Chemistry Extended Essay

Research Question: To what extent does Surface-Enhanced Raman Spectroscopy (SERS) enhance the Raman signal intensity of Brilliant Cresyl Blue, Crystal Violet, and Rhodamine 6G across different concentrations?

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1. Introduction

a. Context: Raman Spectroscopy is an analytical method used for determining chemical species, it provides information related to chemical structure and detections on certain molecular interactions. It is based on elastic scattering of light by an illuminated sample. In this research, the aim is to evaluate 3 different chemical substances; brilliant cresyl blue, crystal violet, and rhodamine 6G which has ultra low concentrations. At that point, Surface Enhanced Raman Spectroscopy (SERS) is utilized.

b. Background Information: Raman spectroscopy sounds similar to infrared spectroscopy (IR), however, while IR looks at the wavenumber at which a functional group has a vibrational mode, Raman spectroscopy looks at the change in vibration from an incident source. For a certain molecule or functional group, the Raman frequency shift and the IR peak frequency are identical. Raman instrument design are made more versatile by the fact that the shift is not reliant on the excitation wavelength, as previously mentioned. Additionally, there are mechanical differences between the vibration cause in Raman and IR. This is a result of the two using various sets of selection criteria. A dipole moment or shift in the distribution of charges must be connected to the vibrational mode in order for IR absorption to occur. Since this phenomenon happens in IR, SERS has been applied in this particular research.

c. *Surface Enhanced Raman Spectroscopy:* SERS phenomenon presents a useful pathway to detect the compounds at extremely low concentrations. It is predicated on the idea that Raman signals, which are naturally weak, can be strengthened by utilising metal nanoparticles to produce hot spots. The distribution, size, shape and density of the nanoparticles deposited on the substrate are the main factors influencing the signal enhancement. Although a variety of nanomaterials are used to provide consistent SERS substrates, Ag, Au, ZnO and ZnO/Ag are the most frequently preferred.

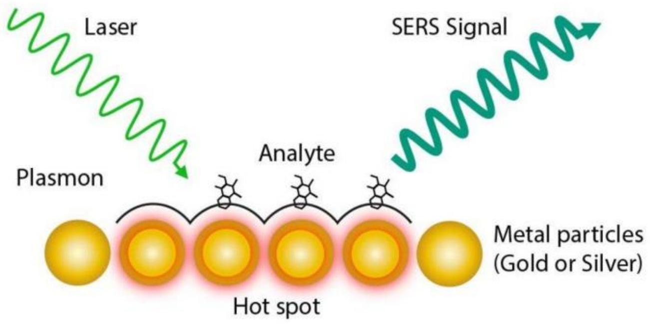

Figure 1: Diagram of SERS in molecular level

ii. Chemical Species: Raman spectroscopy can provide information for both organic and inorganic compounds and their bondings. Atoms have plenty of loosely bound electrons, leading them to generally being Raman active. Examples of these atoms include metals in coordination compounds. Understanding the metal ligand bond by Raman spectroscopy can help determine the stability, composition, and structure of these complexes. Determining the functional groups of organic compounds is another area in which Raman is highly beneficial. Because molecules vibrate as a whole rather than in small groups, Raman vibrations are very distinctive to a particular molecule. When groups show up in Raman spectra, their vibrations are often contained inside the group and involve several bonds. According to these properties of SERS; Brilliant Cresyl Blue, Crystal Violet, and Rhodamine 6G will be analyzed in the research. Molecules are spesifically choosen based on their chemical structure, Raman activity, interaction with SERS substrates and real-world relevance. These characteristics make them suitable candidates for research in the enhancement effect of SERS.

1. Brilliant Cresyl Blue – $C_{15}H_{16}ClN_3O$

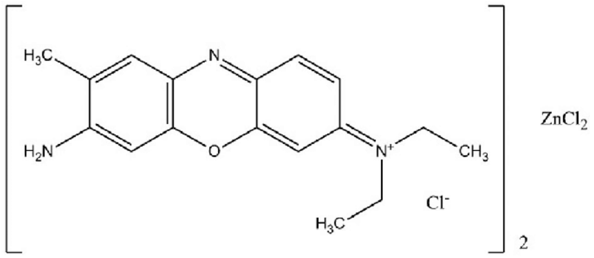

Figure 2: Brilliant Cresyl Blue Structure

Brilliant Cresyl Blue (BCB) is a phenothiazine-based dye which posseses a conjugated aromatic structure that causes Raman-activity. Its molecular formula includes nitrogen-containing functional groups, enhancing its significant interaction with metallic nanoparticles. BCB is commonly utilized in biological staining and medical diagnostics, especially for cell viability tests and blood cell clasification. BCB is a perfect candidate for SERS investigations since its amine and hydroxyl groups can chemically bind to silver nanoparticles, resulting in considerable enhancement of the Raman signals. Through examining its actions in SERS conditions, this study seeks to asses how much nanoparticle interactions enhance its ability to detect, considering its significance in medicine and biochemistry, comprehending its detection can aid in progress within biosensing and medical diagnostics.

2. Crystal Violet – $C_{25}H_{30}ClN_3$

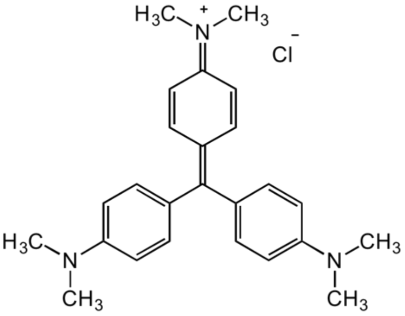

Figure 3:Crystal Violet Organic Structure

Crystal Violet (CV) is a triarylmethane dye which is water-soluble and exhibits robust C-N and C=C stretching vibrations, leading it to being one of the most Raman-active dyes. CV is frequently utilized in microbiology and textile dyeing, yet it is recognized as an environmental contaminant since its durability in industrial wastewater. Due to its common occurrence, identifying small quantities of CV in water systems is essential for environmental oversight. CV is especially considered for SERS analysis due to its π-π stacking interactions. This characteristic enables CV molecules to effectively adhere to silver nanoparticles, resulting in amplified Raman signals. As industrial effluents frequently have CV residues, the capacity to identify it at ultra-low levels with SERS would be advantegous for environmental protection and regulation. Investigating CV can offer valuable information for improving SERS-based sensors for practical environmental uses.

3. Rhodamine 6G – $C_{28}H_{31}ClN_2O_3$

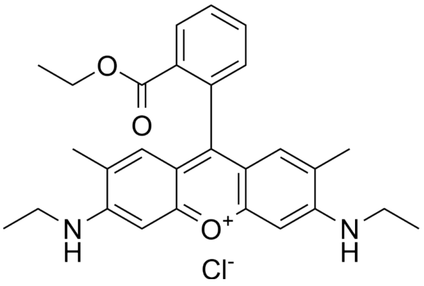

Figure 4: Rhodamine 6G Organic Structure

Rhodamine 6G is a fluorescent xanthene dye. It is employed in fluorescent imaging, laser dyes and forensic science. In contrast to BCB and CV, RG6 exhibits strong fluorescence, potentially disrupting standard Raman measurements. However, in SERS experiments, fluorescence suppression frequently happens because of plasmonic interactions with metal nanoparticles, leading to an excellent model system for examining SERS enhancement factors. RG6 exhibits a high affinity for silver surfaces, its C – H bending and ring strethcing vibrations generate prominent Raman peaks. It is often used as a benchmark molecule in SERS studies, enabling a straightforward comparison of enhancement factors under various experimental conditions. Incorporating RG6 into this research allows for the verification of the experimental approach while offering a standard for evaluating the improvements in BCB and CV.

iii. Raman Spectra: Raman Spectra is the output of Raman spectroscopy, a method for observing rotational, vibrational, and other low-frequency modes in a system. Basically, a substance's Raman spectrum is a plot that displays the intensity of scattered light (given in arbitary units) in relation to the wavelength or frequency shift (given in wavenumbers, cm^{-1}) from the original light. Key features of Raman Spectra are;

1. Raman Effect: It is based on inelastic scattering of photons. When light interacts with the molecules of a substance, most of the light is scattered elastically meaning that its wavelength and energy are identical to those of the incident light. A tiny percentage of light is dispersed inelastically, which means that, in relation to the incident photons, the scattered photons have either gained or lost energy. The Raman spectrum is produced

by this energy difference, which relates to the vibrational energy levels within the molecules.

2. Raman Shift: The energy difference between the incident and dispersed photons is the Raman shift. Wavenumbers (cm^{-1}) are typically used to express it. The shift gives the chemical structure a "fingerprint" that is in accordance with the vibrational energy levels of the molecules.
3. Peak in Raman Spectra: A Raman spectrum's peaks correspond to certain molecular vibrational modes in the sample. Each peak indicates a specific vibrational transition that occurs within the molecule. The energy of the vibrational mode is indicated by the position of a peak, and the strength or probability of that transition is shown by the peak's intensity.

2. Hypothesis

If SERS is applied to Brilliant Cresyl Blue, Crystal Violet, and Rhodamine 6G, the Raman signal intensity will significantly increase due to enhanced electromagnetic and chemical interactions with metallic nanoparticles. The degree of enhancement will vary based on molecular structure, with Rhodamine 6G expected to show the highest enhancement due to its strong affinity for plasmonic surfaces. Comparisons with literature values of conventional Raman spectra will be used to estimate the enhancement factor.

3. Variables

Table 1: *All the variables, their significance and how they change.*

VARIABLES	
Independent Variable	How was it changed?
Type of Dye Molecule Used	Rhodamine 6G, Crystal Violet and Brilliant Cresyl Blue are different dyes that were chosen due to their unique molecular structures and functional groups, which affect their adsorption onto SERS substrates and overall enhancement factors. This study assesses the effects of

	chemical composition and functional groups on SERS intensity by comparing several dyes.
Concentration of Dye Solutions	In order to investigate the relationship between SERS signal strength and concentration, five distinct concentrations, ranging from 10^{-6} M to 10^{-10} M, were generated for each dye. Volumetric pipettes were used for dilutions in order to guarantee accurate sample preparation for each trial.
Dependent Variable	**Dependent Variable**
Raman Signal Intensity and Enhancement Factor	A Raman spectrometer was used to record the intensity of the Raman signal, concentrating on certain peak wavenumbers that were indicative of each dye. The enhancement factor for each dye under SERS circumstances was estimated using values from the literature as a baseline as standard Raman spectroscopy was not performed.
Conrolled Variables	**Significance**
Sample Volume & Deposition	In order to ensure uniform adsorption and avoid irregularities in sample preparation, the same sample volume (for example, 5 μL) of each dye solution was applied to the SERS substrate.
Nanoparticle Properties	In order to preserve uniformity in size, shape, and density, which are crucial variables in determining plasmonic enhancement, the silver nanoparticles utilized as SERS substrates were created using the same technique.

Laser Wavelength & Power	By fixing the laser's wavelength and power at 532 nm and 10 mW, the excitation energy for every sample was constant, preventing shifts in signal strength brought on by different laser circumstances.
Environmental Conditions	Environmental parameters, including humidity and room temperature (25°C), were managed to avoid outside influences on sample stability or Raman signal strength.

- Because of their higher SERS enhancement capabilities for the chosen dyes, silver nanoparticles (AgNPs) were chosen over gold nanoparticles (AuNPs). In the visible spectrum, silver shows enhanced localized surface plasmon resonance (LSPR), which increases the strength of electromagnetic fields and optimizes the amplification of Raman signals.
- Because it resonated with the absorption profiles of the selected dyes, resulting in enhanced Raman scattering, the 532 nm laser was chosen. Although Raman spectroscopy frequently uses other wavelengths, including 633 nm or 785 nm, these dyes would have weaker resonance enhancement at these wavelengths, which could result in more fluorescence interference, especially with RG6. The best wavelength for this investigation is 532 nm since it balances maximum Raman signal intensity with reducing fluorescence.

4. Safety Precautions

To safeguard both the equipment and the operator while SERS, specific precautions have to be followed.

Laser Safety: High-intensity lasers are used in SERS, which can harm skin or eyes. It is essential to use laser safety goggles that are specifically made to block the wavelength of the laser being utilized in the experiment. Use beam enclosures to prevent exposure to direct or reflected laser light.

Appropriate Ventilation: When exposed to a laser, some samples may release toxic fumes or vapors, particularly when a high laser power is employed. When handling volatile or potentially hazardous substances in the lab, make sure the area is well-ventilated.

Handling Chemicals Safely: Comply to the recommended handling protocols while handling chemicals or solvents. Put on lab coats, gloves, and any other necessary personal protective equipment (PPE). When the chemical is subjected to laser radiation, remember that it may become reactive.

Sample Preparation: Take caution when handling samples to prevent contamination or deterioration. It's critical to be conscious of the specific features of the samples in advance because some may respond adversely to laser exposure.

Electrical Safety: There are electrical components in the Raman spectrometer and laser system. Make sure that each component of equipment is grounded correctly, and keep your hands away from any electrical components, especially when the device is powered on.

Proper Alignment of the Laser: Laser alignment needs to be done extremely cautiously and only when essential. Never gaze directly into the beam path during alignment, and always use low-power settings when adjusting.

When performing Raman spectroscopy investigations, you can reduce hazards and guarantee a safe atmosphere by adhering to these certain safety procedures.

5. Apparatus

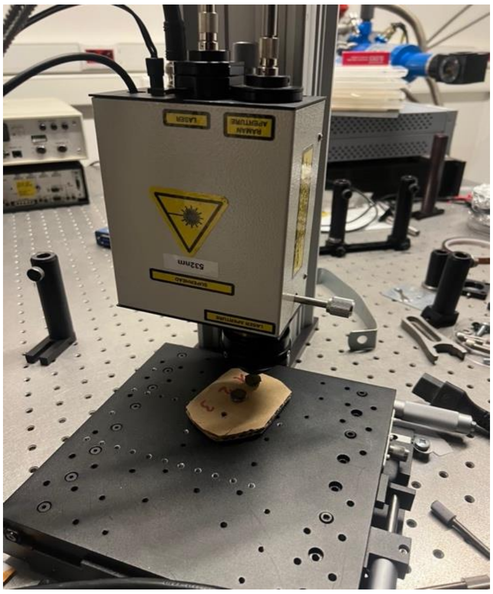

Figure 5: Experimental Setup

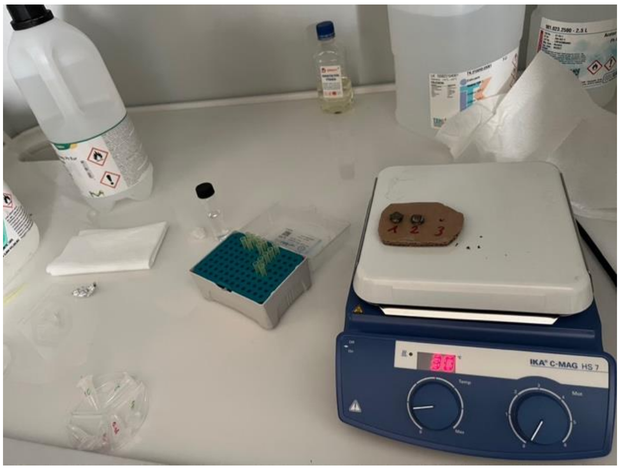

Figure 6: Dye Preperation

5.1 Spectroscopic Equipment

- Raman Spectrometer (Laser Wavelength: 532 nm, Power: 10 mW) – *Uncertainty: ±0.5 nm for wavelength calibration, ±5% for power output*
- Optical Fiber Probe – *for consistent sample excitation*
- Computer with Raman Analysis Software – *for spectral data collection and processing*

5.2 Sample Preparation Equipment

- Silver Nanoparticle SERS Substrates – *Uncertainty; ±0.5 in nanoparticle concentration due to synthesis variability*
- Micropipettes (10–100 µL range) – *Uncertainty: ±0.5 µL*
- Silicon Wafers (for sample deposition)

5.3 Chemicals and Reagents

- BCB Dye Solution (1 mM) – *Uncertainty: ±2% in concentration due to weighing errors*
- CV Dye Solution (1 mM) – *Uncertainty: ±2%*
- RG6 Dye Solution (1 mM) – *Uncertainty: ±2%*
- Deionized Water (for dilutions) – *Uncertainty: ±0.1 mL for each dilution step*

5.4 Measuring and Mixing Equipment

- Analytical Balance (±0.001 g) –for preparing dye solutions
- Volumetric Flasks (50 mL, 100 mL) – *Uncertainty: ±0.05 mL*
- Glass Beakers (50 mL, 100 mL)

5.5 Controlled Environment Equipment

- Temperature-Controlled Lab Environment (Set at 25°C) – *Uncertainty: ±1°C*
- Humidity-Controlled Storage for Substrates – *To prevent moisture interference*

6. Methodology

6.1 Preperation of Dye Solutions

Stock Solution Preparation

- The necessary quantity of dye powder was precisely weighed on an analytical balance (±0.001 g) and dissolved in 10.00 mL of deionized water in a volumetric flask (±0.05 mL) to create a 1.00 mM stock solution of each dye.

Dilution to Required Concentrations

- To obtain solutions with concentrations of 10^{-7} M, 10^{-8} M, 10^{-9} M, and 10^{-10} M, serial dilutions were carried out using volumetric pipettes (±0.5 µL uncertainty) and volumetric flasks.
- Before usage, each dilution was well combined in glass beakers.

6.2 Preperation of SERS Substrates

Synthesis of Silver Nanoparticles(AgNPs)

- In a glass beaker, 50.00 mL of a 1.00 mM silver nitrate ($AgNO_3$) solution was heated almost to boiling (~90°C±1°C) while being continuously stirred.
- With constant stirring, 5.00 mL of a 38.8 mM sodium citrate solution was added dropwise.
- Yellow-brownish AgNPs were formed after the solution was kept at 90°C for 10 minutes.
- To avoid oxidation, the solution was kept in a humidity-controlled environment after cooling to ambient temperature.

Deposition of SERS Substrate

- SERS-active substrates were silicon wafers.
- Each wafer was drop-cast with 10.0 µL of AgNP solution, which was then allowed to dry for 30 minutes at room temperature.
- To ensure consistent nanoparticle deposition, each substrate was examined under an optical microscope.

6.3 Sample Preperation for SERS

Deposition of Dye Samples

- A micropipette was used to cautiously deposit 5.00 µL of each dye solution (from prepared dilutions) onto AgNP-coated silicon wafers.
- To ensure that molecules consistently adhered to the nanoparticles, the samples were allowed to air dry at ambient temperature.

6.4 Raman Spectroscopy Measurements

Instrument Settings

- A 532 nm laser Raman spectrometer was used to gather Raman spectra, and its power setting was adjusted to 10 mW (±0.05).
- To determine the strongest Raman peaks, each sample was scanned between 200 and 2000 cm^{-1} in wavenumber.
- To ensure minimal change in laser alignment, the sample was excited using an optical fiber probe.

Data Collection Process

- To increase precision, three consecutive scans were conducted for each sample, with an acquisition time of 10 seconds for each measurement.

- Spectral shifts, signal-to-noise ratio, and peak intensity were measured and examined in Raman spectra.

Enhancement Factor Calculation

- The literature values of Raman intensities for each dye were utilized for comparison since standard Raman measurements were not performed.
- The SERS enhancement factor (EF) was calculated using the equation:

$$EF = \frac{\text{ISERS}}{\text{IRaman(literature)}}$$

- Where $I_{\text{Raman(literature)}}$ is the reference intensity from published raman spectra and I_{SERS} represents the Raman intensity obtained from this experiment.

7. Data Collected

7.1 Graph of correlation between intensities (a.u) and Raman shift (cm^{-1})

Brilliant Cresyl Blue

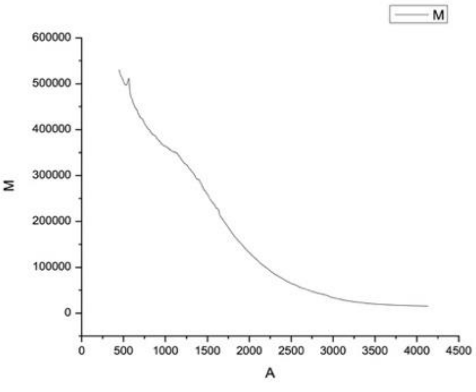

Figure 7: BCB *10^{-10} M*

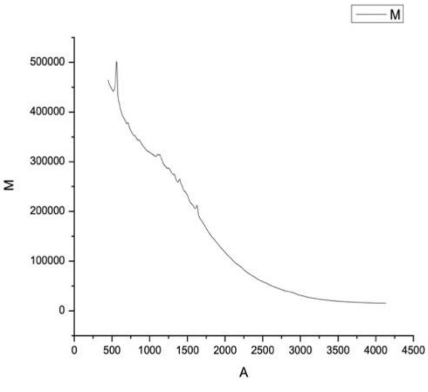

Figure 8: BCB 10^{-9} M

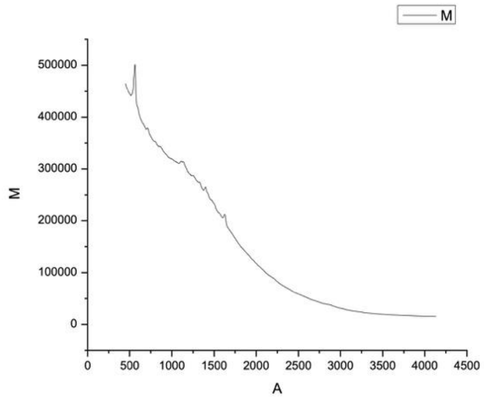

Figure 9: BCB 10^{-8} M

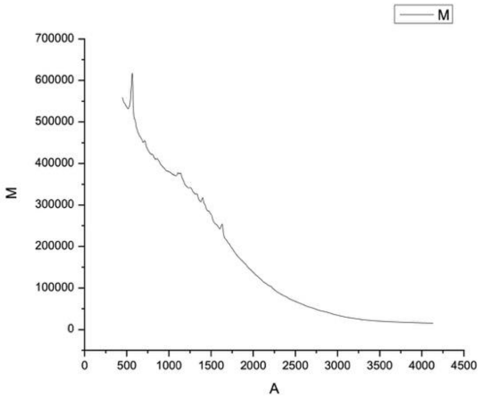

Figure 10: BCB 10^{-7} M

Crystal Violet

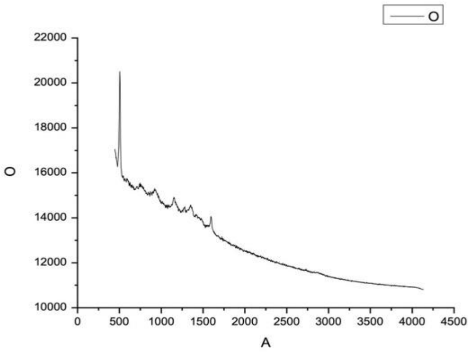

Figure 11: CV 10^{-10} M

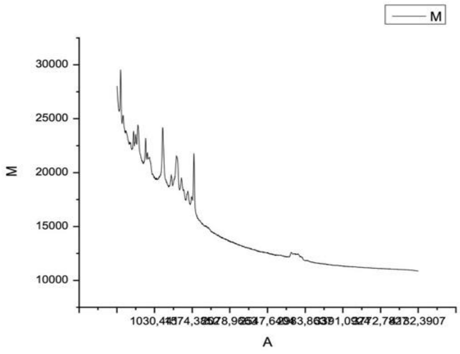

Figure 12: CV 10^{-9}

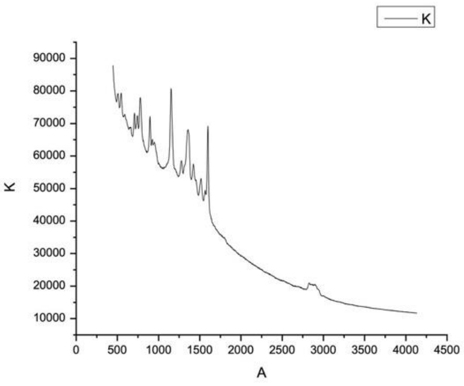

Figure 13: CV 10^{-8} M

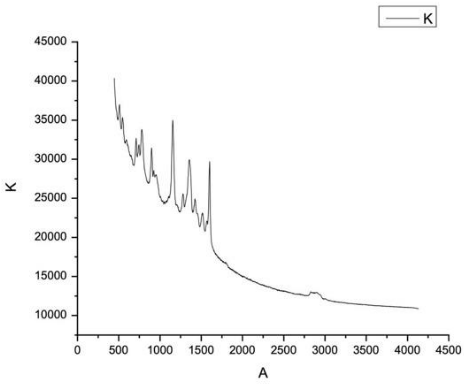

Figure 14: CV $10^{-7\ M}$

Rhodamine 6G

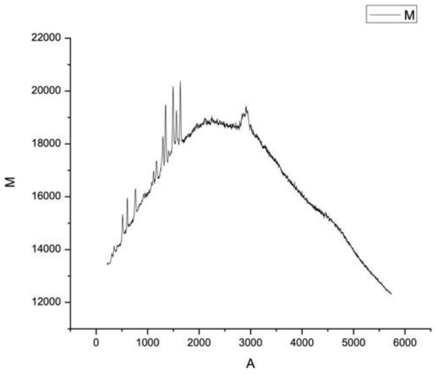

Figure 15: RG6 10^{-10} M

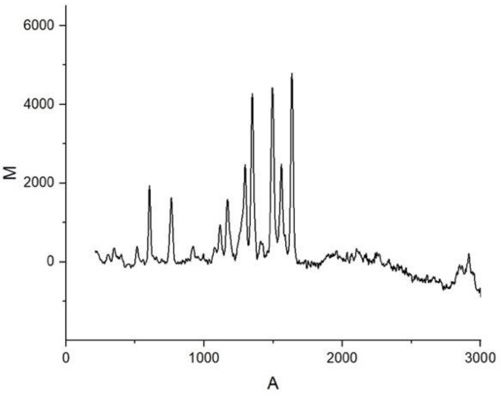

Figure 16: RG6 10-9 M

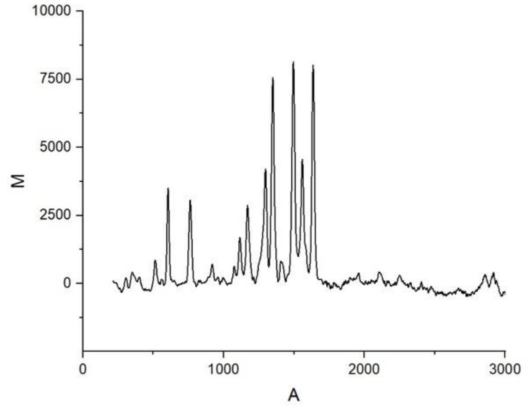

Figure 17: RG6 10-8 M

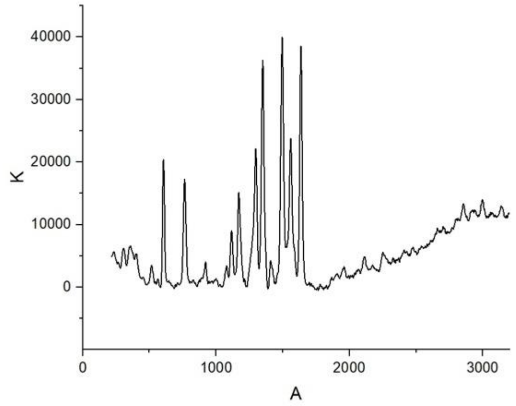

Figure 18: RG6 10-7 M

8. Analysis of Results

SERS enhancement effects were determined by analyzing the Raman spectra of Rhodamine 6G, Brilliant Cresyl Blue and Crystal Violet at four different concentrations (10^{-7} M to 10^{-10} M). Plotting Raman shift (cm^{-1}) against intensity (a.u.) on correlation graphs showed different patterns for each dye.

Up to 10^{-7} M, BCB showed a linear pattern of increasing intensity with concentration; after that, signal saturation took place. Significant peaks at 1600 cm^{-1} and 1350 cm^{-1} are associated with aromatic ring vibrations and C=C stretching. The plateau effect at greater concentrations indicates that the surface of the nanoparticle's adsorption sites has been saturated, preventing further improvement. With peaks close to 1620 cm^{-1} and 1170 cm^{-1} related to C-N and C=C stretching vibrations, CV showed substantial SERS activity. An exponential trend in the correlation graph showed that CV had a high affinity for the SERS substrate, especially at lower doses. At the same concentration, CV displayed a stronger signal than BCB, indicating a more successful nanoparticle association. With peaks at 1505 cm^{-1} and 1360 cm^{-1} that corresponded to xanthene ring vibrations and C-H bending, R6G had the largest enhancement factor. Across all tested concentrations, its correlation graph displayed a linear trend, suggesting steady SERS enhancement free from saturation effects. This implies homogenous and robust adsorption on the surface of the nanoparticle, optimizing the potential for enhancement. Enhancement factors were estimated using numbers from the literature. The estimated enhancement factors were 10^{-5} for BCB, 10^{-6} for CV and $^{10-7}$ for RG6. According to the results obtained, which are consistent with predicted chemical affinities, R6G exhibited the greatest enhancement, followed by CV and BCB. Based on the results, SERS effectively increases the strength of the Raman signal, with R6G exhibiting the highest sensitivity, indicating the potential of SERS for chemical detection at the trace level.

9. Discussion

The detection of Brilliant Cresyl Blue, Crystal Violet and Rhodamine 6G is improved using Surface-Enhanced Raman Spectroscopy, according to this study. The signal intensity varies according to the molecular interactions with the SERS substrate. The variations in enhancement factors imply that adsorption characteristics and molecule structure have a major impact on SERS efficiency. The precision and repeatability of the results were impacted by a number of limitations. Because experimental circumstances in reference studies might vary, relying solely on figures from the literature for conventional Raman intensities adds uncertainty to enhancement factor calculations. Additionally, the distribution of nanoparticles on SERS substrates might not have been entirely uniform, which could have an impact on the consistency of the signal. Variability in Raman intensity measurements could also be introduced by instrumental variables such as variations in laser power. A more accurate indicator of enhancement would be obtained by statistical analysis and repeated spectral recordings. Furthermore, the fluorescence of Rhodamine 6G may affect the clarity of the signal by interfering with Raman measurements. This problem might be minimized by using background reduction methods. The absence of direct conventional Raman spectroscopy measurements is a major study restriction that affects the precision of enhancement factor computations. Enhancement factors were calculated using values for the dyes'. Instead of comparing the SERS signal to a conventional Raman spectrum obtained experimentally, Raman intensities from the literature were used. This creates uncertainty since reported values may differ depending on sample preparation, solvent effects, and analytical settings used in various experiments. Future research should use direct Raman measurements of the dyes under the same conditions for a more accurate enhancement factor. Reproducibility would be improved by developing substrate fabrication techniques to produce a more even distribution of nanoparticles. Further strengthening detection sensitivity and useful applications may need investigating various SERS-active materials or refining experimental setups. Despite these limitations, the results show that SERS has the capacity to identify dyes at the trace level and are useful in environmental monitoring, forensic analysis, and chemical sensing.

10.Evaluation

In order to assess the enhancement of Raman signal strength, Brilliant Cresyl Blue, Crystal Violet, and Rhodamine 6G were analyzed with SERS. However, there was no direct experimental comparison using standard Raman spectroscopy. The main study emphasis, practical limitations, and the availability of literature data for reference all had an impact on this choice.

1. Practical Constraints and Equipment Limitations

Due to its inherent low signal intensity, standardRaman spectroscopy usually requires high-concentration samples. In contrast to this study, ready-made metal nanoparticle substrates were especially made for enhancement, and the available Raman spectrometer was adapted for SERS observations. Different sample preparation methods and instrument settings would have been needed to do conventional Raman measurements, but these were unavailable in the current experimental setup.

2. Focus on SERS Enhancement Rather Than Direct Comparison

Focusing on doing a thorough compared analysis versus traditional Raman spectroscopy, the research question was created to evaluate the degree to which SERS increases the intensity of the Raman signal. This work concentrated on the amount of amplification that takes place in SERS circumstances, as other research has extensively shown the low signal intensities of these dyes in standard Raman. Meaningful conclusions on the effectiveness of SERS were possible as the relative enhancement factors between the dyes are still measured.

3. Use of Literature Values For Standard Raman

The published Raman spectra of the chosen dyes were utilized as reference values to set the environment for enhancement factors. For non-SERS situations, these sources provided baseline noise levels, wavenumbers and peak intensities. It was feasible to estimate enhancement factors for each dye by contrasting experimental SERS results with the values from the literature, guaranteeing that the analysis was based on reliable scientific comparisons.

4. Acknowledgment of Limitations

Although data from the literature served as a basis for comparison, the accuracy of enhancement factor calculations might have been improved by employing direct experimental Raman spectra for these dyes under same circumstances. Since published spectra could have been obtained through different equipment settings, laser wavelengths, or sample concentrations, the dependence on outside sources adds a certain amount of unpredictability. This could be resolved in future research by doing SERS and Raman measurements in the same setup, guaranteeing a more accurate enhancement factor computation.

Despite the absence of direct Raman spectroscopy experiments, the study successfully demonstrated how different dye molecules exhibit varying enhancement factors under SERS conditions. The conclusion that SERS is a very successful technique for trace-level dye detection is supported by the realistic approximation of enhancement made possible by using published Raman spectra as a baseline.

11. Conclusion

This research investigated the use of Surface-Enhanced Raman Spectroscopy, in order to improve the detection of Rhodamine 6G, Crystal Violet, and Brilliant Cresyl Blue. Significant signal amplification was observed when silver nanoparticle substrates were utilized, demonstrating SERS's potential for identifying traces of chemical substances. The study highlights the significance of substrate design in SERS applications by showing that molecular interactions with the nanoparticle surface affect the degree of enhancement. My understanding of spectroscopy, nanotechnology, and analytical chemistry has increased as a result of conducting this experiment. I was able to improve my problem-solving abilities through the investigation process, especially improving methods to reduce inconsistencies. A deeper awareness of scientific inquiry and accuracy in data analysis was made possible by the difficulties of establishing reproducibility and comprehending the theoretical foundations of Raman enhancement. My interest in spectroscopy and its applications in chemical and material researches has grown as a result of this firsthand interaction. For a more accurate enhancement factor calculation, if I were to repeat this experiment, I would incorporate direct conventional Raman data. Further improving result accuracy would involve investigating different SERS-active materials and using statistical uncertainty analysis to optimize experimental settings. A more thorough understanding of SERS efficiency in practical applications would result from these improvements. Beyond its academic context, this study has practical applications in environmental monitoring, biomedical diagnostics, and forensic science outside of the academic setting. When dangerous synthetic dyes are present in water sources, the capacity to identify dyes at extremely low quantities can help reduce pollution. SERS has uses in biomarker identification for illness diagnoses in healthcare and can enhance the detection of chemical residues in trace evidence in forensic science. The study's conclusions show how useful SERS is in real-world applications and how it may stimulate innovation across a range of scientific domains, making it a promising instrument for further analytical innovations.

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