7.15 Determination of serum/ plasma urea

Principle

A pink colour complex is formed when urea is treated with di-acetyl monoxime and thiosemicarbazide in the presence of sulphuric acid, phosphoric acid and ferric chloride which is read at 525 nm in spectrophotometer.

Reagents

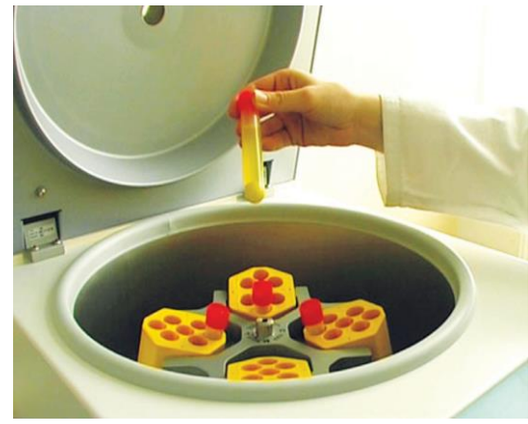

Fig. 7.13 Centrifuging of extracts

1. Reagent A:
   300 ml concentrated H_2SO_4 (95-98%)
   50 ml distilled water
   100 ml concentrated H_3PO_4 (85%)
   100 mg ferric chloride ($FeCl_3$)
   Mix well and volume it to 1 litre with distilled water.
2. Reagent B:
   500 g diacetyl monoxime (DAMO)
   10 mg thiosemicarbazide (TSC)
   Mix well with distilled water to make 100 ml solution
3. Reagent C:
   Prepare immediately before use with Reagents A & B at 2:1 proportion.
4. Trichloro acetic acid (TCA) 5% (w/v) with distilled water.
5. Standard urea solution (0-150 nmol)
   Working standard (50 µg/ml): Dissolve 50 mg urea in 1 litre of distilled water.

Procedure

1. Protein is precipitated out from serum/plasma 0.2 ml mixed with 1.8 ml of 5 per cent TCA.
2. Centrifuge at 2000 rpm for 10 min (Fig. 7.13) and collect supernatant for determination using the protocol given below.
3. Boil in water bath for 5 minutes.
4. Cool to room temperature and read absorbance at 525 nm against reagent blank.
5. Plot a standard curve and read concentration of the sample against absorbance.

Protocol

Reagents/ Solutions (ml)	Blank	Sample	Standard				
			1	2	3	4	5
Protein free sample	0.20	-	-	-	-	-	-
Standard	-	-	0.02	0.04	0.06	0.08	0.10
Distilled water	-	0.20	0.18	0.16	0.14	0.12	0.10
Reagent C	3.00	3.00	3.00	3.00	3.00	3.00	3.00

Calculation

OD unknown

$$\text{Urea (mg/ 100 ml)} = \frac{\text{OD standard}}{\text{concentration of standard}} \times \text{concentration of standard} \times 50$$

Reference: Rahmatullah, M. and Boyde, T.R.C. 1980. An improvement in determination of urea using diacetylmonoxime method with and without deproteinization. *Cli. Chem. Acta,*. 107: 3-9.