

Raman Spectroscopy

Phys. Chem. Lab course, UNIST, 2023 -- Thomas Schultz

Course content

In this course, you will set up a spectroscopic experiment for the measurement of vibrational Raman spectra, you will measure spectra for unknown compounds, and you will identify the compounds based on your measured spectra. Make sure you understand the practical and theoretical aspects of Raman spectroscopy before entering the lab.

The learning goals of this lab course are:

- (1) Understand fundamental concepts of spectroscopy.
- (2) Build and a spectroscopic instrument.
- (3) Measure, analyze and interpret spectroscopic data.
- (4) Write a scientific report.

Introduction

When electromagnetic radiation (light) interacts with matter, it may be absorbed, reflected, or scattered. Spectroscopic experiments investigate this interaction and almost all our understanding about molecular properties and structure originated from spectroscopic experiments. Modern spectroscopy is an important tool to identify known compounds or analyze properties of unknown compounds.

Raman spectroscopy is based on the inelastic scattering of light in molecules (see Fig. 1). In a scattering process, light photons can change their direction and/or impulse. This is different from absorption spectroscopy, where a photon is either absorbed or not absorbed. In an inelastic scattering process, energy is exchanged between the scattered particles. Because molecules can only absorb or emit discrete (quantized) amounts of energy, the scattered *Raman light* is different for each molecular species and therefore helps to identify and characterize molecular samples. Raman spectroscopy was named after C. V. Raman, who discovered the Raman effect in 1928 [1].

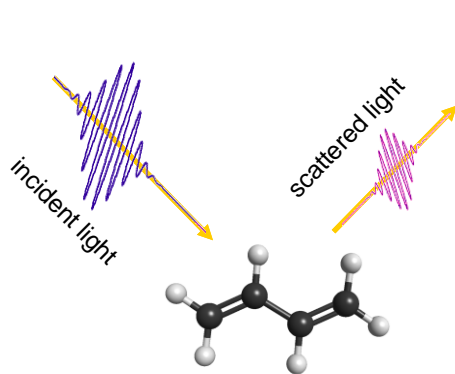


Fig 1: Inelastic scattering: The incident and scattered photons have different momenta and energy.

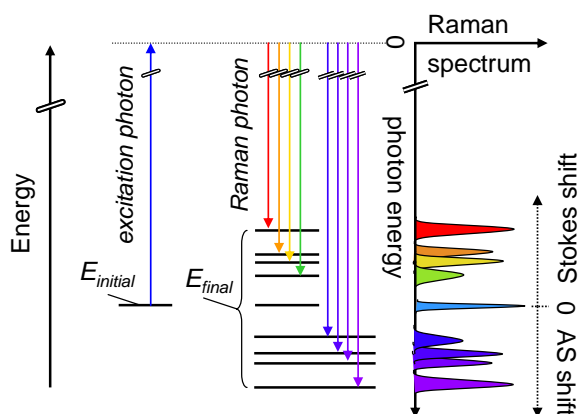


Fig 2: Energy scheme for Raman spectroscopy. An excitation photon (blue) is scattered and produces Stokes photons of lower energy (green-red) or anti-Stokes photons of higher energy (blue-purple).

The shift between the incoming and outgoing photon energy is called *Raman shift*. The Raman process may deposit energy in the molecule (Stokes transition, see Fig. 2)

or remove energy from the molecule (anti-Stokes transition). The Raman shift (difference between incident and Raman photon energy) must be calculated after measuring the Raman light wavelength. This shift is often given in the pseudo-energy unit of wavenumbers $\Delta\omega$ (in units of cm^{-1}): $\Delta\omega = (1/\lambda_{\text{incident}} - 1/\lambda_{\text{Raman}})$. Measured Raman shifts directly correspond to relative molecular energy levels.

Raman spectroscopy is a type of nonlinear spectroscopy and relies on a second order interaction with light: The light electromagnetic field E_{light} polarizes a molecule with polarizability α and induces a dipole $\mu_{\text{ind}} = \alpha \cdot E_{\text{light}}$. The interaction of the induced dipole with the light field can then lead to a Raman transition. The probability P for a Raman scattering event and the corresponding strength of a Raman transition is therefore proportional to the second power of the light electric field intensity $P \propto \mu_{\text{ind}} \cdot E_{\text{light}} = \alpha \cdot (E_{\text{light}})^2$. This is different from ordinary absorption or emission spectroscopy (“linear spectroscopy”), where the light field interacts with an existing dipole μ_0 and the signal power is proportional to the light intensity ($P \propto \mu_0 \cdot E_{\text{light}}$).

Because the signal power depends on the $(E_{\text{light}})^2$ and the light electric fields are generally very small, the intensity of Raman signals is very small. A high excitation light intensity is required to generate any detectable Raman signal. Lasers can be focussed to very small spot sizes, greatly increasing the intensity of the light beam in a small spot. This greatly facilitates the experimental observation of Raman signals.

Vibrational Raman Spectroscopy

In vibrational Raman spectroscopy, the energy difference between incoming and outgoing photon corresponds to exactly one (or multiple) quantum of vibrational energy (see Fig. 3). Because vibrational modes are harmonic and have evenly spaced energy levels between neighbouring quantum numbers v , the signals for all transitions $v \rightarrow v+1$ coincide. We therefore expect only one Raman band for each vibrational mode.

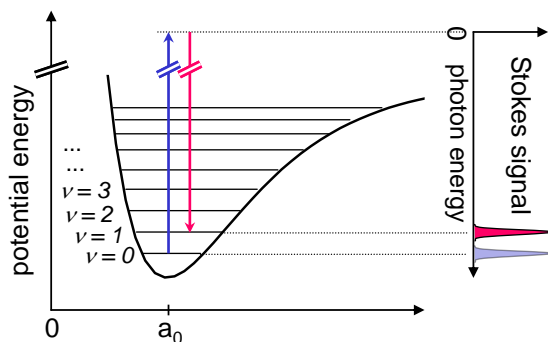


Fig. 3: Energy scheme for vibrational Raman spectroscopy: The photon energy difference corresponds to a vibrational energy quantum.

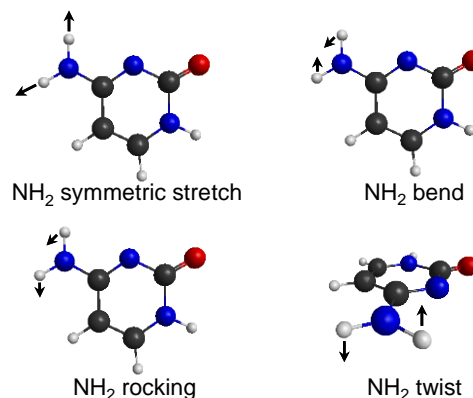


Fig. 4: Some vibrational modes of Cytosine

A non-linear molecule has $3N-6$ vibrational degrees of freedom. Not all vibrational modes are Raman active and we cannot expect to see Raman signals for every molecular vibration. Only vibrations along molecular coordinates that affect the molecular polarizability α can be observed (Fig. 5). Raman spectroscopy and IR absorption spectroscopy are complementary: Raman spectroscopy allows to observe most IR inactive vibrational modes.

The vibrational frequencies observed in IR or Raman spectra are characteristic for the chemical bonds involved in the vibrational motion. This allows the straightforward characterization of *functional groups* in the observed molecules. An IR correlation table is attached to the end of this course guideline and can help you recognize and assign observed vibrational signals. Refer to references [2,3] for more information about IR spectroscopy and spectrum interpretation.

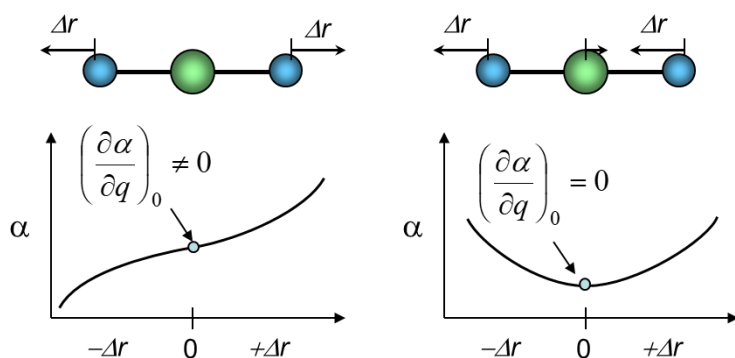


Fig. 5: Example for a Raman active vibration (left) that affects the molecular polarizability α , and a Raman inactive vibration (right).

At room temperature, most molecules are in their vibrational ground state and the anti-Stokes process is very weak. The Stokes photons in the Stokes signal contain less energy than the incoming photons (cf. Fig. 2). This energy difference can be measured as a wavelength difference between excitation- and Stokes- light using a spectrometer. To avoid an overlap of the excitation and Raman wavelength, we require a very monochromatic source of excitation light.

Raman Spectroscopy Set-Up

To acquire Raman spectra, we need a light source for excitation light, an optical system to generate Raman signal in a sample, an optical system to collect the Raman light, and a spectrometer to analyze the Raman light.

We use a laser modules with approx. 532 nm (green) and 638 nm (red) wavelength as excitation light sources. Lasers are monochromatic and can be focussed to very high intensities and are therefore best suited for this type of experiment.

In a first spectrometer set-up (Fig. 6), the laser is focussed by a microscope objective into a sample cuvette. The resulting focus is very small and has the required high intensity to generate detectable amounts of Raman signal.

The Raman signal is collected in a back-scattering geometry by the microscope objective mentioned above. A beam splitter (Semrock FF535-

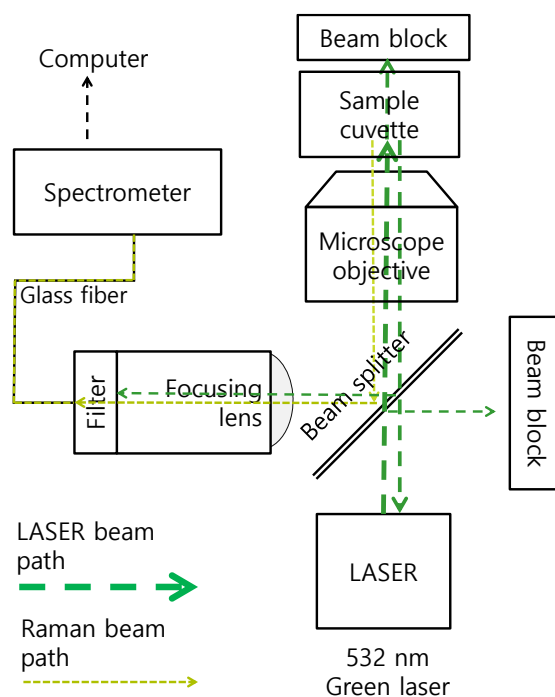


Fig. 6: Experimental set-up for Raman spectroscopy. Laser light is focussed into a sample with a microscope objective. The Raman signal is collimated by the objective and guided into a fiber spectrometer.

SDi01, reflectivity >95% at >539 nm, transmittivity >90% at 532 nm) reflects the Raman signal towards the spectrometer but transmits most of the scattered 532 nm laser light.

The Raman light is focussed with a lens onto the entrance of a fiber spectrometer. An additional cut-off filter (Omega Optical 538ALP) removes residual 532 nm laser light, but transmits the Raman signal with wavelength >539 nm. The spectrometer (Brolight BIM-6 or Thorlabs CCS100) analyzes the wavelength-dependent spectrum and transmits the data onto a computer for further analysis.

A second Raman spectrometer set-up (Fig. 7) uses a red 538 nm laser module. The optical set-up is based on a different beamsplitter (Semrock FF649-Di01, reflectivity >98% at <642 nm, transmittivity >90% at >654 nm) and filter (Omega Optical 648 ALP), but is functionally equivalent to the spectrometer described above.

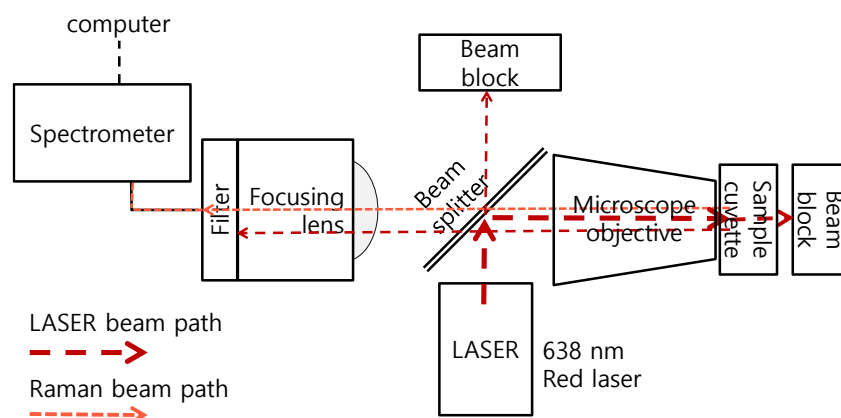


Fig. 7: Experimental set-up for Raman spectroscopy. Laser light is focussed into a sample with a microscope objective. The Raman signal is collimated by the objective and guided into a fiber spectrometer.

You will assemble one of these Raman spectrometers in the laboratory. To succeed, you must understand the measurement principle of Raman spectroscopy and you must understand the role of every optical element in the spectrometer set-up. Please ask questions if anything remained unclear.

Safety Considerations

In this lab course, you will work with class 3B laser modules that can cause lasting damage to your eyes. You need to follow several safety rules to protect yourselves and your colleagues:

- (1) Never look directly into the laser beam.
- (2) Keep all laser beams parallel to the laser table and at a height well-below eye-level. Keep the laser beam within the pre-defined alignment boundaries and ensure that every laser reflection is caught by a beam-block. Never bend down so that your eyes are at a height with the laser beam.
- (3) Only adjust the laser direction if you and exposed colleagues wear laser goggles.
- (4) Remove watches, jewellery, and other objects that could reflect the laser beam.

Spectroscopic Experiment

The Raman spectroscopy set-up will be used to measure Raman spectra of common organic chemicals. Each student will measure the spectrum of a different unknown compound and assign characteristic IR frequencies in the acquired spectrum. The compound should then be assigned based on identified chemical groups and by comparison to literature spectra or calculated data. To help your assignment, you can find an IR correlation table for common organic groups in Appendix 1 or at the CRASY website: <http://crasy.org/WP/archives/1080-08-2016-xk3>.

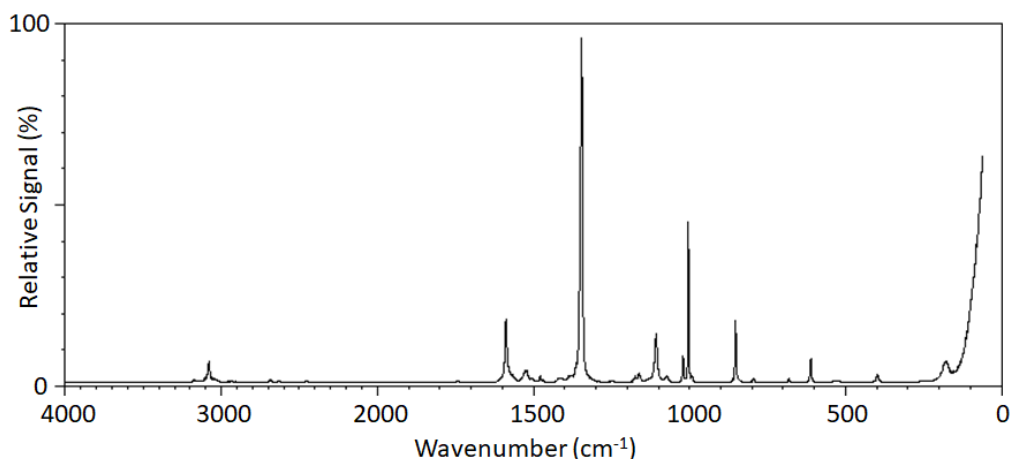


Fig. 8: Vibrational Raman spectrum for nitrobenzene. Note the characteristic aromatic C-H stretch ($>3000\text{ cm}^{-1}$) and N=O stretch ($1500\text{-}1600\text{ cm}^{-1}$ and $1345\text{-}1385\text{ cm}^{-1}$) frequencies. Spectrum reproduced from reference [4].

Writing a Report

You must submit a report describing your experiment and the experimental results in < 5 pages of English text and figures. I summarized some tips on writing and links to further information in Appendix 2 and at: <http://crasy.org/WP/archives/1087-08-2016-xk3>. Please use the linked ACS article template for your report, set the line spacing to double-spaced, and insert page numbers if they are absent. For style and structure, please follow the guidelines of the American Chemical or American Physical Society and maybe refer to the cheat sheet for writing a scientific manuscript.

Test your understanding about Raman spectroscopy with the following comprehension questions:

- (1) Explain the difference between light scattering and light absorption.
- (2) Why is the anti-Stokes transition much weaker than the Stokes transition?
- (3) What is monochromatic light and why is it required for Raman spectroscopy?
- (4) Give an example for a Raman-active and -inactive mode.
- (5) What is the function of the beam splitter and the filter in the Raman set-up?
- (6) Why is the wavenumber a pseudo-energy unit? How could you convert this unit into a true energy unit? Give an example.

References

- [1] Foil A. Miller and George B. Kauffman, "C. V. Raman and the discovery of the Raman effect", *Journal of Chemical Education* **1989** 66 (10), 795, DOI: [10.1021/ed066p795](https://doi.org/10.1021/ed066p795).
- [2] Infrared: Interpretation, <https://chem.libretexts.org/@go/page/1846> (accessed Oct 18, 2023).
- [3] James Ashenhurst, "Infrared Spectroscopy: A Quick Primer On Interpreting Spectra", www.masterorganicchemistry.com/2016/11/23/quick_analysis_of_ir_spectra (accessed Oct 18, 2023).
- [4] K. Tanabe and J. Hiraishi, Spectral Database for Organic Compounds (SDBS) at the National Institute of Advanced Industrial Science and Technology, sdfs.db.aist.go.jp (accessed Aug 30, 2016).

IR Correlation Table

Characteristic IR band positions for important functional groups. IR absorption band intensities (\neq Raman intensities) are given as: s - strong, m - medium, wk - weak, var - variable.

Functional Group	Band position (cm ⁻¹)	Band Intensity	Band Assignment
Alkanes	2850 - 3000	s	CH ₃ , CH ₂ , CH stretch (2 or 3 bands)
	1350 - 1470	m	CH ₂ , CH ₃ deformation
	1370 - 1390	m	CH ₃ deformation
	720 - 725	wk	CH ₂ rocking
Alkenes	3000 - 3100	m	=C-H stretch (usually sharp)
	1630 - 1690	var	C=C stretch
	880 - 995	s	=C-H, =CH ₂ out-of-plane bend
	800 - 840	med	R ₂ CCHR o-o-p bend
	675 - 730	med	cis-RCH=CHR o-o-p bend
	1900 - 2000	s	C=C=C asymmetric stretch
Arenes	3030	var	C-H str. (may be several bands)
	1600 & 1500 & 1450	m-wk	C=C str. (in ring, 2 or 3 bands)
	690 - 900	s-m	C-H bend, ring puckering
Alkynes	3300	s	C-H str. (usually sharp)
	2100 - 2250	var	C≡C (symmetry reduces intensity)
	600 - 700	s	C-H deformation
Alcohols	3580 - 3650	var	O-H str. (free, usually sharp)
	3200 - 3550	var	O-H str. (H-bonded, usually broad)
	1330 - 1430	m	O-H bend (in-plane)
	970 - 1250	s	C-O stretch
	650 - 770	var-wk	O-H bend (out-of-plane)
Amines	3400 - 3500 (dil. soln.)	wk	N-H str. (prim.-amines), 2 bands
	3300 - 3400 (dil. soln.)	wk	N-H str. (sec.-amines)
	1550 - 1650	m-s	NH ₂ bend
	1000 - 1250	m	C-N stretch
	660 - 900	var	N-H wag (shifts when H-bonded)
Aldehydes, Ketones	2690 - 2840 (2 bands)	m	C-H str. (aldehyde C-H)
	1670 - 1820	s	C=O stretch
	1720 - 1740		C=O str. (saturated aldehyde)
	1710 - 1720		C=O str. (saturated ketone)
	1680 - 1700		C=O str. (aryl ketone)
	1665 - 1685		C=O str. (α , β -unsaturated)
	1350 - 1360	s	α -CH ₃ bend
	1400 - 1450	s	α -CH ₂ bend
	1100	m	C-C-C bend
Carboxylic Acids & Derivatives	2500 - 3300 (acids)	s	O-H str. (H-bonded, very broad)
	1705 - 1720 (acids)	s	C=O str. H-bonded
	1210 - 1320 (acids)	m-s	O-C str. (sometimes 2-peaks)
	1395 - 1440	m	COH bend
	1785 - 1815	s	C=O str. (acyl halides)
	1750 & 1820	s	C=O str. (anhydrides, 2 bands)
	1040 - 1100	s	O-C str. (anhydrides)
	1735 - 1750	s	C=O str. (esters)
	1000 - 1300	s	O-C str. (esters, 2 bands)
	1630 - 1695	m	C=O str. (amide band I)
	1590 - 1650 & 1500 - 1560	m	N-H bend (amide band II & amide band III)
	Ethers	1275 - 1020	s-var
1020 - 1075			Ar-O-C-

	1070 - 1150		-C-O-C-
	1200 - 1275		=C-O-C-
Nitriles	2240 - 2260	m	C≡N str. (sharp)
Imine, oxime	1692 - 1640	m	C=N str.
Nitro compounds	1515-1560 1345-1385	s	N-O str. (two bands)
Isocyanates, Diimides, Azides, Ketenes	2100 - 2270	m	-N=C=O, -N=C=S stretch -N=C=N- stretch -N ₃ stretch C=C=O stretch
Halogens	1400-1000	s	C-F
	850-550	s	C-Cl
	690-515	s	C-Br
	600-500	s	C-I

For more extensive IR correlation tables see:

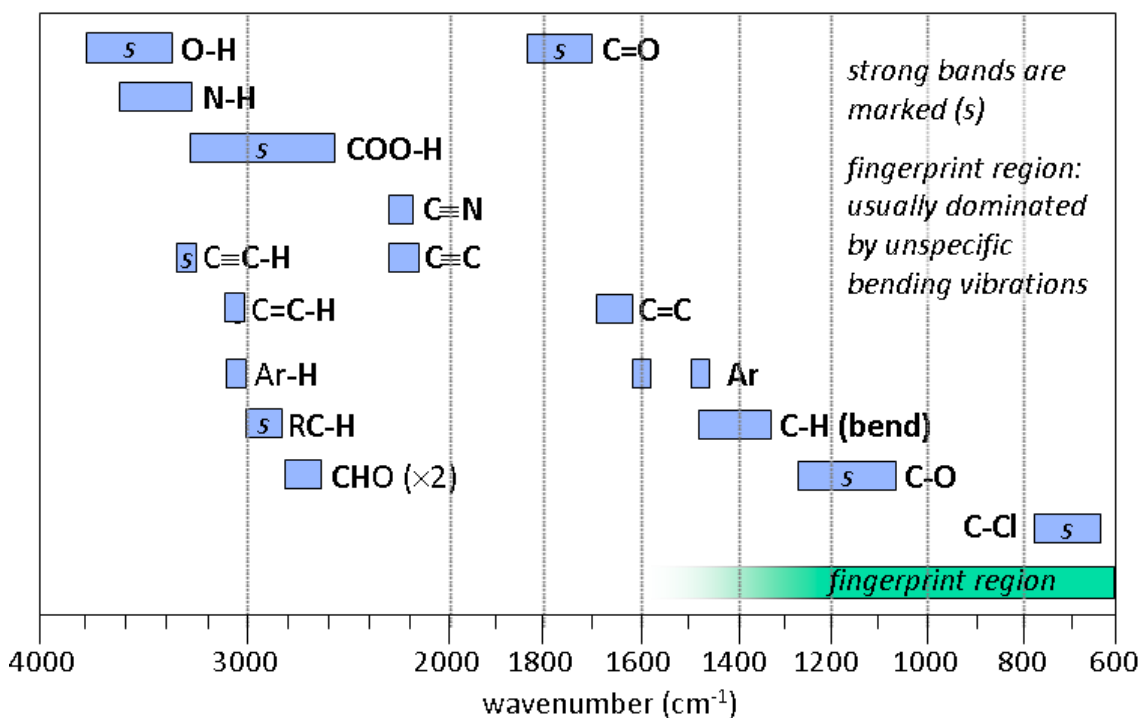
http://en.wikipedia.org/wiki/Infrared_spectroscopy_correlation_table

<http://www2.chemistry.msu.edu/faculty/reusch/VirtTxtJml/Spectrpy/InfraRed/infrared.htm#ir3>

http://www.ochemonline.com/Infrared_spectroscopy_absorption_table

Simplified correlation chart

(adapted from Royal Society of Chemistry website, <http://www.rsc.org/education>)



Fingerprint region

The wavenumber region of 1450 - 600 cm^{-1} is called the *fingerprint region* and should not be used for functional group assignment. The IR spectrum of most compounds is very complex in the fingerprint region. This is due to the presence of numerous, coupled bending and deformation modes. Use the fingerprint region to confirm the assignment of a molecular structure by comparison with reference spectra. A google search can help you find reference spectra.

Guide to Writing in English

Style

1. Each sentence should make a clear statement or ask a clear question. Avoid vague statements and give specific examples. *Bad: To overcome the present limitations of soccer balls, one can modify the ball surface in several ways. Better: Traditional soccer balls flutter when shot at high velocity. Simple modifications of the ball surface stabilize the flight trajectory.*

2. Each paragraph should address one topic or question. The topic should be introduced in the first sentence of the paragraph. *E.g.: The articles in English are a, an, and the. In some cases, no article is necessary. Placing articles correctly can be difficult for English learners, whose native language does not use articles. Fortunately, a few simple rules can help to identify the correct article for every case. ...*

3. Write short and descriptive sentences. Never hide the subject of your sentence and stick to subject-verb-object sentences whenever possible. Use precise language and avoid vague terms. *Bad: To be able to compare the capabilities of the device to measure the photo-electron spectra of high kinetic energy particles, a simulation of the ionization of xenon with a photon energy of 1200 eV is done. Better: A simulation modeled the energy resolution of our spectrometer under realistic experimental conditions. We simulated the ionization of xenon with 1200 eV photons.*

4. Use active tense and strong subjects + verbs

Deliberately choose the subject for your sentence. *Bad: To compare the capabilities of the spectrometer, a simulation is done. Better: A simulation modeled the capabilities of the spectrometer.*

5. Remove unnecessary words and qualifiers

George Orwell: "If it is possible to cut out a word, always cut it out."

~~Much better to be able to describe the fundamental importance in a brief summary.~~

6. Stay positive (use passive tense and negative statements only when necessary)

Bad: Apart from fluoride, CTTS states are not accessible. Better: CTTS states are only accessible in fluorides. Bad: We show that also for fluoride, the excitation of the CTTS state does not play a role. Better: We show that excitation of the CTTS state is negligible even for fluorides.

7. Be precise and specific

Avoid vague statements and give precise information instead. *Bad: spectrometer capability; Better: energy resolution of the spectrometer.*

Structure of a Manuscript (Only some sections may be applicable to your text.)

Abstract (Write this at last): The abstract should accurately reflect the content of the paper.

Introduction: Define the question you will address in the text. Explain what has been done to address similar questions before (literature). Explain why this question is relevant or interesting.

Experimental (Write this first): Describe which tools and methods you used and how you performed your experiment. A skilled reader should be able to reproduce your work based on the information you give -- write no more and no less. Use the past tense.

Results (Write this after the Experimental section): Describe experimental results, plot figures and give tables that summarize all relevant results. Describe your steps to analyze the data.

Discussion: Discuss how (or whether) the results answered your scientific question. Refer to the literature to show whether your results agree, disagree, or complement published work.

Figures: All fonts in a figure must be legible (>11 pt fonts). font. Use only dark colors to allow grayscale reproduction. Check the clarity and legibility of your figures. Give a figure caption that describes the figure content. If you reproduce a figure, cite the reference. -- Don't plagiarize!

Grammar:**8. Check your subjects and verbs**

The verb must stand in proper relation to the Subject (singular/plural, context).

Repeller and extractor needs to be biased. (Two subjects); To shorten the flight tube enhances the spectrometer. --> A shorter flight tube enhances the spectrometer. (Missing subject)

9. Check the articles

"a": Indefinite article. Use when referring to one object the reader does not yet know about.

"the" (also: "that", "this", "my", ...): Definite article. Use when referring to one or multiple objects that the reader knows.

"" (no article): Indefinite quantity of an object that the reader does not yet know about.

E.g.: I teach in a University. (You didn't know about it yet.) The University is new. (I talk about the same University, so you already know about it.) I like a University. (Now I talk about a different University that you don't know about.) That University is in Germany. (I now refer to that last University, not to the one mentioned farther above.) Universities are cool. (I make a general statement about all Universities.) I teach chemistry. (There isn't "one" chemistry.) I go to University. I come from University. (I now refer to a place and I can omit "the"/"a".) Bikes are fast. (General statement about bikes.) The bike is fast. (You should know which bike I talk about.)

10. Put the commas

For a brief explanation of comma rules, visit the [Purdue Online Writing Lab](#).

Put a comma after: *However, ... (Introductory word); As last step, ... (Introductory element); Compared to the last election, ... (Introductory clause); I am here, you are there. (Independent clauses).*

11. Use Dictionaries and Spellcheckers

Dictionaries: www.merriam-webster.com (US-English), [Oxford dictionary](#) (British-English).

12. Cite the literature

You can be guilty of plagiarism even if you rephrased or translated a text into your own words. Clearly mark where you obtained relevant information. Use quotation marks: "You should quote material when you believe the way the original author expresses an idea is the most effective means of communicating the point you want to make" [from Website [P.org: How Do I Cite Sources](#)]. Cite the source of your figures in the figure captions.

13. Further reading

The ACS Style Guide (<http://bit.ly/xF7to8>); AIP Style Manual (<http://bit.ly/AdYsAC>); APS 'Mechanics of Style' (<http://bit.ly/xjjdts>); From Research to Manuscript: A Guide to Scientific Writing (Michael Jay Katz); [The Economist Style Guide](#); ...

Scientific honesty and correctness

Give all results with reasonable precision (significant digits) or confidence range.

Honestly describe your work and results. Others should be able to reproduce your result (including your errors).

If possible, calculate statistical and expected errors and discuss uncertainties.

Compare your results to known/published data. Point out and discuss discrepancies.

Credit contributions from other people and cite other people's work correctly.