

Achieving Very Low Levels of Detection: An Improved Surface-Enhanced Raman Scattering Experiment for the Physical Chemistry Teaching Laboratory

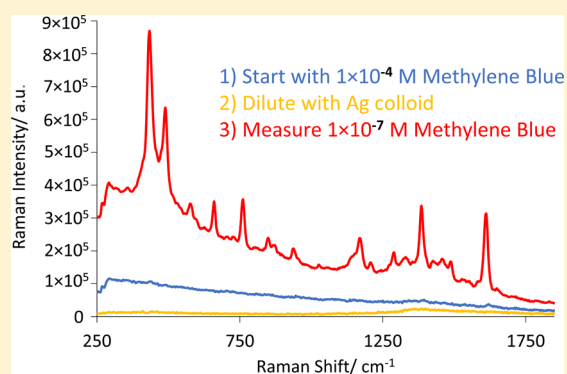
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S Supporting Information

ABSTRACT: This experiment was designed and successfully introduced to complement the nanochemistry taught to undergraduate students in a useful and interesting way. Colloidal Ag nanoparticles were synthesized by a simple, room-temperature method, and the resulting suspension was then used to study the surface-enhanced Raman scattering (SERS) of methylene blue. The colloid was also characterized by UV–visible spectroscopy, and these results were used to help explain some of the observed SERS features. The students looked at the effects of concentration and acquisition time on the measured SERS spectra, and the final part of the experiment was based around using their newly acquired knowledge to investigate the lowest concentration of methylene blue that could be detected. Concentrations of 5×10^{-10} M were routinely achieved. The combination of UV–visible spectroscopy, SERS, and nanochemistry made for an interesting and thought-provoking laboratory experience.

KEYWORDS: Upper-Division Undergraduate, Analytical Chemistry, Laboratory Instruction, Physical Chemistry, Hands-On Learning/Manipulatives, Problem Solving/Decision Making, Colloids, Nanotechnology, Raman Spectroscopy, UV–Vis Spectroscopy



With nanochemistry now a firmly established subject in undergraduate lecture courses, it is important that this learning is consolidated by practical, laboratory work. If such an experiment can also exploit nanochemistry for a useful purpose, then this will further reinforce its relevance and applicability. This experiment combines a simple, room temperature synthesis of a Ag nanoparticle colloid and surface-enhanced Raman scattering (SERS) to achieve levels of detection of methylene blue (MB) down to 5×10^{-10} M. The colloids are also characterized by UV–visible spectroscopy, and this experiment encourages the students to bring together the results of both spectroscopic techniques to help explain what they observe. The students build on their initial results and understanding and then use their newly acquired knowledge to design a short investigation to establish their minimum level of detection of MB. While the experiment was developed and taught from a physical chemistry viewpoint, it also has a clear analytical chemistry theme.

SERS exploits the massive enhancement (up to 10^9 times)^{1,2} of the Raman signal which is observed when a Raman active molecule is adsorbed on a SERS active substrate. Since first being reported in 1974,³ the technique has been widely applied⁴ and the enhancement mechanism has been studied.^{5,6} Current theory suggests that there are two possible mechanisms.⁴ First, an electromagnetic enhancement is caused when a surface plasmon mode of the metallic substrate is

excited by the incident light. This amplifies the local electromagnetic field experienced by the adsorbed analyte molecules.⁷ Preferential amplification occurs in gaps formed when nanoparticles aggregate together, as localized plasmon modes can couple.^{8,9} These “hot-spots” of activity have given rise to single molecule detection.¹⁰ The second mechanism is a chemical one involving charge transfer between the test molecule and the metal;¹¹ the excitation wavelength can resonate with metal–molecule electronic states.^{12,13} The electromagnetic mechanism is thought to be the predominant one,⁸ as SERS is still possible when no charge transfer mechanism exists.

The vivid colors of colloidal Au, Ag, and Cu metal particles are caused by their surface plasmon modes, so these are widely used as SERS substrates.¹⁴ Silver colloidal nanoparticles are highly active,¹⁵ and Ag salts are much less expensive than Au ones, which is an important consideration when designing any teaching experiment.

There are several reported synthetic methods for preparing SERS active colloidal Ag nanoparticles,^{16,17} but not all of them are all accessible or practical for teaching laboratory experi-

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ments. Some have been applied to SERS experiments,^{18–20} but the emphasis of this experiment is different.²¹ Unlike these previously reported experiments, this one focuses on combining a simple, rapid, room-temperature synthesis (based on that developed by Leopold and Lendl)²² with UV–visible and SERS spectroscopy to guide the students toward investigating the minimum level of detection of MB. By reducing the time and complexity of the synthesis step, the students can focus on characterizing and using their colloid. In contrast with the other reported SERS teaching experiments, the students then build on what they learn during these characterization steps to design and implement a short investigation which enables the minimum level of detection of MB to be determined; the levels achieved are impressively low for an undergraduate teaching laboratory experiment. Sequentially introducing the analysis method and instrumental parameters allows the students to glean the important parameters as they progress through the work. This experiment begins as a fully described one, but it then progresses to give the students some freedom to investigate for themselves. Reducing the synthesis time enables this to be completed in a short laboratory session. Instructors can choose how much additional guidance to give to their students when conducting the investigation section of the experiment, allowing the experiment to be tailored to match students' abilities.

In addition to the synthesis advantages listed above, using MB as the analyte is less hazardous, more convenient, and less expensive than other potential test molecules such as pyridine¹⁸ or Rhodamine 6G.²⁰

CHEMICALS AND EQUIPMENT

All chemicals were reagent grade, and all solutions were prepared using deionized water.

UV–visible absorbance spectra were recorded using an Ocean Optics USB2000+ spectrometer, the light source was a tungsten lamp, and the cuvette holder was built in-house. The Raman instrument was a Centice MMS Raman spectrometer with an excitation wavelength of 784 nm. Both of these instruments were controlled using Ocean Optics SpectraSuite software, which offered an additional advantage to the students, as everything was controlled through a single computer interface. This experiment can be adapted and optimized to suit alternative spectroscopic equipment.

COLLOID SYNTHESIS

The Ag colloid was synthesized as described by Leopold and Lendl²² with only slight modification for practical, teaching laboratory purposes. Full details are in the [Supporting Information](#) but, briefly, the students were supplied with stock solutions of 1 mM AgNO₃ and 0.3 M NaOH whereas the hydroxylamine was supplied as a solid, as solutions of it were found to be unstable, lasting no more than a few days. The students prepared the reducing solution and added this to the AgNO₃ (with stirring) in an Erlenmeyer flask. The colloid goes through a series of color changes before finally becoming milky-yellow; it is ready for use in 2–3 min. The advantages of using this process in a teaching experiment are that it can be carried out at room temperature and useable colloid is rapidly prepared—the students know quickly by the color change if their synthesis has been successful and can readily produce another batch if necessary. Other syntheses, in contrast,

generally take significant time¹⁸ and require heating¹⁶ or cooling¹⁷ of the reagents.

TEST REAGENT

Methylene blue (MB) was chosen as the test reagent, as it is both inexpensive and gives reproducible SERS spectra showing many vibrational bands. The normal Raman (non-SERS) spectrum of even the 1×10^{-4} M MB stock solution supplied to the students showed very few features.

UV–VISIBLE CHARACTERIZATION

The absorbance spectrum of the diluted colloid (*ca.* 5-fold dilution—see [Supporting Information](#) for details) was initially measured, and the typical absorbance profile of Ag nanoparticles was observed ([Figure 1](#)).

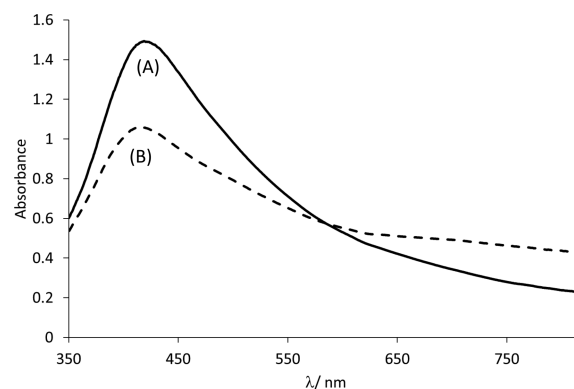


Figure 1. UV–visible spectra of Ag colloid without (A) and with (B) added NaCl solution.

The broad absorption band corresponds to the surface plasmon resonance of the suspended Ag nanoparticles, which is caused by a collective, quantized oscillation of the free electrons in the metal nanoparticles. The resonance is initiated by the electric field of the incident photons. One drop of 2 M NaCl solution was then added to the cuvette, and the spectrum was remeasured after 60 s. The changes in the absorbance are caused by electrostatic aggregation of the colloidal particles^{22,14} with Cl[−] ions bridging between particles, and these changes are used to help explain some of the SERS features recorded during the next part of the experiment. It is noted in passing that the SpectraSuite software continuously acquires and displays the absorption spectra as the aggregation proceeds, allowing the students to watch the changes in real time.

SURFACE-ENHANCED RAMAN SPECTROSCOPY

Initial Measurements and Concentration Dependence

For comparison, the initial Raman spectra of the Ag colloid and the 1×10^{-4} M MB stock solution were measured, and neither of these showed significant Raman scattering ([Figure 2](#)). The SERS spectrum of the colloid with MB (1×10^{-7} M) was then measured and a large increase in the signal was observed. This is also shown in [Figure 2](#).

The SERS intensity as a function of MB concentration was then measured to give the plot in [Figure 3](#), illustrating the linearity of the SERS technique in this concentration range.

For the next measurement, one drop of 2 M NaCl was added to the sample vial containing the final MB concentration and the SERS spectrum was remeasured after 60 s. A further, large

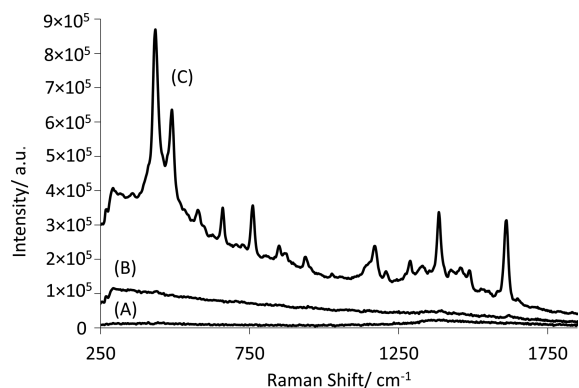


Figure 2. Raman spectra of the Ag colloid (A), 1×10^{-4} M MB solution (B), and SERS of the Ag colloid with 1×10^{-7} M MB (C).

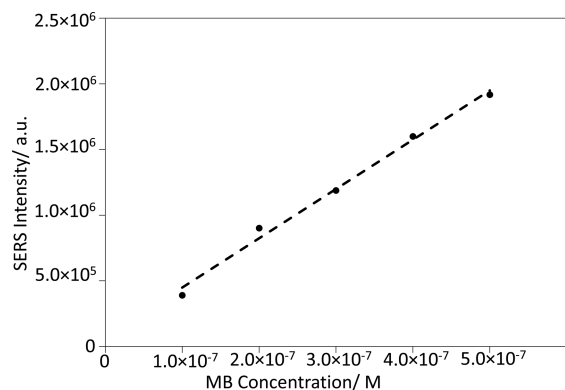


Figure 3. Plot of SERS intensity vs MB concentration.

enhancement of the signal was observed as shown in [Figure 4](#). A NaCl concentration in the 0.1–0.2 M range was found to work well in this experiment.

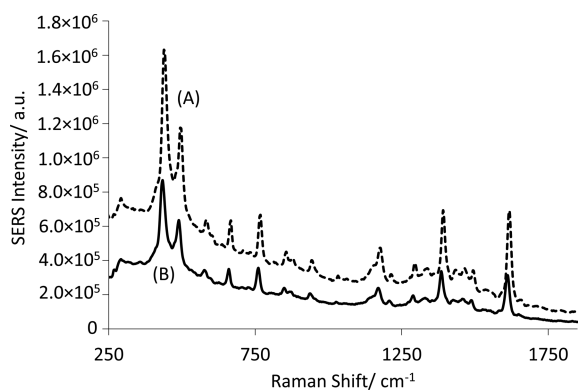


Figure 4. SERS spectra of MB adsorbed onto a Ag colloid with (A) and without (B) added NaCl solution.

Adding NaCl causes the nanoparticles to aggregate,²² which increases the overall scattering intensity as the effective particle size increases. The aggregation is also thought to increase the number of “hot-spots”,⁸ which are formed when plasmon modes of nanoparticles brought together by aggregation couple; preferential electromagnetic amplification occurs here. A higher measured “absorbance” (there will be light lost due to scattering from the larger particles so extinction is more correct) in the UV–visible spectrum at the laser wavelength of 784 nm was observed when NaCl was added. This can

therefore be used, in simple terms, to illustrate why the SERS intensity increases. There are more possible interaction events between the nanoparticles and the excitation light. When synthesized, the colloidal particles are capped and protected from aggregation by the hydroxylamine, as this leaves them with a surface charge.¹⁴ The addition of ions to the suspension screens this charge and allows aggregation. The other potential effects of the chloride ion on the SERS signal (through the charge transfer mechanism) were considered too advanced for this experiment,¹⁴ but this could be further explored and developed. In any case, this illustrates that the SERS intensity also depends on the shape/morphology of the nanostructure.

Spectrometer Acquisition Time Dependence

The dependence of the SERS signal on the instrument’s acquisition time was then measured using a constant MB concentration. The data are plotted in [Figure 5](#).

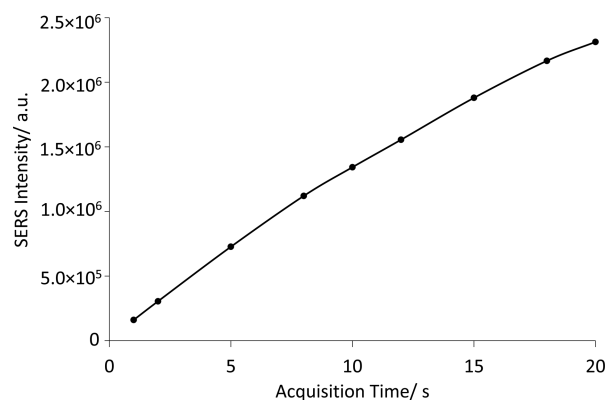


Figure 5. Plot of SERS Intensity vs spectrometer acquisition time for 4×10^{-7} M MB solution.

This part of the experiment was designed to show that the measured Raman scattering intensity could also be improved (up to a point) by changing some of the instrumental parameters. The initial linear increase in intensity cannot be maintained, as the acquisition time continues to increase; the students are asked to comment on this in their laboratory reports. This section of work was specifically included to assist with the final stage of the experiment.

Investigation of the Minimum Level of Detection

Here, the students were asked to bring the previous work together to determine the minimum concentration of MB that they could detect using the SERS technique. Basic instructions were given to them (see [Supporting Information](#)), and the students were asked to decide at what concentration they were sure they were first detecting MB by noting when the main peak at *ca.* 430 cm^{-1} first appeared. This fine qualitative judgment formed part of the assessment of the experiment. Concentrations of MB down to 5×10^{-10} M were detected, and example spectra are shown in [Figure 6](#). These data were recorded with a 20 s acquisition time and show Raman spectra from the colloid itself, together with the first evidence of MB detection at 5×10^{-10} M, where 1 μL of a solution containing 1×10^{-6} M MB was added to 2 cm^3 of colloid. To confirm that this really was MB, a further 1 μL aliquot was added and the increased Raman activity in the region around 450 cm^{-1} was taken as confirmation. Further dilution of the MB and the addition of NaCl may have yielded a lower detection threshold,

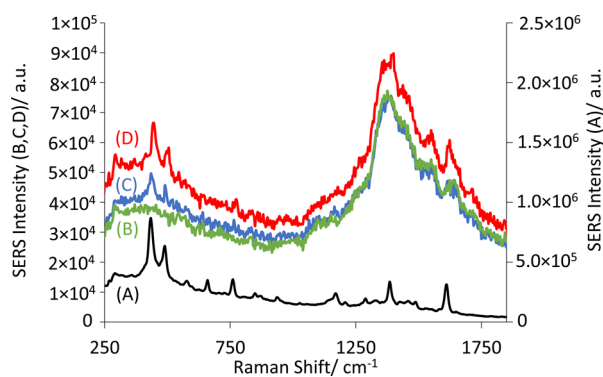


Figure 6. Determining the minimum level of MB that can be detected. Spectrum (A) is the same as spectrum (C) from Figure 2, which is Ag colloid with 1×10^{-7} M MB. This is included to show the peaks developing. Spectrum (B) is the colloid alone, (C) contained 5×10^{-10} M MB, and (D) contained 1×10^{-9} M MB. Acquisition time was fixed at 20 s, and no NaCl was added.

but the recorded spectra were sufficiently good for the purpose of the experiment.

HAZARDS AND PRECAUTIONS

The prepared solutions provided for the students are at low concentration, so standard chemical handling precautions, including laboratory coats, safety glasses, and gloves are appropriate; no special precautions are necessary. Hydroxylamine hydrochloride is used as a solid and is considered harmful and an irritant in this form. Gloves must be worn when weighing out this material. Alternatively, this can be given to the students preweighed in a vial or as a freshly prepared solution (see Supporting Information for details) for use; the required solution concentration is very low.

The Raman spectrometer used in this experiment has a safety interlock which prevents exposure to laser radiation when the cell compartment is open. Other spectrometers and particular Raman microscope equipment might require additional precautions.

DISCUSSION OF IMPACT

During the course of the academic year, over 130 third-year undergraduate students carried out the experiment in the physical chemistry teaching laboratory. The students worked in pairs and took between 2 and 3 h to work through the procedure. Each student was required to submit a laboratory report which included answering “quiz questions” based on their results. A cross-section of the class was also interviewed on completion of the experiment to gauge their interest and understanding.

Examination of the marked reports showed that, on the whole, the students engaged with the experiment, with the class average mark being 81%. The students’ answers to the “quiz questions” showed that their understanding of Raman spectroscopy in general and SERS in particular was enhanced by this practical activity, and the linking of the absorbance and Raman spectra proved to be thought-provoking and encouraged much discussion and interest. The analytical as well as the physical and nanochemistry aspects were also highlighted by the questions asked of the students.

During the course of the year, a few issues came to light. The most common problem encountered was with consistently mixing very small volumes (e.g., 1–2 μL) of liquid into larger

(e.g., 2 cm^3) ones. To counter this, a practical demonstration was given, and the experimental procedure has been updated to stress the need for careful and consistent mixing. This problem caused nonlinearity in the types of plot shown in Figure 3.

The students were particularly interested and somewhat surprised at the low MB detection limit achieved using the SERS effect, particularly given the virtual nonactivity of MB in normal Raman. The stark contrast between the lack of Raman activity from the MB stock solution and the detailed spectrum recorded when the first 2 μL of MB were added to the colloid really caught the students’ attention. The students were also interested in using Raman spectroscopy in general, as while they do learn about the technique, they had never used it before. They were much more accustomed to using FTIR spectroscopy, and the fact that Raman spectra can be collected in aqueous solution was also of interest.

The investigation part of the experiment can be modified to suit the abilities of the students, with more or less help or instruction given on how to complete the task. What is included in the Supporting Information is what was considered appropriate for the class at the time. It is possible to achieve a lower level of MB detection by using a more highly diluted MB stock solution (1 μL was the lowest volume that could be pipetted in the lab), and adding NaCl would further enhance the signal; 1×10^{-11} M was achieved during development. This would, however, add considerable time to the experiment and could change its emphasis too much. It is also noted that the absolute level of detection achievable will be dependent on the available instrumentation.

In conclusion, this experiment was successfully implemented and it did reinforce the lecture classes on nanochemistry, absorbance spectroscopy, and Raman spectroscopy by introducing a relevant practical activity. The investigation part of the experiment encouraged students to think more independently about what they were doing and gave them some experience of designing and implementing a short experimental protocol which required skill and judgment. Minimum MB levels in the 1×10^{-8} M to 5×10^{-10} M range were achieved by all of the students.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.6b00312.

Staff guidelines and marking scheme (PDF, DOCX)

Full laboratory manual (PDF, DOCX)

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Notes

The author declares no competing financial interest.

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