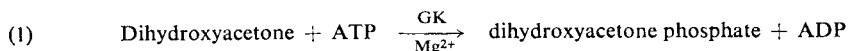


Dihydroxyacetone

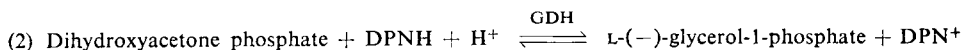
Otto Wieland

Principle

Dihydroxyacetone is phosphorylated by adenosine triphosphate (ATP) in the presence of glycerokinase (GK) to give dihydroxyacetone phosphate.



The dihydroxyacetone phosphate formed is measured by reduction with α -glycerophosphate dehydrogenase (GDH) and reduced diphosphopyridine nucleotide (DPNH).



Each mole of dihydroxyacetone oxidizes 1 mole of DPNH.

Reagents

See determination of glycerol (p. 211), but substitute:

1. Triethanolamine
2. Hydrochloric acid, 2 N
7. Reduced diphosphopyridine nucleotide, DPNH
disodium salt, DPNH-Na₂; commercial preparation, see p. 1011.

Preparation of Solutions

See determination of glycerol (p. 212), but substitute:

- I. Triethanolamine buffer (0.05 M containing 2×10^{-3} M Mg²⁺; pH 7.15):
Dissolve 0.75 g. triethanolamine in distilled water, add 0.2 ml. 1 M MgCl₂ solution, adjust pH to 7.15 with *ca.* 4 ml. 2 N HCl and dilute with distilled water to 100 ml.
- V. Reduced diphosphopyridine nucleotide (*ca.* 0.006 M β -DPNH):
Dissolve 11.2 mg. DPNH-Na₂ in distilled water and make up to 2.0 ml.

Procedure

Experimental material and deproteinization: see determination of glycerol (p. 212, 213).

Spectrophotometric measurements

Wavelength: 366 m μ ; light path: 1 cm.; final volume: 2 ml. Read against air or water.

Pipette the solutions into the cuvette in the following order:

- 1.37 ml. buffer (solution I)
- 0.05 ml. DPNH solution (V)
- 0.05 ml. ATP solution (IV)
- 0.02 ml. GDH suspension (VI)
- 0.50 ml. deproteinized sample.

Proceed as for the determination of glycerol (p. 213). The only difference is that after the addition of

0.01–0.02 ml. glycerokinase suspension (VII)

a decrease in optical density occurs.

Calculations

Since $\frac{\Delta E \times V}{\epsilon \times d} = \mu\text{moles substance/assay mixture}$

where

$$V = 2 \text{ ml.}$$

$$\epsilon_{366} = 3.3 \text{ [cm.}^2/\mu\text{mole]}$$

$$d = 1 \text{ cm.}$$

then $\frac{\Delta E_{366} \times 2}{3.3} = \mu\text{moles dihydroxyacetone/assay mixture.}$