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

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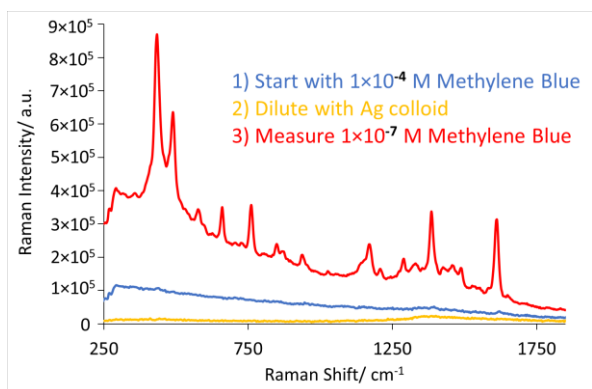
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ABSTRACT

This experiment was designed and successfully introduced to complement the nanochemistry taught to undergraduate students in a useful and interesting way.

- 10 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
- 15 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.

20 GRAPHICAL ABSTRACT



KEYWORDS

Upper-Division Undergraduate, Analytical Chemistry, Laboratory Instruction, Physical
Chemistry, Hands-On Learning/Manipulatives, Problem Solving/Decision Making,
25 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.
30 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 characterised by UV-Visible spectroscopy and this experiment
35 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
40 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 being first reported in 1974,³ the technique has been widely applied⁴
and the enhancement mechanism has been studied.^{5,6} Current theory suggests that
45 that there are two possible mechanisms.⁴ Firstly, 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 one⁸ as SERS is still possible when no charge transfer mechanism exists.

55 The vivid colours 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 active¹⁵ and silver salts are much less expensive than gold 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 experiments. Some have been applied to SERS experiments,^{18,19,20} but the emphasis this experiment is different.²¹ Unlike these previously-reported experiments, this one focusses 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 towards 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

75 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
80 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 deionised
water.

85 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
90 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
95 supplementary material 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
100 becoming milky-yellow; it is ready for use in 2-3 minutes. 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 supplemental material for details) was initially measured and the typical absorbance profile of Ag nanoparticles was observed (Figure 1).

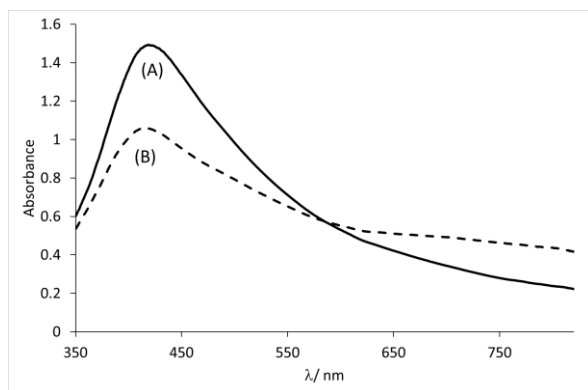


Figure 1. UV-Visible spectra of Ag colloid without (A) and with (B) added NaCl solution.

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 re-measured 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.

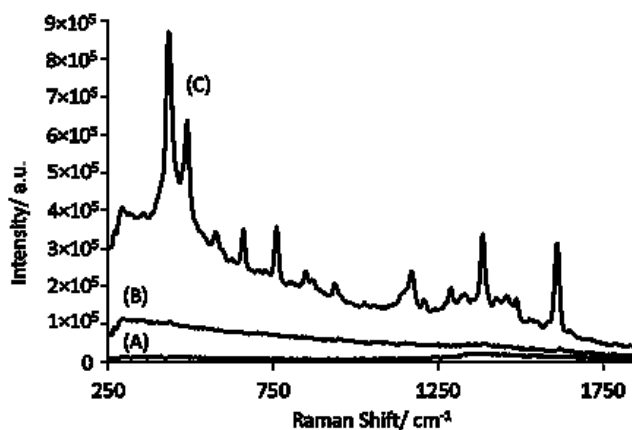


Figure 2. Raman spectra of the Ag colloid (A), 1×10^{-4} M MB solution (B) and SERS of the Ag colloid with 1×10^{-7} M MB (C).

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.

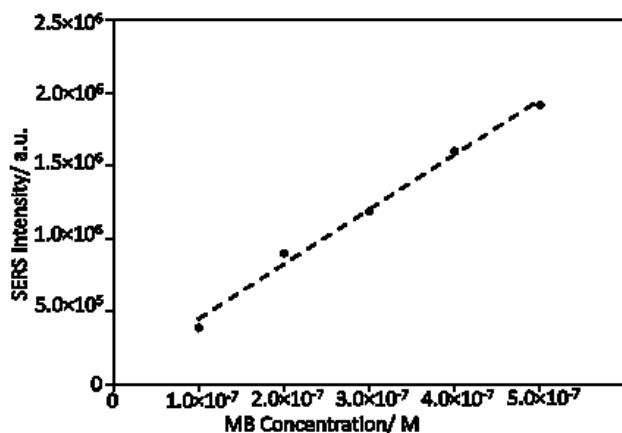
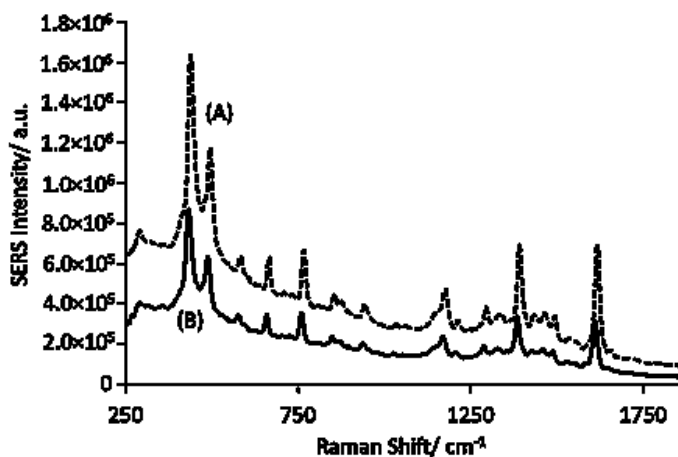


Figure 3. Plot of SERS intensity vs. MB concentration.

For the next measurement, one drop of 2 M NaCl was added to the sample vial
 145 containing the final MB concentration and the SERS spectrum was re-measured after
 60 s. A further, large enhancement of the signal was observed as shown in Figure 4. A
 NaCl concentration in the 0.1 M – 0.2 M range was found to work well in this
 experiment.



150 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
155 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.
160 There are more possible interaction events between the nanoparticles and the excitation
light. When synthesised, 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
165 mechanism) were considered too advanced for this experiment¹⁴ 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

170 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.

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
175 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.

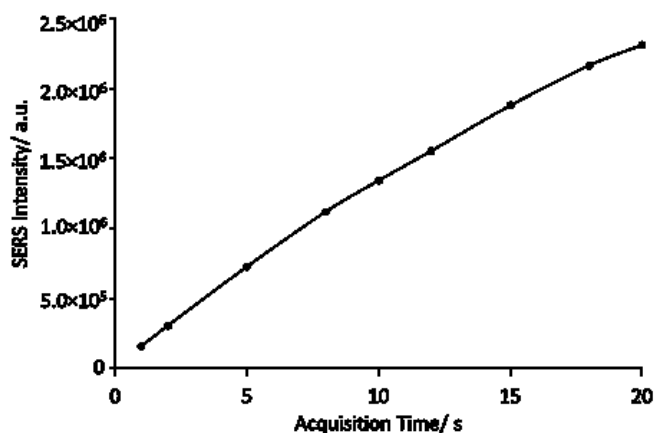


Figure 5. Plot of SERS Intensity vs. Spectrometer Acquisition Time for 4×10^{-7} M MB solution.

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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 supplemental 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 judgement 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 $1 \mu\text{l}$ 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 $1 \mu\text{l}$ 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, but the recorded spectra were sufficiently good for the purpose of the experiment.

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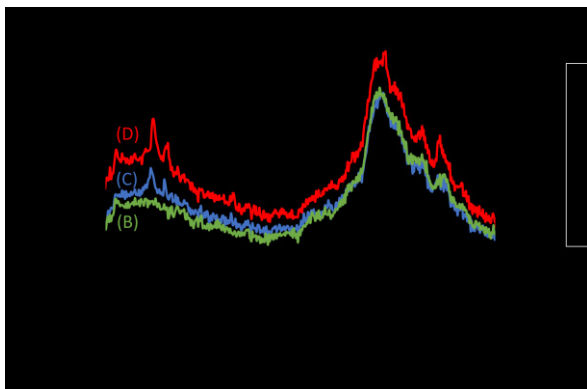


Figure 6. Determining the minimum level of MB that can be detected. Spectrum (A) is the same as Spectrum (C) from Figure 2, which is Ag colloid with 1×10^{-7} M MB. This is included to show the peaks developing. Spectrum (B) is the colloid alone, (C) contained 5×10^{-10} M MB and (D) 1×10^{-9} M MB.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

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 irritant in this form. Gloves must be worn when weighing out this material. Alternatively, this can be given to the students pre-weighed in a vial or as a freshly-prepared solution (see supplemental 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, 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 two and three hours to work through the procedure.

Each student was required to submit a laboratory report which included answering
220 '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
225 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
230 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 non-linearity in the types of plot shown in
Figure 3.

235 The students were particularly interested and somewhat surprised at the low MB
detection limit achieved using the SERS effect, particularly given the virtual non-activity
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 also were
240 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 supplemental 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 enhances 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 to 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 judgement. 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 full laboratory manual, staff guidelines and marking scheme are provided.

AUTHOR INFORMATION

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Demonstrators' Notes

Preliminary:

- Ensure that the PC, Raman and UV-Vis spectrometer are on with the black cuvette in the cell holder to block the beam.
- Ensure that the stock solution bottles (1 mM AgNO₃, 0.3 M NaOH, 1 × 10⁻⁴ M methylene blue and 2 M NaCl) are topped up and that there are sufficient micropipette tips, sample vials and hydroxylamine hydrochloride. There is spare colloid in the cupboard.
- There should also be: 1 × 100 ml Conical Flask, 2 × 100 ml volumetric flasks, 1 × 50 ml pipette, 1 × 10 ml pipette, 1 × 5 ml pipette, 1 × 1 ml pipette, 1 × 1000 μl micropipette, 1 × 20 μl micropipette, 1 glass weighing boat, the marked cuvette and some beakers.

Points to be note:

- Ensure that gloves are being worn.
- Try to get to the students after they have made their colloid and make sure it looks correct.
- Remind them to take two samples of their colloid at the start so that they have a 'clean' one for Part C, explaining that methylene blue contamination is common and that the experiment is sensitive to this.
- The students tend not to mix the solutions enough when adding methylene blue to the colloid – highlight this and demonstrate if needed. Recommend at least 10 inversions of the vial per mixture.
- Try to find the opportunity talk the students through what they are seeing after they measure the Raman spectrum of the colloid + methylene blue. Highlight the lack of peaks in the colloid, methylene blue stock and the intense spectrum with the very dilute methylene blue and the colloid.
- For Part C, ask the students what their plan is for achieving the minimum level of detection and be prepared to guide them. As a guide, a few μl of 1 × 10⁻⁶ M methylene blue in 2 ml colloid is required. If their blank shows methylene blue peaks check that they are using the clean colloid sample taken at the start. If they are, give them a vial of the spare colloid to use.
- The Raman peak assignments are on display on the wall should anyone ask!

Experiment 3C Marking Scheme (25 marks)

- An Excel spreadsheet can be used to calculate the answers quickly.
- Remember that Excel calculates to many decimal places, so answers may vary slightly.
- If a student miscalculates one part of a question or simply uses the wrong value, remember that they may have calculated the rest correctly using this wrong value *e.g.* density = mass/volume and if they use the wrong volume then the density will be wrong. In such cases please do the following:
 1. Mark the initial mistake as wrong and deduct the mark(s) associated with it.
 2. Manually input their incorrect answer into the marking spreadsheet and proceed to mark with these answers (pointing out to student that you have taken their error into account).
 3. If any further calculation mistakes are made, do not update spreadsheet answers. Mark them wrong and deduct marks associated. The students only get one 'free' mistake!
 4. This method also applies to error calculations.
 5. Above all, please use your discretion and seek advice if necessary.
- Do not use negative marks, once a student has got zero for one part of a question move on to the next part.

Report Quality

If untidy, poorly explained, poorly structured, no aims *etc.* deduct 1. (2)

Results/Calculations

If there are poor practical results, results not clearly presented *e.g.* data not tabulated, no example of each calculation, unclear calculations *etc.* deduct as appropriate. (2)

Q1 – The students are asked to plot their two UV-VIS spectra on the same chart and to rescale the x-axis to range from 350 – 820 nm. The spectra should be labelled to show which has the NaCl added. Typical spectra are shown in Fig. 1 below.

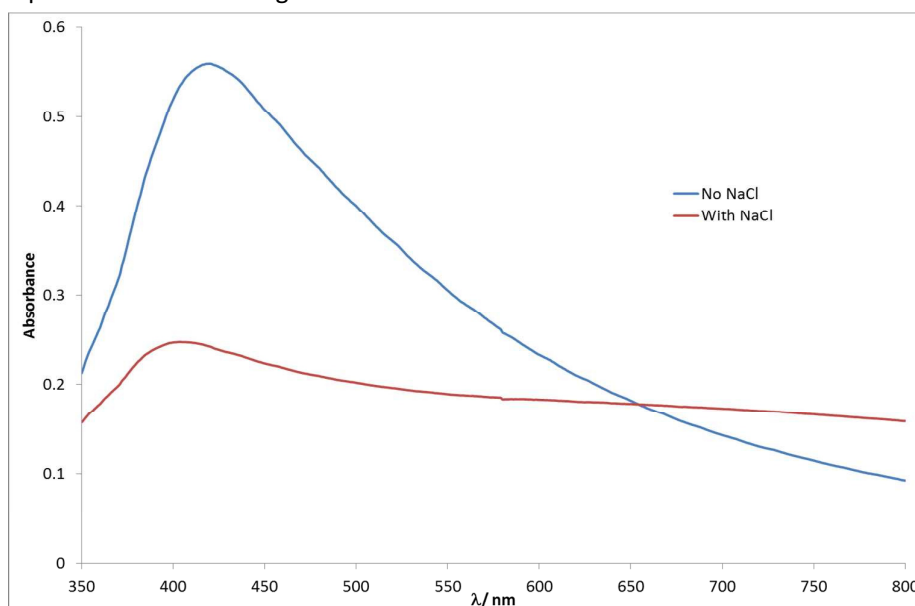


Figure 1 – UV-VIS Spectra

(2 marks for correctly labelled plot)

Q2 – This question asks the students to plot their Raman spectra for the Ag colloid and the methylene blue (MB) stock solutions and to highlight some Raman bands in the MB spectrum. Typical data in Fig. 2 below.

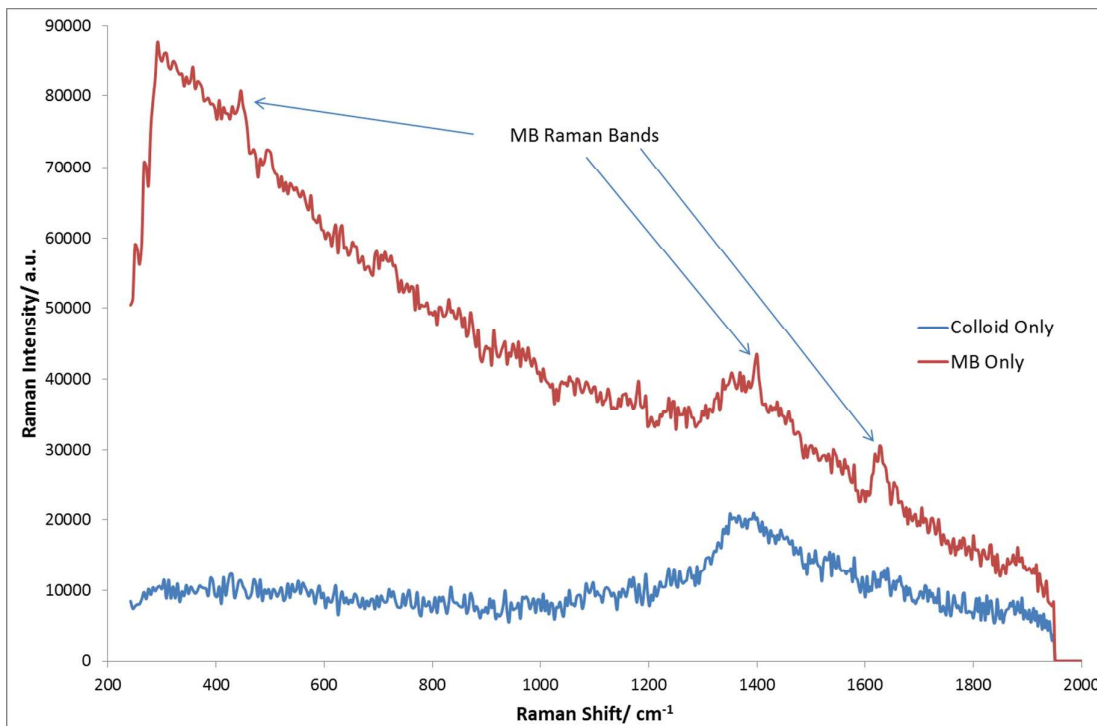


Figure 2 – Raman spectra of Ag colloid and MB stock solution

Please note that sometimes, the large fluorescence background in the MB spectrum is sometimes absent and that the Raman bands are not particularly obvious – credit reasonable attempts to identify peaks.

(2 marks for correctly labelled plot, 1 mark for labelling some peaks)

Q3 – The students are asked to plot the MB stock solution spectrum, their Ag Colloid/10 μ l MB spectrum and their Ag Colloid/10 μ l MB/NaCl spectrum. Typical spectra are shown in Fig. 3 below. The students are asked to comment on the differences between the spectra.

The key features are that the combined colloid/MB spectrum shows a great surface enhanced Raman (SERS) effect caused by the adsorption of the MB molecules onto the surface of the Ag nanoparticles. Virtually nothing is observed in the 'normal' Raman spectrum of the MB stock solution but the SERS spectrum contains many features, even at the much reduced concentration used.

The addition of NaCl causes the SERS signal intensity to increase further.

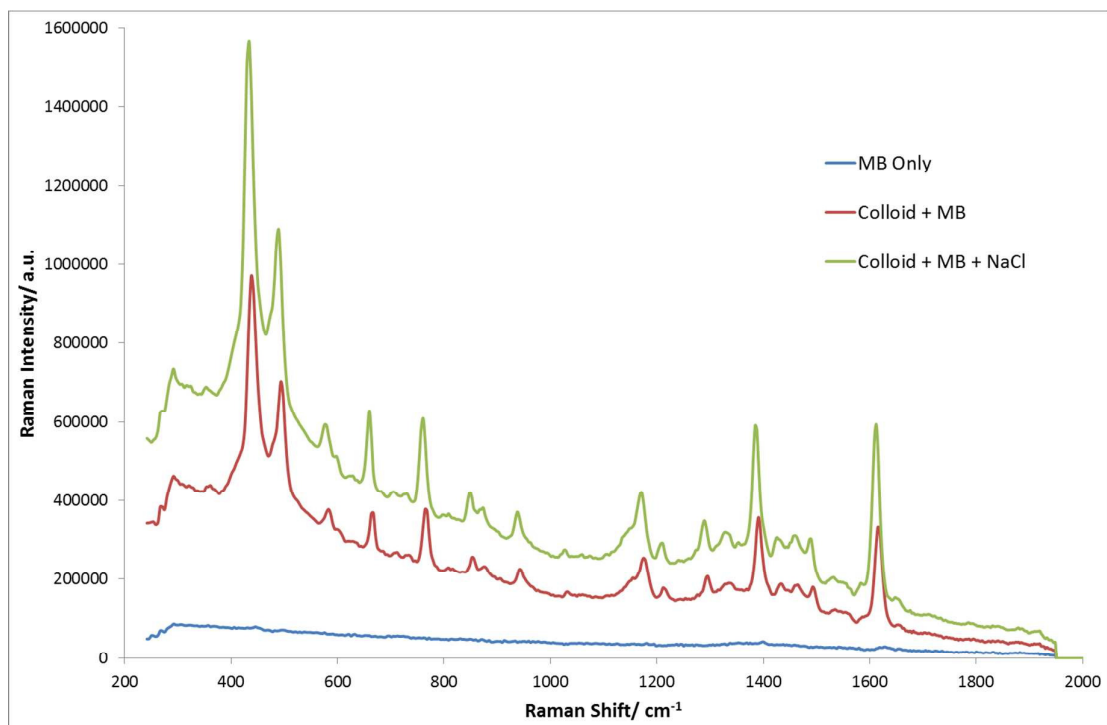


Figure 3 – SERS Spectra

(2 marks for correctly labelled plot, 1 mark for discussion of features)

Q4 – The students are asked to explain why the signal intensity increases on the addition of NaCl. The students are asked to make reference to their UV-VIS data and the laser wavelength used, which was 784 nm. The explanation is that the NaCl causes aggregation of the nanoparticles. The addition of the NaCl causes a general overall reduction and broadening of the UV-VIS absorption feature, but the absorption at the laser wavelength is actually higher when NaCl is added. This indicates that the number of particles interacting with the laser wavelength is higher in the aggregated colloid and thus the SERS enhancement is greater. The students are given all of this information in the script, but they will have to piece it together to get to the answer!

(2 marks for the explanation)

Q5 – The Students are asked to include their tabulated data as below:

MB Volume/ μl	MB Molarity/ mol dm^{-3}	Raman Intensity/ a.u.
2	1.00E-07	190635
4	2.00E-07	335023
6	3.00E-07	445216
8	4.00E-07	633892
10	5.00E-07	820034

Table 1 – Raman Intensity and concentration

They are asked to plot and comment on the data. The plot is shown in Fig. 4, it should be a straight line.

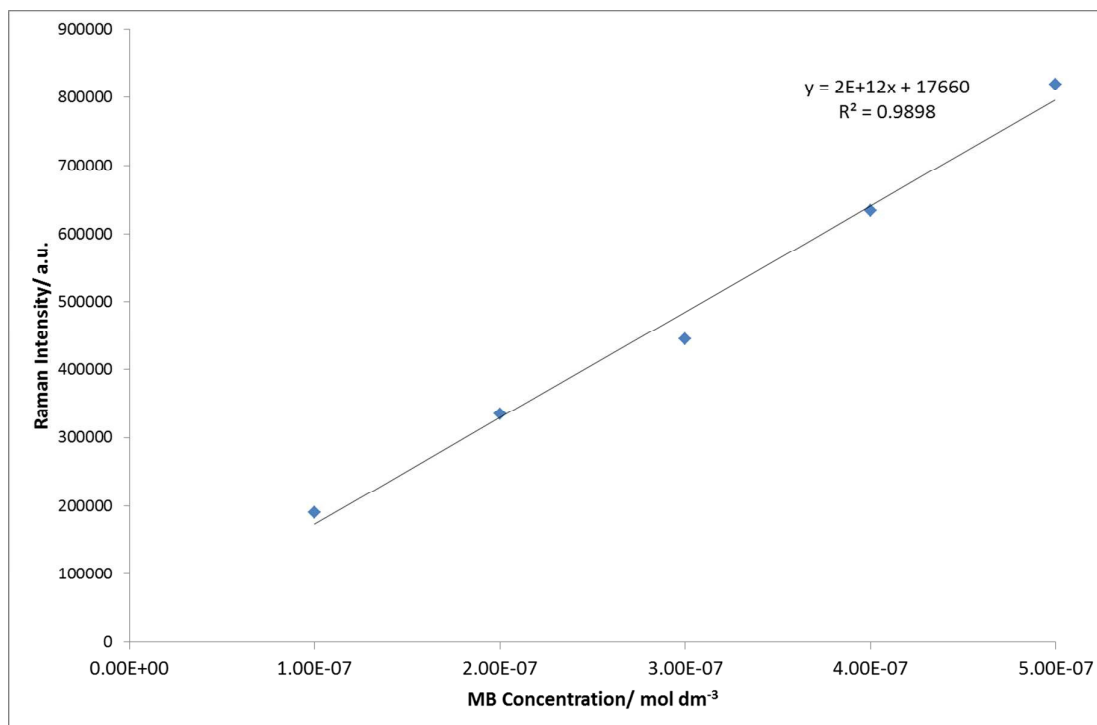


Figure 4 – Raman Intensity vs. Concentration.

(1 mark for table, 2 marks for plot, 1 mark for comment)

Q6 – The students are asked to include their tabulated data as below:

Integration Time/ s	Raman Intensity/ a.u.
1	78113
2	148778
5	372879
8	577631
10	720117
12	821703
15	986437
18	1126670
20	1192454

Table 2 – Raman Intensity and integration time

They are asked to plot these data (Fig. 5) and comment on the plot. The plot should initially be linear, but should flatten out at the higher integration times.

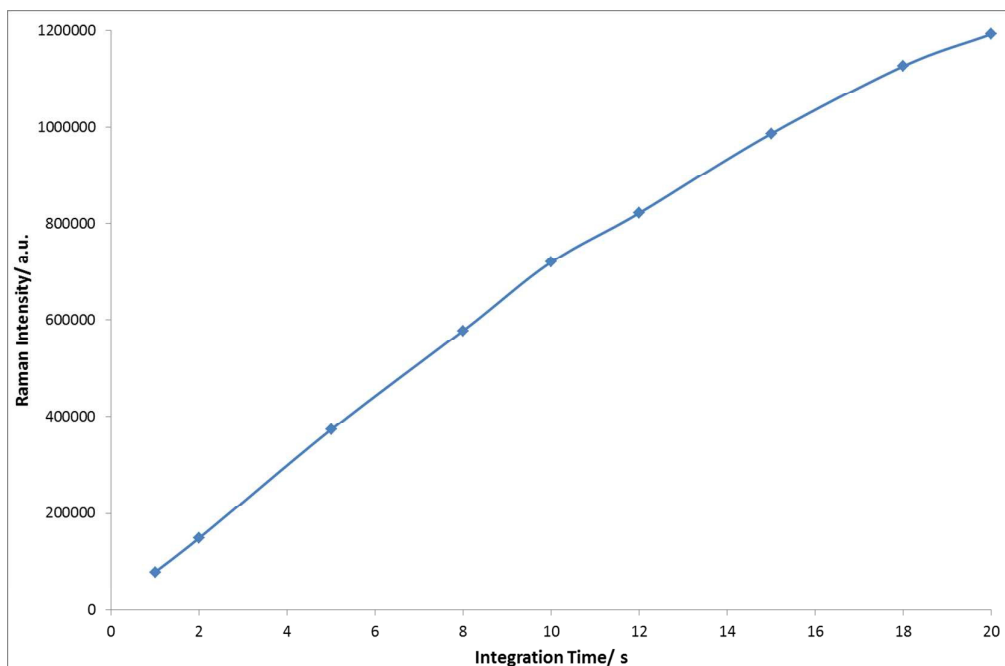


Figure 5 – Raman Intensity vs. Integration time

(1 mark for table, 2 marks for plot, 1 mark for comment)

Q7 – The final part of the experiment is short investigation which asks the students to determine the lowest concentration of MB that they can measure. They are asked to calculate this concentration and show the Raman spectrum. In this example, the lowest *reliable* signal was achieved by adding 2 μl of $1 \times 10^{-6} \text{ mol dm}^{-3}$ MB (10-fold dilution of the stock solution supplied) to 2 cm^3 of the colloid. Amount of MB added = $2 \times 10^{-6} \times 1 \times 10^{-6} = 2 \times 10^{-12} \text{ mol}$. Volume = 2 $\text{cm}^3 = 2 \times 10^{-3} \text{ dm}^3$. MB Concentration = $2 \times 10^{-12} / 2 \times 10^{-3} = 1 \times 10^{-9} \text{ mol dm}^{-3}$. The Raman Spectrum is shown in Fig. 6 below.

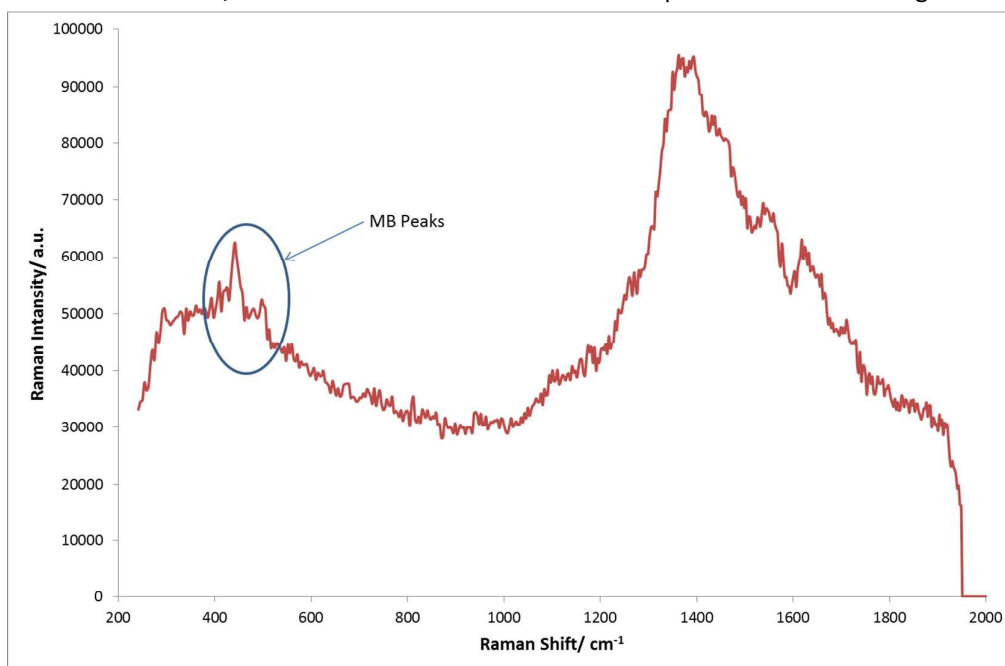


Figure 6 – Raman spectrum of $1 \times 10^{-9} \text{ mol dm}^{-3}$ MB

(2 marks for chart, 1 mark for reasonable estimate of lowest MB level)

DEPARTMENT OF PURE AND APPLIED
CHEMISTRY

PHYSICAL AND APPLIED CHEMISTRY
LABORATORY

**EXPERIMENT 3C – SURFACE ENHANCED
RAMAN SPECTROSCOPY**

RISK ASSESSMENT

EXPERIMENT 3C – SURFACE ENHANCED RAMAN SPECTROSCOPY

Compound	Hazard	Handling	Spillage	Disposal
Sodium Chloride	Irritant	No special instructions	Flush with water	Wash down sink
Sodium Hydroxide	Corrosive Irritant	Wear gloves and wash hands after using	Flush with water	Wash down sink
Silver Nitrate	Toxic Corrosive	Wear gloves	Flush with water	Wash down sink
Hydroxylamine Hydrochloride	Harmful Corrosive	Wear gloves	Flush with water	Wash down sink
Methylene Blue	Harmful	Wear gloves	Flush with water	Wash down sink

EXPERIMENT 3C

SURFACE ENHANCED RAMAN SPECTROSCOPY (SERS)

AIMS

- 1) To gain an understanding of the potential uses of Raman and UV-VIS spectroscopy.
- 2) To gain some experience in preparing nanoscale materials.

THEORY

When a photon interacts with a molecule it can excite it into an electrically polarised virtual energy state. In the *vast* majority of cases, the excited molecule will rapidly revert to its original energy state and a photon of the same energy is re-emitted/scattered. Occasionally, this excited polarisation state will couple with one of the normal modes of vibration of the molecule. When this happens, the re-emitted photon will have a different energy to the original one and this energy difference will correspond to a vibrational transition: this is known as the Raman Effect. Like IR spectroscopy, Raman Spectroscopy gives information on molecular vibrations. Raman active vibrational modes require a change in molecular polarisability whereas IR active vibrational modes involve a change in dipole moment; this means that IR active modes are not necessarily Raman active and *vice versa*. These two techniques can therefore offer complementary information on the vibrational modes of molecules.

Historically, the Raman Effect has had limited applicability as only around 1 in 10^7 photons are scattered in this way. Laser technology has changed this, however, as sources of intense, monochromatic light are now readily available.

Recently, substantial research effort has been devoted to nanoscale materials as their properties can be very different from the everyday, large-scale ones. An example is the colloidal suspensions of metal nanoparticles. When metal particles are sufficiently small, the free electrons at the particle's surface can be collectively excited to oscillate by an incoming photon. This collective oscillation is called a *surface plasmon*, which gives rise to a strong absorption band in the UV-VIS region; these colloidal suspensions are often coloured. The precise frequency of the surface plasmon depends on the size and shape of the metal nanoparticle, so the UV-VIS spectrum of a colloidal suspension will generally show a broad absorption band, with the absorption at each wavelength being proportional to the number of particles having a surface plasmon there. This effect is not observed for large metal particles, as the free electrons cannot be collectively excited by an incoming photon.

If a Raman active molecule is adsorbed on the surface of metal nanoparticle and an incoming photon excites **both** the surface plasmon **and** an active vibrational mode then the Raman Effect can be enhanced by a factor of 10^9 times. This effect is called 'Surface Enhanced Raman Spectroscopy' (SERS) and its discovery has allowed single-molecule detection to be achieved. It is important to note that unlike IR spectroscopy, Raman spectroscopy is not a form absorption spectroscopy. Light of a **single** wavelength is used to excite all of the Raman active vibrational modes, so the Raman scattered, or 'shifted', photons detected will come from all of the allowed vibrational modes.

EXPERIMENTAL

Synthesis of the Ag Nanoparticle Colloidal Suspension

The chemical reaction scheme is complex and unresolved, but the hydroxylamine initially reduces the silver ions to form colloidal nanoparticles. The excess hydroxylamine then adsorbs onto the surface of the nanoparticles, both capping their size and preventing aggregation - the capped particles will carry the same electrical charge and hence repel each other. The suspensions remain stable for many months.

In common with many experiments involving nanoscale materials, this experiment involves handling small volumes of dilute solutions. It is very important to avoid unwanted contamination. To help this:

- Do not pipette the stock solutions directly from the bottles – pour the approximate amounts required into small beakers and pipette from these.
 - Do not return excess solutions to the bottles, dispose of these down the sink.
 - It is recommended that you give all glassware a rinse with deionised water before use. Tap water contains chlorine which will precipitate **ALL** of the dissolved Ag^+ as AgCl before anything else happens...you don't know how thorough the people doing the experiment before you were at rinsing things, especially in their rush to leave the lab at the end of the day!
 - Having said that, please help you fellow students by giving all glassware a final rinse with deionised water before you leave.
1. Pipette 10 cm^3 of the NaOH stock solution into the 100 cm^3 volumetric flask.
 2. Using the glass weighing boat provided, weigh out $(0.100 \pm 0.005) \text{ g}$ of hydroxylamine hydrochloride. **WEAR GLOVES.**
 3. Carefully rinse the hydroxylamine directly into the NaOH using deionised water and make the solution up to the mark. Invert the flask several times to fully dissolve the salt.
 4. Pipette 50 cm^3 of the AgNO_3 stock solution into a 100 cm^3 conical flask, add a magnetic stirrer bar and set the stirring rate to be slow and steady.
 5. Pipette 5 cm^3 of your Hydroxylamine/ NaOH solution into the AgNO_3 and continue the stirring for the rest of today's experiment. The colour should initially change to grey and then become milky yellow/brown – ask if unsure, the success of the experiment depends on the colloid.

UV-VIS

1. Open the SpectraSuite software from the desktop: you should see the screen shown in Fig 1.
2. Close the 'Graph (A)' tab as shown.

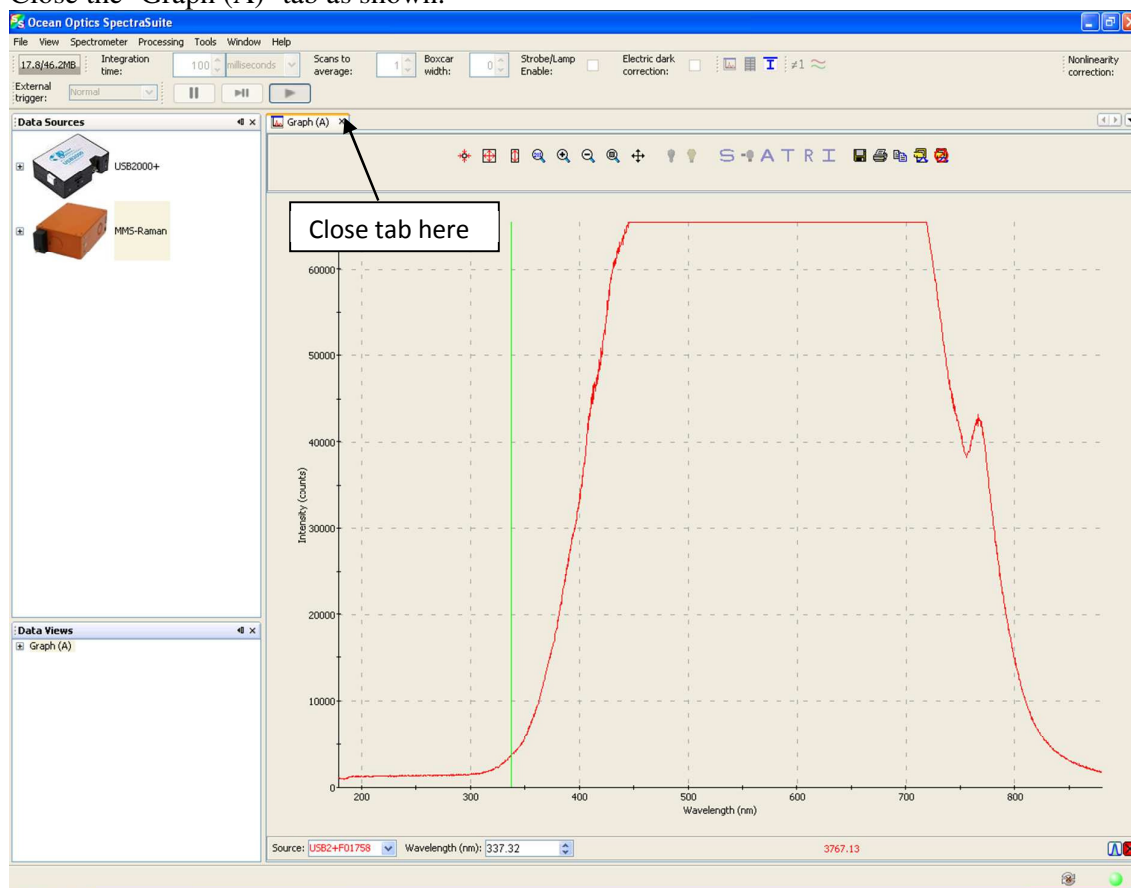




Figure 1 – SpectraSuite Screen Shot

3. From the 'File' menu select 'New Absorbance Measurement'.
4. Ensure that the USB2000+ instrument is highlighted then click 'Next'.
5. Set 'Integration time' to 10 ms, 'Scans to average' to 20, 'Boxcar width' to 20 and click 'Next'.
6. Fill the cuvette to the black line with deionised water, place it into the cell holder, press the yellow light bulb icon to record the baseline spectrum and click 'Next'.
7. Remove the cuvette and replace it with the darkened one. Press the grey light bulb icon to measure a dark spectrum and then click 'Finish'.
8. Press the  button and under X-Axis Range set Minimum to 350 nm and Maximum to 820 nm. Under the Y-Axis Range set Minimum to 0.0 and Maximum to 1.8. Click 'Apply' then 'Close'.
9. Attach a new 1 cm³ tip to the large micropipette and ensure that the pipette is set to read '100', this sets the pipette to deliver 1 cm³. Pipette 1 cm³ of your prepared Ag colloid into the cuvette of deionised water. To do this, slowly depress the plunger down until it *first* stops, insert the tip into the suspension and **slowly** raise the plunger to fill the tip. Discharge the suspension into the water by **FULLY** depressing the plunger. Invert several times to mix.
10. Place the cuvette into the spectrometer; the absorbance spectrum should appear.

11. Click  to copy the data. Open Excel and paste the data into a spreadsheet under the heading UV-VIS NO NaCl.
12. Add 2 drops of NaCl solution to the cuvette, mix and replace in the spectrometer. Wait for 1 minute and copy and paste these data into your spreadsheet under the heading UV-VIS AND NaCl.
13. Save your spreadsheet, remove and rinse the cuvette and switch off the UV-VIS lamp.
14. Close the 'Graph (B)' tab on the SpectraSuite window.

Raman: Part A – Concentration Dependence

1. Turn on the MMS-RAMAN Spectrometer by turning the key on the front clockwise. Make sure the red light on the front comes on (see Fig. 2).

Indication that the laser is on, key in ON position



Figure 2 – Photograph of MMS-RAMAN Spectrometer

2. Open Excel and set up a spreadsheet with the following headings:

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	Colloid		MB				10 µl MB			10 µl MB + NaCl			Most Dilute Spectrum	
2	Raman Shift/cm ⁻¹	Intensity/a.u.	Raman Shift/cm ⁻¹	Intensity/a.u.	Raman Shift/cm ⁻¹	Intensity/a.u.	Raman Shift/cm ⁻¹	Intensity/a.u.	Raman Shift/cm ⁻¹	Intensity/a.u.	Raman Shift/cm ⁻¹	Intensity/a.u.	Raman Shift/cm ⁻¹	Intensity/ a.u.
3														
4														

Figure 3 – Excel Spreadsheet

3. In SpectraSuite, click the picture of the MMS-Raman instrument to highlight it as shown in Fig 4.

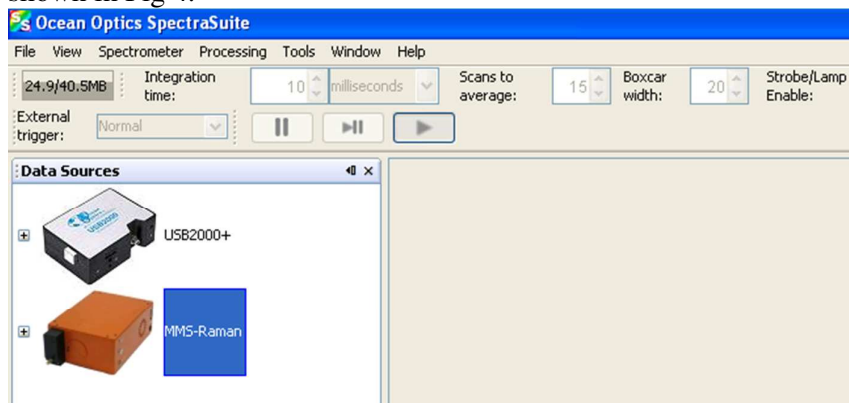


Figure 4 – Screen Shot

4. From the 'File' menu, select 'New Spectrum Graph'. Set the Integration time to 5 s (select 'seconds' from the dropdown menu before setting the number '5'). See Fig. 5.

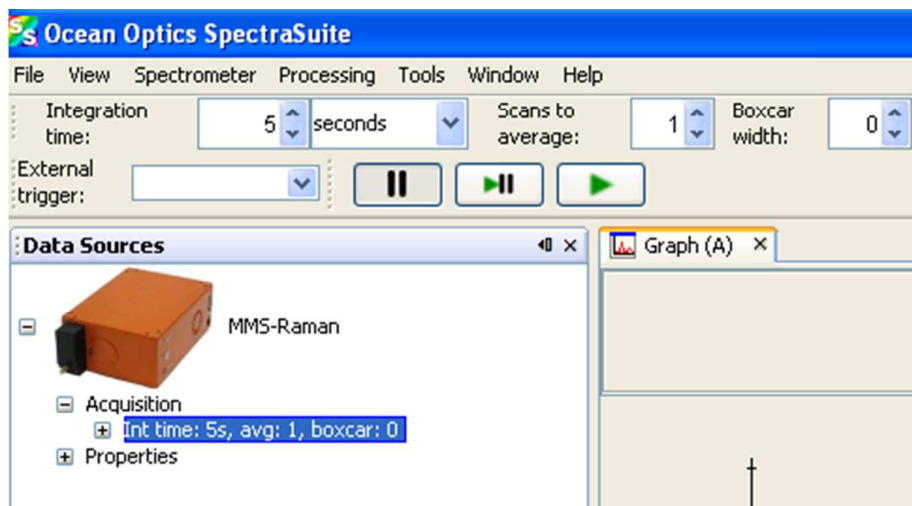


Figure 5 – SpectraSuite Interface

5. From the 'Processing' menu, select 'Processing Mode' then 'Raman Shifts'.
6. Pipette 2 cm³ of your colloid into each of two 2 cm³ glass vials. **Place one aside for use in Part C.**
7. Lift off the black metal cover on the spectrometer. This will automatically turn off the laser (the red light on top turns green). Place your sample vial into the sample bay and replace the cover. The light on top should now again be red, indicating that the laser is on. This is all illustrated in Fig. 6.

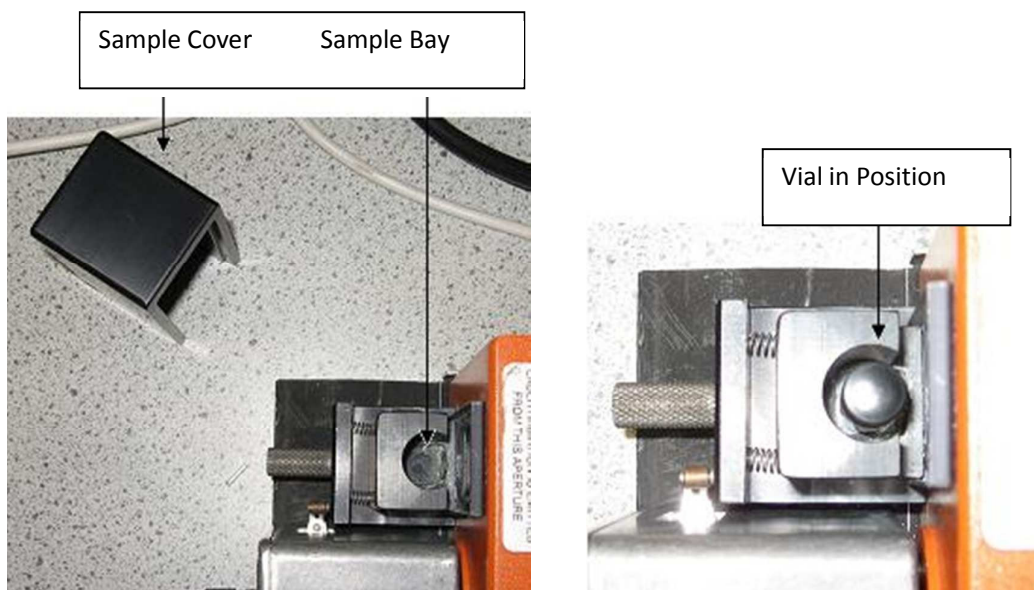











Figure 6 – Sample Placement

8. Click the  button next to the picture of the Spectrometer on the left of the screen to expand the menu. Now click the  next to 'Acquisition'. Click to highlight the line that says 'Int time: 5s, avg: 1, boxcar: 0'. It should turn blue as in Fig. 5.
9. Measure the spectrum by clicking the  button.

10. Once the spectrum has been measured, click the  button to resize it to full screen and then click  to copy the data to the clipboard. Bring up Excel and paste the data into the spreadsheet. **Save your spreadsheet now and after every change you make.**
11. Retain your vial of Ag colloid for later.
12. Using a clean, glass Pasteur pipette, fill a new vial with the 1×10^{-4} M methylene blue stock solution and measure its Raman spectrum. You will have to click to highlight the 'Int time: 5s, avg: 1, boxcar: 0' line every time you want to run a spectrum.
13. Copy and paste these data into your Excel spreadsheet.
14. Place a new tip onto the smaller micropipette and set this to read '020'. This delivers $2 \mu\text{l}$ ($2.0 \times 10^{-3} \text{ cm}^3$) of solution.
15. Pipette $2 \mu\text{l}$ of the methylene blue stock solution into your vial of Ag colloid and invert several times to thoroughly mix. (NOTE: When pipetting $2 \mu\text{l}$, the first stop of the pipette plunger will occur very quickly, it is therefore important to do this slowly. Consult a demonstrator if you are unsure.)
16. Measure the Raman spectrum of your sample, clicking  to resize your spectrum. *Do not copy and paste the spectrum at this stage.*
17. Click on the spectrum and then click on the integration button  at the bottom right of the screen.
18. Double click first main peak at *ca.* 430 nm to move the green cursor there and then use   buttons to position the cursor exactly on the peak. Read off the Raman Intensity as shown in Fig. 7 and write this in Table 1.

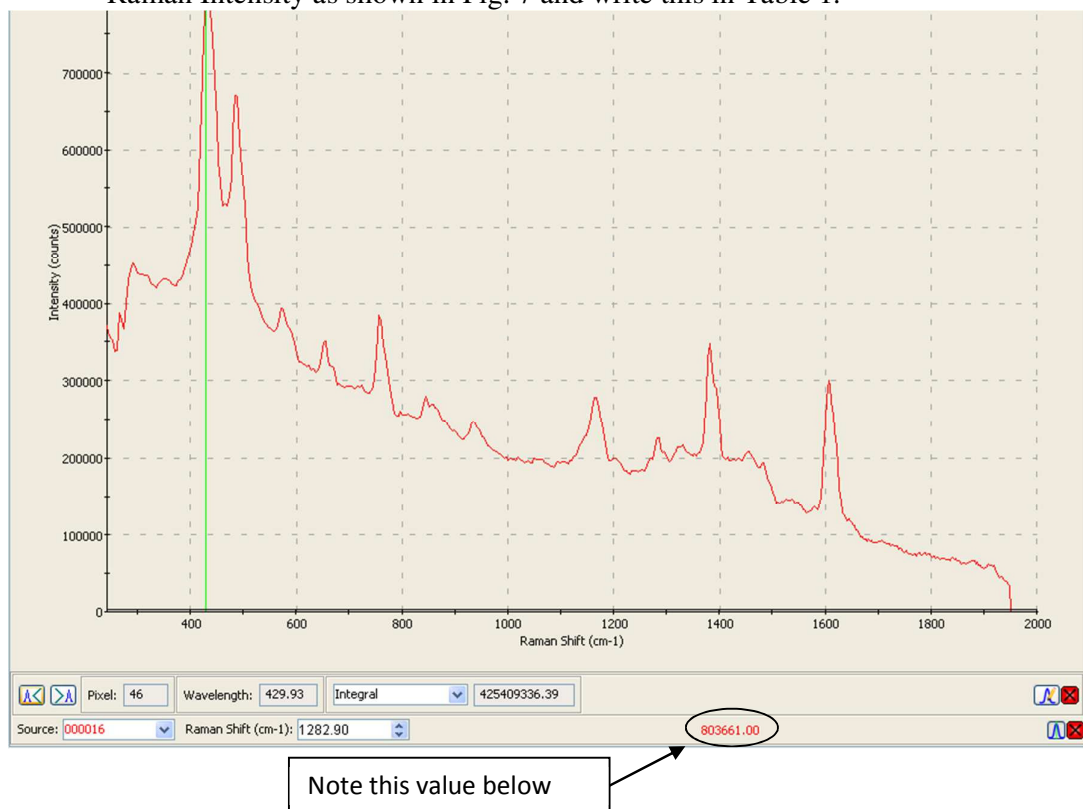


Figure 7 – Raman Peak Evaluation

Volume Added/ μl	Concentration/ mol dm^{-3}	Raman Intensity/ a.u.
2.0	1.0×10^{-7}	
4.0	2.0×10^{-7}	
6.0	3.0×10^{-7}	
8.0	4.0×10^{-7}	
10.0	5.0×10^{-7}	

Table 1 – Raman Intensity & Methylene Blue Concentration

- Repeat steps 15 – 18 a further 4 times until you have added a total of 10 μl of methylene blue to your sample. **Only copy and paste your 10 μl sample data into Excel.**
- Add one drop of NaCl solution to the vial, mix well and re-measure the Raman Spectrum after ~ 1 minute. Resize, and copy and paste your data. NaCl causes aggregation of the colloidal particles.

Raman: Part B – Integration Time Dependence

- Pipette 2 cm^3 of your colloid into a new sample vial and add 4 μl of methylene blue.
- Change the Integration time to 1 s and record the Raman Spectrum as before, noting the integration value of the large peak in the table below. There is no need to copy and paste your spectral data.
- Complete Table 2 for the given integration times. Do not remove the sample from the spectrometer, just change the integration time.

Integration Time/ s	Raman Intensity/ a.u.
1	
2	
5	
8	
10	
12	
15	
18	
20	

Table 2 – Raman Intensity & Integration Time

Raman: Part C – Investigation

What is the lowest methylene blue concentration that you can reliably measure using this technique? Here is a guide on how to proceed.

- Dilute the methylene blue stock solution by e.g. pipetting 0.5 or 1 cm^3 into a 100 cm^3 volumetric flask and making up to the mark.
- Place the vial of colloid that you set aside in Part A into the spectrometer, select your integration time and record a blank spectrum (if it's not blank, consult a demonstrator).
- Add 1 μl of your diluted methylene blue solution to the colloid. Do you see a *definite* methylene blue signal? Add further 1 μl aliquots and measure a spectrum after each

addition until you see the first definite signal. What else could you add to the colloid to confirm that you were measuring the SERS spectrum? With this in mind, could you improve the detection level further?

- **Copy and paste your most dilute, yet positive, response into your spreadsheet and explain what you did in your report.**

At the end of the experiment, rinse all glassware and cells with deionised water – please help us to avoid contaminating the glassware (particularly with NaCl) for the next students.

DATA RETRIEVAL

You should have your UV-VIS and Raman data on spreadsheets. In your UV-VIS data, the first column is λ (nm) and the second is absorbance. Each Raman spectrum has two columns of data. The first column is the Raman Shift (cm^{-1}) and the next is the Raman Intensity (a.u.). Your spectra will simply be plots of Raman Intensity vs. Raman Shift.

QUESTIONS AND ANALYSIS

- Q1** On the same chart, plot your UV-VIS spectra for the diluted Ag colloid both with and without added NaCl. **You should plot absorbance vs. wavelength in the 350 nm - 820 nm range only.**
- Q2** On a new chart, plot the Raman spectra of both the Ag colloid and the methylene blue stock solution. These are the first two spectra that you recorded. **Indicate some of the (weak) methylene blue Raman bands on your spectrum.**
- Q3** On a new chart, plot the Raman spectra of the methylene blue stock solution, the 10 μl methylene blue/Ag colloid and the 10 μl methylene blue/Ag colloid/NaCl. Comment on the differences between the three spectra.
- Q4** Given that the laser wavelength is 784 nm, explain the difference in the Raman scattering intensity when the NaCl is added. You should refer to your UV-VIS spectra.
- Q5** Include Table 1 in your report and plot Raman Intensity vs. Concentration – comment on these data.
- Q6** Include Table 2 in your report and plot Raman Intensity vs. Integration Time – comment on these data.
- Q7** What is the lowest methylene blue concentration that you were able to detect (in mol dm^{-3})? Remember that the volume of colloid added was 2 cm^3 and this needs to be accounted for when calculating your final concentration. Show your calculation and your Raman spectrum.

