Practical No. 2: To Prepare Phosphate Buffer Saline for Animal Cell Culture

Materials required: NaCl, KCl, Na2HPO4 · 2H2O, KH2PO4, Double distilled water, HCl for pH adjustment, Glassware, Pipettes, Weighing Machine, pH meter, Stirrer, 0.45micron Filters.

Principle: Phosphate buffered saline (abbreviated as PBS) is a buffer solution commonly used in biological research. It is a salty solution containing sodium chloride, sodium phosphate, and (in some formulations) potassium chloride and potassium phosphate. The buffer helps to maintain a constant pH. The osmolarity and ion concentrations of the solution usually match those of the human body (isotonic). PBS has many uses because it is isotonic and non-toxic to cells. It can be used to dilute substances. It is used to rinse containers containing cells. PBS can be used as a diluent in methods to dry biomolecules, as water molecules within it will be structured around the substance (protein, for example) to be 'dried' and immobilized to a solid surface. The thin film of water that binds to the substance prevents denaturation or other conformational changes. Carbonate buffers may be used for the same purpose but with less effectiveness. PBS can be used to take a reference spectrum when measuring the protein adsorption in ellipsometry. Additives can be used to add function. For example, PBS with EDTA is also used to disengage attached and clumped cells. Divalent metals such as zinc, however, cannot be added as this will result in precipitation.

Procedure:

1. Add the following compounds as mentioned in the table below

Reagent	Amount to add (for 1× solution)	Final concentration (1×)	Amount to add (for 10× stock)	Final concentration (10×)
NaCl	8 g	137 mM	80 g	1.37 M
KCl	0.2 g	2.7 mM	2 g	27 mM
Na_2HPO_4	1.44 g	10 mM	14.4 g	100 mM
KH_2PO_4	0.24 g	1.8 mM	2.4 g	18 mM
If necessary, PBS may be supplemented with the following:				
$CaCl_2 \cdot 2H_2O$	0.133 g	1 mM	1.33 g	10 mM
$MgCl_2 \cdot 6H_2O$	0.10 g	0.5 mM	1.0 g	5 mM

2. PBS can be made as a 1× solution or as a 10× stock.

3. To prepare 1 L of either 1× or 10× PBS, dissolve the reagents listed above in 800 mL of H_2O.
4. Adjust the pH to 7.4 (or 7.2, if required) with HCl, and then add H_2O to 1 L.
5. Dispense the solution into aliquots and sterilize them by autoclaving for 20 min at 15 psi (1.05 kg/cm^2) on liquid cycle or by filter sterilization.
6. Store PBS at room temperature.

Precautions:

a. The PBS can be prepared in 10X concentration stock solution and working solution (1X) can be prepared by diluting the stock solution with sterile autoclaved double distilled water.
b. Autoclave should be used in presence of lab technician, trained staff or teacher.
c. HCl should be added dropwise for pH adjustment. Never add more HCl than required and then add NaOH for adjusting pH again.