Determination of Total Alkaloid Content in Cortex Chinae by Spectrophotometric Method (European Pharmacopoeia)

PHARMACOGNOSY-III PRACTICE

2020

Spectroscopic methods

•Spectrophotometry is a method which is based on transmittance (permeability)/absorbance and emission of the light beam passing through a sample solution.

➢**Most commonly used spectroscopic methods:**

- •Spectrophotometry (UV-Visible, IR, X-ray)
- •Colorimetry
- •Mass Spectroscopy
- •NMR Spectroscopy

- Method: Spectrophotometry
- Device: Spectrophotometer

- Spectrophotometry is an analysis method based on the absorption of light energy.

- Wavelength;
  - 200-400 nm: Ultraviolet (UV = ultraviolet);
  - 400 - 750 nm: Visible

Spectrophotometer

Deuterium (D2) lamp : For UV light,
Tungsten (W) lamp : For visible light.

- The ray emitted by the lamp is converted into a single wavelength ray (monochromatic ray).
- This beam passes through the sample.
- The intensity of the light passing through the sample is detected by the detector and sent to the recorder or printer.

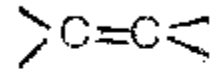
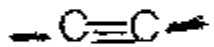
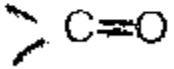
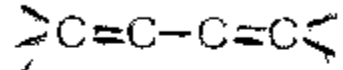
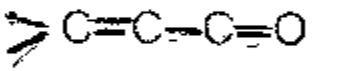
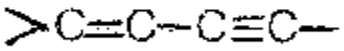
- Light can be absorbed by atoms, ions and molecules. The absorbtion of the light at a specific wavelength leads to the electron's rising to a higher energy orbit (passing excited state from ground state). Thus the absorption peak occurs.

- Each functional group (eg C = C double bond) always makes absorbtion in the same range of wavelength.

Absorption Spectrum

- The graph of the amount of absorption versus the wavelength of light is called the absorption spectrum.

In any molecule, the functional group responsible for the absorption of light in a certain wavelength range is called the **chromophore group**; the groups that change the wavelength and absorption coefficient of the light absorbed by chromophore groups, although they do not absorb light, are called **oxochrome group**s.

Chromophore Group	Oxochrome Group
	CH_3
	I, Cl, Br
	OH
NO_2	OCH_3
	SO_2NH_2
	COOH
	NH_2
	CHO
$N\equiv N$	

Absorbtion changes caused by absorption of oxochrome groups

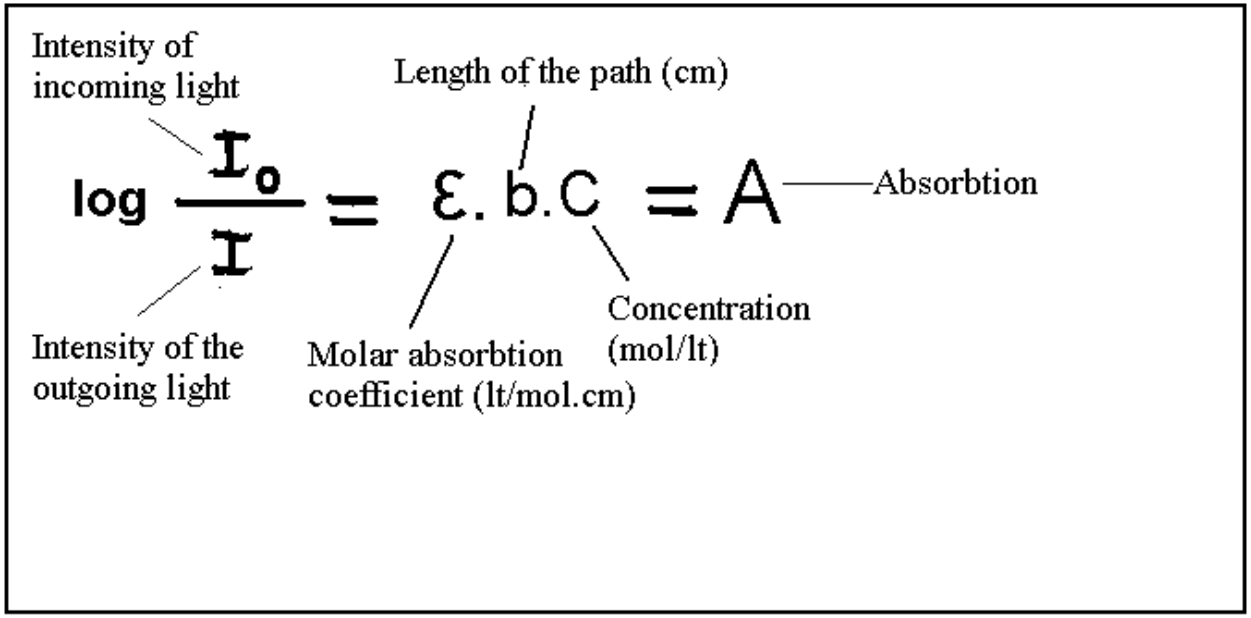
1) Batochromic shift : The shift of the absorption band of a molecule to a longer wavelength range

2) Hypsochromic shift : The shift of the absorption band of a molecule to a shorter wavelength range.

Increased intensity of the absorption band is called **hyperchromic effect**, its decrease is called **hypochromic effect.**

- Monochromatic light with an intensity value of I_0;
  - is absorbed by any molecule in the solution which has a path length of b cm and
  - leaves the tube with an intensity of I.
  - decrease in light intensity due to absorption by the molecule;
- The reduction in light intensity due to absorption by the molecule is explained by the Lambert-Beer equation.

According to this equation; The difference in logarithms of light intensities entering and leaving the sample container is directly proportional to the concentration.



Experimental Procedure

0.2 g C. Chinae + 2 ml water + 1.4 ml dil. HCl

↓ Cool after 15 min. on waterbath

5 ml $CHCl_3$ + 10 ml ether + 1 ml 20% NaOH

↓ Shake vigorously for 20 min.

0.6 g Gum tragacanth

↓ Shake until the solution becomes clear

Filtrate + Chlorofom: ether (1:2)

↓ Evaporate on waterbath

Residue + 2 ml ethanol (dissolve)

↓

↓ Evaporate on waterbath

Residue is dissolved in 0.1 M HCl and filled upto 50 ml in volumetric flask

Preparation of Reference Solutions

15 mg quinine is dissolved in 0.1 M HCl and filled upto 50 ml.

15 mg of kinkonin dissolved in 0.1 M HCl and filled upto 50 ml.

$$x = \frac{[A_{316} \times A_{348c}] - [A_{316c} \times A_{348}]}{[A_{316q} \times A_{348c}] - [A_{316c} \times A_{348q}]} \times \frac{100}{M} \times \frac{2}{1000}$$

$$y = \frac{[A_{316} \times A_{348q}] - [A_{316q} \times A_{348}]}{[A_{316c} \times A_{348q}] - [A_{316q} \times A_{348c}]} \times \frac{100}{M} \times \frac{2}{1000}$$

c= cinchonine

q= quinine

M = weight of the drug (g)
x = percentage of quinine type alkaloid
y = percentage of cinchonine type alkaloid
A316 = absorbance of test solution at 316 nm
A348 = absorbance of test solution at 348 nm
A316c = absorbance of the reference solution containing cinchonine at 316 nm
A348q = absorbance of the reference solution containing quinine at 348 nm
A316q = absorbance of the reference solution containing quinine at 316 nm
A348c = the absorbance of the reference solution containing cinchonine at 348 nm

$$\frac{100\ x}{x + y} = \text{(Relative percentage of quinine type alkaloids)}$$