2.2.2.1. Preparation of insulin-chitosan polyelectrolyte complex (PEC) aqueous phase

The aqueous phase was composed of chitosan and insulin mixture in solution state. The preparation of the aqueous phase was carried out in two stages. The first stage represented the preparation of chitosan solution while the second stage involved preparation of insulin solution.

In order to prepare the chitosan solution, in clean glass vial, the low molecular chitosan (13KDa and 99% DAA) in weight of 125 mg was dissolved in 4 ml deionized water. By using pH meter the pH of the solution was adjusted to 5.5 using 0.2 M of NaOH added drop by drop under stirring using magnetic stirrer and the final volume should be completed to 5 ml using water.

Conversely, the insulin solution was prepared by weighting 25 mg of recombined insulin (rh-insulin) in another clean vial. The insulin powder was dissolved in 250 µl of 0.1 M HCL under gentle hand shaking then 750 µl of 1 M modified Tris (hydroxymethyl)-aminomethane buffer pH 6.95.

The modified Tris buffer was composed of 400 mg of hydroxyl propyl ß-cyclo dextrin (HPßCD) dissolved in 10 ml of 1 M tris buffer.

The polyelectrolyte complex solution was constructed by the addition of 1 ml insulin solution to 1 ml chitosan solution drop wisely in new vial. The equal volumes of the chitosan and insulin were shaked gently for 15 minutes at room temperature.