary pretreatment procedures to yield distinctive spectral fingerprints of PQ. Noteworthy sensitivity is evident with MDLs of 1.36 and 4.05 μg/L in urine and gastric juice, respectively. The expeditious completion of the entire POCT process within 1 min ensures rapid analysis, and the satisfactory recoveries achieved, ranging from 73.55 to 103.99%, stand as a crucial metric for clinical diagnosis. Compared to conventional approaches employed in clinical settings, this SERS-based POCT kit provides real-time determinations, even in non-laboratory settings. The main shortcoming of this study is that the established POCT method is mainly used for detecting PQ in gastric and urine samples. Further research is needed on blood and other biofluids. In brief, the presented SERS-based POCT strategy holds considerable promise as a practical and user-friendly method for swiftly analyzing PQ in complex clinical biosamples.

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Author Contributions

W.L.: methodology, data curation, writing—original draft. Y.Z.: data curation. W.Z.: sample collection. P.H.: writing—review and editing. M.Z.: validation. X.M. and X.Z.: software. M.S.: funding acquisition, project administration. X.D.: software. C.W.: conceptualization, writing—review and editing, funding acquisition, supervision. W.L. and Y.Z. contributed equally to this work.

Notes

The authors declare no competing financial interest.

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