Spectrophotometer measurements

MM/2021

1. Introduction

Spectrophotometers are used for measuring reflectance or transmittance of the sample. In this case we will measure transmittance. Some spectrophotometers are equipped with integrating sphere. They can measure also diffuse reflectance/transmittance, but our device measures only light propagating as a beam through the sample.

Aims of this work are to a) define concentration of a chemical in liquid sample, b) compare UV-transmittance of visually transparent materials, c) study IR-transmittance of visually opaque materials, d) learn how to recognize different types of plastics from IR spectrum and d) check applicability of laser safety glasses.

2. Spectrophotometer

The spectrophotometer used in this work is Perkin-Elmer Lambda 9. It looks like this, not very beautiful or modern, but actually very good and accurate device.

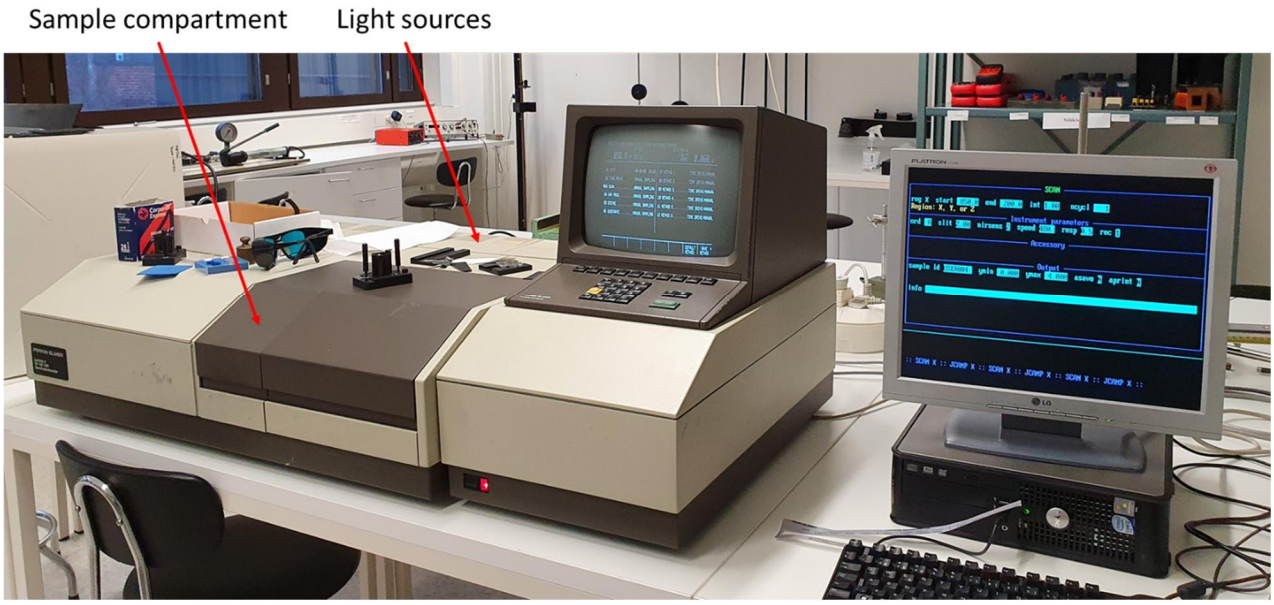


Figure 1. Perkin-Elmer Lambda 9 spectrophotometer.

The spectrophotometer has two light sources, halogen lamp for visible light and IR and deuterium lamp for UV. It also has two detectors, photomultiplier tube for UV and visible and lead sulfide detector for IR. It is possible to measure wavelength range 170 – 3200 nm.

The light sources are shown in Figure 2. Light is guided to diffraction grating, which separates different wavelengths to different directions. By rotating the optical components, it is possible to select which wavelength is incident to the sample. When measuring, the device automatically scans through the given wavelength range. Since the device scans through the wavelengths, measurement takes typically some minutes. Other alternative would be to illuminate the sample with white light and analyze

transmitted/reflected light with a diffraction grating + row detector combination (own detector element for every wavelength). That kind of devices are faster, but less accurate.

Figure 2. Light sources of spectrophotometer. Violet bulb is deuterium lamp, halogen is in the lower middle part of the picture (covered by the edge).

Sample compartment of the Spectrophotometer is shown in Figure 3. Monochromatic beam of light comes from left (two circular things on the left wall), transmits the sample, and goes to the detector (two circular things on the right wall). As you can see, there are two channels for the light. The channel closer to camera is used for measuring the sample. The channel further away is reference channel, which is used to monitor stability of the light sources. If the power of light source happens to change for some reason, it is automatically compensated by using information of the reference channel.

Figure 3. Sample compartment.

Square areas in Figure 3 are places of sample holders (rarely needed in reference channel). There are two kind of sample holders, one type for thin flat plate-like objects and one for liquid cuvettes. They are not suitable for all kind of samples, so sometimes you must improvise by putting extra objects to the sample compartment for supporting your sample. It is fine as long as measuring beam goes through your sample and you don't block the reference beam.

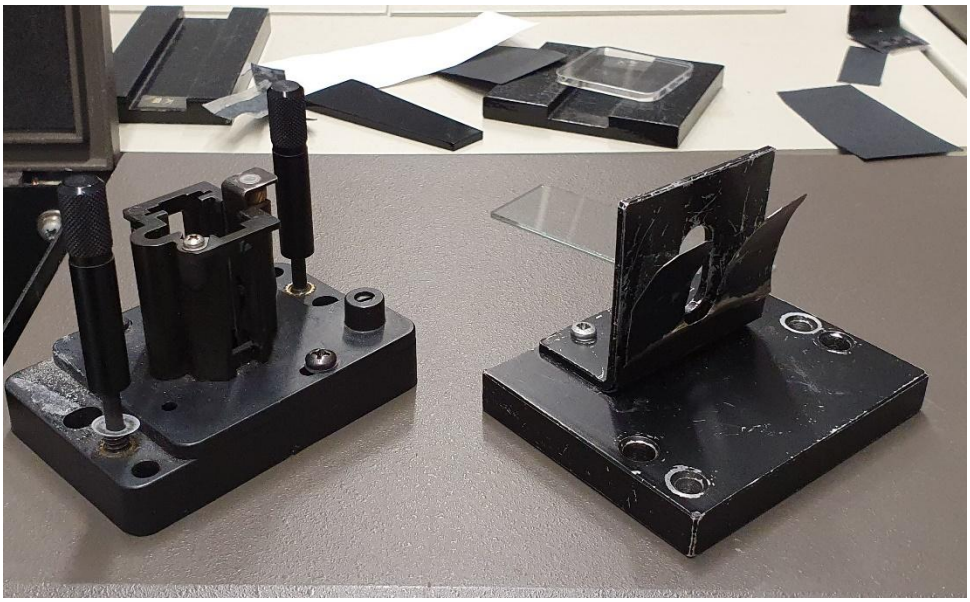


Figure 4. Sample holders. On left holder for cuvettes, on right for flat samples.

The screws shown in liquid cuvette holder (left) fit holes in the bottom of the sample compartment. Liquid cuvettes used in this work are 10 mm thick (light transmits through fluid layer of 10 mm) and they are made of quartz glass. If the fluid absorbs relatively strongly, thinner cuvette may work better: more light is transmitted through 5 mm or 1 mm layer than through 10 mm layer. Cuvettes have two diffuse and two clear sides. The light must go through the clear sides, so pay attention when putting cuvette to the sample holder. You can touch the diffuse sides, but if you make the clear sides dirty, clean them with suitable solvent. You don't want to measure transmittance of your fingerprints.

Figure 5. Quartz cuvette for measuring fluids.

3. Software of the spectrophotometer and measurements

A little bit before extinction of the dinosaurs, about 67 million years ago, Microsoft released operating system called MS-DOS. Software of the spectrophotometer is DOS-based, which doesn't matter very much. However, compared to e.g. modern Windows, there are many limitations for the acceptable file names.

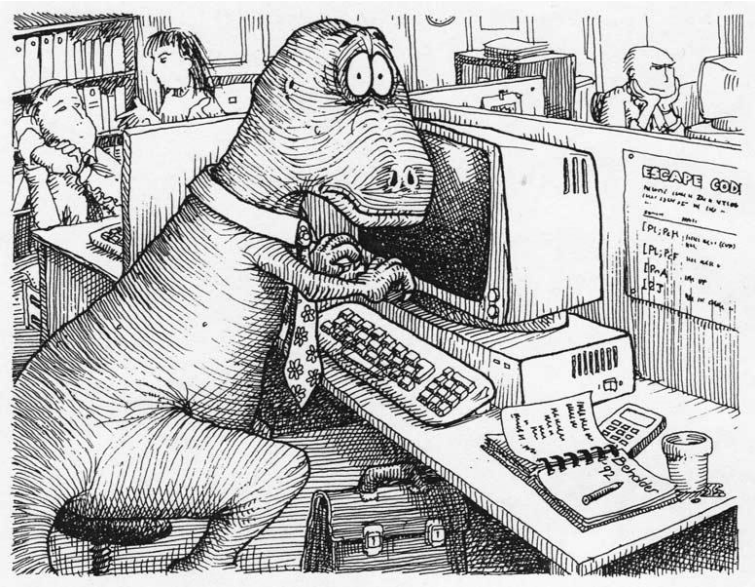


Figure 6. Tyrannosaurus rex writing a report with DOS-based Word Perfect 5.0.

In practice you may need some help from the supervisor, but in theory software and measuring with the spectrophotometer works like described below.

Start the software by clicking icon "Perkin-Elmer c-asemalle tallennus" (saying in Finnish "saving to drive c"). The files you measure will be saved to hard disc of the computer, folder c:\elmeri. After the measurements you can copy them to OneDrive or send them to yourself as attachments of e-mail.

Setting measurement parameters

Press F10 and window shown in Figure 7 will open. Most of the parameters are OK for us by default, so we will check only few of them (surrounded by red line). You can use arrow keys to move between the fields.

"Start" and "end" define wavelength range we measure. The device always starts from the longest wavelength, and measures towards shorter wavelengths. Therefore, in Figure 7 "start" is 850 and "end" 200 nm. Parameter "int" is interval. Default value 1 nm is OK for us, so the measurement would have 651 data points in this case (850 nm, 849 … 200 nm).

"Ord" selects if we want to measure transmittance (T) or absorbance (A). Well, actually the device always measures transmittance, and absorbance is calculated from transmittance as explained later. If you use wrong mode, or you don't know which one would be better, don't worry. It is always possible to convert absorbance to transmittance or transmittance to absorbance afterwards in Excel or Matlab.

"Speed" defines how fast the device scans through the wavelengths. In case of Figure 7 we would measure 650 nm range (200 – 850 nm) with speed of 480 nm/min, so the measurement would last somewhat over a minute. Slower you scan, more accurate the results, but longer the measurement time. Minimum value is 0.9 nm/min and maximum 960 nm/min. Measuring the full wavelength range 170 – 3200 nm with the slowest scanning speed would take over two days! However, in this work measurements last few minutes.

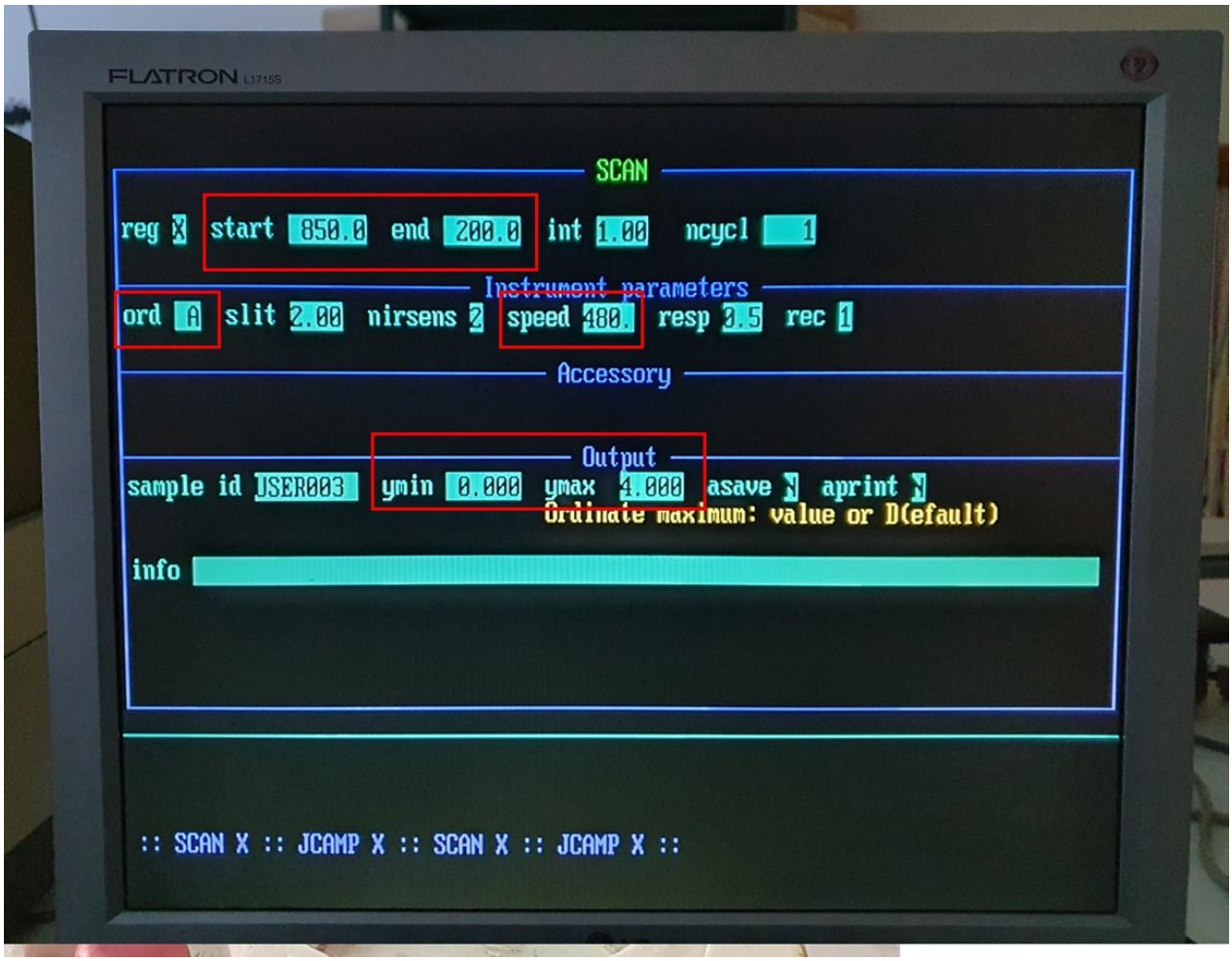


Figure 7. Measurement parameters of the software. Red lines are added for emphasizing important things.

Values “ymin” and “ymax” are for scaling the graphics on display, they don’t change anything in the measurement itself. In case of transmittance default is 0 – 100, which is kind of natural (something between 0 % and 100 % of the light must go through the sample). However, since absorbance is logarithmic entity, there is no natural upper limit. Default setting is 0 – 1, but then you may not see what happens during the measurement. After the values are OK, press enter to accept them.

Calibration

Now you will see this:

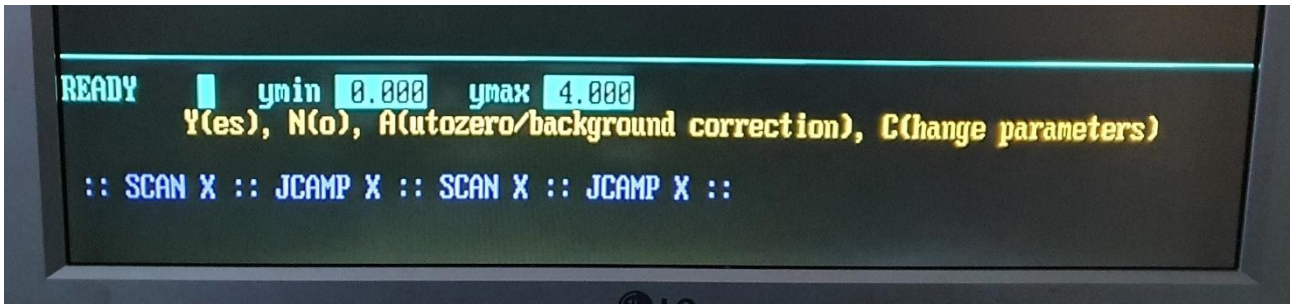


Figure 8. Measurement mode.

The spectrophotometer must be calibrated before the measurement. Make sure the sample compartment is empty (empty sample holder can be there). Press “a” for autozero. Now the spectrophotometer calibrates

transmittance of the empty compartment to be 100 % (or absorbance to be 0). This will take same time as the measurement itself. You need to do this only once if you keep measuring with the same parameters. If you change the parameters, then you have to calibrate the device again. When the calibration is ready, you will see the same selections as in Figure 8 again.

Measurement

Now the device is ready. Put the sample to its place, and press “y” for yes. The spectrophotometer starts to measure, and you can see the results from the display in graphical form. So, all you need to do is to watch curve updating on display… When the measurement is ready, you see word “ready” in the lower left corner. Like in Figure below.

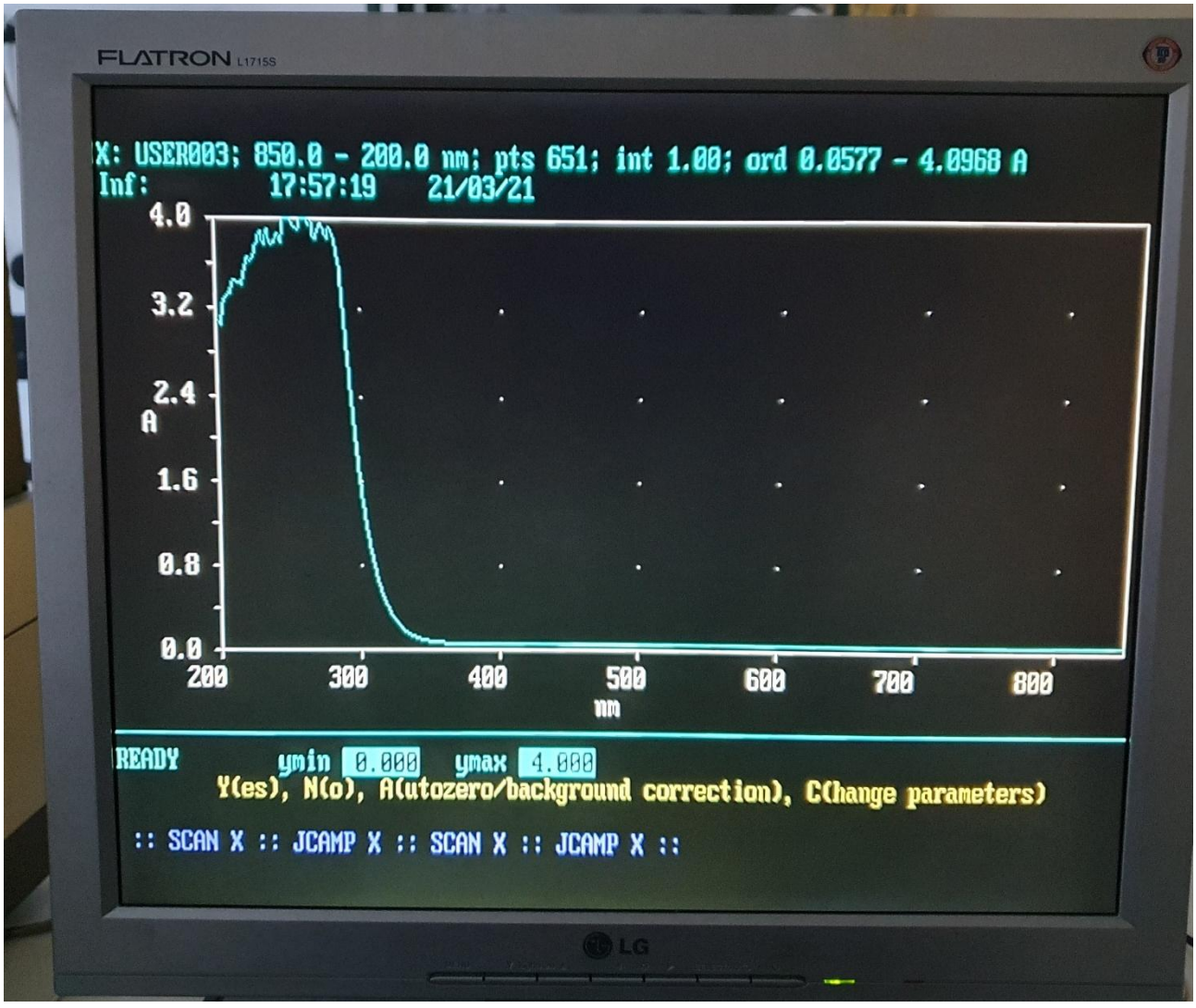
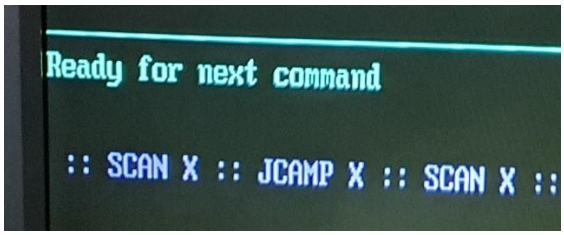


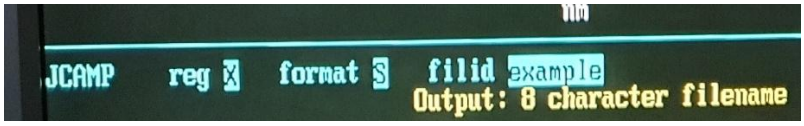
Figure 9. Display after finishing an absorbance measurement.

Saving the results

The measured data is not automatically saved, you need to do it yourself. When the measurement is ready (Figure 9), press esc key. You will see in the left bottom corner:



Then press shift + F6 (sort of difficult to guess!). You will see this:



Go to field "filid" field and write file name there (here "example").

Note: here you have to pay attention due to the limitations of MS-DOS system, it is a little bit tricky for those who have never used DOS:

- File name may have only eight characters.
- File name may have numbers, but it can't start with a number: A123 is OK, but 123A is not.
- You can't use some special characters, e.g. dot and comma are not accepted. So just use numbers and alphabets to be sure.
- If the file name is not acceptable, the software will tell you… "Unexpected error: person under 45 years old is trying to use the spectrophotometer"… or something like that.

Then just press enter, and the file will be saved to folder c:\elmeri. File extension is .dx, so in the final file name would be in this case "example.dx".

After saving the file, press F10. You will be back in window of Figure 7. If you continue with the same parameters, just accept them by pressing enter… and repeat the same procedure.

The files are standard ASCII format, so you can open them with any software (Word, Excel, Matlab etc.). There is about 15 - 20 lines of text in the beginning of the file and one line in the end of file. You need to remove them if you bring the data to Matlab with "load" command. "Import" works without removing the text. Structure of the files is like this, first column wavelength, second column transmittance or absorbance. In this case transmittance was measured from 380 to 780 nm. Values are absolute, not in per cents (first value would be 15.31 %).

```
##TITLE= punainen
##JCAMP-DX= 4.24
```

[some 15 lines more text]

```
380 0.1531
381 0.1538
382 0.1540
```

[and so on some 400 lines more…]

```
779 0.5761
780 0.5756
##END=
```

4. Theory

All the variables in this chapter are functions of wavelength, but that is omitted from the equations. Let us assume that light with power I is propagating through an infinitesimally thin layer of material. If absorption coefficient of the material is α and thickness of the material dz, amount of absorbed light is (sign negative because we lose power dI from power I)

$$-dI = I\alpha dz. \tag{1}$$

By rearranging the terms, we get

$$-\frac{dI}{I} = \alpha dz. \tag{2}$$

Now let us suppose that material has finite thickness d. If the power of the incident beam is I_0 and power of the beam transmitting the material I_T, we get by integrating both sides (from I_0 to I_T and from zero to thickness d) Beer-Lambert law

$$I_T = I_0 e^{-\alpha d}. \tag{3}$$

Transmittance of the material is defined as

$$T = \frac{I_T}{I_0} = e^{-\alpha d}. \tag{4}$$

Absorption coefficient can be written in terms of concentration of absorbing material (we consider now small amount of absorbing material mixed with non-absorbing material)

$$\alpha = \varepsilon c, \tag{5}$$

where ε is molar absorption coefficient and c is concentration. If the unit of thickness d is cm and unit of concentration moles per liter (mol/l), unit of ε is obviously l/(mol*cm). However, other unit systems work too. We will define in this work "concentration" as relative volume of the colorant and ignore mols.

Absorbance A of the material can be defined two different ways, which may be occasionally a little bit confusing. One way is to define it is (indices 1 and 2 just for clarity, they are not used in "real life")

$$A_1 = \ln\left(\frac{1}{T}\right) = \alpha d. \tag{6}$$

Then of course transmittance in terms of absorbance is

$$T = e^{-A}. \tag{7}$$

However, often it is defined (our spectrophotometer uses this definition)

$$A_2 = \log_{10}\left(\frac{1}{T}\right). \tag{8}$$

The latter definition is also called "optical density". It can be easily shown that

$$A_2 = A_1 \log_{10}(e) \cong 0.434 * A_1, \quad (9)$$

i.e. they are linearly proportional to each other.

Bringing the theory to practice, even if your sample is not absorbing light at all, you will not get 100 % transmittance with a spectrophotometer. If a beam of light is incident from the direction of normal from material 1 to material 2, reflectance from the boundary is

$$R = \left(\frac{n_2 - n_1}{n_2 + n_1}\right)^2, \quad (10)$$

where n_1 and n_2 are refractive indices of the materials. A clear non-absorbing glass plate with refractive index 1.5 reflects 4 % from air-glass boundary and again 4 % from glass-air boundary. Therefore, measured transmittance would be about 92 %.

If we define absorbance according to Eq.(8), A = 0 means 100 % transmittance, A = 1 means 10 % transmittance, A = 2 means 1 % transmittance etc. Due to the surface reflections mentioned above, apparent absorbance of non-absorbing sample is not zero.

5. Measurements and tasks

This work has five separate tasks.

Defining chemical concentration of a sample

Use following values

- Start 800 nm
- End 200 nm
- Ord = A(bsorbance)
- Speed 240 nm/min
- Ymin 0, Ymax 4 (just for seeing the results, some absorbance values are higher than default value ymax = 1)

The samples contain

- Blueish chemical disodium;2-[[4-[ethyl-[(3-sulfonatophenyl)methyl]amino]phenyl]-[4-[ethyl-[(3-sulfonatophenyl)methyl]azaniumylidene]cyclohexa-2,5-dien-1-ylidene]methyl]benzenesulfonate
- Dihydrogen monoxide as a solvent

Measure absorbance of reference samples (0, 0.5, 1, 1.5 and 2 % concentration of blueish chemical) and unknown sample. Supervisor will show you how to get the sample into the cuvette safely. Be careful, dihydrogen monoxide causes tens of deaths in Finland yearly.

First task after the measurements is to find which wavelengths are suitable for our calculations. For doing that, it is useful to plot the absorbance curves as a function of wavelength.

- The spectrophotometer is unable to measure absorbance values over 4 (it means that only 0.01 % of light transmits the sample!). Select a wavelength where absorption of the colorant is not too high.
- On the other hand, you can't use a wavelength where the bluish chemical absorbs very little.

The figure below tries to explain the principle of selecting suitable wavelength.

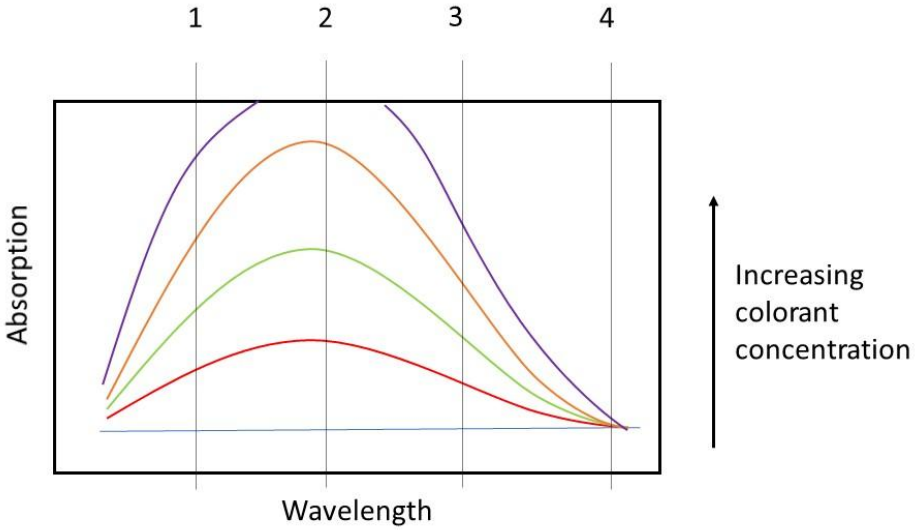


Figure 10. Selection of wavelength for defining concentration.

Wavelength 2 is not suitable because absorbance is too high (over 4) for defining real absorbance value of high concentration sample. Wavelength 4 is not suitable either because the colorant doesn't absorb there. Wavelengths 1 and 3 would be suitable: we see the difference between the samples, but absorbance is not too high.

According to the Eqs.(6) – (9), absorbance is linearly proportional to the concentration of the colorant. After finding a suitable wavelength, pick up absorbance values of reference samples and plot concentration as a function of absorbance. Add a fitting line (you need the equation), and you will get something like in the figure below.

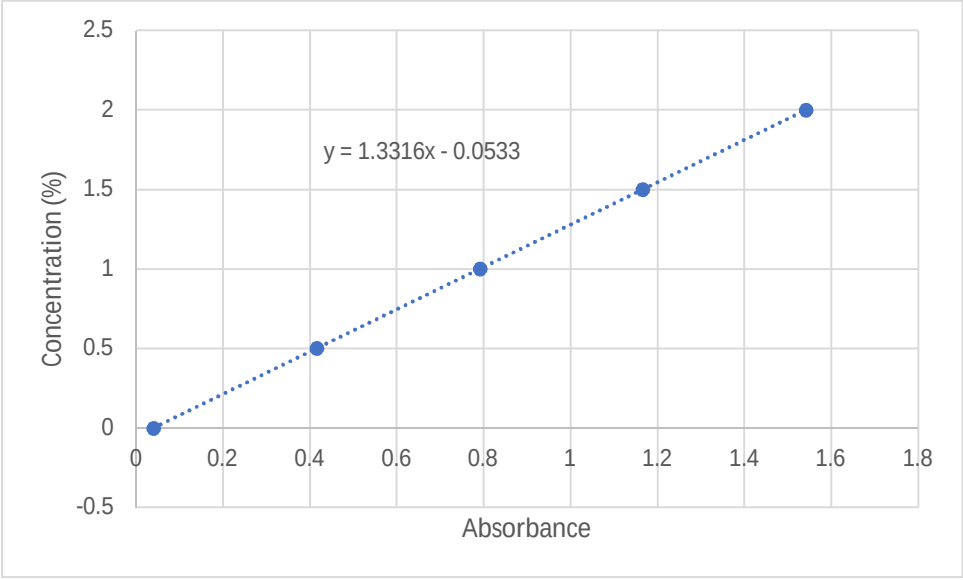


Figure 11. Fitting line of absorbance-concentration data.

Pick up absorbance of the unknown sample at the same wavelength. By using the equation of the fitting line, you can calculate concentration of the unknown sample from its absorbance. In principle this should work with any suitable wavelength, but in practice there are always some random errors. Therefore, repeat the procedure with three wavelengths and see if you will get the same result.

The bluish chemical with very long name is blue food colorant E133 and dihydrogen monoxide is of course water. It just sounded more exacting with fancier names, didn't it? The samples are completely harmless, but the blue color is difficult to clean from the fingers. And yes, tens of drunken Finnish men drown every year, so I didn't lie very much.

Finding material for UV-protection

Mercury discharge lamp radiates lots of UV light. In case of fluorescent lamp UV radiated by mercury is converted to visible radiation with fluorescent materials. However, in some applications UV light is used directly. For example, radiation peak 254 nm is very effective for killing bacteria, and therefore it can be used for sterilizing materials. This kind of UV radiation is dangerous for eyes, and if the lamp is powerful, also for skin.

Let us now suppose that your task is to build a transparent box, which has mercury discharge lamp inside. The walls should stop 254 nm radiation, otherwise it would be dangerous to use the device. So now we use spectrophotometer for checking which materials would be suitable for stopping the radiation. You have following materials available:

- Normal glass (microscope slide)
- Quartz glass (cuvette of spectrophotometer)
- Acrylic (Polymethyl methacrylate = PMMA, thicker piece of transparent plastic)
- Polycarbonate (PC, thinner piece of transparent plastic)

Use following values (we will use IR area for other purpose)

- Start 3200 nm
- End 200 nm
- Ord = T(ransmittance)
- Speed 960 nm/min
- Graphics will be automatically scaled right (0 – 100 % transmittance)

Note: quartz cuvette has two walls, so there are four boundary reflections instead of two. Therefore, even if the material doesn't absorb anything, measured transmittance is about 85 %.

Based on the measurements, which one of the materials would be the best? Mercury discharge lamp radiates also less harmful 365 nm radiation, it is a bonus, if the material stops that wavelength too.

Finding material blocking visible light, but transmitting IR

In the previous task some of the transparent materials absorb UV-light strongly. Similarly, some materials are opaque for us, but they transmit infrared light. Water absorbs strongly at wavelengths 1450 and 1950 nm, so those wavelengths can be used for measuring moisture of materials. As an example, we can illuminate the object with 1850 nm and 1950 nm light, and measure reflectance. Since water absorbs more 1950 nm than 1850 nm light, proportion of the measurements tells us moisture content of the material.

In this task we have to design a device that measures moisture content. Let us suppose that our detector is sensitive to visible light, and the device will be installed in brightly illuminated area. Therefore, we have to cover the detector with a material that transmits IR (either around 1450 or 1950 nm, or both) but blocks visible light and UV. Let's measure following materials.

- Polished silicon disc
- Green polypropylene (PP)

Use the same measurement parameters as in the previous task.

Which one would be better material for this application?

Recognizing type of plastic

Absorption in IR area is connected to molecular structure of the materials. Since different material have different molecular structure, it is possible to recognize material by finding absorption wavelengths. Now we have measured transmittance of three different kind of plastics, acrylic (PMMA), polycarbonate (PC) and polypropylene (PP). What wavelengths might be used to recognize these three types of plastics? Low transmittance of course means high absorbance.

Note: color of the plastic doesn't usually have strong effect on IR reflectance or transmittance. Black plastic is exception. Black colorant absorbs also IR light, and therefore it is difficult to recognize type of black plastic with IR light.

Laser safety glasses: are they safe on not?

Laser safety glasses are used for protecting eyes while working with lasers. Quite obviously the glasses are designed only for a certain type of laser, i.e. they absorb only a certain wavelength band. If they absorbed all the wavelengths, they wouldn't be transparent at all… which might be a little bit inconvenient.

Measure transmittance of lens of the safety glasses by using the same parameters as before. Find a way to put them to sample compartment without blocking the reference channel*. Check if these glasses would be suitable, when working with the following lasers?

- Xenon chloride laser, 308 and 459 nm
- Argon laser, 488 and 514 nm
- Helium-neon laser, 633 nm
- Ruby laser, 694 nm
- Nd:Yag laser, 1064 nm
- Er:YAG laser, 2940 nm

*If you block the reference beam with non-transparent material, you will get some nonsense like million per cent transmittance, because measurement beam is so much stronger than reference beam. If other lens of the glasses happens to block the reference beam, you will get about 100 % transmittance: both beams go through a lens, so they are attenuated same way.

Summary of measurements

Part 1: fluids

Calibrate the spectrophotometer before the measurement!

Parameters

- Start 800 nm
- End 200 nm
- Ord = A(bsorbance)
- Speed 240 nm/min
- Ymin 0, Ymax 4 (just for seeing the results, some absorbance values are higher than default value ymax = 1)

Samples

- Reference samples, 0, 0.5, 1, 1.5 and 2 % of colorant
- Unknown sample

Part 2: solid materials

Calibrate the spectrophotometer again before the measurement!

Parameters

- Start 3200 nm
- End 200 nm
- Ord = T(ransmittance)
- Speed 960 nm/min
- Graphics will be automatically scaled right (0 – 100 % transmittance)

Samples

- Normal glass (microscope slide)
- Quartz glass (cuvette of spectrophotometer)
- Acrylic (Polymethyl methacrylate = PMMA, thicker piece of transparent plastic)
- Polycarbonate (PC, thinner piece of transparent plastic)
- Polished silicon disc
- Green polypropylene (PP)
- Laser safety glasses

In the last measurement, be careful not to block the reference channel!