Polarimetric determination of glucose

THEORY

Polarimetry is the method used for the determination of concentration of optically active substance dissolved in the solution. The optical activity of the substance is caused by the presence of a chiral, i.e. asymmetric carbon, with four different substituents. It is mostly organic substances – for example some sugars, amino acids, or alcohols. Polarimeter is a device which consists of two Nicol prisms honed from Iceland spar. The first prism is firmly positioned and passes only light in one plane of oscillation, thus polarizes the light. Polarized beam passes through the sample and rotates the plane of oscillation. The angle of rotation (unit: °, degrees) is dependent on the measured substance, its concentration, the ambient temperature and the distance the beam passes, i.e. the length of the polarizing tube. The second nicol prism is connected with a scale and is used to measure the optical rotation of the beam. If optically inactive substance is measured, for example distilled water, the polarized beam passes through in the same plane and the scale reads 0°. As soon as the beam passes through an optically active sample, we turn the second nicol prisms on so many degrees so the beam can pass through, i. e. to see the uniformly illuminated background – half-shade. As a source of light in polarimetry sodium lamp with a constant wavelength is used.

The dependence of the angle of rotation on the concentration of optically active substance in the solution can be expressed as:

$$\alpha = \alpha_D^{20} \cdot l \cdot c ,$$

where $\boldsymbol{\alpha_D^{20}}$ is specific rotation, characteristic for the substance; it is the angle of rotation of the polarized light plane when the length of the cuvette is l = 1 dm and concentration of solution is c = 1 g · ml^{-1}. The value is usually given for the sodium doublet line D (λ = 589.3 nm) and temperature 20 °C.

TASK

- Construct a calibration curve of dependence of rotation angle on the glucose concentration in the solution. Determine the concentration of unknown glucose samples using polarimetry.

EQUIPMENT AND CHEMICALS

- Manual polarimeter + light source (sodium lamp); 2× polarimetric cuvette; 6× 50ml volumetric flask; graduated pipette (25 ml); manual propipetter; 3× beaker; stock solution of glucose (2.5 mol · dm^{-3}); unknown samples I and II.

PROCEDURE

- Using stock solution of glucose (2.5 mol · dm^{-3}) prepare 5 calibration solutions of concentrations 0.5; 1.0; 1.5; 2.0 and 2.5 mol · dm^{-3} into 50ml volumetric flasks. **Mix the stock solution thoroughly before the pipetting!**
- Fill the polarimetric cuvette as follows: At first unscrew the cuvette cap on one side and be careful not to let the circular slide loosely embedded in the cap fall off. Then fill the cuvette with measured solution (**at first with distilled water, then gradually with all calibration solutions and finally with unknown samples** – use the same unknown samples as for refractometry). Then put the slide on the cuvette from side and close it with cap. Finally dry the cuvette with a cellulose wadding. Especially the slides through which the light beam pass should be clean and dry. Properly filled cuvette must not contain air bubbles! – or place the bubbles to the bulging space in the cuvette – to **prevent light beam from passing through the bubbles**.
- Carry out measurement using **manual polarimeter**: Try to find half-shade for a sample of distilled water, the scale should read 0 ± 2°. For other solutions **slightly** turn the screw to reach the half-shade (towards gradual rising values, not back through 180°).
- After the measurement rinse the polarimetric cuvette thoroughly with distilled water.

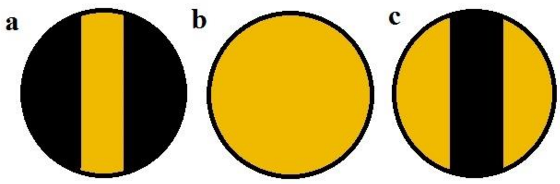
PROTOCOL

- Calculation of dilution for preparation of all solutions.
- For manual polarimeter: Measured values for all solutions. Calibration table and graph: the dependence of the angle of rotation of glucose on the concentration of the calibration solutions *c* (mol · dm^{-3}). Calculation of the concentration of the unknown samples using the linear regression equation of the calibration curve.

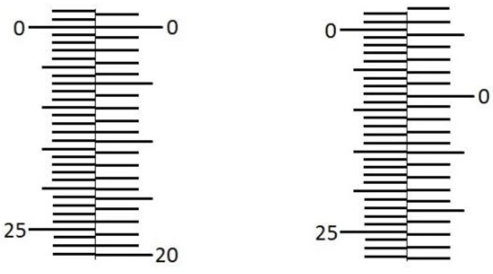
Operating instructions for manual polarimeter

- Manual polarimeter with rotating prism: The angle of rotation is read off on a circular scale by setting an appropriate half-shade through the eyepiece.
- The apparatus contain eyepiece (1), scale for measuring the angle of rotation (2), polarimetric screw (3), cuvette space (4), light source (5).

- Connect the light source to the mains supply.
- Insert the cuvette filled with distilled water into the cuvette space and close the cuvette space by a lid.
- Set the polarimetric screw to a position to reach the half-shade in eyepiece.
- Transmitting of the light beam through the polarimeter: a, c – light does not pass through, b – light passes through (desired half-shade):



- Then read off the angle of rotation **to 1 decimal place**. The polarimetric scale consists of two parts. The scale on the left is fixed; scale on the right is movable using polarimetric screw. At first find the value 0° on the left scale, then read off the angle of rotation from the right scale (the value that corresponds to the zero on the left scale). It should be read off approximately 0° for a blank (distilled water). For the sample of glucose it can be for example 5.5°.



$\alpha = 0°$ $\alpha = 5.5°$

- After the measurement disconnect the light source from the mains supply.

Refractometry

THEORY

Monochromatic light, which passes from one medium to another, changes its speed. The ratio of the speed of light in vacuum *c* to the speed of light in another medium *v* is called absolute refractive index *n*.

$$n = \frac{c}{v}$$

In the case of passing from the medium with refractive index n_1 to the medium with refractive index n_2, relative refractive index is used. This variable is corresponding to the ratio: the sine of the angle of incidence divided by the sine of the angle of refraction at the interface of media.

$$n = \frac{n_1}{n_2} = \frac{v_2}{v_1} = \frac{sin\alpha_2}{sin\alpha_1}$$

The speed of the light in air and in vacuum are only little different, therefore the refractive index at the interface of the test substance and air can be considered as absolute refractive index.

For the refraction measurement instruments called refractometers are used. In laboratory practice hand refractometer is available, it is used especially in the food industry for determining the sugar content in fruit, juices and musts etc. Brix scale in percentage (which is a recalculation of refractive index) represents the weight percentage of sugar in the solution, i.e. grams of sugar per 100 g of solution. The device includes automatic temperature correction.

TASK

- Determine values of % Brix for all prepared samples and construct the graph of the dependence of % Brix on the glucose concentration in the solution.

EQUIPMENT AND CHEMICALS

- Refractometer; 5× 50ml volumetric flask; 2× beaker; graduated pipette (25 ml); manual propipetter; plastic dropper; stock solution of glucose (2.5 mol · dm^{-3}); unknown samples I and II.

PROCEDURE

- Using stock solution of glucose (2.5 mol · dm^{-3}) prepare 5 calibration solutions of concentrations 0.50; 0.75; 1.00; 1.25 and 1.50 mol · dm^{-3} into 50ml volumetric flasks. **Mix the stock solution thoroughly before the pipetting!**
- The procedure for measuring using refractometer: Add a few drops of distilled water to the prism with a dropper (or a pipette) and cover the cover plate so as to avoid bubble formation. Turn the refractometer on the light source (towards the window) and observe a sharp interface intersecting

the scale in the point of 0. Then add a few drops of prepared sample to the prism and read off the value in % Brix from the left scale. After each measurement dry the surface of the prism with a cellulose wadding. Carry out the measurement **three times for each measured solution** (**all calibration samples and unknown samples** – use the same unknown samples as for polarimetry).

PROTOCOL

- Calculation of dilution for preparation of all solutions.
- Table of measured values for each sample and calculated means.
- Graph of the dependence of % Brix on the glucose concentration. Determined concentrations of unknown samples.