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Computer aided desk-top scale-up and optimisation of chromatographic processes

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Abstract

Further experimental validation of an existing set of computer programs, called Simulus, will be presented. This validation includes predicting the performance of the recovery of immunoglobulin G from a crude, unclarified tissue culture using a Prosep-G fluidised bed and the recovery of amylase from a crude feedstock using a Streamline DEAE expanded bed. The mathematical theory for these programs will be highlighted. It is not possible to obtain analytical solutions to these sets of equations defining the mass transfer processes within a column and its media. In the prediction of column breakthrough, a well established coarse numerical solution is sufficient to achieve good results, however, when attempting to predict sharp elution profiles, the model fails. A unique discretisation of the same equations will be presented, giving dramatically improved modelling capabilities. The experimental validation, using an amylase/Sephacryl gel filtration chromatography system, gave convincing results.

1. Introduction

The use of adsorption and chromatographic techniques throughout the chemical and pharmaceutical industry is extensive. However, a full process optimisation of high-value products, which are often proteinaceous, is often restricted by the value of the products involved. Furthermore, the commercial environment necessitating "first-to-the-market" competitiveness, often restricts research programmes to process validation and not to the level of efficiency in the full-scale purification facility.

Previous work [1–11] has shown that mathematical equations which model the diffusion, adsorption and convection of target and contaminant molecules within a process can be solved to give accurate predictions of the purity and yield of the final product.

In the past we have shown that a set of programs could be used strategically to assess the effect of operational parameters, such as flow-rate or column aspect ratio, on the performance of a process [3,6,10]. Moreover, it has been shown that complex equilibria existing in ion-exchange and other chromatographic columns could be modelled using simple equations.

Although the theoretical credibility of computer-aided design packages is unquestioned [2], its use will not be widespread until numerous criterion are met: (i) generic programs are written with diverse capabilities, (ii) extensive experimental validation work is undertaken, and (iii) simple, user-friendly packages are compiled.

To this end a design package was developed by BIOSEP [12] and tested on real biological

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systems. This design package is incorporated into a proprietary design report available to members of BIOSEP. This paper, however, will seek to address some of the criteria highlighted.

The two sets of equations which we have used model the dynamic equilibrium between molecules in the adsorbed and desorbed states and the rates of diffusion across particle surface boundary layers and through the pores. These equations using a simple discretisation will be used to predict the performance of fluidised and expanded bed chromatography.

One major limitation to modelling elution chromatography is the ability to characterise the sharp peak which often results. With existing models, the column is normally broken down into a number of discrete cells divided equally down the column, however, to model the movement of a sharp peak as it moves down the column requires a highly concentrated discretisation. For the entire column to be discretised in such a fashion would be immensely computer intensive and therefore inviable. This study therefore seeks to address these problems.

In the latest stage of the work, to develop the programs into a useful tool for the chromatographer, we have produced a Windows-compatible version of their suite of adsorption programs, which is called Simulus. Validation will be given in several new key areas of purification.

2. Theory

2.1. Existing models

The basic models and equations which Simulus uses were transferred directly from the original suite of BIOSEP programs. This suite of programs, and their equations, is well documented [12,13] and their worthiness for modelling a number of systems is now well established. The equations for these models are given by Cowan et al. [11], so will not be given again here. The validation of the use of these equations, however, is ongoing and, since several new purification techniques are now commercially available, further validation of their capability to model such new adsorption processes is sought.

2.2. Moving grid strategy

The importance of the model's capability to characterise sharp elution peaks, is paramount for a detailed and accurate estimation of a process's efficiency [14]. The original models, in their simplicity, were proficient at modelling these types of experimental data, but required an immense discretised grid (often over a 200 cells per cm of column). Furthermore, the computational processing power required made the feasibility of using such models impractical. To approach this problem a new model was included into Simulus, called the "moving grid" [15]. This program enables a highly localised discretisation to be positioned around the peak and to subsequently track the theoretical peak as it moves down the column.

In order to validate the application of this moving grid technique, the simplest form of elution chromatography was selected. In size-exclusion chromatography, or gel filtration, a sample is loaded onto the top of a column, and the mixture is separated by diffusion effects only. Here the need for modelling adsorption can be removed which simplifies the initial validation of the program.

The resulting equations which model the process are given below.

When considering a gel filtration column the convection of adsorbate along the length of the column can be described by Eq. 1.

$$\frac{\partial c_b}{\partial t} = D_a \cdot \frac{\partial^2 c_b}{\partial z^2} - \nu \cdot \frac{\partial c_b}{\partial z} - \frac{3k_f V_S}{RV_L} \cdot \left(c_b - c_j|_{r=R}\right) \tag{1}$$

Here the last term represents the quantity of the species under consideration which diffuses across the particle boundary layer. The adsorbate concentration at the surface of the particle is given by Eq. 2:

$$\frac{\partial c_j}{\partial r}\Big|_{r-R} = \frac{k_f}{D_e \epsilon_i} \cdot \left(c_i - c_j|_{r=R}\right) \tag{2}$$

The diffusion of adsorbate into the pores of the particle is described by Eq. 3:

$$\frac{\partial c_j}{\partial t} = D_e \cdot \left(\frac{\partial^2 c_j}{\partial z^2} + \frac{2}{r} \cdot \frac{\partial c_j}{\partial r}\right) \tag{3}$$

assuming the boundary condition holds that the rate of mass transfer at the centre of the particle is zero (Eq. 4).

$$\frac{\partial c_i}{\partial r}\Big|_{r=0} = 0 \tag{4}$$

The simultaneous solution to these differential equations cannot be undertaken analytically, but instead relies upon numerical solution techniques. In past modelling programs, the numerical solution involves dividing the column into identical "cells". Eq. 1 can be solved using the "method of lines" [16] to convert the partial differential equation into a set of ordinary differential equations. This is carried out using a "finite volume discretisation" [16] to integrate over each cell volume, which then gives the required ordinary differential equations. In the moving grid model, each cell is set up as in Fig. 1.

The grid is set up in a similar way to past numerical solutions except that the entire grid is localised to one tenth of the loading length, with the first cell boundary at the inlet to the column. Typically a tenth of the loading length is around 2% of the column length. As the integration proceeds, however, the bulk fluid concentration, c_b, reaches the last cell at which point the outer grid boundary begins to move. As loading finishes, the inner boundary similarly moves as in Fig. 2.

These boundaries are then fixed to ensure that there is no mass flux across each external boundary, i.e. z_0 and z_n, where n is the number of cells, such that there is 98% mass containment. The solution for cases where D_a is negligible (as it is in most cases) is:

$$\frac{dc_i}{dt} = \frac{3k_f V_s}{RV_L} \cdot (c_i^{surface} - c_i) + \frac{1}{h_i} \cdot \left[(\dot{z}_{i+1} - v) \cdot c|_{z^{i+1}} - (\dot{z}_i - v) \cdot c|_{z^i} - (\dot{z}_{i+1} - \dot{z}_i) \cdot c_i\right] \tag{5}$$

The program employs instantaneous re-gridding which is carried out at each time step along the integration to maintain 98% of the loaded mass within the confines of the gridded volume (Fig. 2). Increasing this "captured" percentage only results in elongation of the grid and a corresponding reduction in resolution. The remaining grid is divided evenly between the inner and outer boundaries such that for all i, h_i is constant, as in Fig. 3. This movable, expanding grid allows maximum resolution of the peak with a minimum of processing power. The corresponding scenario with the fixed grid (also shown) cannot adequately characterise the diffusive mass transfer due to the prohibitive nature of the discretisation.

The homogeneity of the media within a gel filtration column, allows it to be modelled as a

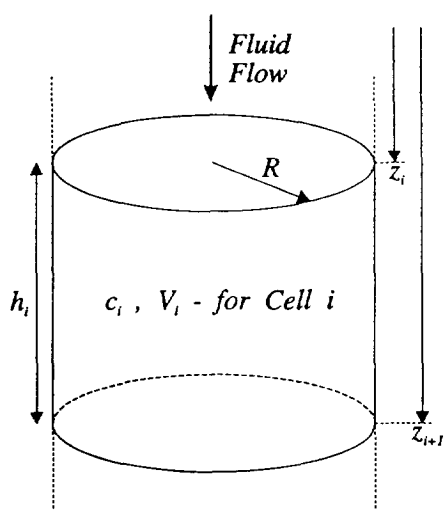

Fig. 1. The cell divisions in the moving-grid discretisation.

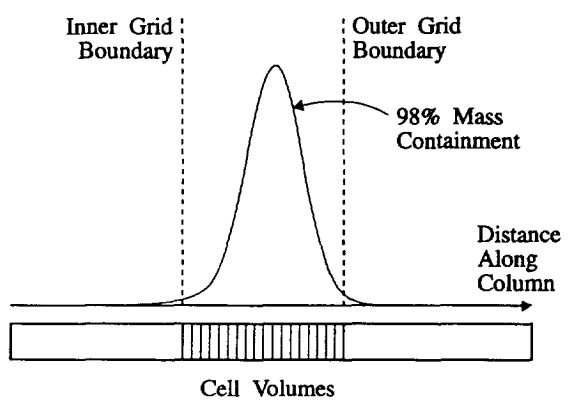

Fig. 2. Grid boundaries tracking the peak movement.

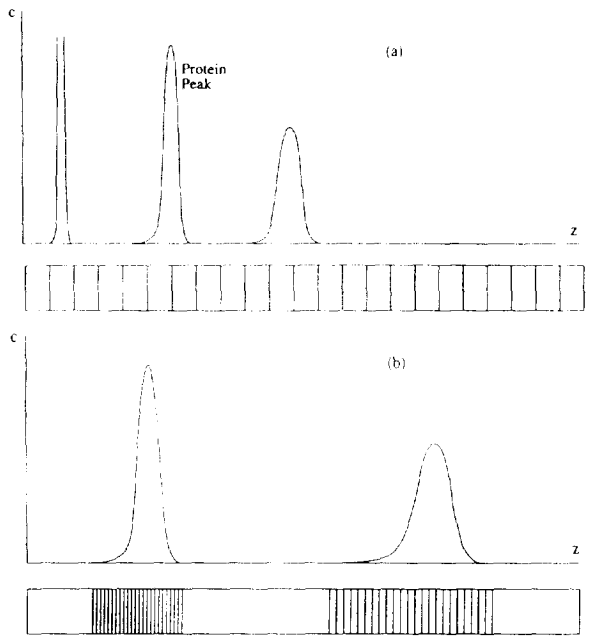

Fig. 3. A comparison of (a) standard grid and (b) moving grid discretisations.

continuous medium. In this case it can modelled as one single particle of radius R split into a number of shells (Fig. 4).

The solution to this problem for a fixed grid system is well documented [14], however, to allow for the movement of the cell grid boundaries, an expansion term (or a shrinking term) must be added to allow for the transfer of particulate phase concentration within the media, from one cell to another. The solution is slightly more complicated though nevertheless

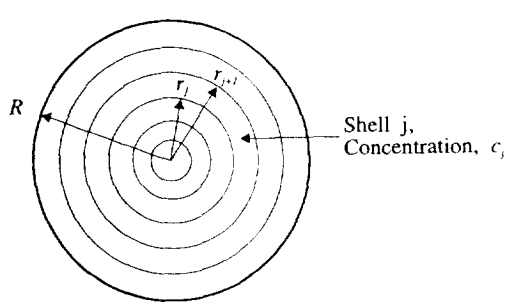

Fig. 4. The particle discretisation.

time consuming to browse through and will hence be excluded from this paper.

In initial studies the difference in processing time, and accuracy, heavily favoured the use of the moving grid (Fig. 5).

In these predictions, 25 cells where used for the moving grid, and 800 cells for the fixed grid giving comparative run times of 1.2 min and 1 h and 49 min respectively (on a 486 DX33 computer with 8 MB of RAM). The predicted peak for the fixed grid could be improved by increasing still further the number cells is the column, however, due to memory limitations of the computer this could not be carried out.

3. Materials and methods

Amongst many others, previous validation work included the purification of aspartic acid using Duolite A162 [11], the adsorption of an intracellular enzyme leucine dehydrogenase onto DEAE-Spherodex [17] and the uptake of α-amylase by QMA-Spherosil [17]. The three processes modelled in this paper have been selected to reinforce the validation of Simulus.

The method employed throughout the modelling of the data was first elucidated by Noble et al. [10]. In short, the strategy adopted was to use experimental results from a small-scale run, which would typically be obtained when assessing the process at laboratory scale, and use characteristic parameters to then predict scale-up and contactor configuration. In many cases it is possible to obtain parameters from, say, a packed bed experiment, and then to predict the outcome of an expanded or fluidised bed process. For the initial validation of the moving grid model results were simply taken from a small column, and used to predict what would happen at a much larger scale.

In each case given below, the analysis using Simulus gives the parameters which best characterise the adsorption process. Simulus also calculates confidence limits [18] to assess the simulation dependency on the parameter in question.

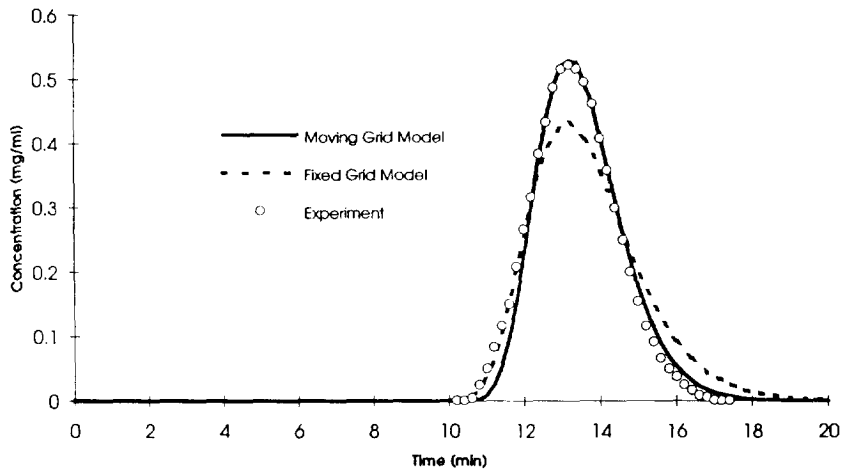

Fig. 5. A comparison between the fixed grid model and the moving grid model using approximate parameters to obtain a close comparison to real experimental results.

3.1. Expanded bed adsorption

The first process is the purification of α-amylase using expanded bed adsorption (or EBA, Pharmacia Biotechnology, Uppsala, Sweden). The unclarified mixture was prepared by dissolving 2 mg ml^{-1} of a crude, bacterial source α-amylase (Sigma, UK) into Tris·HCl buffer at pH 9. Dried yeast (5 g l^{-1})was added to simulate a fermentation broth. The clarified solution was loaded at 10 ml min^{-1} onto a 10 × 1.6 cm column packed with Streamline DEAE media (Pharmacia Biotechnology). The unclarified "broth" was loaded at 100 ml min^{-1} onto a 55 × 5 cm expanded bed (also using Streamline DEAE).

The results from the packed bed study were analysed using Simulus to obtain the parameters which characterise the adsorption process (Fig. 6a). These parameters were then taken and used to predict the outcome of the expanded bed study. The comparison between the real and the predicted results are given in Fig. 6b. This prediction represents approximately a 20-fold scale-up in capacity.

3.2. Affinity fluidised bed adsorption

The second of the two processes is the purification of a monoclonal antibody using affinity

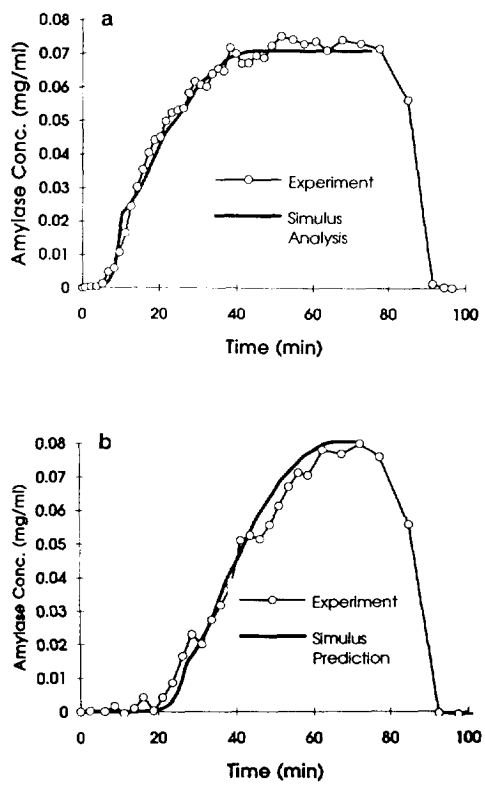

Fig. 6. (a) Comparison between the adsorption of α-amylase onto Streamline DEAE and the results fitted by Simulus. (b) Comparison of adsorption of α-amylase onto Streamline DEAE using EBA technology and the prediction given by Simulus.

fluidised bed adsorption. The approach for this modelling study was the same as for the expanded bed analysis. In the initial packed bed experiments an ideal system was selected. A solution of 20 m*M* trisodium phosphate containing 0.27 mg ml^{-1} of the antibody (cultured at AEA Technology) was loaded onto a 5 × 0.5 cm Prosep-G packed bed at 0.49 ml min^{-1}. The resulting frontal curve was analysed to obtain parameters which characterise the adsorption kinetics. The comparison of the experimental and the fitted curve is shown below (Fig. 7a).

A similar solution, at 0.275 mg ml^{-1} was then applied to a fluidised bed process with the same type of media, Prosep-G. The bed was fluidised to 16.2 × 1.0 cm at a constant flow-rate of 1.95 ml min^{-1}. Due to the particle size variation, the bed was observed to be relatively stable with little axial motion. The results from the experiment were compared to predictions given by Simulus (Fig. 7b).

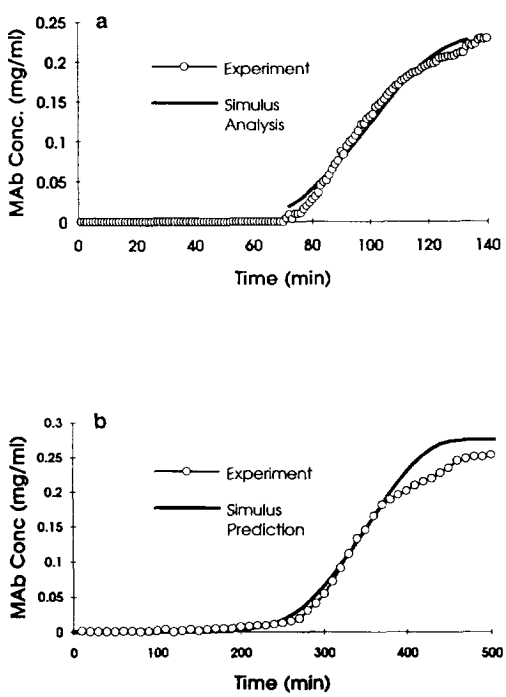

Fig. 7. (a) Comparison between the adsorption of IgG onto Prosep-G and the results fitted by Simulus. (b) Comparison of adsorption of IgG onto Prosep-G using an affinity fluidised bed and the prediction given by Simulus. MAb = Monoclonal antibody.

3.3. Gel filtration

The final model system seeks to validate the use of the moving grid strategy for gel filtration. In the initial studies the selected system was pure α-amylase dissolved into Tris · HCl buffer at pH 7.2. This solution was loaded onto various size columns packed with Sephacryl S100HR. The packing followed the instructions given by the manufacturers [19]. To alleviate non-specific binding, 50 m*M* sodium chloride was also added to the loading and elution buffers. The analysis of the mass transfer processes was undertaken using results from the smallest column used which was 11.2 × 1.6 cm where the linear flow-rate was 30 cm h^{-1} (Fig. 8a).

The elution profiles of the remaining experiments were then compared to the predictions by Simulus. Accurate correlations were found between the simulation and reality. One such comparison is given in Fig. 8b. In this experiment the amylase solution was loaded onto a 87.7 × 2.6 cm column (465.2 ml) at 60 cm h^{-1}, corresponding to an increase in throughput of 4000%.

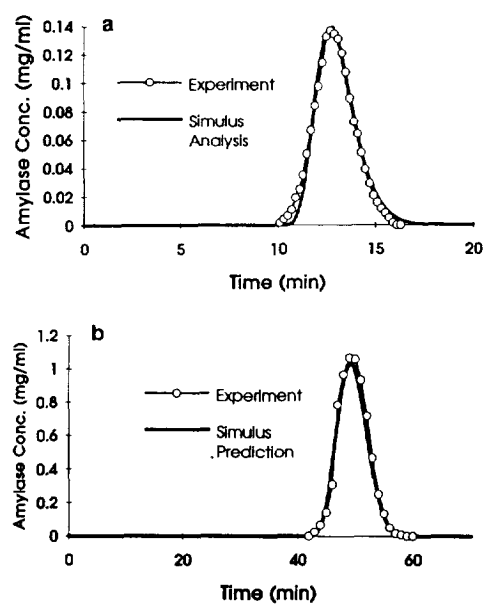

Fig. 8. (a) Comparison between the elution profiles for α-amylase and the results fitted by Simulus for a 22.5-ml column. (b) Comparison between the elution profiles for α-amylase and the prediction given by Simulus for a 465.2-ml column.

In further validation work an industrial gel filtration process was modelled which gave equally convincing results.

4. Conclusions

It is apparent that the inclusion of the moving grid strategy into Simulus will be of great benefit in the modelling of separation processes. The accuracy of the validation with gel filtration, the simplest form of elution chromatography, strengthens this claim.

With the incorporation of new modelling techniques into the current programs, Simulus's overall performance was significantly enhanced and will revolutionise chromatographic modelling techniques.

Symbols

c_b	Bulk phase concentration (kg m^{-3})
c_i	Bulk cell concentration (kg m^{-3})
c_j	Particle shell concentration (kg m^{-3})
$c\|_{z^i}$	Estimated concentration at the z_ith boundary calculated by upwind (or upstream) differencing
D_a	Axial dispersion coefficient (m^2 s^{-1})
D_e	Pore diffusion coefficient (m^2 s^{-1})
ε_i	Effective intraparticle porosity
h_i	Height of the ith cell
k_f	Liquid film diffusion coefficient (m s^{-1})
r	Radial distance (m)
R	Particle radius (m)
t	Time (s)
v	Calculated interstitial velocity of the loading fluid
ν	Superficial bulk fluid velocity (m s^{-1})
V_L	Liquid phase volume (m^3)
V_S	Solid phase volume (m^3)
z	Axial distance (m)
$\dot{z}_i$	Velocity of the ith boundary

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