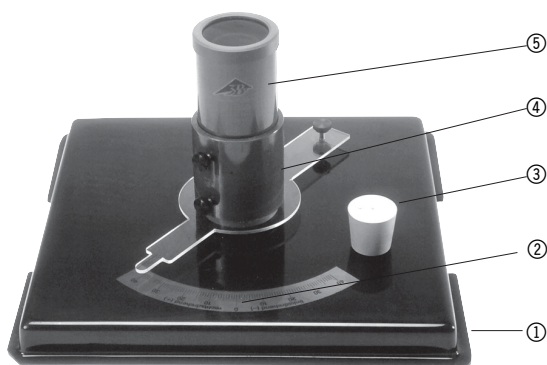


U14390 Polarization Demonstration Device

Operating instructions

3/03 ALF



- ① Base plate
- ② Angular scale
- ③ Plug
- ④ Rotary grip with a pointer
- ⑤ Analyzer

The U14390 polarization device is designed for use on a daylight projector as part of qualitative and quantitative experiments conducted before large audiences at schools and universities, for example in order to demonstrate optical activity as well as determinations of specific angles of rotation, or concentrations if these angles are already known.

1. Safety notes

- Do not clean the polarization demonstration device with aggressive agents.
- Do not fill the cuvette with liquids which attack plexiglass.
- Ensure that the filters do not get scratched.

2. Description, technical data

A yellow filter and a polarizer are set in the middle of a black, plastic base plate. A cuvette marked at 50 mm and 100 mm and containing a solution of the substance to be examined is inserted into the inner holder and subsequently covered by an analyzer mounted on an outer holder equipped with a rotary knob and a pointer. Turning the analyzer allows the angle of rotation to be read on a transparent scale ranging from $+40^\circ$ to -40° and having divisions of 1° .
 Dimensions: 370 mm x 330 mm x 190 mm

Illustration 1:

- ① Yellow filter
- ② Polarizer
- ③ Holder for the cuvette
- ④ 50-mm mark
- ⑤ Holder with rotary knob and pointer
- ⑥ 100-mm mark
- ⑦ Cuvette

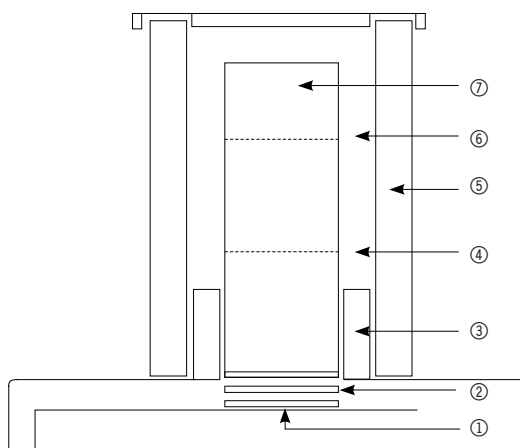
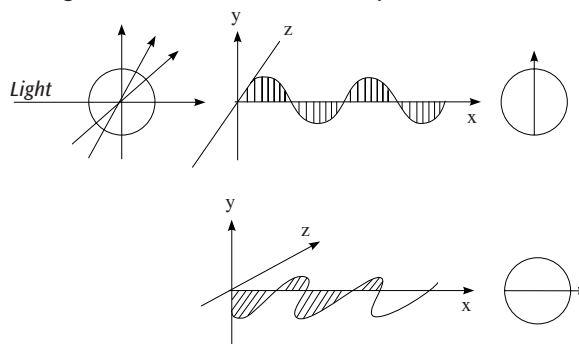


Illustration 1

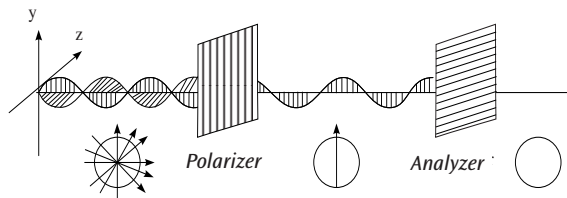
2. Operating principle

Light (visible electromagnetic radiation) emitted by the daylight projector is made to pass through a yellow filter, as yellow light by definition increases the measurement accuracy.

This light oscillates in a number of planes:



The first polarization filter, or polarizer, preferentially allows the passage of one of the oscillation planes, thus polarizing the light. If a second polarization filter (analyzer) rotated by 90° with respect to the first one is connected in series, the polarized light is largely absorbed, because the lattices formed by these mutually perpendicular filters are transverse with respect to the oscillation planes. The result is maximum extinction.



If the light path is made to pass through a substance (comprising the solution in the cuvette) which rotates the oscillation plane of the polarized light either to the left or the right, i.e. an optically active substance, the analyzer needs to be turned accordingly in order to maximize extinction again.

The angle (in degrees) between maximum absorption with and without the cuvette solution, or between pure solvent and solution, is determined by turning the analyzer; this angle is a decisive parameter, in addition to the concentration of the solution and the filling level of the cuvette.

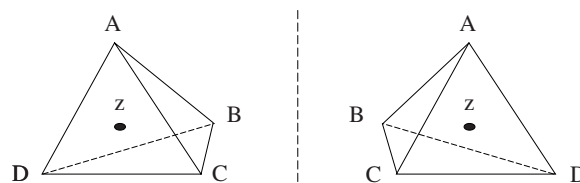
4. Operation

- Place the polarization demonstration device on the daylight projector and focus the image of the scale.
- Set the pointer to zero. Rotate the analyzer so that the extinction is maximized. No light spot from the light path should be visible on the projection area.
- Fill the cuvette with the pure solvent and insert it into its holder.
- Turn the pointer to the left and the right until a light spot just becomes visible again on both these sides of the scale. The value located exactly between these two measurement results serves as the zero-point or reference point for further measurements. Ideally, it coincides with the zero mark on the scale. Example: Measurement limits of -6° and $+4^\circ$ result in a reference value of -1° .
- After that place the cuvette with a solution of the optically active substance in the light path, and note the filling level for future calculations.
- As in the case of the pure solvent, establish the points on either side at which maximum absorption occurs, i.e. at which the light spot just appears again. This will allow you to determine the angle of rotation.
For instance, limits of -21° and -11° would result in a reference value of -16° here. If the reference value of the pure solvent was -1° , the measured angle of rotation α is -15° .

5. Polarimetry

Compounds which carry four different substituents or ligands at a center (activity center) and which can be reflected on a mirror plane are termed optically active (chiral).

Mirror plane



Such compounds behave like objects and their mirror images, and are not superimposable (enantiomeric forms). Optically active substances rotate the oscillation plane of light. If 50% of each form is present in the mixture (racemate), rotation is cancelled. If one of the two forms predominates, the oscillation plane is rotated as a whole. The angle of rotation α is a material constant which depends on the following conditions, in addition to the nature of the particles:

- Wavelength of the light: As the general convention is to use the sodium-D line of the emitted light (Na vapor discharge lamps) for exact measurements, the bottom of the device is fitted with a yellow filter to approximate this spectral range.
- Temperature: 20°C are usually specified for measurements.
- The number of rotating particles: Dependence on the concentration of the dissolved substance and the layer thickness of the solution (= filling level of the cuvette); proportional relationship.
- Solvent.

Rotation expressed with respect to a particular quantity of optically active substance (right-handed = +, left-handed = -; angle of rotation) is a material constant termed specific rotation (specific angle of rotation).

$$[\alpha]_D^{20} = \frac{\pm \alpha \cdot 100}{c \cdot d}$$

- $[\alpha]_D^{20}$ = Spec. angle of rot. for the Na-D line at 20°C
 α = Measured angle of rotation (scale reading)
 c = Concentration in grams/100 ml (g/0.1 dm³) of solution
 d = Layer thickness (filling level) in dm.

5.1 Examples

Examples of specific angles of rotation $[\alpha]_D^{20}$ (End rotation) in degrees:

D-glucose: +52.7; D-fructose: -92.4; D-mannose: +14.6;
 D-galactose: +80.2; D-xylulose: +33.1; D-ribose: -23.7;
 Saccharose: +66.5; Maltose: +130.4; Lactose: +52.5
 (values provided by Aebi, Einführung in die praktische Biochemie, Karger 1982)

α -D-glucose 113.0 (crystallized from water); α -D-glucose +19.0 (crystallized from pyridine); α -Hydroxybutyric acid -24.8; Protein -52.8
 (values provided by Rapoport/ Raderecht, Physiologisch-chemisches Praktikum, VEB Verlag Volk u. Gesundheit, 1972).

6. Experiment examples

6.1 Specific angle of rotation of saccharose

Initial weight: Dissolve 50 g of saccharose in water in a volumetric flask and fill up to the 100 ml mark. The resulting solution is poured into the cuvette to a height of 10 cm (1 dm). The following angle of rotation is measured: 32°, right-handed.

$$\text{Specific angle of rotation: } [\alpha]_D^{20} = \frac{+32 \cdot 100}{50 \cdot 1} = +64$$

The determined specific angle of rotation is thus of the same order of magnitude as the bibliographic value.

Note: Even high-precision polarimeters are not always able to achieve bibliographic values. Due to tautomerism or mutarotation (α - or β - form), it may be necessary for a certain amount of time to elapse before equilibrium is reached. Solutions of mutarotating sugar should be left standing for extended periods (overnight) following their preparation.

Watch out for yeast and bacteria after long periods of storage! When weighing sugars (for instance, glucose), carefully read the label on the chemical bottle. Any crystal water (monohydrate) must be indicated on the label, and either compensated by means of an additional calculated dose or subtracted during the calculation later (g/100 ml).

6.2 Measurement of concentration

The specific angle of rotation of a substance is measured first. After that, a solution with an unknown concentration of this substance (or known only to the trainer) is prepared. Filling level $d = 1$ dm,

$$c = ? \quad [\alpha]_D^{20} = +64^\circ$$

$$\alpha \text{ (measured)} = +14^\circ$$

The concentration c in g/0.1 dm³ is calculated as follows:

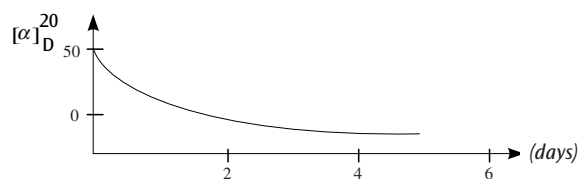
$$c = \frac{\alpha \cdot 100}{[\alpha]_D^{20} \cdot d} = \frac{+14 \cdot 100}{+64 \cdot 1} = 21,9 \text{ g/100ml}$$

6.3 Inversion of saccharose

Acid can be used to split the disaccharide saccharose into D-glucose and D-fructose. The solution of these fission products – also optically active – has a different angle of rotation compared with saccharose (inversion). A glucose-fructose mixture with a molar ratio of 1:1 is therefore termed invert sugar (for instance, in artificial honey). At room temperature, the specific angle of rotation changes over a period ranging between several hours and several days, depending on the acid concentration. Higher temperatures notably accelerate the inversion process (to a matter of hours). The specific angle of rotation changes from +66° to roughly –22° (saccharose: +66°; glucose („equilibrium glucose“): +52°; D-fructose: -92.4°).

Recommendation: Dissolve up to 50 g of saccharose in a little water, and top the solution up to 100 ml with

more water and 5 - 20 ml of dilute hydrochloric acid. At room temperature, perform measurements initially at 10-minute intervals, then at hourly intervals; convert the read angles of rotation into specific angles of rotation, and plot these values in a diagram.



If inversion is to be performed at higher temperatures, it is advisable to use a thermostatted solution (water bath) of a higher volume (1-2 l). Before performing the measurements, draw samples, allow them to cool quickly, and pour them into the measuring cuvette.

6.4 Wine

Wine exhibiting right-handed rotation may have been mixed with glucose before or after fermentation, or with saccharose after fermentation. Wine exhibiting left-handed rotation is natural (according to Dr. Steeg & Reuter).

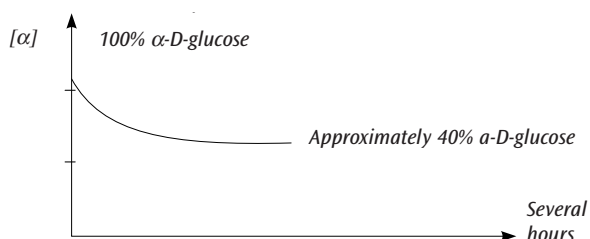
6.5 Mutarotation in the case of anomeric C-atoms

Mutarotation occurs when a solution of an optically active substance changes its angle of rotation, gradually leading to a state of equilibrium.

D-glucose is weighed and dissolved quickly by shaking. The angle of rotation is determined at regular time intervals, converted immediately into the specific angle of rotation, and plotted in a diagram.

$[\alpha]_D^{20}$ of α -D-glucose: 112-113°; after equilibrium has been reached (several hours): +52°

A mixture of α - and β -D-glucose is now present. Mutarotation in the case of fructose takes place much more quickly.



5. Care

The Perspex cuvette is only suitable for liquids which do not attack it. However, the focus of interest here in any case is aqueous solutions. Before inserting the cuvette, always ensure that it is clean and dry! The cover must on all accounts be closed if the measurement will take long or if the cuvette is to remain inside the device (as in the case of mutarotation, refer to 4.5). Cleaning should be performed with a soft, dust-free cloth. Do not scratch the filters! It is advisable to store the device under dust-free conditions (in an anti-dust jacket).