DETERMINATION OF MAGNESIUM

OBJECTIVE: <u>Determine magnesium concentration in unknown sample</u>

THEORETICAL PREPARATION FOR PRACTICAL LECTURE:

Magnesium - significant intracellular cation. Mg^{2+} as a cofactor in enzymatic systems, its function in a cell, its importance for the organism. Complex ATP-Mg^{2+}, examples of where it works. Hypermagnesemia, hypomagnesemia - causes. Knowledge of the principle of spectrophotometric measurement (Lambert-Beer's law and its use).

PRINCIPLE OF THE METOD:

Magnesium in the sample reacts with xylidyl blue in an alkaline medium forming a color complex that can be measured spectrophotometrically. EGTA is included in a reagent to remove calcium interference.

REAGENTS:

Reagent (A): 0.1 M sodium carbonate, 0.1 M EGTA, 0.1 M sodium triethanolamine, 7.7 mM potassium cyanide, 0.95 g / l sodium azide.
DANGER: **The agent causes severe skin burns and eye damage. Wear protective gloves / protective clothing.** In case of skin or hair contact: Immediately remove / dispose of all contaminated clothing. Rinse skin with water / shower

Reagent (B): Glycine 25 mmol / L, xylidyl blue 0.5 mmol / l, chloroacetamide 2.6 g / l.

Preparation of the working reagent – PREPARATION IS DONE BY THE LEADER OF PRACTICAL LECTURE: Pour the reagent B content into Reagent A. Mix gently. The working agent is stable for 15 days at 2-8 ° C.

<u>**Standards:**</u>		
standard 1 -	0,5 mg/dL
standard 2 -	1 mg/dL
standard 3 -	2 mg/dL
standard 4 -	4 mg/dL

MATERIAL (samples)

List of equipment and chemicals Required: unknown sample, different concentration of magnesium for calibration curve, working reagent, water, ELISA reader, pipettes, tips, microtubes, tubes, 96-well plate.

PROCEDURE

1. Mark the test tubes 1-12 and prepare the reactions according to the pipetting scheme.
2. Pipette **samples** (water, standards and unknown sample) – **10 µL** (small tips) to the bottom of tubes.
3. Pipette **working reagent** to each tube – **240 µL** (large tips) to the tubes containing samples.

4. Pipetting scheme

	Mg²⁺ concentration (x- axis)	A_{520}	Mean value A_{520}	$A_{520\ sample}$ - $A_{520\ BLANK}$ (y - axis)	Calculated concentration
1	Blank (water)				-
2					
3	Standard 1 **0,5 mg/dl**				-
4					
5	Standard 2 **1 mg/dl**				-
6					
7	Standard 3 **2 mg/dl**				-
8					
9	Standard 4 **4 mg/dl**				-
10					
11	**Unknown sample**				
12					

5. Mix thoroughly on VORTEX
6. Pipette 200 µL of solution into 96 well microtitrate plate and record the plate number and well position (eg. plate 1; A1 - B2)
7. Moving to the central lab
8. Measure on an ELISA reader at 520 nm

From the measured absorbance values and known concentrations of magnesium, create a calibration curve and from the calibration equation calculate the unknown sample concentration where y is $A_{520\ sample}$ - $A_{520\ BLANK}$ value

Comparing with the atest

Compare the result of the magnesium concentration in the unknown sample obtained from your calculated values from the calibration curve with the magnesium concentration specified in the certificate (calibration package insert). In the case of a different result, analyze the possible causes (eg. errors in the magnesium determination) and state them at the end of the report.
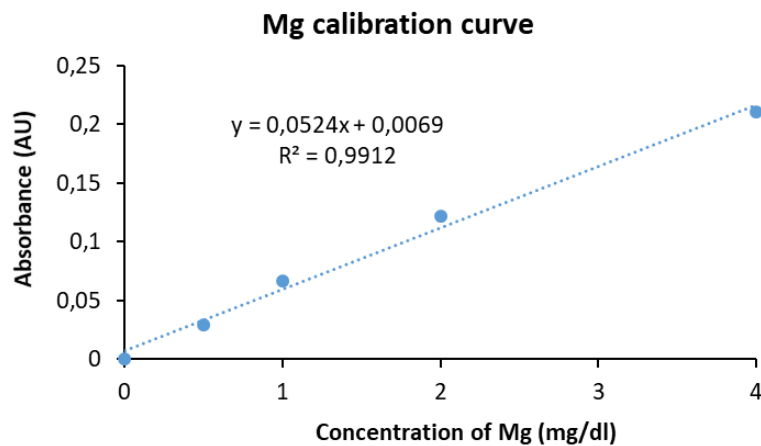
Unknown sample=

Preparation of calibration standard curve - line

Plot graf in excel – linear regression,

<u>**Preparation of calibration standard curve**</u>

A **standard curve** is a type of graph used as a quantitative research technique. Multiple samples with known properties are measured and graphed, which then allows the same properties to be determined for unknown samples by interpolation on the graph. The samples with known properties are the standards, and the graph is the standard curve.

Standard curves are most commonly used to determine the concentration of a substance such as glucose, protein or DNA. For example, a standard curve for protein concentration is often created using known concentrations of bovine serum albumin. The property measured may be absorbance, optical density, luminescence, fluorescence, radioactivity, or other parameters.

Mg calibration curve

$y = 0,0524x + 0,0069$
$R^2 = 0,9912$

Absorbance (AU)

Concentration of Mg (mg/dl)

PREPARE PROTOCOL, compare your calculated concentration of unknown sample with table values.

PROTOCOL

Objective :

Principe – summary:

Results - measured values :

Calculations:

Graph – calibration curve

Comparison of results:

Summary:

Answer the test questions:

1. What is blank?

2. Explain the principle of magnesium determination. What will you measure? At which wavelength will you measure?

3. What is spectrophotometry?

4. Give Beer-Lambert law?

5. Give the principle of determination of unknown concentration with standard calibration curve

Magnesium

Magnesium is one of the most abundant cations in the body. It is stored mainly in the bone, but significant amounts of magnesium are present in gastric and biliary secretions. Magnesium acts as an essential cofactor for enzymes concerned with cell respiration, glycolysis, and transmembrane transport of other cations. Plasma magnesium concentration is normally kept within narrow limits. The kidneys are the main organ of magnesium homeostasis in maintaining plasma concentrations.

Reference values – human:

Magnesemia – serum, plasma: 0.70 – 0.98 mmol/L

Hypermagnesemia:

Causes - acute and chronic renal failure, decreased magnesium excretion, increased magnesium intake with diet - dietary supplements, medication, dehydratation

Hypomagnesemia:

Causes - heart disease, ventricular arrhythmia, gastrointestinal malabsorption, fluid loss

Spectrophotometry is the quantitative measurement of the reflection or transmission properties of a material as a function of wavelength.

Spectrophotometry involves the use of a spectrophotometer. A spectrophotometer is a photometer that can measure intensity as a function of the light source wavelength. Important features of spectrophotometers are spectral bandwidth and linear range of absorption or reflectance measurement.

The Beer-Lambert law states that the absorbance of a solution is directly proportional to the concentration of the absorbing species in the solution and the path length. Thus, for a fixed path length, UV/Vis spectroscopy can be used to determine the concentration of the absorber in a solution. It is necessary to know how quickly the absorbance changes with concentration. This can be taken from references (tables of molar extinction coefficients), or more accurately, determined from a calibration curve.

THE BEER-LAMBERT LAW relates the absorption of light to the properties of the material through which the light is travelling.

$$A = \epsilon c l,$$

where A is the **absorbance**, c is the concentration of the solution, l is the path length and the constant ϵ - **extinction coefficient** of the absorber (properties of material).
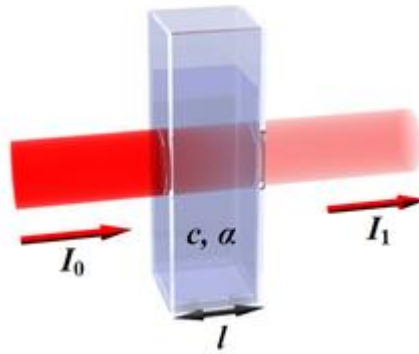
BLANK

Blank (solution), a solution containing **no analyte (sample)**, typically used to zero an analytical instrument and ensure that any reagents used do not contribute to overall measurements. Very often it is solvent in case of direct spectroscopy (UV-spectroscopy of DNA, proteins) or analytical reagent with solvent.

- A spectrophotometer is a device which measures the absorbance of a solution as light of a specified wavelength is passed through it.
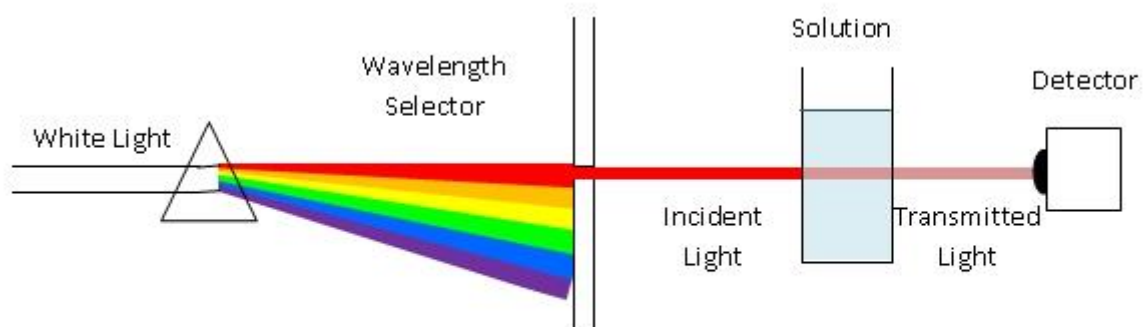

The difference between the incident and transmitted light indicates the absorbance

- If we measure the absorbance of a solution containing a known concentration of an analyte, we can use this value to estimate the concentration of the analyte in an unknown solution by comparing the two absorbance values

- The range over which absorbance is proportional to concentration varies according to the analyte and the wavelength of light used. To ensure that there is a direct relationship between absorbance and concentration, we must prepare a standard curve. Despite its name, the part of the standard curve that gives a proportional relationship is a straight line.

- To prepare a **standard curve**, we prepare a series of dilutions of a standard solution of our analyte of known concentration. The first tube always contains none of the analyte (eg. a concentration of 0g/L) and we call this a blank. We use this to calibrate the

spectrophotometer to take into account the natural absorbance of the diluents. Each of the following tubes contain increasing concentrations of the analyte. The absorbances of each of the standards are read using the spectrophotometer.

| 0g/L (Blank) | 1g/L Std 1 | 1g/L Std 2 | 1g/L Std 3 | 1g/L Std 4 | 1g/L Std 5 |
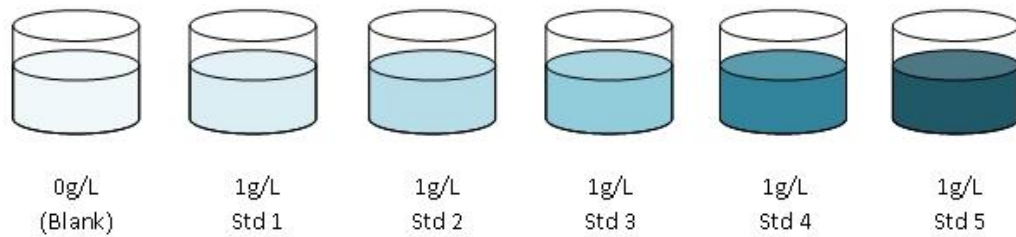
- To create the standard curve, we plot a line graph of Absorbance (Y axis) vs Concentration (X Axis) for each of the standards. A line of best fit is then drawn through the points.
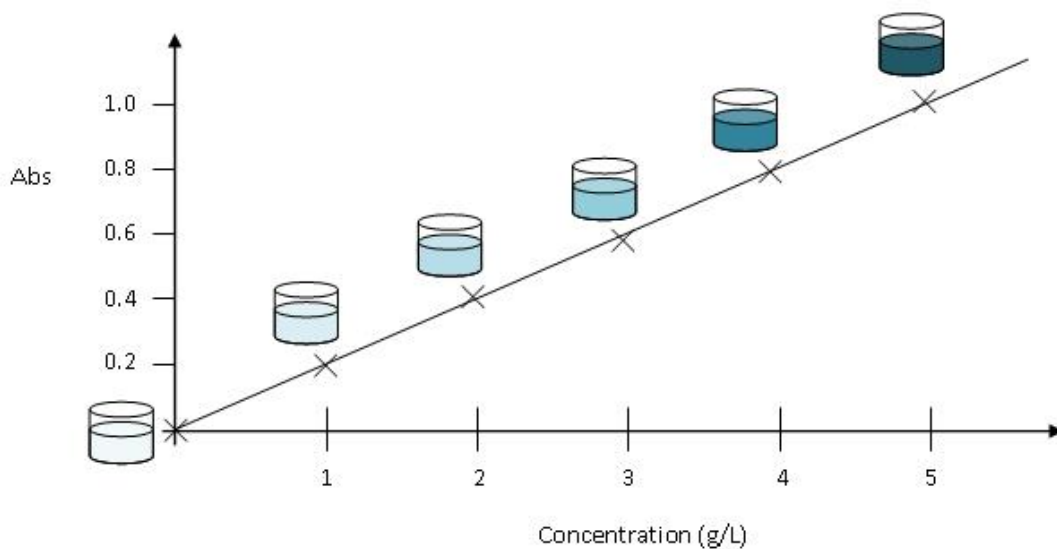
- To estimate the concentration of an unknown solution of the analyte, we read the absorbance, and then use the standard curve: