SPECTROPHOTOMETRY

In spectroscopy, the absorbance A is defined as:

$$A_{\lambda} = log_{10}(I_0 / I)$$

where I is the intensity of light at a specified wavelength λ that has passed through a sample (transmitted light intensity) and I_0 is the intensity of the light before it enters the sample or incident light intensity. According to Lambert-Beer law, the absorbance of a sample is proportional to the thickness of the sample and the concentration of the absorbing species in the sample, in contrast to the transmittance I / I_0 of a sample, which varies exponentially with thickness and concentration.

Lamber-Beer law:

$$A = \alpha l c$$

l is the distance that the light travels through the material (the path length)

c is the concentration of absorbing species in the material

α is the absorption coefficient or the molar absorptivity of the absorber

Procedure

Detection of absorption maximum, $A = f(\lambda)$:

Pipette 5 ml of standard solution of $CuSO_4$ (c = 0,05 mol/l) and 10 ml of solution of chelatone 3 (CH3, c = 0,05 mol/l) in volumetric flask and fill it with buffer pH 6,0 (created complex between copper and chelatone is stable at pH 5 -10, whereas pH 6 is optimal for measuring absorbance of this complex. Absorbance of prepared solution is measured at 500 - 800 nm in 20 nm intervals. Absorption maximum is found out.

Needed amount of solution of chelatone 3:

Pipette 5 ml of solution of $CuSO_4$ in five 50 ml volumetric flasks. Using byrette, add 2; 3,5; 5; 7 and 10 ml of solution of CH3 respectively in each flask. Fill them with buffer up to mark, measure the absorbance and make a graph $A = f$ (ml CH3). Find out an amount of reagent which guarantee stechiometric reaction between Cu^{2+} and CH3).