Summary & Conclusions
Suspension culture systems (derived from mammalian cells) are being used increasingly in the biotechnology industry due to the thorough understanding of their engineering, standardization of process at large scale and well establishment of automation and cleaning procedures. Mammalian cell suspension systems offer the possibility of quick implementation of production protocols due to their ability to be scaled-up easily, once the basic culture parameters are optimized (Birch and Arathoon, 1990).

There are many advantages of suspension cultures including simplicity and homogeneity of culture. Suspension systems are very adaptable (e.g., for microcarrier, micro encapsulation, or other methods of culture) and it provides opportunity to study interactions of metabolic and production phenomena in chemostat or turbidostat steady-state systems. Furthermore, suspension culture systems have additional advantage of (i) cell number and cell mass measurements are easy to obtain and (ii) rigorous and quantitative estimations of the effects of growth conditions or perturbations of metabolic homeostasis can be assessed with minimal effort. Newer inputs on these lines can speed up the development of optimal biotechnological processes (Birch and Arathoon, 1990).

Despite these many advantages, many biotech industries are continuing to use adherent cells, with many disadvantages like loss of cells due to poor trypsinization, cell death and loss of properties due to over trypsinization and handling problems at large-scale.

In the previous study (Sreedhar Reddy, 2008), the adherent r-CHO cells were adapted to suspension form with 7.5 % serum concentration, and rhEPO was successfully produced. The rhEPO produced was characterized by SDS-PAGE, RP-HPLC and the biological activity was assessed by using TF-1 cell line.

In the present study, serum concentration was further reduced from 7.5 % to 2.5 %. Additional to serum, 0.1 % pluronic F-68 was used for the growth of the cells. The produced rhEPO was compared with the partial adapted cells (7.5 % serum), which was characterized RP-HPLC, SDS-PAGE and biological activity was evaluated by TF-1 cells.

This was further followed by the optimization of composition of production medium and growth conditions and the optimal parameters was selected based on the amount of rhEPO produced at each condition. The amount of rhEPO produced was
estimated by RP-HPLC analysis. At this stage rhEPO was not subjected to SDS-PAGE and *in vitro* bioassay as the objective was to screen the optimized conditions for maximum yield of EPO.

The cells (suspension adapted with 2.5 % serum + 0.1 % pluronic F-68) were scaled up and rhEPO was produced with the optimized conditions. The harvest collected from these cells was then subjected to purification and then the purified rhEPO was characterized extensively.

- Among the different serum concentrations tested (10 %, 7.5 %, 5 % & 2.5 %) in conical flasks at different rpm’s viz., 150, 300 and 450 that were tried, 7.5 % serum concentration was found to be optimal at 300 rpm shaker speed. There were low cell clumps and greater viability at this condition. Adherent cells served as control in the experimental investigation (Sreedhar Reddy, 2008).

- Among the different serum concentrations (7.5 %, 5 %, & 2.5 %) with combination of different factors tested (Serum, Pluronic F-68 & Insulin) in different containers (Conical flask & Spinner flask) at single rpm (300), cells with 2.5 % serum + 0.1 % Pluronic F-68 in spinner flask was found to be optimal. Suspension cells that were adapted to 7.5 % serum served as control in the experimental investigation (Sreedhar Reddy, 2008).

- During the rhEPO production period (day-2 to day-6) the confluency of adherent cells was observed to be 95 %, while the adapted cells (suspension cells with 7.5 % serum) showed viability in the range of 90 % to 70 %. The cell viability decreased as a function of time in case of suspension-adapted cells. During the rhEPO production period with 2.5 % serum + 0.1 % Pluronic F-68 suspension adapted cells (day-2 to day-6), the viability of the cells was observed to be 95.7 to 89.3 %.

- Based on the RP-HPLC analysis it was concluded that the concentration of Erythropoietin elaborated by adherent cells was 5.7 ± 0.25 mg/L, while suspension adapted cells with 7.5 % serum concentration produced 2.9 ± 0.4 mg/L and with suspension adapted cells with 2.5 % serum + 0.1 % pluronic F-68 produced 6.6 mg/L.
In the present study, following optimization with 2.5 % serum cells + 0.1 % pluronic F-68 (test), observed ~ 1.15 fold increase in the yield of rhEPO when compared with the results obtained with the control (adherent) cells (Sreedhar Reddy, 2008).

The relative retention time was recorded to be 4.0 ± 0.5 minutes. All the recorded peaks for control and test samples were well within the system suitability limits (95 % confidence limit).

Recovery at different purification steps viz., Affinity Chromatography, Dia-filtration, Cation Exchange Chromatography, Anion Exchange Chromatography-I, Anion Exchange Chromatography-II, Gel filtration & Membrane filtration was 89.9, 85.4, 55.5, 41.6, 33.3, 31.6 & 30.6 respectively.

Characterization of purified rhEPO derived from both control (with 7.5 % serum) and test (suspension adapted cells with 2.5 % serum + 0.1 % Pluronic F-68) by SDS-PAGE (non-reducing condition) indicated a single broad band with an apparent molecular weight of 38 kDa. The observed molecular weight is in close agreement with the commercial reference rhEPO (Eprex).

The Western blot and peptide mapping profile of test protein was found to be comparable with the reference standard, Eprex.

The N-terminal amino acid sequence of test protein was found to be acceptable to that of the acceptance criteria laid down by European Pharmacopoeia.

The isoform profile of test protein was similar to that of the Eprex in terms of distribution and was found to be acceptable to that of the acceptance criteria laid down by European Pharmacopoeia.

The number of moles of sialic acid /mole of EPO obtained with test protein was found to be comparable with the reference standard. A minimum of 10 moles of sialic acids per mole of erythropoietin was observed.

In vitro bioassay based on use of TF-1 cells indicated that the biological activity of rhEPO derived from test cells to be 0.993 x 10^5 IU/mg (99.3 % potent), which is comparable to the biological activity of the WHO reference standard, with a labeled value of 1x10^5 IU/mg (100 % potent) (European Pharmacopoeia) protein.
Parallel line assay analysis confirm a linear dose response relationship (five doses), similar to the one reported by WHO (NIBSC) standard for rhEPO.

The cost benefit analysis indicated that rhEPO produced from suspension adapted cells to be ~85% cheaper (by way of cost reduction in materials required and handling of large volumes) as compared to the rhEPO production through adherent cells.

The outcome of the present study paves new avenues to develop mammalian cell culture platform with suspension cells and minimum serum to enhance production of recombinant proteins and to enable handling of large volumes and easy scale up.

The rhEPO produced in the present investigation after adapting the adherent cells to suspension form further requires undergoing pre-clinical studies in animals, clinical studies in humans prior to the commercialization and release into the market.