DETERMINATION OF GLUCOSE

OBJECTIVE: Determine glucose concentration in urine and control solution

TOPICS FOR REPETITIONS:
Glycolysis, hormonal regulation of glucosemia, glycemic curve, diagnostic importance, diabetes mellitus, Beer-Lambert law and its use in practice

PRINCIPLE OF THE METOD:
Glucose in the sample is measured by means of the coupled reactions described below, a coloured complex can be measured by spectrophotometry (diagnostic kit by BioSystems).

$$\text{glucose} + \tfrac{1}{2}\,O_2 + H_2O \xrightarrow{\text{glucose oxidase}} \text{gluconate} + H_2O_2$$

$$2\,H_2O_2 + 4\text{-aminoantipyrine} + \text{phenol} \xrightarrow{\text{peroxidase}} \text{quinoneinimine} + 4\,H_2O$$

The enzyme glucose oxidase catalyses oxidation of glucose by the oxygen. In this reaction arises gluconate and hydrogen peroxide. It becomes the substrate for consequential reaction catalyzed by peroxidase and substituted phenol and 4-aminoantipyrine is copulated and form red colored complex, which can be measured spectrophotometrically. The intensity of color is proportional to concentration of glucose in sample.

REAGENTS:

Reagent (A) : phosphate 70 mmol/L, phenol 5 mmol/L, glucose oxidase > 10U/mL, peroxidase > 1 U/mL, 4-aminoantipyrine 0,4 mmol/L, pH = 7,5

Standard (S) : Biosystems (glucose in concentration of 100 mg/dl (**5,55 mmol/L)**

Own urine (U): Urine for analysis bring with you to exercise!

MATERIAL (samples)

We will analyze the samples of own urine and control solution (controlled solution of serum, with attested glucose value) based on the standard recommended for calibration of the method on automatic analyzers at a concentration of 5.55 mmol / L (unless otherwise specified by the supervisor).

PROCEDURE

1. Prepare the test tubes – mark 1-8.
2. Pipette blank (water), standard and samples (urine, unknown calibrator) to the bottom of tubes according to a table in duplicates, use the pipette with fixed volume 10 µl (small tips)
3. Pipette Reagent A to each tube - 1ml with automatic pipette (for 100-1000 µl, large tips)

4. TABLE FOR PIPETTING

Test tube	Sample	Volume of sample	Reagent A	**A_{500} measurement**	**A_{500} mean of 2 measurements**	**A500sample-A500blank Y axis**
1	Blank **water**	10µl	1000µl			
2						
3	Standard **5,55 mM**	10µl	1000µl			
4						
5	Sample **urine**	10µl	1000µl			
6						
7	Calibrator **unknown**	10µl	1000µl			
8						

5. Mix thoroughly on VORTEX and incubate the tubes for 10 minutes at room temperature (16-25°C).
6. Measure the absorbance of blanks (A_{Bl}), samples (A_{Sam}) (standard, urine and calibrator) and standards (A_{St}) **at 500 nm** against deionized water.
7. Use spectrophotometer **HELIOS.** First water, than samples in order 1,2,5,6,3,4,7,8
8. Calculate concentrations of glucose in urine and control solution (calibrator) using the following formula:

CALCULATION METHOD 1) Method is based on an attested standard

$$C_{Sam} = \frac{A_{Sam} - A_{Bl}}{A_{St} - A_{Bl}} \times C_{St}(Glu) = GLUCOSE \quad (mmol/L)$$

For calculation use concentration of standard: **$C_{St}(Glu)$ = 5,55 mmol/L**

Make calculation:

Urine =

Control solution=

9. PREPARE PROTOCOL, compare your calculated concentrations of urine and control solution/calibrator with table values and physiological concentrations

Glycosuria

Glycosuria or **glucosuria** is the excretion of glucose into the urine. Ordinarily, urine contains no glucose because the kidneys are able to reclaim all of the filtered glucose back into the bloodstream. Glycosuria is nearly always caused by elevated blood glucose levels, most commonly due to untreated diabetes mellitus. Rarely, glycosuria is due to an intrinsic problem with glucose reabsorption within the kidneys themselves, a condition termed renal glycosuria. Glycosuria leads to excessive water loss into the urine with resultant dehydration, a process called osmotic diuresis.

Pathophysiology

Blood is filtered by millions of nephrons, the functional units that comprise the kidneys. In each nephron, blood flows from the arteriole into the glomerulus, a tuft of leaky capillaries. Bowman's capsule surrounds each glomerulus, and collects the filtrate that the glomerulus forms. The filtrate contains waste products (e.g. urea), electrolytes (e.g. sodium, potassium, chloride), amino acids, and glucose. The filtrate passes into the renal tubules of the kidney. In the first part of the renal tubule, the proximal tubule, glucose is reabsorbed from the filtrate, across the tubular epithelium and into the bloodstream. The proximal tubule can only reabsorb a limited amount of glucose. When the blood glucose level exceeds about 160 – 180 mg/dl, the proximal tubule becomes overwhelmed and begins to excrete glucose in the urine. Normally value in urine is 0,72 mmol/day (in 24 hours).

This point is called the renal threshold of glucose (RTG). Some people, especially children and pregnant women, may have a low RTG (less than ~7 mmol/L glucose in blood to have glucosuria).

If the RTG is so low that even normal blood glucose levels produce the condition, it is referred to as renal glycosuria (failure of renal tubular cells).

PROTOCOL

Objective :

Principe – summary:

Results - measured values :

Calculations:

Urine
Control solution

Results:

Summary:

Answer the test questions:

1. What is blank?

2. Explain the principle of glucose measurement. What will you measure? At which wavelength will you measure?

3. What is spectrophotometry?

4. Give Beer-Lambert law?

HELP

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

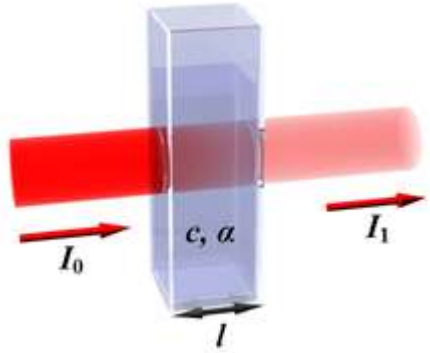
Spectrophotometry involves the use of a spectrophotometer. A spectrophotometer is a photometer that can measure intensity as a function of the light source wavelength. Important features of spectrophotometers are spectral bandwidth and linear range of absorption or reflectance measurement.

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)



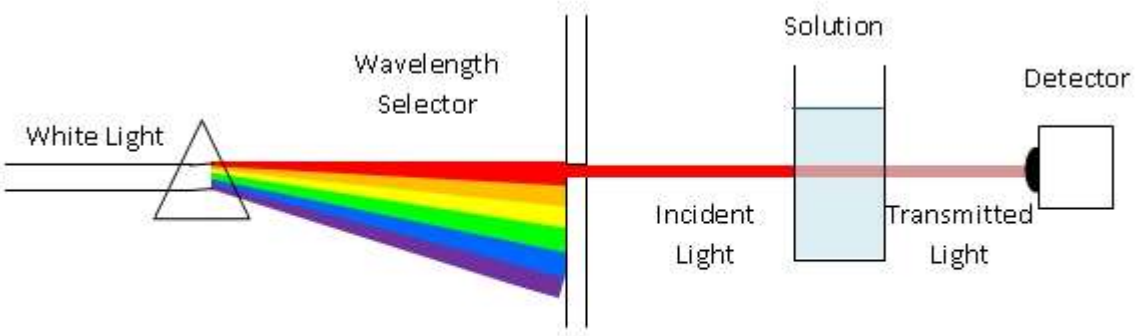
BLANK

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.

- Spectrophotometry
-
- **Spectrometry**
- Spectrophotometry is a method used to estimate the level of an analyte in solution. It relies on the principle that materials absorb light of a certain wavelength as it passes through the solution.

- Beer's Law states that the amount of light of a particular wavelength absorbed by a substance across a constant distance (lightpath) is proportional to the concentration of that substance.
-
- **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)

- 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.