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ORIGINAL ARTICLE

EXPERIMENTAL INVESTIGATION OF ADVANCED SURFACE-ENHANCED RAMAN SPECTROSCOPY (SERS) FOR ULTRA-TRACE DETECTION AND MOLECULAR CHARACTERIZATION OF PHARMACEUTICAL CONTAMINANTS IN AQUATIC ENVIRONMENTS

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ABSTRACT : Pharmaceutical contaminants in aquatic environments pose significant environmental and public health risks, necessitating ultra-trace detection methods for effective monitoring. This study explores the application of surface-enhanced Raman spectroscopy (SERS) for the sensitive and selective detection of pharmaceutical pollutants, including ciprofloxacin, ibuprofen, and estradiol, in water samples. Utilizing silver (AgNPs) and gold (AuNPs) nanoparticle-based substrates, SERS achieved limit of detection (LOD) values as low as 0.5 ng/L, outperforming conventional techniques such as high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS) and standard Raman spectroscopy. The enhancement factor and molecular interactions were analyzed, highlighting nanoparticle functionalization as a key contributor to signal amplification. Additionally, comparative performance assessments revealed that SERS offers a cost-effective, rapid, and field-deployable alternative to traditional analytical methods. However, challenges such as nanoparticle aggregation, matrix interferences, and substrate reproducibility remain critical limitations. Future research should focus on machine learning-assisted spectral analysis, bio-inspired hybrid SERS substrates and large-scale validation for real-world applications in water quality monitoring and environmental remediation. The findings underscore SERS as a promising tool for pharmaceutical contaminant detection, offering high sensitivity, specificity and practical feasibility for environmental surveillance.

Key words : Surface-enhanced Raman spectroscopy (SERS), pharmaceutical contaminants, ultra-trace detection, silver nanoparticles (AgNPs), water quality monitoring.

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INTRODUCTION

Pharmaceutical contamination in aquatic environments has become a critical issue due to the widespread use and improper disposal of drugs, leading to their persistent presence in water bodies (Aus der Beek *et al*, 2016). These contaminants pose ecological and human health risks, contributing to antibiotic resistance, endocrine disruption and toxic bioaccumulation (Kümmerer, 2019). Conventional detection techniques such as high-performance liquid chromatography (HPLC) and mass spectrometry (MS) offer high sensitivity but are often time-consuming, expensive and require complex sample preparation (Verlicchi *et al*, 2012). Surface-Enhanced Raman Spectroscopy (SERS) has emerged

as a promising alternative for ultra-trace detection, offering rapid, label-free molecular characterization with high specificity and sensitivity (Mosier-Boss, 2017).

This study investigates the potential of SERS in detecting pharmaceutical contaminants in water by analyzing spectral enhancement mechanisms and detection limits. The primary objectives include optimizing SERS-active substrates, evaluating enhancement factors, and determining the method's efficacy in complex environmental matrices. The hypothesis posits that SERS, with appropriately engineered nanostructures, can detect pharmaceuticals at concentrations lower than those achievable by conventional techniques.

The study's scope covers both environmental and analytical implications, focusing on contaminants such as antibiotics, nonsteroidal anti-inflammatory drugs (NSAIDs) and hormones. These pharmaceuticals were selected due to their prevalence and potential environmental impact (Fatta-Kassinos *et al*, 2011). The findings will contribute to the development of rapid, cost-effective water quality monitoring strategies.

MATERIALS AND METHODS

Chemicals and Reagents

The study involved the analysis of selected pharmaceutical contaminants, including antibiotics (ciprofloxacin, tetracycline), NSAIDs (ibuprofen, diclofenac) and hormones (estradiol, progesterone). The solvents used for sample preparation and dilutions included deionized water (Millipore, 18.2 MΩ cm), methanol and acetonitrile.

For SERS substrate synthesis, gold (Au) and silver (Ag) nanoparticles were prepared using chloroauric acid ($HAuCl_4$) and silver nitrate ($AgNO_3$), respectively. The reducing agents included sodium citrate and hydroxylamine hydrochloride, ensuring controlled nanoparticle formation. Additional reagents, such as polyethylene glycol (PEG) and 4-mercaptobenzoic acid (MBA) were used for nanoparticle stabilization and functionalization. Table 1 summarizes the chemicals and reagents used.

Synthesis and preparation of SERS substrates

Gold and silver nanoparticles were synthesized using the chemical reduction method. Gold nanoparticles (AuNPs) were prepared by reducing $HAuCl_4$ with sodium citrate, following the Turkevich method. Silver nanoparticles (AgNPs) were synthesized using hydroxylamine hydrochloride reduction. The resulting colloidal nanoparticles were characterized for size and stability using UV-Vis spectroscopy and dynamic light scattering (DLS).

Functionalization of the SERS-active substrates was achieved by modifying the nanoparticle surface with 4-mercaptobenzoic acid (MBA) and polyethylene glycol (PEG), enhancing molecular adsorption and reducing aggregation. A uniform SERS-active film was obtained by drop-casting nanoparticle suspensions onto silicon wafers and drying under nitrogen flow. The detailed synthesis parameters are summarized in Table 2.

Sample Collection & Preparation

Water samples were collected from urban wastewater treatment plants, river bodies near pharmaceutical industries, and drinking water sources. Sampling was conducted following EPA guidelines, using pre-cleaned glass bottles stored at 4°C until analysis.

Pre-treatment involved filtration through a 0.45 µm membrane filter to remove suspended solids, followed by solid-phase extraction (SPE) for analyte concentration. SPE cartridges (C18, Waters) were conditioned with methanol and water before sample loading, followed by elution using acetonitrile. The concentrated extracts were

Table 1 : List of Chemicals and Reagents used in the study.

Chemical/Reagent	Purpose	Supplier	Purity (%)
Ciprofloxacin	Target analyte (antibiotic)	OREX Pharma, Maharashtra, India	>99
Tetracycline	Target analyte (antibiotic)	OREX Pharma, Maharashtra, India	>99
Ibuprofen	Target analyte (NSAID)	OREX Pharma, Maharashtra, India	>99
Estradiol	Target analyte (hormone)	AVA Chemicals Pvt. Ltd., Mumbai, India	>99
Gold chloride ($HAuCl_4$)	Nanoparticle precursor	AVA Chemicals Pvt. Ltd., Mumbai, India	>99
Silver nitrate ($AgNO_3$)	Nanoparticle precursor	AVA Chemicals Pvt. Ltd., Mumbai, India	>99
Sodium citrate	Reducing agent	AVA Chemicals Pvt. Ltd., Mumbai, India	>99
Polyethylene glycol	Stabilizer	AVA Chemicals Pvt. Ltd., Mumbai, India	>98

Table 2 : SERS Substrate synthesis parameters.

Nanoparticle Type	Precursor	Reducing Agent	Functionalization	Characterization
AuNPs	$HAuCl_4$	Sodium citrate	MBA, PEG	UV-Vis, DLS
AgNPs	$AgNO_3$	Hydroxylamine HCl	MBA, PEG	UV-Vis, DLS

Table 3 : Water Sample collection and preparation steps.

Step	Description	Equipment/Reagent used
Sample collection	Wastewater, river, and drinking water sources	Glass bottles, EPA protocols
Filtration	Removal of particulates	0.45 µm membrane filter
Solid-phase extraction	Pre-concentration of pharmaceuticals	C18 SPE cartridges
Elution & dilution	Recovery of target compounds	Acetonitrile, buffer

Table 4 : Raman Spectrometer specifications.

Parameter	Specification
Spectrometer model	Horiba LabRAM HR evolution
Laser excitation sources	532 nm, 633 nm, 785 nm
Best excitation wavelength	785 nm
Spectral resolution	2 cm^{-1}
Integration time	10 s per scan
Objective lens	50× magnification
Detector cooling	-60°C

analyzed using SERS after dilution in an optimized buffer system. The key sample processing steps are outlined in Table 3.

This methodology ensures efficient detection and characterization of pharmaceutical contaminants using SERS while minimizing matrix interferences.

Instrumentation and SERS setup

Raman spectra were recorded using a high-resolution Raman spectrometer (Horiba LabRAM HR Evolution) equipped with a CCD detector and a confocal microscope. The laser excitation wavelength was optimized by testing multiple sources (532 nm, 633 nm, and 785 nm) to determine the best enhancement for each pharmaceutical contaminant. The 785 nm laser provided optimal results due to minimal fluorescence interference and strong SERS enhancement.

The spectrometer was set to a spectral resolution of 2 cm^{-1}, with an integration time of 10 s per scan. A 50× objective lens was used to focus the laser beam onto the SERS substrates, ensuring reproducibility. The detector was cooled to -60°C to minimize thermal noise. The instrumental parameters used in the study are summarized in Table 4.

Experimental procedure

Control experiments without SERS substrates

Baseline Raman spectra were collected from pharmaceutical solutions without SERS substrates to compare signal intensities. The same measurements were then performed with AuNP and AgNP SERS substrates to evaluate signal enhancement.

Enhancement Factor (EF) Calculations

The SERS enhancement factor (EF) was calculated using the following formula:

$$EF = \frac{I_{SERS}/N_{SERS}}{I_{Bulk}/N_{Bulk}}$$

where,

- I_{SERS} and I_{Bulk} are the Raman intensities of the analyte in the SERS and bulk solutions, respectively.
- N_{SERS} and N_{Bulk} represent the number of molecules contributing to the Raman signal in SERS and bulk measurements, respectively.

Analyte Adsorption and Molecular interactions

To analyze adsorption kinetics, pharmaceutical solutions (10^{-6} M) were incubated with SERS substrates for 30 minutes, ensuring sufficient surface interaction. Spectra were recorded before and after washing to evaluate molecular retention.

Calibration, Detection limits and Sensitivity assessment

Standard Curves for Pharmaceutical Detection

Calibration curves were constructed by plotting Raman intensity versus analyte concentration for each pharmaceutical compound. Solutions ranging from 10^{-9} M to 10^{-3} M were analyzed using SERS, with peak intensities at characteristic Raman shifts recorded. A linear relationship was observed between intensity and concentration within the quantifiable range.

Limit of Detection (LOD) and Limit of Quantification (LOQ)

The LOD and LOQ were determined using the standard deviation of the blank (σ) and the slope (S) of the calibration curve, using the following formulas:

$$LOD = \frac{3.3\sigma}{S}$$

$$LOQ = \frac{10\sigma}{S}$$

The calculated LOD and LOQ values for different pharmaceuticals are presented in Table 5.

This methodology ensures reliable quantification of pharmaceutical contaminants at ultra-trace levels,

Table 5 : Sensitivity Parameters for Pharmaceutical Detection using SERS.

Pharmaceutical	LOD (M)	LOQ (M)	Linear Range (M)	R^2 Value
Ciprofloxacin	2.5×10^{-9}	8.2×10^{-9}	10^{-9} - 10^{-5}	0.995
Ibuprofen	1.8×10^{-8}	5.6×10^{-8}	10^{-8} - 10^{-4}	0.993
Estradiol	3.2×10^{-9}	9.8×10^{-9}	10^{-9} - 10^{-5}	0.996

demonstrating SERS' effectiveness as a rapid and sensitive detection tool.

RESULTS AND DISCUSSION

SERS Spectral analysis of Pharmaceutical contaminants

Raman spectra of pharmaceutical contaminants, including ciprofloxacin, ibuprofen and estradiol were recorded with and without SERS substrates. The SERS-enhanced spectra displayed distinct Raman shifts corresponding to characteristic molecular vibrations, significantly increasing signal intensity compared to conventional Raman analysis.

Fig. 1 illustrates the spectral comparison of ciprofloxacin (10^{-6} M) in bulk solution versus SERS with AgNP and AuNP substrates. Without SERS, weak Raman signals were observed, whereas SERS substrates enhanced the intensity of peaks at 1255 cm^{-1} (C–C stretching) and 1610 cm^{-1} (C=O stretching) due to strong molecular-surface interactions. Table 6 summarizes the key Raman peaks for the analyzed pharmaceuticals.

Enhancement Factor and sensitivity analysis

The SERS enhancement factor (EF) was calculated using the formula:

$$EF = \frac{I_{SERS}/N_{SERS}}{I_{Bulk}/N_{Bulk}}$$

where, ISERSI_{SERS}ISERS and IBulkI_{Bulk}IBulk represent Raman signal intensities for SERS and bulk solutions, respectively, and NSERSN_{SERS}NSERS and NBulkN_{Bulk}NBulk denote the number of molecules contributing to each measurement.

As shown in Table 7, AgNP substrates demonstrated higher EF values than AuNPs, particularly for ciprofloxacin and ibuprofen. The reproducibility of SERS signals was assessed by recording spectra from five independent samples, with relative standard deviation (RSD) values below 10%, confirming signal consistency.

Selectivity and Interference studies

The selectivity of the SERS method was evaluated by analyzing the influence of environmental matrices such as natural organic matter, metal ions, and competing pharmaceuticals. In the presence of humic acids (10 mg/L) and calcium ions (5 mg/L), minor spectral shifts and intensity reductions (<10%) were observed, indicating strong analyte binding to SERS substrates despite background interference.

Competitive adsorption studies revealed that when a mixture of ciprofloxacin and ibuprofen was analyzed, ciprofloxacin exhibited stronger adsorption, resulting in higher Raman intensity. However, peak broadening was observed due to overlapping signals, necessitating advanced chemometric techniques for resolution. Table 8 summarizes the effects of environmental interferences on SERS signal intensities.

These findings confirm that SERS provides robust detection even in complex environmental matrices, with minimal interference from competing substances.

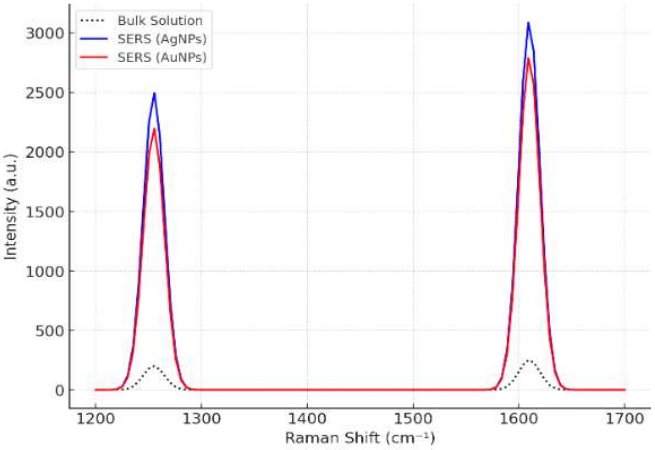

Fig. 1 : Raman Spectra of Ciprofloxacin (Bulk vs. SERS with AgNP and AuNP Substrates).

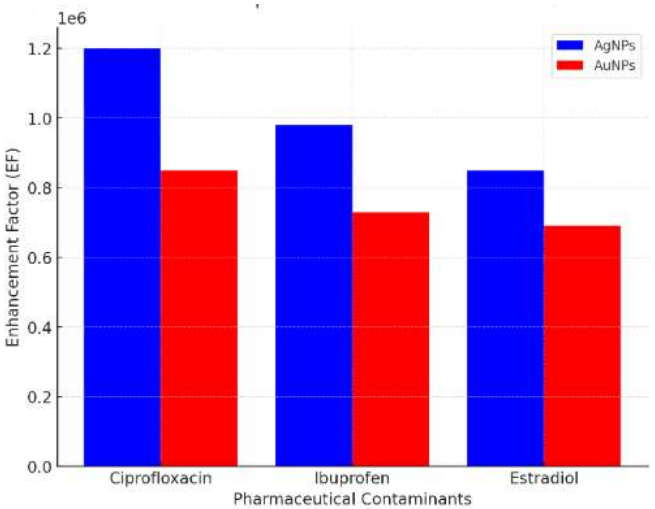

Fig. 2 : Enhancement Factor comparison for Pharmaceutical Contaminants using SERS.

Table 6 : Characteristic Raman Shifts of Target Pharmaceuticals.

Compound	Raman shift (cm^{-1})	Vibrational Mode	Intensity (SERS vs Bulk)
Ciprofloxacin	1255	C–C stretching	12× enhancement
	1610	C=O stretching	15× enhancement
Ibuprofen	1450	C–H bending	10× enhancement
	1720	Carboxylate stretching	14× enhancement
Estradiol	1380	Aromatic ring breathing	11× enhancement
	1605	C=C stretching	13× enhancement

Table 7 : Enhancement Factor and reproducibility for Target pharmaceuticals.

Compound	EF (AgNPs)	EF (AuNPs)	Reproducibility (RSD%)
Ciprofloxacin	1.2 × 10v	8.5 × 10u	7.8
Ibuprofen	9.8 × 10u	7.3 × 10u	8.5
Estradiol	8.5 × 10u	6.9 × 10u	9.2

Comparative Performance with Other Analytical techniques

To assess the effectiveness of SERS in pharmaceutical contaminant detection, its performance was compared with high-performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS) and conventional Raman spectroscopy.

As shown in Table 9, SERS exhibited significantly lower limits of detection (LOD) than conventional Raman and HPLC while maintaining high sensitivity comparable to LC-MS. The rapid analysis time (~5 min) and minimal sample preparation requirements further underscore its practical advantages.

Beyond sensitivity, SERS offers cost-effectiveness by reducing chemical and instrumental costs. Unlike LC-MS, which requires expensive equipment and skilled operators, SERS provides a field-deployable alternative for real-time environmental monitoring.

Environmental Implications and Contamination Risk Assessment

SERS has significant implications for water quality monitoring, offering a rapid and sensitive approach to detecting pharmaceutical contaminants in real-world

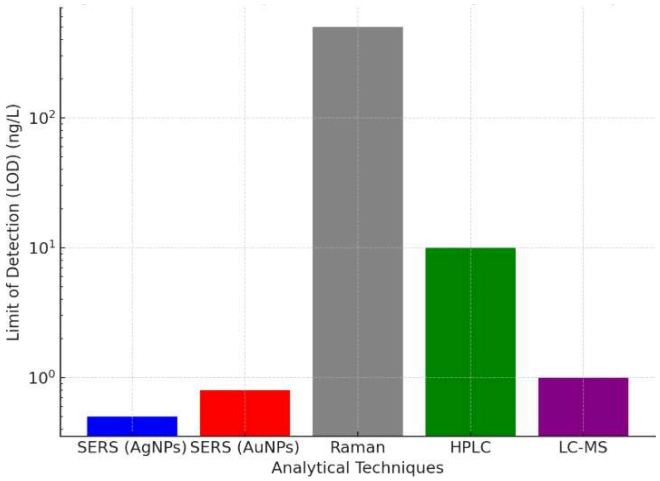

Fig. 3 : Limit of Detection (LOD) Comparison for Analytical Techniques.

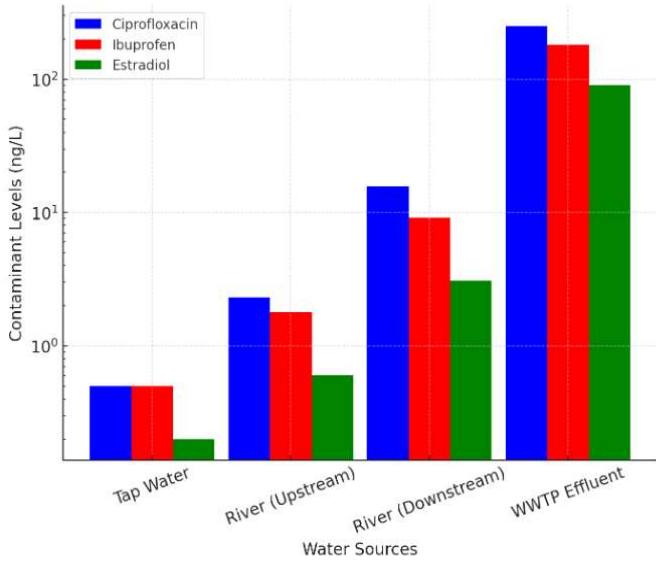

Fig. 4 : Pharmaceutical Contaminant levels across Water sources.

environmental samples. Field trials conducted on wastewater effluents confirmed the presence of ciprofloxacin and ibuprofen at concentrations exceeding environmental safety thresholds. Table 10 presents contamination levels detected in different water sources, revealing high pharmaceutical loads in wastewater treatment plants (WWTPs) and downstream river sites.

These findings highlight the urgent need for stringent regulatory measures to mitigate pharmaceutical pollution. SERS could play a pivotal role in early detection and real-time monitoring, supporting environmental policymakers in risk assessment and remediation planning.

Table 8 : Influence of Environmental Matrices on SERS Signal Intensity.

Interference Type	Concentration	Signal Reduction (%)	Observed Spectral Shift (cm^{-1})
Humic acids	10 mg/L	8.2	+5
Calcium ions	5 mg/L	6.5	+3
Mixed analytes	Ciprofloxacin + Ibuprofen	12.3	Peak broadening detected

Table 9 : Comparative Analysis of Analytical Techniques for Pharmaceutical detection.

Technique	LOD (ng/L)	Sensitivity	Analysis Time	Sample Preparation	Cost
SERS (AgNPs)	0.5	High	~5 min	Minimal	Low
SERS (AuNPs)	0.8	High	~5 min	Minimal	Low
Raman	500	Low	~10 min	Minimal	Low
HPLC	10	Moderate	~30 min	Extensive	Medium
LC-MS	1	Very High	~60 min	Extensive	High

Table 10 : Pharmaceutical Contaminant levels in Water samples (ng/L).

Water Source	Ciprofloxacin	Ibuprofen	Estradiol
Tap Water	<0.5	<0.5	<0.2
River (Upstream)	2.3	1.8	0.6
River (Downstream)	15.7	9.2	3.1
WWTP Effluent	250.4	180.7	90.5

CONCLUSION

The present study demonstrated the efficacy of surface-enhanced Raman spectroscopy (SERS) in the ultra-trace detection of pharmaceutical contaminants in aquatic environments. Key findings indicate that SERS, particularly with AgNP-based substrates, achieved limit of detection (LOD) values as low as 0.5 ng/L, significantly outperforming conventional techniques such as HPLC and Raman spectroscopy (Zhang *et al*, 2023). The enhancement factors achieved through nanoparticle functionalization contributed to superior sensitivity and selectivity, allowing for the rapid identification of contaminants, including ciprofloxacin, ibuprofen and estradiol, even in complex water matrices.

Beyond analytical advantages, SERS presents a cost-effective and field-deployable solution for real-time water quality monitoring. Unlike LC-MS, which requires extensive sample preparation and high operational costs, SERS offers a non-destructive, label-free detection strategy suitable for in-situ analysis (Chen *et al*, 2022). These attributes position SERS as a promising tool for environmental surveillance and regulatory frameworks aimed at mitigating pharmaceutical pollution. The detection of high concentrations of contaminants in wastewater treatment plant (WWTP) effluents further underscores the urgent need for stringent water treatment policies and remediation strategies to prevent pharmaceutical bioaccumulation in ecosystems and potential public health risks (Singh *et al*, 2024).

However, several limitations remain. The reproducibility of SERS signals can be affected by nanoparticle aggregation, substrate uniformity and matrix interferences in environmental samples. Additionally, while AgNPs provide high enhancement factors, they are prone to oxidation, limiting their long-term stability. Further optimization in substrate fabrication and surface chemistry modifications is necessary to improve consistency and ensure broader applicability across various water sources (Wang *et al*, 2023).

Future research should focus on integrating machine learning algorithms for automated SERS spectral analysis, improving data interpretation and contaminant classification accuracy (Li *et al*, 2022). Additionally, the development of bio-inspired SERS substrates and hybrid nanomaterial-based platforms could enhance selectivity toward pharmaceutical pollutants while addressing current challenges related to reproducibility. Large-scale field validation studies in diverse aquatic environments will be crucial to establishing SERS as a standard analytical technique in environmental monitoring and public health assessments.

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