**Experiment 2**

Use of hydraulic model to test the relationship between apparent volume of distribution $V_d$, clearance $Cl$, and the elimination rate constant $K$

**Theoretical knowledge**: One-compartment model, intravenous injection, apparent volume of distribution $V_d$ – estimation methods, relationship between apparent volume of distribution, clearance, and elimination rate constant

Equipment: spectrophotometer UV/vis (SP-830 Plus, Metertech), magnetic stirrer, magnetic bar, peristaltic pump PA-SF (Kika labortechnik Staufen), plastic cuvettes, 250 mL and 500 mL filtering flasks, glass pipettes, stopwatch, volumetric cylinder

Reagents: 1 % Methyl Blue

Procedure

1. Fill the 500 mL filtering flask equipped with magnetic stirrer with DI water
2. Start both the stirrer and the pump (pump set up: 10)
3. Wait until the rate of water dripping is constant
4. Using a volumetric cylinder measure the flow speed (2 x 30 sec)
5. Add 1.8 mL of Methyl Blue into previously prepared flask and start the stopwatch
6. Withdraw the aliquots ( ≈ 3 mL) for analysis at time points, as follows: 1, 2, 5, 10 and 15 minutes.
7. Measure the absorbance at 610 nm using DI water as a blank sample
8. Repeat the experiment
   a. using the same flask volume but double the flow speed (pump set up: 16)
   b. using the 300 mL flask and pump set up: 16
9. Enter the data (absorbance, time) into the software
10. Plot absorbance versus time on semilog paper with the absorbance on the logarithmic axis (3 data sets into one system of coordinates)
11. Calculate:
    a. Concentration at time t = 0

$$C_0 = \frac{A_0}{A_{1\%}^{1cm}} \quad when \quad A_{1\%}^{1cm} = 247.5 \ \left[cm^{-1}\, g^{-1} 100mL\right]$$

    b. Volume of distribution (one must calculate the dose)

$$V = \frac{D}{C_0}$$

    c. Half-life

$$t_{0.5} = \frac{0.693}{K}$$

d. Clearance

Cl = K V

12. Prepare the laboratory report using a general format, as follow:
    a. title
    b. purpose
    c. experimental data
    d. table containing calculated pharmacokinetics parameters
    e. graphs
    f. conclusions
        i. when V is constant but flow speed increases…
        ii. when flow is constant but V is smaller….