Appendix
APPENDIX

Reagents for determination of carbonic anhydrase activity

1. **Bromothymol blue indicator in ethanol (0.002%)**
   
   0.002 g bromothymol blue was dissolved in approximately 100 mL DDW.

2. **Cystein hydrochloride solution (0.2 M)**
   
   48 g cystein hydrochloride was dissolved in sufficient DDW and final volume was made up to 1000 mL with DDW.

3. **Hydrochloric acid (0.05 N)**
   
   4.3 mL pure hydrochloric acid was mixed with 0.057 mL DDW.

4. **Phosphate buffer (0.2 M) for pH 6.8**
   
   This was prepared by dissolving 27.8 g sodium dihydrogen orthophosphate and 53.65 g di-sodium hydrogen orthophosphate in sufficient DDW separately and final volume of each was maintained up to 1000 mL with DDW. To get pH 6.8, 5 mL of monobasic sodium phosphate solution was mixed with 49 mL of dibasic sodium phosphate solution and diluted to 200 mL with DDW.

5. **Sodium bicarbonate solution (0.2 M) in 0.02 M sodium hydroxide solution**
   
   16.8 g sodium bicarbonate was dissolved in sodium hydroxide solution (0.8 g NaOH/l) and final volume was maintained up to 1000 mL with the sodium hydroxide solution.

Reagents for the estimation of nitrogen, phosphorus and potassium

1. **Aminonaphthol sulphonic acid**
   
   500 mg l-amino-2-naphthol-4-sulphonic acid was dissolved in 195 mL 15% sodium bisulphite to which 5 mL 20% sodium sulphite solution was added. The solution was kept in an amber coloured bottle.

2. **Molybdic acid reagent**
   
   6.25 g ammonium molybdate was dissolved in 175 mL 10 N H₂SO₄.
3. **Nessler's reagent**

3.5 g potassium iodide was dissolved in 100 mL DDW in which 4% mercuric chloride was added with stirring until a slide red precipitate remains, then 120 g NaOH was mixed with 250 mL DDW. The mixture was kept in an amber coloured bottle.

4. **Sodium hydroxide solution (2.5 N)**

100 g sodium hydroxide was dissolved in sufficient DDW and final volume was maintained up to 1000 mL with DDW.

5. **Sodium silicate solution (10%)**

109 sodium silicate was dissolved in sufficient DDW and final volume was made up to 100 mL with DDW.

6. **Sulphuric acid (10 N)**

27.2 mL sulphuric acid was mixed with 72.8 mL DDW.