OPTIMISATION OF SCALE-UP OF CHROMATOGRAPHY

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ABSTRACT

Chromatography has become an integral part of biotechnology. It plays an important role in the purification of biological compounds from natural sources, as well as those produced from recombinant DNA and hybridoma techniques. Unfortunately, it is widely regarded as difficult and uneconomic to scale-up from the laboratory, where it is employed for analytical and preparative purposes, to larger-scale systems, where cost-effective commercial-scale manufacture is important. While simulation is a common tool for scale-up of chromatographic systems in other industries, its application in biotechnology has been limited by the availability of protein properties capable of describing essential parameters in the complex mathematical models which must be used. Consequently, alternative scale-up methodologies for chromatographic systems have been developed. These employ a combination of heuristics and time consuming experimentation. A generic approach is to arbitrarily increase flowrate to maximise productivity (the product yield per unit resin volume per unit time). Normally, this is unsuccessful. It does not guarantee optimal cost during scale-up.

The optimal scale-up of an ion-exchange system is investigated in this thesis. The ion-exchange system is used to separate a whey growth factor extract (WGFE) from cheese whey. A laboratory-scale ion-exchange system has already been the subject of a scale-up study. A heuristic technique was employed to devise an industrial-scale design. Flowrate was arbitrarily increased to improve productivity (WGFE yield per unit resin volume per unit time).

Key scale-up problems in the ion-exchange system were identified. The impact of resin compressibility on pressure drop during scale-up was examined. Pressure drop is an important constraint to the successful scale-up of a chromatographic system. It restricts the combination of flowrates and bed heights that can be utilised. Sepharose Big-Beads SP Pharmacia, Uppsala, Sweden) is the chromatographic resin employed in the ion-exchange system. It is compressible. During scale-up, the pressure drop across a compressible packed bed will increase in larger (diameter) columns. This is a result of wall friction effects. The implications of such a change for the ion-exchange system are potentially
catastrophic. Some form of prediction is essential. However, existing models for pressure-drop prediction in compressible packed beds are inadequate. They cannot account for wall friction effects.

A new model for pressure-drop prediction in compressible packed beds of chromatographic resin is developed. It combines a common theoretical thread between the multiphase theory of filtration and the method of differential slices which predicts wall friction effects in hoppers. Its key parameters were determined for Sepharose Big-Beads SP. These were used to validate model predictions against experimental packed-bed pressure-drop data. The model can successfully predict packed-bed pressure drop during scale-up of the ion-exchange system. Furthermore, the model is a significant advance in analysis of fluid flow in particulate systems. It is the first to allow true a priori prediction of pressure drop for compressible packed beds where wall friction effects are significant.

The new model was used to investigate the packed-bed pressure-drop behaviour of Sepharose Big-Beads SP in a production-scale chromatographic column, namely, a Pharmacia (Uppsala, Sweden) BioProcess™ Glass (BPG) 450/500 column. The results were combined with column pressure-loss data to anticipate the pressure-drop behaviour of the ion-exchange system during scale-up. The possible combinations of superficial velocity and packed-bed height are restricted by a pressure constraint to a feasible region. The current operating conditions being considered for the ion-exchange system are a superficial velocity and packed-bed height of 8.5 cm/min and 21 cm, respectively. These do not compromise the pressure constraint. Furthermore, a proposed increase in superficial velocity to 14 cm/min can be sustained by the ion-exchange system during scale-up.

The impact of compression on porosity and resin particle properties in the packed bed of the ion-exchange system during scale-up was examined. Porosity can decrease in a production-scale column by up to 36% compared to 9% in a laboratory-scale column. Similarly, Sauter-mean particle diameter can also decrease by 5 and 2%, respectively. This increased compression during scale-up can deleteriously influence ion-exchange system behaviour and performance.

As a consequence, compression effects on the behaviour and performance of the ion-exchange system were investigated. A non-linear multicomponent rate-equation
chromatography model was modified. The modifications account for the variation in porosity and Sauter-mean particle diameter that can occur in a packed bed during compression. Two major whey proteins, namely, lactoperoxidase and lactoferrin, were used to simulate the ion-exchange system behaviour. The variation in porosity and Sauter-mean particle diameter during scale-up was estimated. An indirect technique using the new model for pressure-drop prediction described above was employed. Model simulations were performed. The results were compared. System behaviour was largely independent of compression for both frontal adsorption and step elution. Increasing compression generated only small and trivial variations in effluent concentration profiles. Compression effects on system behaviour and performance do not need to be considered during scale-up of the ion-exchange system.

The general non-linear multicomponent rate-equation chromatography model used to simulate compression effects in the ion-exchange system can be also be used as a tool to optimise its scale-up. Model simulations were employed to examine the sensitivity of system behaviour during frontal adsorption to superficial velocity and packed-bed height. Lactoperoxidase and lactoferrin were employed to imitate the two key fractions in the microfiltered whey: the WGFE product and lactoferrin. Intraparticle-diffusion limitations were found to play an important role in determining the whey volume that can be sustained by the packed bed during frontal adsorption. This has an important impact on the cyclic WGFE product yield of the ion-exchange system. An increase in superficial velocity can produce a dramatic decline in WGFE yield per cycle.

Simulation results were combined with pilot-plant data for other steps in the ion-exchange system to examine the impact of packed-bed height and frontal-adsorption superficial velocity on productivity. An optimal region of productivity exists. It corresponds to a band of superficial velocities and packed-bed heights which rise from 5 cm/min at a bed height of 10 cm to nearly 20 cm/min at 30 cm. There are multiple design scenarios for the superficial velocity in the frontal-adsorption step and the packed-bed height which will maximise productivity of the ion-exchange system. This is a direct result of intraparticle-diffusion limitations and the decline in cyclic WGFE yield it induces with an increase in superficial velocity. The proposed increase in superficial velocity to 14 cm/min for the ion-exchange system (at a packed-bed height of 21 cm) corresponds to an optimal region of productivity.
These results have important implications for scale-up of other chromatographic systems in biotechnology. Standard industry practice involves a heuristic technique which arbitrarily increases flowrate to increase productivity. However, this study shows increasing flowrate does not always necessarily improve productivity.

The frontal-adsorption superficial velocity and packed-bed height which will optimise the cost of the ion-exchange system during scale-up was investigated. A comprehensive design for the ion-exchange system in a proposed commercial-scale facility was developed. The design was correlated with an objective function describing annualised dimensionless cost. There is an optimal design for the ion-exchange system which will minimise cost. It is located at a superficial velocity and packed-bed height of 2 to 6 cm/min and 25 to 30 cm, respectively. While the cost of chromatographic resin and columns were important, the cost of the ion-exchange system was dominated by consumable costs, in particular, those for eluent and sanitisation solutions. This is an important observation. Maximising productivity to minimise resin volume in a chromatographic system will not always optimise cost. Furthermore, a heuristic of increasing flowrate inadvertently acts to increase consumable costs. This is a result of the rise in cycle frequency which inevitably accompany higher superficial velocities and increase the volumetric consumption of wash, buffer, eluent and/or sanitisation solutions. Existing heuristic techniques which rely on maximising productivity are not always suitable for chromatographic scale-up where optimal cost is the key objective.

Consequently, the current operating conditions considered for the ion-exchange system are sub-optimal. They will result in annual scale-up costs more than 15 to 20% greater than if a superficial velocity and packed-bed height in the optimal-cost region are employed. Furthermore, the proposed increase in superficial velocity to 14 cm/min should be avoided. While it will improve system productivity, it will lead to an annual scale-up cost that exceeds its optimal counterpart by more than 50%.

Further work to optimise scale-up of the ion-exchange system should be considered. In particular, the use of a perfusion chromatographic resin or smaller resin particle size to alleviate intraparticle-diffusion limitations may yield significant performance and cost benefits.
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Nomenclature

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