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# Simulation of chromatographic processes applied to separation of proteins

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**Abstract**

The advantages of using a detailed mathematical model for fixed bed chromatography is demonstrated by the personal computer program SIMCHROM. The chromatography model includes axial dispersion in the bulk liquid, external and internal mass transfer resistances and an instationary non-linear adsorption model. Frontal and pulse chromatography can be studied for single and multicomponent systems. The simulation program can easily be used to make parametric evaluations to study the influence of variations in physical, kinetical and operating parameters. The special features of the present intrinsic model is demonstrated by comparing the SIMCHROM results with simulations using simplified lumped models. Experimental data describing affinity chromatography of lysozyme on Cibacron Blue Sepharose CL-6B is used as a model system. The intrinsic model is able to describe variations in the physical, kinetic and operating parameters better than the simplified models. This results in a more reliable prediction of the performance of the chromatography process as well as a better understanding of the underlying mechanisms responsible for the separation. 

*Keywords:* Mathematical modelling; Computer simulation; Proteins

## 1. Introduction

When applying a chromatographic operation to a new system or when scaling up the process it is common practice to perform numerous tedious experiments. As the products often are valuable and available only in small quantities, the experiments are expensive to perform. This is especially true for the separation of proteins. This makes it important to be able to predict the performance of the process by mathematical modelling and computer simulation in order to minimize the number of experiments required.

Depending on the type of chromatographic process different parameters are important. In gel chromatography or gel filtration the separation depends on the different abilities of the substances to enter the pores of the gel beads. Large molecules, which cannot enter even the largest pores, are thus not retained by the slow transport through the gel beads. Thus the diffusion rate is the most important parameter.

In ion-exchange chromatography the biomolecules are instead separated according to their charge. The adsorption is not specific and, in contrast to affinity chromatography, a number of contaminant solutes in the sample, as well as the desired product, are adsorbed in the column. This makes the desorption procedure more difficult, since the composition of the elution liquid must be varied in order to desorb the adsorbates at different times. This is done to obtain a fraction containing only the desired product. Both the adsorption and the desorption procedures are important and complex processes. This makes ion-exchange chromatography more difficult to de-

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scribe in mathematical terms and to simulate compared with affinity chromatography.

Simulation of chromatographic processes has become an interesting tool along with the development of efficient computer hardware and software [1–12]. Advanced numerical methods are still required to solve the more sophisticated mathematical models. Often, simplified models, neglecting some of the mass transfer resistances, are used. The applicability of these models is limited as lumped constants are used to describe the combined effects of diffusion, dispersion, kinetics and external mass transfer.

In this study the intrinsic model is based on basic physical and chemical principles. Thus, both external and internal mass transfer resistances, as well as the sorption kinetics, are considered. The complete mathematical model is solved [8,9] by using the personal computer program SIMCHROM [8,9] applying the method of orthogonal collocation utilizing a moving window technique [13–15]. The program can be used to study frontal or pulse chromatography for single and multicomponent systems.

In this study the frontal chromatography computer model is compared with simplified models describing the kinetics only [16,17] and with a diffusion-equilibrium model [3]. All these are compared with experimental data from an affinity chromatography experiment describing the adsorption of lysozyme to Cibacron Blue Sepharose CL-6B [16].

The models are evaluated by studying the sensitivity of the respective model to changes in the physical, kinetic and operating parameters. A parametric analysis of some of the physical parameters demonstrates how a better understanding of the underlying mechanisms for the separation is achieved. This approach demonstrates how a sensitivity analysis can be carried out if the basic intrinsic parameters have been determined in previous experiments. It is therefore important to organize the experimental work to determine the physical constants in such a way that they will be independent of the operating conditions.

## 2. Mathematical model

It is assumed in this study that the chromatographic separation is performed in a column of uniformly packed beads of equal size. The fluid flowing through the column is subjected to intermixing. This is defined as the axial dispersion and is described by an axial dispersion coefficient, $D_{AX}$. The mass transfer from the mobile phase through the external boundary layer to the surface of the beads is described by a film mass transfer coefficient, $K$. The mass transfer within the beads to the adsorption sites is a diffusive process described by an effective diffusion coefficient, $D_E$. The adsorption can be described by different kinetic models. In this study an instationary adsorption–desorption model of the Langmuir type is used.

### 2.1. Equations

To describe the concentration change of adsorbate with time in the mobile phase, the following equation can be derived by performing a mass balance over a differential volume of the column:

$$\frac{\partial C_L}{\partial T} = D_{AX} \cdot \frac{\partial^2 C_L}{\partial l^2} - \nu_{INT} \cdot \frac{\partial C_L}{\partial l} - Ka \cdot \frac{1-\epsilon_C}{\epsilon_C} \cdot (C_L - C_{Pr=R}) \quad (1)$$

The left-hand side describes the accumulation of adsorbate in the mobile phase. On the right-hand side, the first term describes the variation in concentration with time caused by dispersion. The second term describes the convective flow through the column. $\nu_{INT}$ is the interstitial velocity of the flow, i.e. the linear velocity of the flow between the beads. The last term accounts for the uptake of adsorbate by the beads. It describes the mass transferred from the mobile phase (concentration $C_L$) to the surface of the bead (concentration $C_{Pr=R}$).

The following two boundary conditions, for the inlet and the outlet of the column, have been used.

$$\frac{\partial C_{inlet}}{\partial l} = \frac{\nu_{INT}}{D_{AX}} \cdot (C_{inlet} - C_0) \quad (2)$$

$$\frac{\partial C_{outlet}}{\partial l} = 0 \quad (3)$$

$C_{inlet}$ is the concentration just inside the column at the inlet, and $C_{outlet}$ is the concentration just inside the column at the outlet from the column.

To describe the change in concentration of adsorbate with time in the pore liquid of the beads, the following equation can be derived by constructing a mass balance over a differential volume of the bead:

$$\frac{\partial C_P}{\partial T} = \frac{D_E}{\epsilon_P} \cdot \left( \frac{\partial^2 C_P}{\partial r^2} + \frac{2}{r} \cdot \frac{\partial C_P}{\partial r} \right) + \frac{r_P}{\epsilon_P} \quad (4)$$

The left-hand side describes the accumulation of adsorbate in the pore liquid. On the right-hand side, the first term describes the change in concentration with time caused by the diffusion in the particle. $D_E$ is the effective diffusion coefficient. The second term describes the adsorption of product in the bead. $r_P$ is the net production of adsorbate in the particle liquid, and during adsorption this term will be negative.

The following boundary condition has been used.

$$\frac{\partial C_P}{\partial r}\Big|_{r=R} = \frac{K}{D_E} \cdot (C_L - C_E) \quad (5)$$

Several equations describing the adsorption kinetics can be found in the literature. In this study an adsorption–desorption model of the Langmuir type has been chosen:

$$-r_P = \frac{\partial q}{\partial T} = k_{ads} C_P (q_m - q) - k_{des} q \quad (6)$$

where $\partial q/\partial T$ is the accumulation of the adsorbed product. On the right-hand side the first term describes the adsorption in the beads, determined by the concentration of product in the pore liquid ($C_P$) and the concentration of free sites in the bead ($q_m - q$). The second term describes the desorption rate from the beads determined by the concentration of the adsorbed product, $q$.

### *2.2. Method of numerical solution*

The complete model results in two partial differential equations, which are the normalized Eqs. (1) and (4), and an ordinary differential Eq. (6). No analytical solution to the problem exists. It can only be solved by advanced numerical techniques such as orthogonal collocation, which has been used in this study. The method is described in detail by Villadsen and co-workers [13,14] and has successfully been applied to the modelling of bioreactors with immobilized enzymes and cells [18,19]. In the orthogonal collocation method the unknown solutions, e.g. the concentration profiles in the beads and in the column, are expanded into trial functions. These are chosen to be sets of orthogonal polynomials (in this case of the Jacobi type) satisfying the boundary conditions. The roots of the polynomials give the collocation points, which are discrete points at which the differential equations are satisfied. The complete concentration profiles are obtained by interpolating between these collocation points. The resulting ordinary differential equations, which are normally stiff differential equations, are solved with a numerical solver using Gear's method [20].

In the present model the column has been divided into three segments. The middle segment, which has a large number of collocation points, is moving along the column following the steep concentration profile, which gives an improved numerical stability.

The physical description of the process can be simplified by certain assumptions, which leads to more simple differential equations that can be solved analytically. These are often referred to as Thomas' model [16,17] and Arnold's model [3]. These are described in more detail in Section 4.

## 3. Input data for the study

The adsorption of lysozyme to Cibacron Blue Sepharose CL-6B has been chosen as a model system. The operating parameters were obtained from a study made by Chase as described in Ref. [9], in which batch experiments, as well as frontal chromatography experiments, were performed. In that study, values of the maximum adsorption capacity ($q_m$), the rate constant for adsorption ($k_{ads}$) and the rate constant for desorption ($k_{des}$) were determined. However, the rate constants found by Chase [16] are lumped constants, i.e. they include not only the adsorption/desorption kinetics but also the internal mass transfer resistance. In order to obtain basic values for the simulation of the intrinsic model in the present study, new values of $k_{ads}$ and $k_{des}$ were determined by fitting the experimental data to the simulated breakthrough curve. The values had to be increased by a factor of four compared to the lumped constant values when an effective diffusion coefficient value of $5.3 \cdot 10^{-11}$ $m^2/s$ was used. The maximum adsorption capacity reported by Chase

[16] is recalculated with respect to bed void ($\epsilon_b$) and available volume to the protein (i.e. bead void for the protein) to 1.22 mol protein/$m^3$ gel. A very good overlap is obtained, except at the very end of the breakthrough curve. The discrepancy between experiments and model for the longest column is obtained (see Fig. 1). The sensitivity of the breakthrough time for the maximum binding capacity show the importance of initial experiments to determine the gel volume of the actual column.

The size of the beads and the void of the packed bed have been given values obtained from Pharmacia [21]. The void in the beads has been determined by Horstmann et al. [22]. The mass transfer parameters were obtained from the literature (see Section 3.4).

### 3.1. Axial dispersion coefficient

The axial dispersion coefficient for liquids in packed beds has been studied by several researchers. Their results have been collected and presented graphically [23,24], with the Peclet number (Pe) vs. Reynolds number (Re).

$$\text{Pe is defined by } \mathrm{Pe} = \frac{\nu_{\text{int}} d_P}{D_{AX}} \tag{7}$$

$$\text{Re is defined by } \mathrm{Re_P} = \frac{\nu d_P}{\nu} \tag{8}$$

An alternative to determine the axial dispersion is using an empirical correlation. Eq. (9) gives the Peclet number as a function of the Reynolds number [25]:

$$\mathrm{Pe} = \frac{0.20 + 0.011\mathrm{Re_P}^{0.48}}{\epsilon_C} \tag{9}$$

This relation is based on numerous experiments

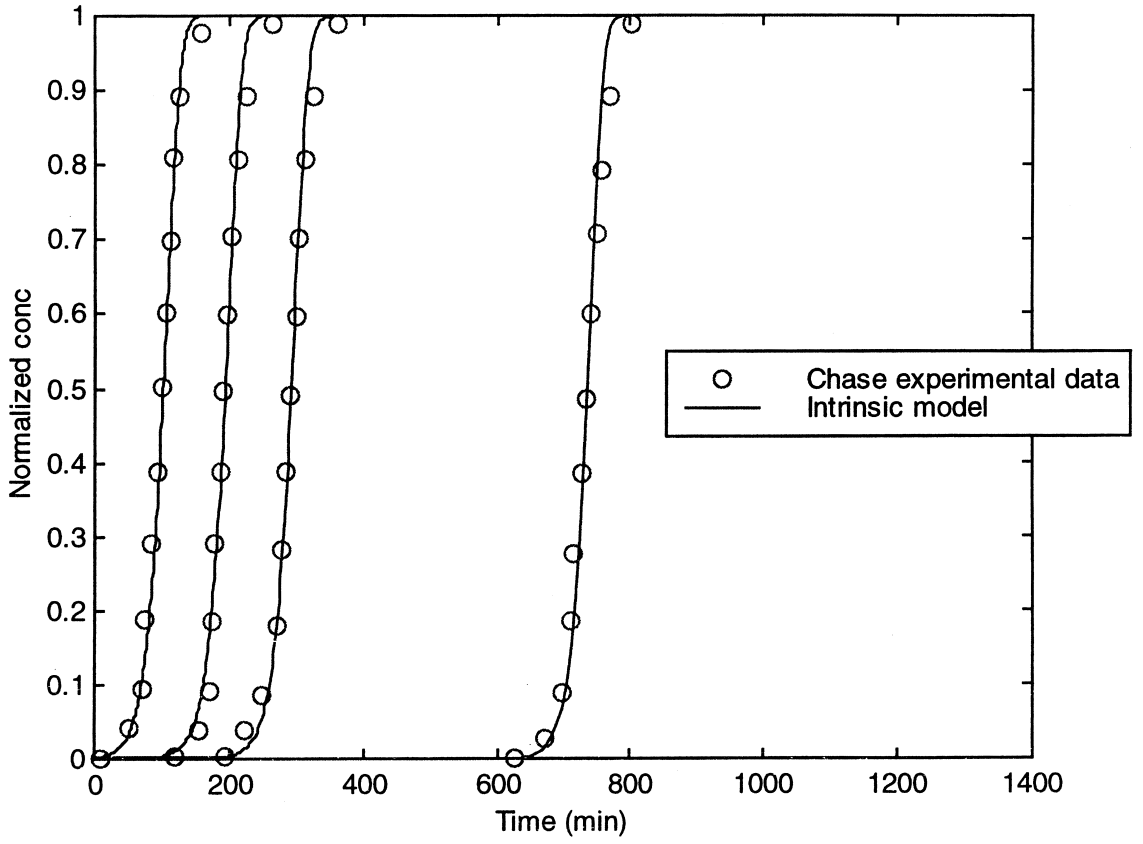


Fig. 1. Intrinsic SIMCHROM model compared with experimental data (Chase [16]). The column lengths are: 14, 27, 41 and 104 mm. Operating condition according to Table 1.

Table 1
Base case data used in simulations

| Variables | Values |
|---|---|
| Inlet concentration | $7.14 \cdot 10^{-3}$ mol/m$^3$ |
| Flow-rate | $1.67 \cdot 10^{-8}$ m$^3$/s |
| Column length | 0.104 m |
| Column diameter | 0.01 m |
| Bed porosity | 0.39 |
| Bead porosity | 0.75 |
| Bead radius | 50 μm |
| Axial dispersion | $5.75 \cdot 10^{-8}$ m$^2$/s |
| Mass transfer rate | $6.9 \cdot 10^{-6}$ m/s |
| Effective diffusion coefficient | $5.3 \cdot 10^{-11}$ m$^2$/s |
| Adsorption rate coefficient | 1.144 m$^3$/(mol·s) (SIMCHROM model) |
| Desorption rate coefficient | $2.0 \cdot 10^{-3}$ s$^{-1}$ (SIMCHROM model) |
| Maximum adsorption capacity | 1.22 mol/(m$^3$ gel) |
| Adsorption rate coefficient | 0.286 m$^3$/(mol s) (Thomas' model) |
| Desorption rate coefficient | $0.5 \cdot 10^{-3}$ s$^{-1}$(Thomas' model) |
| Effective diffusion coefficient | $1.3 \cdot 10^{-11}$ m$^2$/s (Arnold's model) |

over a broad range of $Re_P$ numbers ($10^{-3}$ to $10^3$). Using data from Table 1 the $Re_P$ number is calculated to be 0.057 which together with a bed void of 0.37 results in a Pe number of 0.55.

In the present study, the value of the Pe number was set to 1. The axial dispersion coefficient can then be calculated from the Pe number by using Eq. (9). Using the data from Table 1 the axial dispersion coefficient was determined to be $5.75 \cdot 10^{-8}$ m$^2$/s.

### 3.2. Diffusion coefficient

The diffusion coefficient for lysozyme in water ($D_{AB}$) has been determined by several researchers. In three of these investigations [26–28] a value of around $10.6 \cdot 10^{-11}$ m$^2$/s was obtained for pH ranging from 4.2 [26] to 6.8 [28].

Investigations performed by Sophianopoulos and Van Holde [29] and Bruzzesi et al. [30] indicated that lysozyme monomers associated to dimers at pH>4.5. However, calculations based on data from Sophianopoulos and Van Holde [29], showed that only approximately 1/400 is present as dimers at the low concentrations prevailing in the present study.

### 3.3. Effective diffusion coefficient

Moussaoui et al. [32] studied the diffusion of proteins in Sepharose gel (CL-6B). They compared their experimental data with theoretical equations and found that a model by Ogston et al. [33] was the most appropriate for the estimation of diffusion coefficients in sepharose CL-B gels.

$$\frac{D_E}{D_{AB}} = Ae^{(-B \cdot R_S)} \tag{10}$$

$D_E$ and $D_{AB}$ are the effective diffusion coefficient of the solute in the gel and the diffusion coefficient in free solvent, respectively. $A$ and $B$ are parameters describing the diffusional properties of the gel. $R_S$ is the Stokes radius of the protein. This correlation (Eq. (10)) has been used in the present study to calculate the value of the effective diffusion coefficient. A value of $5.3 \cdot 10^{-11}$ m$^2$/s was calculated for the effective diffusion coefficient.

### 3.4. Mass transfer coefficient

The mass transfer coefficient, $K$, describes the external mass transfer resistance in the film surrounding the beads. This resistance can be estimated by numerous correlations which all give mass transfer coefficients of the same order of magnitude.

The correlation used here is the correlation of Foo and Rice [31]:

$$Sh = 2 + 1.45 Re_P^{1/2} Sc^{1/3} \tag{11}$$

where

$$Sh = \frac{Kd_P}{D_{AB}} \quad (12)$$

$$Sc = \frac{\nu}{D_{AB}} \quad (13)$$

Using the data from Table 1, a value of $6.9 \cdot 10^{-6}$ m/s is obtained for the film mass transfer coefficient.

## 4. Comparison with simplified models

The present intrinsic model (SIMCHROM) has been compared with two simplified models.

The first is often referred to as Thomas model [16,17] and considers only the kinetics together with the convective transport through the column. Thus, all effects of internal and external diffusion within and outside the beads as well as any dispersion in the column are lumped together with the kinetics. This model is referred to as a kinetic model.

The second model according to Arnold, considers a diffusion within the beads and an irreversible linear adsorption that is so fast that an equilibrium is obtained between the pore liquid and the pore surface. Any effect of axial dispersion is lumped together with the diffusion [3]. This model is referred to as a mass transfer model. The low complexity of these simplified models makes them possible to solve analytically. The simplified solutions have been solved using MATLAB [34] (Fig. 2).

Thomas' model describes the performance of the different columns fairly well using the lumped kinetic constant as determined by Chase. In order to fit the model of Arnold to the present experimental data the effective diffusion coefficient had to be

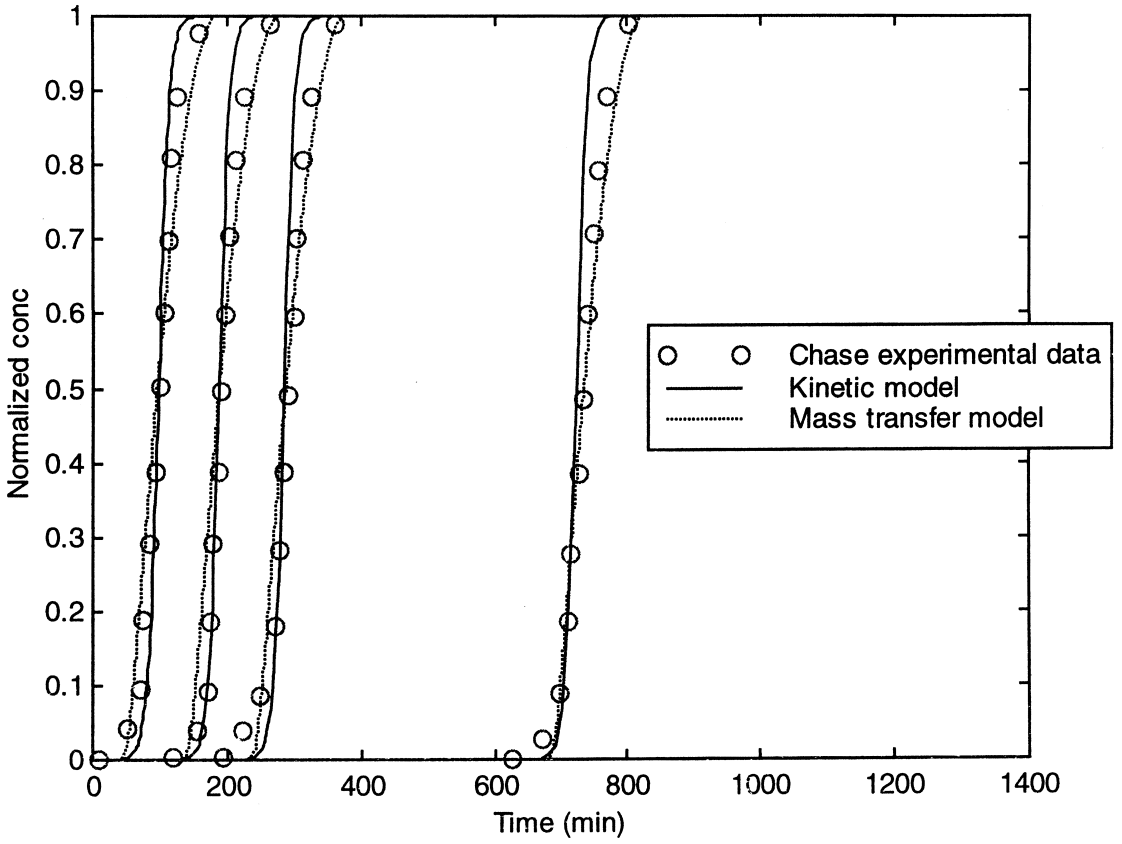


Fig. 2. Thomas' and Arnold's models compared with experimental data (Chase [16]). See Fig. 1 for column lengths.

changed to $1.3 \cdot 10^{-11}$. In this case the fitted effective diffusion coefficient is a lumped constant including the kinetic effects.

### 4.1. Influence of the inlet concentration

In order to study how the different models are able to describe variations in the physical, kinetic and operating parameters the inlet concentration was varied. The concentration is 104 $g/m^3$ in the base case which should be compared with the $K_D$ value of 26 $g/m^3$.

In Fig. 3 the three models have been compared when the inlet concentration has been decreased to be the same as the $K_D$ value of 26 $g/m^3$. The intrinsic and the mass transfer model are more influenced than Thomas' model as they include the internal diffusion as an extra mass transfer resistance.

A decreased inlet concentration gives a later breakthrough curve. The slope is slightly flattened for the lower inlet concentration. Note that the concentration is normalized. The difference in steepness would have been even more apparent if absolute concentrations had been used. This difference in steepness can be explained by the following reasoning.

A decreased inlet concentration results in a flattened concentration profile in the bead. The lower concentration gradient causes a slower transport, just as a decreased diffusion coefficient or a decreased mass transfer coefficient would cause a slower transport. This results in a slower saturation of the beads. When the beads become saturated more

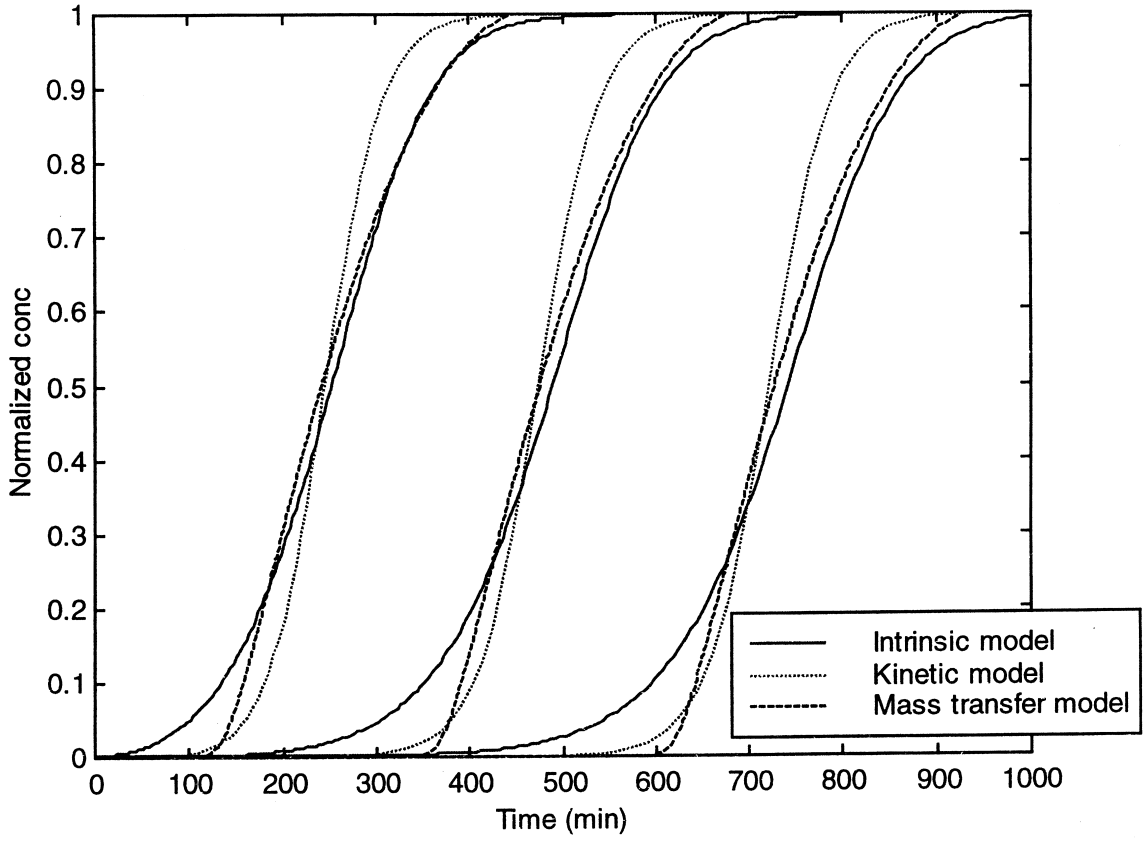


Fig. 3. Decreased inlet concentration of 26 $g/m^3$ compared to 104 $g/m^3$ in the base case. Comparison of Thomas', Arnold's and the intrinsic SIMCHROM models.

slowly, they will extract protein from the mobile phase for longer, resulting in a flatter breakthrough curve.

### *4.2. Influence of the effective diffusion coefficient of lysozyme*

Depending on the operating conditions for the chromatographic separation, i.e. pH and ionic strength, the diffusivity of a protein can vary a lot. In order to study the influence of changed operating conditions, the three models were compared when the diffusivities had been doubled (Fig. 4).

The increase of the diffusion coefficient results in steeper breakthrough curves for SIMCHROM and Arnold's model. No change in the Thomas' model can be seen as the diffusion is not explicitly included in the model. However, if new lumped kinetic parameters are determined for the new operating conditions good agreement can be accomplished using Thomas' model. Arnold's mass transfer model is very sensitive for changes in the diffusion coefficient as this contains the whole mass transfer resistance. The lumped kinetic parameters used in Thomas' model are only valid for a certain operating condition. By instead using the intrinsic model, the decrease of the diffusion coefficient, due to for example an increased crosslinking of the gel matrix, can easily be studied.

### *4.3. Influence of the bead radius*

The influence of the bead radius has been studied by increasing the diameter from 100 to 200 μm (Fig. 5). The increased radius results in an increased distance for the protein to diffuse within the beads. At the same time, the area/volume ratio for a single bead decreases, giving a decreased mass transfer area between the surrounding mobile phase and the bead.

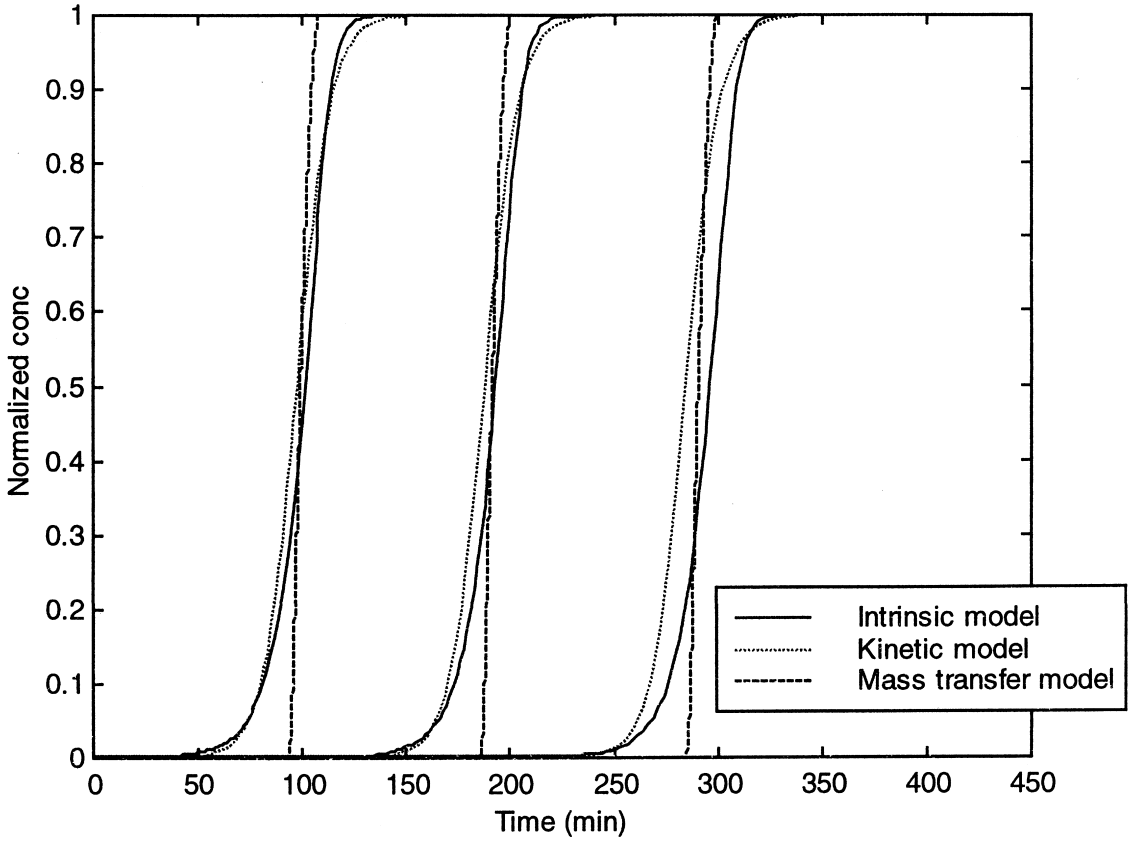


Fig. 4. Doubled diffusion coefficient for lysozyme. Comparison of Thomas', Arnold's and the intrinsic SIMCHROM models.

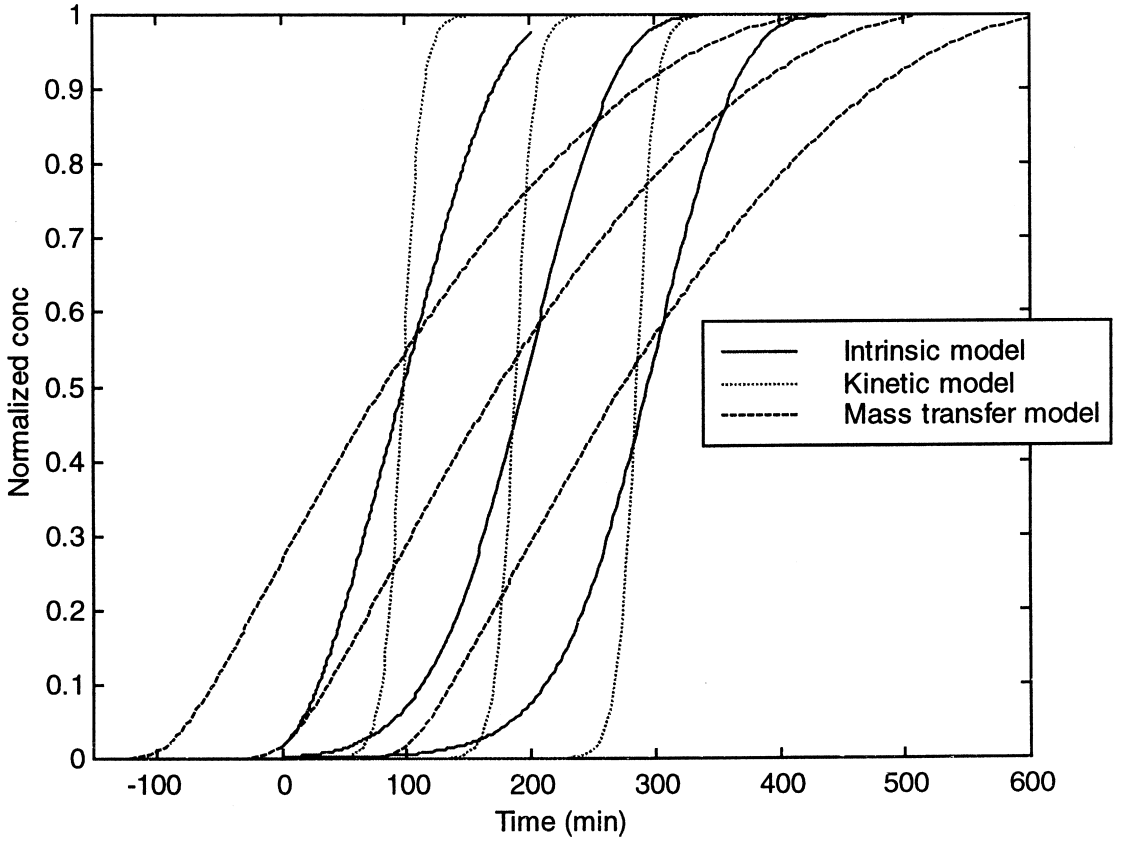


Fig. 5. Doubled bead diameter from 100 to 200 μm. Comparison of Thomas', Arnold's and the intrinsic SIMCHROM models.

Both these factors contribute to the decrease in the total adsorption rate. The difference in the slope of the breakthrough curve obtained when the bead diameter is varied between 50 and 100 μm is much smaller than the difference obtained between 100 and 200 μm (not shown). This implies that there is not much to gain in reducing the particle diameter below 100 μm in this case.

Dramatic differences can be seen for the three models. Again since the particle size and any mass transfer hindrance is lumped in the kinetic parameters in the model, no difference is seen for Thomas' model. However, if new lumped kinetic parameters are determined for the altered particle size, good agreement can be accomplished. This means that a new set of physical lumped parameters have to be determined for each new operating condition. On the other hand Arnold's model results in an overestimation of the flatness of the curve due to the lumped character of the Arnold diffusion coefficient. The same effect was seen in Section 4.2 when the diffusion coefficient was changed.

The present simulation demonstrates clearly the advantages of using an intrinsic model.

### *4.4. Influence of the axial dispersion*

The only model which includes the axial dispersion, $D_{AX}$, is the intrinsic SIMCHROM model. The influence of the axial dispersion was studied by varying the axial dispersion coefficient in this model. It was decreased one hundred times and increased ten times from the basic value (Fig. 6).

The dispersion is expressed by the Pe number, defined by Eq. (7). As mentioned in Section 3.1 Pe values around 1 are common in these packed beds [12–15]. From Fig. 6 it can be seen that a decrease in the Peclet number by a factor of 10 from the base

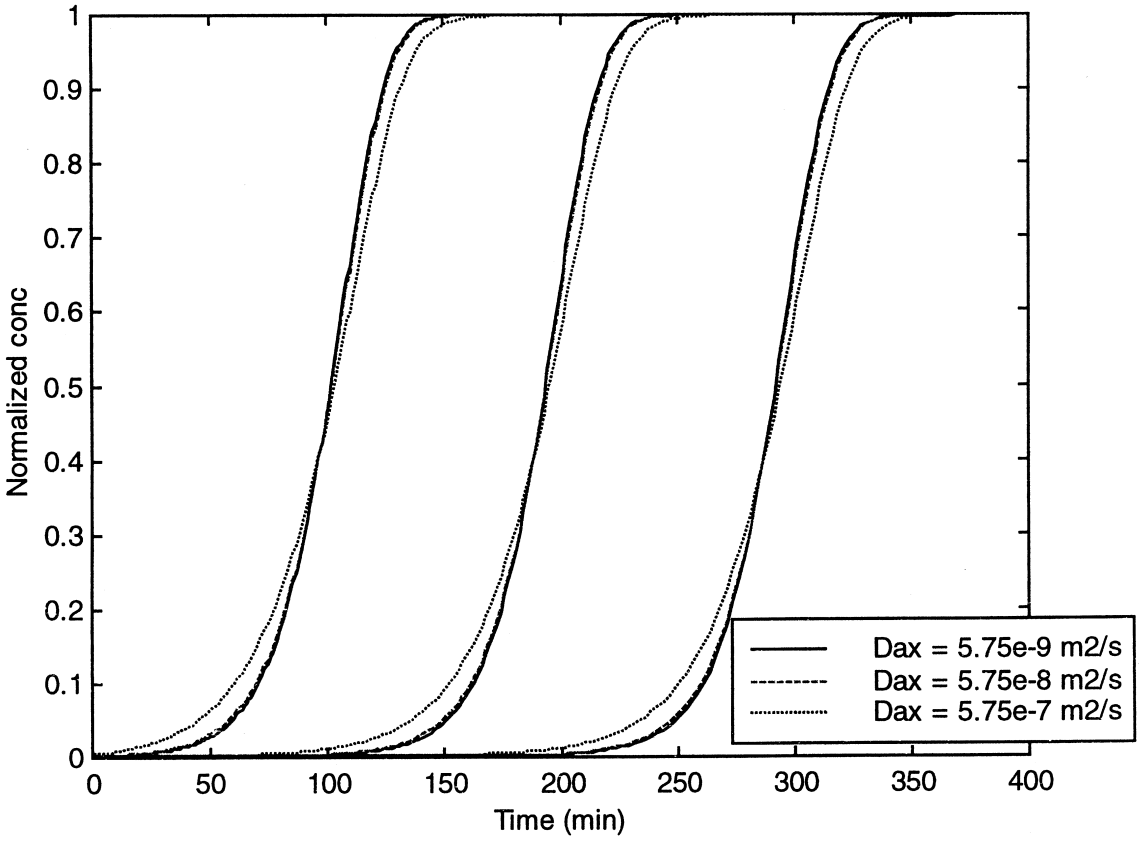


Fig. 6. Influence of the axial dispersion coefficient in the intrinsic SIMCHROM model.

case value of 1, influences the shape of the breakthrough curve very little, and a increase of 100 times produces no difference at all. An increased backmixing expressed as an increase in the dispersion coefficient, and thus a decrease in the Pe number, causes no detrimental effect to the breakthrough curve in the case above. It can be generally concluded that backmixing will not affect the breakthrough curve to any large extent, as long as the column is thoroughly packed and the liquid evenly distributed over the packing material.

## 5. Discussion and conclusions

The advantages of using an intrinsic mathematical model for fixed bed chromatography is demonstrated by the PC program SIMCHROM. The program is based on a model including axial dispersion in the bulk liquid, external and internal mass transfer resistances and an instationary non-linear adsorption model. Frontal and pulse chromatography can be studied for single and multicomponent systems.

The present intrinsic model has been compared with simplified models: a kinetic model (Thomas' model) considering only the kinetics and a diffusion/equilibrium model (Arnold's model) considering only diffusion and adsorption equilibrium. It is shown that although the resemblance between the three models in the base case is fairly good the models differ a lot when the operating conditions for the chromatography process is changed. This results in a more reliable prediction of the performance of the chromatography process as well as a better

understanding of the underlying mechanisms responsible for the separation.

Thus, it can be concluded that simplified models utilizing lumped physical constants are of less value when new operating conditions are looked for. Furthermore, parametric studies have to be performed on an intrinsic model to gain qualitative information of good quality.

The SIMCHROM program can thus easily be used to make parametric evaluations to study the influence of variations in physical, kinetical and operating parameters.

In a parameter study it is shown that the axial dispersion coefficient influences the breakthrough curve very little. $D_{AX}$ must be decreased an order of ten times from the basic case value to detect any difference (not shown). The axial dispersion influences the breakthrough curve very little in this case and the value obtained from empirical relations is accurate enough to give a reliable breakthrough curve.

An important process parameter is the bead size. It was shown that a bead diameter of 200 μm instead 100 μm will result in an extended breakthrough curve. To predict the performance the intrinsic model has to be applied as Thomas' model does not include the particle size and Arnold's model overestimates the effect of the diameter change.

It can also be concluded that the axial dispersion coefficient does not have to be extremely accurate for the design of the chromatography process for the conditions prevailing in this study. Even a large error in the range of ±50% in the axial dispersion coefficient would have very little influence on the result.

Finally, in order to obtain good simulation results, accurate experimental data for the basic physical parameters are required.

## 6. Symbols

| | |
|---|---|
| $a$ | specific area ($m^2/m^3$) |
| $C_0$ | inlet concentration to column (mol/$m^3$ solution) |
| $C_L$ | concentration in mobile phase (mol/$m^3$ solution) |
| $C_P$ | concentration in pore liquid (mol/$m^3$ pore liquid) |
| $C_{Pr=R}$ | concentration at surface of particle (mol/$m^3$ pore liquid) |
| $C_{inlet}$ | concentration in mobile phase entering the column (mol/$m^3$ solution) |
| $C_{outlet}$ | concentration in mobile phase leaving the column (mol/$m^3$ solution) |
| $D_{AB}$ | diffusion coefficient in free liquid ($m^2/s$) |
| $D_{AX}$ | axial dispersion coefficient ($m^2/s$) |
| $D_E$ | effective diffusion coefficient ($m^2/s$) |
| $d_p$ | bead diameter (m) |
| $K$ | mass transfer coefficient (m/s) |
| $k_{ads}$ | adsorption rate coefficient ($m^3/(mol \cdot s)$) |
| $k_{des}$ | desorption rate coefficient ($s^{-1}$) |
| $L$ | column length (m) |
| $l$ | length coordinate in column (m) |
| $q$ | adsorbed adsorbate concentration in beads (mol/$m^3$ gel) |
| $q_m$ | maximum adsorbed adsorbate concentration in beads (mol/$m^3$ gel) |
| Pe | Peclet number (dimensionless) (Eq. (7)) |
| $R$ | bead radius |
| $Re_p$ | particle Reynolds number (dimensionless) (Eq. (8)) |
| $r$ | length coordinate in bead (m) |
| $r_P$ | negative adsorption rate (mol/$m^3$ gel s) |
| Sc | Schmidt number (dimensionless) (Eq. (13)) |
| Sh | Sherwood number (dimensionless) (Eq. (12)) |
| $t$ | time (s) |
| $v$ | superficial velocity (m/s) |
| $v_{INT}$ | interstitial velocity (m/s) |

Greek symbols

| | |
|---|---|
| $\epsilon_c$ | void in bed ($m^3$ mobile phase/$m^3$ column) |
| $\epsilon_p$ | void in bead ($m^3$ pore liquid/$m^3$ bead) |
| $\nu$ | kinematic viscosity ($m^2/s$) |

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