Spectroscopy II

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Transmission, Absorption, Fluorescence, Beer’s law and Scattering

In this experiment you will use the SpectroVis Plus (a small computer-controlled Spectrophotometer + Fluorometer) to:

• Measure the Transmission and Absorbance spectra of some common liquids from 380-950 nm.
• Measure the Fluorescence spectrum of Olive Oil for excitation at 405 and 500 nm.

In the process you will learn about:

• Beer’s law and verify its dependence on the number density of particles.
• Light scattering from small particles and molecules and how to detect it, as well as how to estimate the size of the scatterers.
Sample Cuvettes:
Contain a “smooth” side and a “grooved” side

Important! Read note for proper cuvette handling
Part I

Transmission, Absorbance, and Fluorescence

Wikipedia: Beer-Lambert Law
The **Beer-Lambert Law** helps to correlate the intensity of absorption of UV-visible radiation to the amount of substance present in a sample. The Beer-Lambert law has been widely used in many fields of pharmaceutical sciences, chemistry and quantification testing. It allows UV-visible spectroscopy to be useful as not just a qualitative but also a quantitative tool. In physics, it is often used to calculate the attenuation in optical fibers and materials, as well as the atmosphere.
The transmission coefficient $T$ is given by the ratio of the transmitted intensity to the incident or initial intensity (often specified in percent as Transmittance) by

$$T = \frac{I}{I_0}$$  \hspace{1cm} (1.)

A schematic of the situation for spectrophotometry is

The absorbance is defined as

$$A = \log_{10} \left( \frac{1}{T} \right) = \log_{10} \left( \frac{I_0}{I} \right)$$  \hspace{1cm} (2.)
For the Beer Lambert law, the transmitted intensity $I$ is given in terms of the initial intensity $I_0$ by the relation

$$I = I_0 e^{-\alpha l}$$

(3.)

where $\alpha$ is the absorption coefficient and $l$ is the path length in the absorbing/scattering medium. The absorption coefficient can be written in terms of the number density $n$ (number/volume) of particles and the absorption/scattering cross section $\sigma$ as $\alpha = n \sigma$, so that the absorbance can be written as

$$A = \log_{10} \left( \frac{1}{e^{-n \sigma l}} \right) = \log_{10}(e^{n \sigma l}) = n \sigma l \log_{10}(e)$$

(4.)

or

$$A = 0.4343 \ n \sigma \ l$$

(5.)

The importance of this last result is that it tells us that the absorbance is proportional to the path length $l$ and the number of particles per unit volume $n$. The standard cuvette length is $l = 1$ cm.

In the Beer’s law part of this experiment, we will decrease the number density by successive known dilutions of a solution of Green Tea, holding the number of molecules constant, and thus show that the absorbance is proportional to the number density, or equivalently, that the inverse absorbance, $1/A$, is proportional to the volume of the solution when the number of molecules is held constant.
Procedure 1

1.) In the first part of the experiment, you will measure the Transmission, Absorbance, and Fluorescence spectra using 405nm and 500nm excitation light of a sample of Extra Virgin Olive Oil and compare the various features of each type of spectrum. You should make some thoughtful comments and discussion on the differences between the graphs and what they mean. Read the referenced articles for additional information. What color is the Chlorophyll fluorescence? Note that shining a 405nm or 532nm laser on green plant leaves produces a bright chlorophyll fluorescence. Try this on spinach lettuce, regular lettuce, spinach juice, and some (living) green plant leaves.

To make a measurement you just select:

>Experiment Menu
>Change Units  Spectrometer

(choose the appropriate spectrum type)

>Transmission, Absorbance, Fluorescence 405nm or Fluorescence 500nm.

Make sure you select **Calibration** before taking any spectra. The software will prompt you to put in a blank cuvette.

**Important!** Read note for proper [cuvette handling](#) before continuing!!
Copy your 4 screen graphs with appropriate ranges (make sure you vertically expand your vertical scales so that the features of the spectra are well displayed and take up most of the vertical scale) into a word document. Similarly, be sure you have wavelength plots over the entire 380-950nm range. If you want to examine a feature in more detail, you can expand the horizontal wavelength scale to be a smaller range around the feature you are interested in (i.e., exclude the Infrared portion).

See Examples Below
Transmission - Olive Oil
Absorbance – Olive Oil
Procedure 2
(more detailed procedure in following slides)

2.) In the second part of this experiment we will measure the absorbance spectrum of a solution of Green tea as a function of the volume of the solution, starting from a high concentration and adding known volumes of water to dilute it.

In most cases, you will be provided with 5 cuvettes of green tea solutions, each having the same number of particles but increased volume of water and thus decreased concentrations. The volumes are 1, 2, 3, 4, and 5 in relative units of the concentration of the stock solution, which is all that is necessary to make Beer’s law plots. Click here if students are to make their own Solution

• Insert the Cuvette labeled “1” and click “run”. Allow the program to run long enough to get a stable graph and completed data table (about a minute.) Click “Stop” and carefully remove the cuvette.
• Repeat for cuvettes 2-5

Hint: When you click on “Run” again, you can choose to “store latest run” and it will conveniently put all the data on one graph.
Make data tables of Absorbance A vs. Volume V and plot 1/A vs. the volume for a wavelength which starts out with an absorbance of about 1 (428.8 nm in the following example), and also for the peak wavelength of about 397nm. (use your actual values)

Hint: Export your *.cmbl (Logger Pro) file to Excel (*.csv) then copy and paste data into sample Spreadsheet provided.

<table>
<thead>
<tr>
<th>(off peak)</th>
<th>A</th>
<th>V(relative)</th>
<th>1/A</th>
</tr>
</thead>
<tbody>
<tr>
<td>lamda(nm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>0.996</td>
<td>1</td>
<td>1.004</td>
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<tr>
<td></td>
<td>0.526</td>
<td>2</td>
<td>1.901</td>
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<tr>
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<td>3</td>
<td>2.564</td>
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<td></td>
<td>0.292</td>
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<tr>
<td></td>
<td>0.218</td>
<td>5</td>
<td>4.587</td>
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<table>
<thead>
<tr>
<th>(peak)</th>
<th>A</th>
<th>V(relative)</th>
<th>1/A</th>
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<tr>
<td>lamda(nm)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>397</td>
<td>1.532</td>
<td>1</td>
<td>0.653</td>
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<tr>
<td></td>
<td>0.959</td>
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<td></td>
<td>0.729</td>
<td>3</td>
<td>1.372</td>
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<tr>
<td></td>
<td>0.549</td>
<td>4</td>
<td>1.821</td>
</tr>
<tr>
<td></td>
<td>0.42</td>
<td>5</td>
<td>2.381</td>
</tr>
</tbody>
</table>
Beer’s Law Experiment

Green Tea Absorbance vs. Wavelength

Note: Your volumes will be relative to the stock solution and will have (dimensionless) values of 1, 2, 3, 4, and 5 labeled on the bottom of the cuvettes.

Analysis & Discussion
To analyze the data on Absorbance as a function of dilution volume

Plot the 1/A vs. wavelength data from the tables for the off peak and peak wavelengths for each of the sample volumes, including the starting stock solution (i.e. 1, 2, 3, 4, 5).

For the off-peak wavelength, fit a linear trend line with the option “set intercept = 0” ENABLED. (this is a trendline option which forces the intercept to be zero)

For the on-peak wavelength, fit a linear trend line with the option “set intercept = 0” DISABLED. (this is a trendline option, default does not force the intercept to zero).

Make sure your graphs are properly formatted and titled and the data vertical range adjusted so as to make the data spread out over most of the range of the axis, as in the following examples
Off peak wavelength obeys Beer’s law over this range of concentration. Note the forced fit with intercept of zero fits the data nicely.
On peak wavelength deviates noticeably from Beer’s law over this range of concentration. Note the non-zero intercept required to fit the data. Fit with zero intercept gives lower $R^2$ value with poor fit.
So the off peak wavelength produces a good fit to Beer’s law, while the peak wavelength requires a non-zero intercept, although the concentration dependence was still linear.

There are several factors which can produce deviations from Beer’s law.

Under certain conditions Beer-Lambert law fails to maintain a linear relationship between absorbance and concentration of analyte. These deviations are classified into three categories:

Real Deviations - These are fundamental deviations due to the limitations of the law itself.

Chemical Deviations- These are deviations observed due to specific chemical species of the sample which is being analyzed.

Instrument Deviations - These are deviations which occur due to how the absorbance measurements are made.
There are at least six conditions that need to be fulfilled in order for Beer’s law to be valid. These are:

1.) The absorbers must act independently of each other;

2.) The absorbing medium must be homogeneous in the interaction volume;

3.) The absorbing medium must not scatter the radiation – no turbidity (not true, can have single scattering, just not multiple scattering effects);

4.) The incident radiation must consist of parallel rays, each traversing the same length in the absorbing medium;

5.) The incident radiation should preferably be monochromatic, or have at least a width that is narrower than that of the absorbing transition; and

6.) The incident flux must not influence the atoms or molecules; it should only act as a non-invasive probe of the species under study. In particular, this implies that the light should not cause optical saturation or optical pumping, since such effects will deplete the lower level and possibly give rise to stimulated emission.

If any of these conditions are not fulfilled, there will be deviations from Beer’s law. Some of them are discussed in more detail here.

Part 3

Scattering
A piece of blue glass, through which the light shines orange, seeming to behave like the sky at sunset. The website shares a long commentary on why the sky is blue.
Rayleigh Scattering

Rayleigh scattering, named after the British physicist Lord Rayleigh, is the elastic scattering of light or other electromagnetic radiation by particles much smaller than the wavelength of the light. The particles may be individual atoms or molecules. It can occur when light travels through transparent solids and liquids, but is most prominently seen in gases and suspensions of particles. Rayleigh scattering is a function of the electric polarizability of the particles. (direct quote with minor modification from Wikipedia article on Rayleigh scattering)

For our purposes, the Rayleigh Scattering cross section $\sigma_s$ can be written as $\sigma_s = \frac{C}{\lambda^4}$, where $C$ is a constant proportional the square of the polarizeability and the diameter to the sixth power of the molecule or particle. As a specific example for the case of a conducting particle of radius $R$,

$$\sigma_s = \frac{144\pi^5 R^6}{\lambda^4}$$

In the Rayleigh scattering regime, the scattering cross section is orders of magnitude smaller than the geometric cross section of the scatterer.

Suffice to say, there are many important practical applications and consequences of Rayleigh scattering, among them are the color and polarization of the blue sky and orange red sunset, the color of opalescent glasses and nanoporous solids, the ultimate distance limit of optical transmission in optical fibers, and critical opalescence, a topic that Einstein showed was related to Rayleigh Scattering and the color of the daytime sky.
Rayleigh Scattering (continued)

In the scattering part of this experiment, we will measure the absorbance spectrum of a solution of distilled water with differing number of drops of non-fat milk. We use non-fat milk to insure that the particles are small compared to the wavelength. In unhomogenized cow's milk, the fat globules have an average diameter of two to four micrometers and with homogenization, average around 0.4 micrometers. The fat-soluble vitamins A, D, E, and K along with essential fatty acids such as linoleic and linolenic acid are found within the milk fat portion of the milk. Use of non-fat milk eliminates essentially all fat molecules (0.0-0.5% by weight), with the remaining molecules small compared to the wavelength of most visible and near infrared light.

So after removal of the fat, what is left in the milk? Fat-free skimmed milk has only the casein micelles to scatter light, and they tend to scatter shorter-wavelength blue light more than they do red, giving skimmed milk a bluish tint.

Caseins

The largest structures in the fluid portion of the milk are "casein micelles": aggregates of several thousand protein molecules with superficial resemblance to a surfactant micelle, bonded with the help of nanometer-scale particles of calcium phosphate. Each casein micelle is roughly spherical and about a tenth of a micrometer across. There are four different types of casein proteins: αs1-, αs2-, β-, and κ-caseins. Collectively, they make up around 76–86% of the protein in milk, by weight. Most of the casein proteins are bound into the micelles. There are several competing theories regarding the precise structure of the micelles, but they share one important feature: the outermost layer consists of strands of one type of protein, k-casein, reaching out from the body of the micelle into the surrounding fluid. These kappa-casein molecules all have a negative electrical charge and therefore repel each other, keeping the micelles separated under normal conditions and in a stable colloidal suspension in the water-based surrounding fluid.
Rayleigh Scattering (continued)

Milk contains dozens of other types of proteins beside the caseins including enzymes. These other proteins are more water-soluble than the caseins and do not form larger structures. Because they proteins remain suspended in the **whey** left behind when the caseins coagulate into curds, they are collectively known as whey proteins. Whey proteins make up approximately 20% of the protein in milk, by weight. **Lactoglobulin** is the most common whey protein by a large margin.

**Particle Size Effects**

As a final note, when the wavelength becomes short enough to approach the particle size, the scattering of the light is called Mie scattering, and is no longer proportional to the inverse 4\(^{th}\) power of the wavelength. At shorter wavelengths, the scattering cross section reaches a maximum and then has minor, damped oscillations, approaching a constant equal to the geometric cross section of \( \sigma_g = \pi R^2 \), where \( R \) is the particle radius. The normalized scattering cross section as a function of the inverse wavelength is shown for a spherical conducting particle in the following graph.

Note that the primary peak scattering cross section occurs very near \( \lambda_p = 2\pi R \), thus providing an estimate of the size of the scattering particles from the (measured) peak wavelength. In addition, the full Mie scattering solution provides an explanation for a characteristic peak in the spectrum having to do with the size of the scattering particles.

Using the measured absorbance peak wavelength of \( \lambda_p = 398.7\) nm we find the particle diameter is

\[
2R = \frac{\lambda_p}{\pi} = \frac{398.7}{3.1416} = 127\) nm .
\]

This is in good agreement with the 0.1 \( \mu \)m size estimate of the milk Casien micelles, which vary somewhat, depending on the cows genetic strain and breeding.
Procedure 3

3.) Measure the absorbance spectra and observe Rayleigh scattering of a solution of distilled water and non fat milk by placing successive drops of milk in a cuvette (1-5). Show that the spectrum is mostly Rayleigh Scattering by fitting the absorbance for 4 or 5 drops to an inverse 4th power of the wavelength using Excel Solver. See example spreadsheet on website.

Start with a clean, empty cuvette and add just enough distilled water so the level sits flush with the spectrometer (about ¾ full.) Add milk one drop at a time using an eyedropper or plastic pipette. Cover the cuvette with cap, shake gently to mix solution, and carefully place in spectrometer. Make sure to wipe the cuvette using a slightly damp paper towel. Click here for proper cuvette handling.
Absorbance of Nonfat Milk Drops Added to Distilled Water

Pink line shows fit to $\text{Abs} = \frac{2.157}{\lambda^4}$ for 5 drops spectrum

Demonstrates an excellent Rayleigh Scattering spectrum!

Casein particle diameter estimate from peak wavelength

$$2R = \frac{\lambda_p}{\pi} = \frac{398.7}{3.1416} = 127\text{nm}$$

1/14/2013 KHW data
Cuvette Handling

Please be Careful!!!

Cuvettes are delicate: they are easily scratched, chipped, and/or broken!

- Cuvettes need to be clean and free of scratches and smudges or they will give false readings.
- Hold them by the “grooves” side
- Cuvettes should be placed only in the cuvette holder or spectrometer. **Do Not** place them (or stand them up) on the table. They will fall over and get scratched, chipped and/or broken!!
- Clean them with soap and water and rinse them with distilled water.
- Wipe them with a clean, slightly damp paper towel. Never use a dry towel to clean them to avoid scratching.
- Always use a cap and wipe off excess moisture/liquid before placing in spectrometer. Any liquid in the Spectral Chamber will damage the device!
Read the following slide only if students make up their own solutions.

It is important in this part of the experiment NOT TO SPILL OR LOSE ANY OF THE SOLUTION, OTHER THAN THE FIRST REDUCTION OF SOLUTION AFTER THE FIRST ABSORBANCE SPECTRUM TO A STANDARD VOLUME. The experiment assumes that the number of molecules in the total volume of solution (that is cuvette plus graduated cylinder) remains constant while the total volume is increased.
Procedure:
Prepare a green tea solution of sufficient concentration to produce a peak absorbance of at least 1.5 around 397 nm. This should already be available in class. Verify your initial solution has sufficient absorbance.

Procedure… (from Steve Mahrley, 11/2/2012)
A cuvette holds approximately 3.6 mL.
- I initially ran Absorbance with full cuvette/concentration and saved the file as * 2 mL.
- I filled the (10 ml) Graduated Cylinder to 2 mL and dumped the rest of the cuvette contents down the drain (see note!). I added water to 4 mL, ran the program again and saved it as *.4 mL.
- I poured the entire cuvette into the GC, checked to make sure the volume was still 4 mL, then added water to 6mL.
- Ran the program, saved as 6 mL.
- Repeated this for 8mL and 10mL
- I did this for both green tea and Gatorade fruit punch

NOTE: After the first reduction of the initial cuvette volume to 2mL, DO NOT THROW AWAY OR LOSE ANY SOLUTION! The laboratory experiment assumes that the total number of molecules remains constant while the volume is increased.
How Does the SpectroVis Plus Work?

Light from the LED and tungsten bulb light source passes through a solution. Emerging light goes through a high-quality diffraction grating, then the diffracted light is collected and sorted by the CCD array detector.

Fluorescent light is scattered at right angles (RA fluorescence) to the excitation light sources (LED’s) to minimize light detected at the excitation wavelength(s).
Specifications for the Spectro Vis Plus

Wavelength Range: 380 nm–950 nm

Support for fluorescence (two excitation sources centered at 405 nm and 500 nm)

Reported Wavelength Interval: 1 nm between reported values (collects 570 values)

Optical Resolution: ~ 2.5 nm

Dimensions: 15 cm x 9 cm x 4 cm

Light Sources: Incandescent white bulb, approximately 8000 hour lifetime, LED-based, approximately 100,000 hour lifetime

One-step calibration

No external power required
More Reading
(Quizable?)
Fluorescent molecules are compounds that absorb light of one wavelength, then re-emit light at a longer wavelength. This emitted light can be quantified using fluorescence spectroscopy. Molecular and cellular biologists use fluorescent compounds to label proteins, gels, and even cellular organelles. In many ways, fluorescent compounds have revolutionized research in the life sciences. Fluorescence spectroscopy is also used in the characterization of laser materials.

Jablonski diagram.
After an electron absorbs a high energy photon the system is excited electronically and vibrationally. The system relaxes vibrationally, and eventually fluoresces at a longer wavelength. In this lab you will observe the fluorescence of extra virgin Olive Oil and compare to its transmission and absorption spectrum.

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

Fluorescence is the emission of light by a substance that has absorbed light or other electromagnetic radiation. It is a form of luminescence. In most cases, the emitted light has a longer wavelength, and therefore lower energy, than the absorbed radiation. However, when the absorbed electromagnetic radiation is intense, it is possible for one electron to absorb two photons; this two-photon absorption can lead to emission of radiation having a shorter wavelength than the absorbed radiation. The emitted radiation may also be of the same wavelength as the absorbed radiation, termed “resonance fluorescence”.\(^1\)

The most striking examples of fluorescence occur when the absorbed radiation is in the ultraviolet region of the spectrum, and thus invisible to the human eye, and the emitted light is in the visible region.

Fluorescence has many practical applications, including mineralogy, gemology, chemical sensors (fluorescence spectroscopy), fluorescent labelling, dyes, biological detectors, and, most commonly, fluorescent lamps.
Fluorescence spectroscopy

Fluorescence spectroscopy aka fluorometry or spectrofluorometry, is a type of electromagnetic spectroscopy which analyzes fluorescence from a sample. It involves using a beam of light, usually ultraviolet light, that excites the electrons in molecules of certain compounds and causes them to emit light; typically, but not necessarily, visible light. A complementary technique is absorption spectroscopy.

Devices that measure fluorescence are called fluorometers or fluorimeters.

Theory

Molecules have various states referred to as energy levels. Fluorescence spectroscopy is primarily concerned with electronic and vibrational states. Generally, the species being examined has a ground electronic state (a low energy state) of interest, and an excited electronic state of higher energy. Within each of these electronic states are various vibrational states.

In fluorescence spectroscopy, the species is first excited, by absorbing a photon, from its ground electronic state to one of the various vibrational states in the excited electronic state. Collisions with other molecules cause the excited molecule to lose vibrational energy until it reaches the lowest vibrational state of the excited electronic state. This process is often visualized with a Jablonski diagram.
Chlorophyll fluorescence

Chlorophyll fluorescence is light that has been re-emitted after being absorbed by chlorophyll molecules of plant leaves. By measuring the intensity and nature of this fluorescence, plant ecophysiology can be investigated.

Assessing plant physiology with Chlorophyll fluorescence

Light energy that has been absorbed by a leaf will excite electrons in chlorophyll molecules. Energy in photosystem II can be converted to chemical energy to drive photosynthesis (photochemistry). If photochemistry is inefficient, excess energy can damage the leaf. Energy can be emitted (known as energy quenching) in the form of heat (called non-photochemical quenching) or emitted as chlorophyll fluorescence. These three processes are in competition, so fluorescence yield is high when less energy is emitted as heat or used in photochemistry.
Space-filling model of the chlorophyll a molecule (from Wikipedia)

Structure of chlorophyll a
The absorption peaks of chlorophyll $a$ are at 665 nm and 465 nm. Chlorophyll $a$ fluoresces at 673 nm (maximum) and 726 nm. The peak molar absorption coefficient of chlorophyll $a$ exceeds 105 M$^{-1}$ cm$^{-1}$, which is among the highest for small-molecule organic compounds.
Fluorescence of Vegetable Oils: Olive Oils

Maurizio Zandomeneghi,* Laura Carbonaro, and Chiara Caffarata

Dipartimento di Chimica e Chimica Industriale, Università di Pisa, Via Risorgimento 35, 56126 Pisa, Italy

Fluorescence spectra of undiluted extra virgin olive oil obtained with the traditional setup (right-angle fluorescence) show considerable artifacts and deformations due to self-absorption phenomena, even when the spectra are corrected for inner filter effects. On the other side, front-face fluorescence spectra are much less affected by self-absorption. Front-face fluorescence of native olive oil reveals the presence of different fluorophores and can provide information about their amount. From the intense emission at ca. 315–330 nm, it is possible to detect fluorescent polyphenols and pherols and to evaluate their overall content. Low-intensity emission bands at 350–600 nm are correlated to vitamins and other important molecules. Among them, the fluorescence of the riboflavin fluorophore can be used to evaluate its concentration. The intense emission of chlorophyll derivatives, measured in the 640–800 nm spectral region, can provide information on their concentration.

KEYWORDS: Front-face fluorescence; olive oil; polyphenols; vitamins; pheophytins
Figure 3. (A) RA fluorescence spectra of the extra virgin olive oil O2 where $\lambda_{\text{exc}}$ is (a) 350 nm; (b) 320 nm; (c) 380 nm; (d) 410 nm; (e) 300 nm; (f) 280 nm; (B) (a) 580 nm; (b) 550 nm; (c) 510 nm; (d) 620 nm; (e) 530 nm. The excitation and emission bandwidths are 1.5 and 3 nm, respectively.

Figure 4. (a) Absorption spectrum of olive oil O2. (b) Absorption spectrum of a solution of maize oil in $n$-hexane (1:100) v/v. The spectrum intensity is multiplied by 100.
Rayleigh scattering

From Wikipedia, the free encyclopedia

This article is about the optical phenomenon. For the magnetic phenomenon, see Rayleigh law. For the stochastic distribution, see Rayleigh distribution. For the wireless multipath propagation model, see Rayleigh fading.

Rayleigh scattering, named after the British physicist Lord Rayleigh,\(^1\) is the elastic scattering of light or other electromagnetic radiation by particles much smaller than the wavelength of the light. The particles may be individual atoms or molecules. It can occur when light travels through transparent solids and liquids, but is most prominently seen in gases. Rayleigh scattering is a function of the electric polarizability of the particles.

Rayleigh scattering of sunlight in the atmosphere causes diffuse sky radiation, which is the reason for the blue color of the sky and the yellow tone of the sun itself.

Scattering by particles similar to or larger than the wavelength of light is typically treated by the Mie theory, the discrete dipole approximation and other computational techniques. Rayleigh scattering applies to particles that are small with respect to wavelengths of light, and that are optically "soft" (i.e. with a refractive index close to 1). On the other hand, Anomalous Diffraction Theory applies to optically soft but larger particles.

References


Rayleigh scattering also occurs from individual molecules. Here the scattering is due to the molecular polarizability $\alpha$, which describes how much the electrical charges on the molecule will move in an electric field. In this case, the Rayleigh scattering intensity for a single particle is given by $^4$

$$I = I_0 \frac{8\pi^4 \alpha^2}{\lambda^4 R^2} \left(1 + \cos^2 \theta\right).$$

The amount of Rayleigh scattering from a single particle can also be expressed as a cross section $\sigma$. For example, the major constituent of the atmosphere, nitrogen, has a Rayleigh cross section of $5.1 \times 10^{-31}$ m$^2$ at a wavelength of 532 nm (green light) $^5$ This means that at atmospheric pressure, about a fraction $10^{-5}$ of light will be scattered for every meter of travel.

The strong wavelength dependence of the scattering ($\sim \lambda^{-4}$) means that shorter (blue) wavelengths are scattered more strongly than longer (red) wavelengths. This results in the indirect blue light coming from all regions of the sky. Rayleigh scattering is a good approximation of the manner in which light scattering occurs within various media for which scattering particles have a small size parameter.

**Reason for the blue color of the sky**

*Further information: Diffuse sky radiation*

A portion of the beam of light coming from the sun scatters off molecules of gas and other small particles in the atmosphere. It is this scattered light that gives the surrounding sky its brightness and its color. As previously explained, Rayleigh scattering is inversely proportional to the fourth power of wavelength, so that shorter wavelength violet and blue light will scatter more than the longer wavelengths (yellow and especially red light). The resulting color, which appears like a pale blue, actually is a mixture of all the scattered colors, mainly blue and green. Conversely, glancing toward the sun, the colors that were not scattered away — the longer wavelengths such as red and yellow light — are directly visible, giving the sun itself a slightly yellowish hue. Viewed from
Reason for the blue color of the sky

Further information: Diffuse sky radiation

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The reddening of sunlight is intensified when the sun is near the horizon, because the volume of air through which sunlight must pass is significantly greater than when the sun is high in the sky. The Rayleigh scattering effect is thus increased, removing virtually all blue light from the direct path to the observer. The remaining unscattered light is mostly of a longer wavelength, and therefore appears to be orange.

Rayleigh scattering primarily occurs through light’s interaction with air molecules. Or, from a purely macroscopic point of view, blue sky comes from microscopic density fluctuations, resulting from the random motion of molecules in the air. A region of higher or lower density has a slightly different refractive index from the surrounding medium, and therefore it acts like a short-lived particle that scatters light in random directions. Smaller regions fluctuate more than larger ones, and, since short wavelengths are disturbed by small regions more than longer wavelengths, they are scattered more.

Some of the scattering can also be from sulfate particles. For years after large Plinian eruptions, the blue cast of the sky is notably brightened by the persistent sulfate load of the stratospheric gases. Some works of the artist J. M. W. Turner may owe their vivid red colours to the eruption of Mount Tambora in his lifetime.

In locations with little light pollution, the moonlit night sky is also blue, because moonlight is reflected sunlight, with a slightly lower color temperature due to the brownish color of the moon. The moonlit sky is not perceived as blue, however, because at low light levels human vision comes mainly from rod cells that do not produce any color perception. [citation needed]
In optical fibers

Rayleigh scattering is an important component of the scattering of optical signals in optical fibers. Silica fibers are disordered materials, thus their density varies on a microscopic scale. The density fluctuations give rise to energy loss due to the scattered light, with the following coefficient:

$$\alpha_{\text{scat}} = \frac{8\pi^3}{3\lambda^4} n^4 p^2 k T_f \beta$$

where $n$ is the refraction index, $p$ is the photoelastic coefficient of the glass, $k$ is the Boltzmann constant, and $\beta$ is the isothermal compressibility. $T_f$ is a fictive temperature, representing the temperature at which the density fluctuations are "frozen" in the material.

In porous materials

$\lambda^4$ Rayleigh-type scattering can also be exhibited by porous materials. An example is the strong optical scattering by nanoporous materials. The strong contrast in refractive index between pores and solid parts of sintered alumina results in very strong scattering, with light completely changing direction each 5 micrometers on average. The $\lambda^4$-type scattering is caused by the nanoporous structure (a narrow pore size distribution around $\sim$70 nm) obtained by sintering monodispersive alumina powder.

See also

- Rayleigh Sky Model
- Rayleigh fading
- Ricean fading
- Raman scattering
- Optical phenomenon
- Dynamic light scattering
- Tyndall effect
- Critical opalescence
Small size parameter approximation

The size of a scattering particle is parameterized by the ratio $x$ of its characteristic dimension $r$ and wavelength $\lambda$:

$$x = \frac{2\pi r}{\lambda}.$$ 

Rayleigh scattering can be defined as scattering in the small size parameter regime $x \ll 1$. Scattering from larger spherical particles is explained by the Mie theory for an arbitrary size parameter $x$. For small $x$ the Mie theory reduces to the Rayleigh approximation.

The amount of Rayleigh scattering that occurs for a beam of light depends upon the size of the particles and the wavelength of the light. Specifically, the intensity of the scattered light varies as the sixth power of the particle size, and varies inversely with the fourth power of the wavelength. [2]

The intensity $I$ of light scattered by a single small particle from a beam of unpolarized light of wavelength $\lambda$ and intensity $I_0$ is given by:

$$I = I_0 \left(1 + \cos^2 \theta \right) \frac{1}{2R^2} \left(\frac{2\pi}{\lambda}\right)^4 \left(\frac{n^2 - 1}{n^2 + 2}\right)^2 \left(\frac{d}{2}\right)^6 [3]$$

where $R$ is the distance to the particle, $\theta$ is the scattering angle, $n$ is the refractive index of the particle, and $d$ is the diameter of the particle.

The Rayleigh scattering cross-section is given by

$$\sigma_s = \frac{2\pi^5}{3} \frac{d^6}{\lambda^4} \left(\frac{n^2 - 1}{n^2 + 2}\right)^2$$

The Rayleigh scattering coefficient for a group of scattering particles is the number of particles per unit volume $N$ times the cross-section. As with all wave effects, for incoherent scattering the scattered powers add arithmetically, while for coherent scattering, such as if the particles are very near each other, the fields add arithmetically and the sum must be squared to obtain the total scattered power.

From molecules
Milk

From Wikipedia, the free encyclopedia

This article is about the fluid produced by mammals, via the mammary glands. For other uses of the word, see Milk (disambiguation).

Milk is a white liquid produced by the mammary glands of mammals. It is the primary source of nutrition for young mammals before they are able to digest other types of food. Early-lactation milk contains colostrum, which carries the mother's antibodies to the baby and can reduce the risk of many diseases in the baby. It also contains many other nutrients.[1]

As an agricultural product, milk is extracted from mammals and used as food for humans. World's dairy farms produced about 730 million tonnes of milk in 2011.[2] India is the world's largest producer and consumer of milk, yet neither exports nor imports milk. New Zealand, the European Union's 27 member states, Australia, and the United States are the world's largest exporters of milk and milk products. China and Russia are the world's largest importers of milk and milk products.[3][4]

Throughout the world, there are more than 6 billion consumers of milk and milk products, the majority of them in developing countries. Over 750 million people live within dairy farming households. Milk is a key contributor to improving nutrition and food security particularly in developing countries. Improvements in livestock and dairy technology offer significant promise in reducing poverty and malnutrition in the world.[5]
Importance of casein micelle size and milk composition for milk gelation.

Glantz M, Devold TG, Vegard GE, Lindmark Månsson H, Stålhammar H, Paulsson M.
Department of Food Technology, Engineering and Nutrition, Lund University, PO Box 124, SE-221 00 Lund, Sweden. maria.glantz@food.lth.se

Abstract
The economic output of the dairy industry is to a great extent dependent on the processing of milk into other milk-based products such as cheese. The yield and quality of cheese are dependent on both the composition and technological properties of milk. The objective of this study was to evaluate the importance and effects of casein (CN) micelle size and milk composition on milk gelation characteristics in order to evaluate the possibilities for enhancing gelation properties through breeding. Milk was collected on 4 sampling occasions at the farm level in winter and summer from dairy cows with high genetic merit, classified as elite dairy cows, of the Swedish Red and Swedish Holstein breeds. Comparisons were made with milk from a Swedish Red herd, a Swedish Holstein herd, and a Swedish dairy processor. Properties of CN micelles, such as their native and rennet-induced CN micelle size and their zeta-potential, were analyzed by photon correlation spectroscopy, and rennet-induced gelation characteristics, including gel strength, gelation time, and frequency sweeps, were determined. Milk parameters of the protein, lipid, and carbohydrate profiles as well as minerals were used to obtain correlations with native CN micelle size and gelation characteristics. Milk pH and protein, CN, and lactose contents were found to affect milk gelation. Smaller native CN micelles were shown to form stronger gels when poorly coagulating milk was excluded from the correlation analysis. In addition, milk pH correlated positively, whereas Mg and K correlated negatively with native CN micellar size. The milk from the elite dairy cows was shown to have good gelation characteristics. Furthermore, genetic progress in relation to CN micelle size was found for these cows as a correlated response to selection for the Swedish breeding objective if optimizing for milk gelation characteristics. The results indicate that selection for smaller native CN micelles and lower milk pH through breeding would enhance gelation properties and may thus improve the initial step in the processing of cheese.
Determination of the size distribution of bovine casein micelles using photon correlation spectroscopy

by David S Horne

Engineering > Chemical Engineering Papers

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Abstract

Photon correlation spectroscopy may be used to determine particle diffusion coefficients. By calculations involving two parameter Schulz-Zimm size distributions contaminated with trace amounts of larger particles, it has been demonstrated that the effects of the large particle component on the measured diffusion coefficient are observed first at small angles and that diffusion coefficients at wider angles depart from their zero contaminant values only at much higher levels of contamination. Provided the particles described by the Schulz-Zimm distribution are large enough to exhibit intramolecular interference, it has been confirmed that size distribution parameters can be extracted from the slope of a linear fit to this wide-angle data and its intercept at zero-angle. The casein micelle system from skim-milk contaminated by much larger fat globules exemplifies the systems described in these model calculations. Diffusion
Fig. 2. Casein micelles in cow milk by metal shadowing with platinum and carbon. False colour TEM. Bar: 0.5 μm.

Casein micelles and their internal structure

Cornelis G. de Kruijf, Thom Huppertz, Volker S. Urban, Andrei V. Petukhov

NIZO food research, PO Box 20, 6710 BA, Ede, The Netherlands
Van't Hoff laboratory for Physical and Colloid Chemistry, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands
Center for Structural Molecular Biology, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831, USA

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Monostatic radar cross section [RCS] of a perfectly conducting metal sphere as a function of frequency (calculated by Mie theory). In the low frequency Rayleigh scattering limit where the circumference is less than the wavelength, the normalized RCS is $\sigma/(\pi R^2) \sim 9(kR)^4$. In the high frequency optical limit $\sigma/(\pi R^2) \sim 1$

http://en.wikipedia.org/wiki/Mie_theory
Selected References