

24. UV-Vis spectra of plant dyes. Colors

Dyes were used by man since antiquity. Until 19th century they were mostly derived from plant sources. A large variety of plants were used as for example:

- Yellow: fruit of bearberry (*Ramnus*) species
- Brown: acacia wood
- Red: dried flower head of safflower
- Black: wood of diverse trees

With only few exceptions, natural dyes could be used with the aid of auxiliary inorganic chemicals (mordants). The mordants precipitate the colouring substance in order to be used. Together with the development of synthetic dyes, theories were also developed trying to explain the relationship between structure and color of compounds. The "chromophore" theory assumes the existence of associated groups that intensify colors. First, chromophores were identified connected with quinones (oxidized structures derived from benzene ring). Nowadays it is rather recognized that a chromophore causes a strong absorption of radiation in the ultraviolet or in the visible region of the spectrum.

According to the quantum theory the absorption of visible radiation raises the electronic energy of the molecule to what is called "excited state". That energy must be after dissipated as heat, phosphorescence or fluorescence, or in a chemical reaction with the surroundings. Nevertheless, a substance is colored because it selectively absorbs the visible light. Thus red substance absorbs green and blue light, the green substance absorbs red and blue, etc.

The example is chlorophyll, the green dye of organisms which are able for photosynthesis. There are few kinds of chlorophyll. The most common in nature are *chlorophyll a* and *chlorophyll b*, present in all photosynthesing organisms. Other chlorophylls, *c* and *d*, can be found in algae. Chlorophylls are well soluble in lipids, acetone, alcohols, but they are almost insoluble in water. More chlorophyll in plant mean more effective photosynthesis and, consequently, better quality and condition of the plant. The color of chlorophyll is green because it absorbs light in the red and blue regions but not in the green one, see the spectrum below.

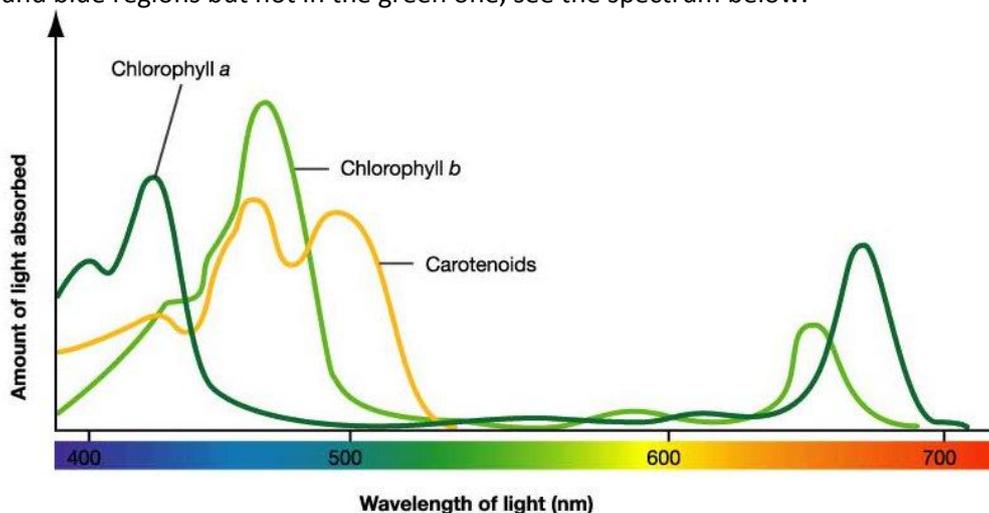


Fig.1. The absorption spectra of carotenoids, chlorophyll a and chlorophyll b.

The most important plant dyes are:

- Carotenes and flavones, which absorb yellow and red parts of the visible spectrum. They are present in the root of carrot, in the flowers of buttercup (*Ranunculus*), primrose (*Primula*) etc. causing their yellow or orange color (see Fig. 1);
- Anthocyanins are dissolved in the milieu intérieur (the extra-cellular fluid environment), they are responsible for red color. They are chemically very close to flavones, and because of that closely related plants have a variety of colors: from yellow and red to blue. Their color depends also on pH and in alkaline environment the red color turns to blue. Moreover, it depends also on the quality of soil and the content of metals (aluminum and others).

Interestingly, the white color is caused by complete reflection of light by miniature bubbles of air present in the extra-cellular spaces of living tissues. It happens in petals.

In autumn, the wonderful colors of leaves are formed by anthocyanins and carotenes, at that time not masked by chlorophylls.

However, the intense red color of beetroot is not caused by anthocyanins but a separate group of dyes, betalains, also present in spinach or goosefoot (*Chenopodium*). These dyes are not resistant to high temperature and oxygen, their stability is higher at pH 4-5, alkaline environment causes them to crack. The strong anti-oxidant character of the beetroot dye makes this vegetable extremely important in healthy diet.

The aim of this exercise is to make comparative analysis of UV-VIS spectra of different plant dyes and finding the relation between the observed spectrum and the color of the natural dyes.

Students are requested to collect before and to bring to lesson a variety of samples: color roots, petals, fruits. At least three different samples (of different colors) are necessary. The most desirable are carrot (carotene), beetroot (betalains) and petals of different flowers (anthocyanins and others), the more different in color the better. The dyes will be extracted or washed out, depending on their properties.

Chemicals and equipment:

- petals (roses, tulips, meadow flowers etc., but not pinks), fruits (cherry, raspberry, redcurrant, blackcurrant etc.), roots (carrot, beetroot etc.)
- pH buffers of pH = 1, 4.5, 8.3
- acetone, n-propyl alcohol, n-hexane or n-heptane
- mortar
- volume flasks 50 mL or smaller
- Büchner vacuum funnel with the vacuum flask and paper filters (medium)
- pipettes

Procedure:

Portion (ca. 1 g) of fresh fruits (roots, petals, leaves) pulp in a mortar, add ca. 80 mL solvent^{*/} and leave for 10 min. A portion of this liquid filter through cotton wool to the volume flask (if necessary, use the vacuum funnel) and add water to the mark. This point should be adapted to the type of sample – ask your teacher for advice.

Pour 1 mL of the solution to the beaker and add 10 mL of pH=1 buffer. Mix the mixture. In the same way prepare solutions using pH=4.5 and 8.3 buffers.

Take the UV-Vis spectra of the prepared solutions. Note also the color as observed. Attention: when operating with spectrometer, ask your teacher for assistance.

The above procedure should be done for three different samples, preferably with different original colors and origin.

For more aspiring students: Try with hortensia (*Hydrangea*) petals, adding at different pH=4.5 also solutions of Al³⁺ or Sn²⁺ salts. Hortensia is known to change the color of petals after adding these metals to soil. Try to interpret this phenomenon in the context of soil pH.

Report:

The report should contain the measured spectra and interpretation concerning relation between their shape and the observed color of samples. Try to explain the changes observed for different pH.

^{*/} *The solvent can be aqueous solution of acetone or alcohol, or a hydrocarbon – students have to find the best one by trials-and-errors procedure – the aim is obtaining clear colored extract.*

Sources:

- Leo M.L. Nollet, "Handbook of Food Analysis", Second Edition, Volume 1: "Physical Characterization and Nutrient Analysis", Marcel Dekker, Inc., USA 2004.
- P. Kafarski, P. Wieczorek, "Ćwiczenia laboratoryjne z chemii bioorganicznej", Wrocław Technical University, 1997
- D. M. Mulati, N. S. Timonah and W. Bjorn, "The absorption spectra of natural dyes and their suitability as a sensitizer in organic solar cell application", JAGST 14(1) 2012, p. 45-60.
- D.A. Skoog, D.M. West, F.J. Holler, S.R. Crouch, "Fundamentals of Analytical Chemistry", Thomson Brooks/Cole (2000).
- Materials of Opole University
- <http://kobiocypracownik.pl/>
- <http://portalwiedzy.onet.pl>
- <http://www.readbag.com/staff-amu-pl-wlodgal-antocyjany>
- http://cct.me.ntut.edu.tw/ccteducation/chchting/aiahtm/pub_cct/paper/Ting07g.pdf
- <http://www.webexhibits.org/causesofcolor/7.html>
- <http://www.newton.dep.anl.gov>
- http://cfb.unh.edu/phycokey/Choices/Chlorophyceae/Chloro_key.html