14.2 Determination of uric acid by uricase method

Principle

Uric acid absorbs UV at 293 nm, although other compounds may also absorb at this wavelength. When samples are treated with uricase, uric acid is converted to allantoin and other compounds that do not absorb UV at 293 nm. Therefore, the reduction in OD reading after treatment with uricase is correlated with the concentration of uric acid in the sample. After treatment, the OD of the standards should be zero if the conversion is complete.

Reagents

- KH_2PO_4 buffer, 0.67 M, pH 9.4. Adjust the pH with KOH.
- Uricase from porcine liver. Prepare an enzyme solution of 0.12 unit/ml buffer.
- Uric acid.

Equipment

Spectrophotometer

Water bath

Ultrasonic bath (optional)

Standards

Prepare uric acid standard working concentrations of 5, 10, 20, 30 and 40 mg/L.

Fig. 14.2 Water bath

Procedure

- Pipette 1 ml of urine or standard or blank (distilled water) into 10 ml tubes. Mix with 2 5 ml phosphate buffer. Prepare two sets of tubes.
- To one set, add 150 ul buffer and to the other add 150 ul of uncase solution. Mix well.
- Incubate in the water bath at 37ºC for 90 min (Fig. 14.2).
- Remove from water bath, mix and transfer the solutions to cuvettes and read the OD at 293 nm.
- If the enzymatic conversion is complete, the OD of the standards with uncase added should be zero If not, incubate in water bath for an additional 30 min and read again

Standard curve and calculation

- Standard curve is curvilinear. When both X and Y are transformed to Ln functions, Ln (Y) is linearly correlated to Ln (X).
- Use the OD reading of the set without addition of uncase for the construction of standard curve.
- Calculate the net reduction in OD (AOD) for the samples due to uricase treatment OD = OD without enzyme - OD with enzyme.
- Calculate the uric acid concentration from OD based on the established standard equation.

Reference: I.A.E.A. 1997. Estimation of rumen microbial protein production from purine derivatives in urine. A laboratory manual, IAEA – TECDOC – 945. IAEA, Vienna.