APPENDIX-II

BUFFER USED

1. **Phosphate Buffer**: (0.12 M/l, pH 7.2 (37°C) for 1l of buffer, 16.33 g KH2PO4 was dissolved in 800 ml water, pH adjusted to 7.2 (at 37°C) with NaOH, 1 M/l and diluted to 1 l with water.

2. **Phosphate buffered saline**: 9.85 g of phosphate buffer (Hi-Media, India) was dissolved in distilled water and pH adjusted to 7.2. Total volume was raised to 1000 ml.

3. **Phosphate buffer with 0.2M NaCl**
   Stock solution A : 0.2 M solution of monobasic sodium phosphate (31.2 g NaH2PO4 2H2O in 1000 ml).
   Stock solution B : 0.2 M solution of dibasic sodium phosphate (28.39 g of Na2HPO4 or 71.7 g of Na2HPO4 12H2O in 1000 ml).
   Stock solution C : 58.5 g of NaCl was dissolved in distilled water and volume was made to 1000 ml.
   39 ml of A + 61 ml of B and 200 ml of C, diluted to 1000 ml.
   Filtered through sintered glass funnel (G2) and used for chromatography.

4. **Sodium acetate buffer**: 6.8 g of sodium acetate trihydrated (Qualigens, India) was dissolved in 900 ml of distilled water and pH of solution was adjusted to 6.5 by adding glacial acetic acid. Total volume of the solution was raised to 1000 ml.

5. **Tris Buffer** (0.5 M/l): 6.05 g Tris base in 70-80 ml water. pH was adjusted to 7.5 with HCl. Made up to 100 ml with water.