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# Modeling and simulation of breakthrough curves of recombinant 503 antigen using immobilized metal affinity expanded bed adsorption chromatography

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ABSTRACT

In this study a mathematical model was used to simulate the breakthrough curves of the recombinant 503 antigen of *Leishmania infantum chagasi* using immobilized metal affinity expanded bed adsorption chromatography. Initially, experimental curves were carried out in different conditions such as superficial liquid velocity, settled bed height as well as the initial 503 antigen concentration. The particle swarm optimization (PSO) algorithm was used for global parameter estimation by a general rate model, which proved to be an efficient tool for parameter estimation in chromatographic processes. Modeling and simulation of the 503 antigen breakthrough curves displayed a good performance during the estimation and validation stages. The highest global process efficiencies obtained was 88.8%. Finally, the validated model was used in the optimization stage, showing the process and column efficiencies of 89.2% and 75.9%, respectively. The results showed the applicability of this approach for modeling and optimization of chromatographic processes.

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## 1. Introduction

Visceral leishmaniasis (VL) is a disease caused by infection with the protozoa *Leishmania infantum chagasi* in the New World. An estimated 300,000 new cases of VL and over 20,000 deaths occur worldwide each year [1]. The main problems in the control of this disease are the difficulty in access to health care, toxicity and expense of treatment regimens, and a lack of a protective vaccine [2]. The 503 antigen is a protein that displays 100% identity with the elongation factor 1ɣ (EF-1ɣ) of *L. infantum* and constitute a probable target for the immune response during the human infection, suggesting its use as a candidate for the development of serological tests or of vaccines against VL [3].

On the other hand, the downstream stage can correspond from 50% to 90% of the production cost of a biomolecule [4,5]. Thus, more studies are needed to minimize the costs associated with the purification stage in order to guarantee the economic feasibility of the process [5]. Expanded bed adsorption (EBA) is a bioseparation technology that provides significant advantages compared to traditional chromatographic processes in terms of process robustness, flexibility, and economy. EBA can capture target proteins from particle-containing feedstocks without any pre-clarification steps, which is helpful from the industrial viewpoint [6,7].

Immobilized metal affinity (IMA) chromatography is an important technique for purification of His-tagged proteins employed from bench scale to industrial scale [8]. A chromatographic separation, such as IMA-expanded bed adsorption, shows sometimes as a quite complex purification process. It is developed through a series of measures that include the choice of high performance resins showing higher binding capacities to the target-biomolecule [9]. Usually during adsorption process development is quite common to start with experiments in batch mode. At this stage characteristics of the resin such as pore distribution, i.e., mono or di-dispersion as well as particle size distribution play a key role on the kinetics of adsorbate [10,11]. In a column process, the breakthrough curve serves as the dynamic evaluation of the adsorption process, which shows the increase of concentration of the

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**Nomenclature**

| | | | |
|---|---|---|---|
| $C$ | 503 antigen concentration dispersed in the fluid (mg mL$^{-1}$) | $k_2$ | desorption constant (s$^{-1}$) |
| $C_o$ | initial the 503 antigen concentration at the inlet of the column (mg mL$^{-1}$) | $k_f$ | mass transfer coefficient (cm s$^{-1}$) |
| $D_{ax}$ | axial dispersion coefficient (cm$^2$ s$^{-1}$) | $q_{\max}$ | maximum adsorption capacity (mg mL$^{-1}$ of adsorbent) |
| $H$ | height of the expanded bed (cm) | $Vz$ | superficial liquid velocity (m s$^{-1}$) |
| $H_o$ | settled bed height (cm) | | |
| $k_1$ | adsorption constant (mL mg$^{-1}$ s$^{-1}$) | *Greek letters* | |
| | | $\varepsilon$ | bed voidage |

adsorbate in the effluent with time until it reaches the same concentration in the feed. The breakthrough point is considered as the time of completion of the adsorption cycle in industrial applications [12,13]. The breakthrough curves could be mathematically modeled by equations that describe the hydrodynamic behavior and the adsorption profile of the expanded bed. This model can be an advantageous tool to simulate the process in conditions different than used to generate data, without the necessity of carrying out new experiments [14,15].

During model development, it can be observed that some parameters cannot be estimated or require hydrodynamic and kinetic assays. In order to estimate these parameters and to improve the model reliability, model parameter values must be estimated from available experimental data through minimization of some objective function that weighs the distance between model predictions and available experimental results [16].

In this context, the objective of the present study was to develop a mathematical model to simulate the breakthrough curves of the recombinant 503 antigen of *Leishmania i. chagasi* using immobilized metal affinity expanded bed adsorption chromatography. Initially, the global parameter estimation was performed by a general rate model using the particle swarm optimization (PSO) algorithm and the validation of parameters was carried out using a new experiment. Then, the validated model was employed as a process simulator, and the operating conditions were optimized in order to maximize the performance of EBA.

## 2. Material and methods

### 2.1. Microorganism and culture conditions

*Escherichia coli* M15 expressing the His-tagged 503 antigen of *Leishmania i. chagasi* was provided by Dr. Mary Wilson from the University of Iowa (Iowa, USA). The *E. coli* strain was cultivated in 2xTY medium (16 g L$^{-1}$ tryptone, 10 g L$^{-1}$ yeast extract, and 5 g L$^{-1}$ NaCl, pH 7.0) containing 0.1 g L$^{-1}$ ampicillin and 0.025 g L$^{-1}$ kanamycin at 37 °C and 400 rpm on a bioreactor (Biostat B., B. Braun Biotech International) with a working volume of 1.5 L. The 503 antigen was expressed as intracellular protein using induction with lactose by a final concentration of 10 g L$^{-1}$ when the optical density at 600 nm reached 0.5 [17]. The cells were harvested with centrifugation at 1500 × g and 4 °C for 30 min. The wet cells were subjected to cell disruption using urea lysis buffer to release inclusion bodies (10 mM imidazole, 8 M urea, 50 mM $NaH_2PO_4$, 0.5 M NaCl, pH 8.0) as carried out by Vaz et al. [17].

### 2.2. Adsorbent and column

The Streamline chelating was purchased from GE Healthcare (Uppsala, Sweden). This adsorbent is composed of highly cross-linked 6% agarose, which entraps an inert quartz core to give a mean particle density of 1.2 g mL$^{-1}$ and a distribution size ranging of 100–300 μm (manufacturer's manual). A glass custom-made column (30.0 cm × 2.6 cm I.D.) was fitted with an adjustable piston to minimize the headspace over the expanded bed. A ruler was placed on the column wall to record the bed height [4,9].

### 2.3. Breakthrough curves

The adsorption performance of the 503 antigen onto the Streamline chelating adsorbent using the expanded bed was evaluated at different superficial liquid velocities ($27.8 \times 10^{-4}$ to $69.4 \times 10^{-4}$ cm s$^{-1}$), settled heights of adsorbent (4–8 cm) and initial 503 antigen concentrations (0.104–0.357 mg mL$^{-1}$). The conditions employed in the experiments are shown in Table 1. All the experiments were carried out at room temperature (25 ± 2 °C). For each experiment, the adsorbent was initially equilibrated with binding buffer previously optimized by Sousa Junior et al. [18] (20.0 mM $NaH_2PO_4$, 2.4 M NaCl, and 10.0 mM imidazole, pH 8) at the desirable superficial velocity for 30 min. Subsequently, the unclarified feedstock of *E. coli* containing the 503 antigen was pumped into the column. Samples were collected in fractions (10 mL per fraction) and analyzed to obtain the amounts of total protein and 503 antigen. Total protein was quantified according to the Lowry method [19], using Folin phenol reagent and bovine serum albumin (BSA) as standard. Protein expression was evaluated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) [20] and the gels were photographed to estimate the 503 antigen amount by densitometry employed the ImageJ software [21]. The amount of 503 antigen was calculated based on the relative quantity of the 503 antigen band on the SDS-PAGE against the amount of total protein obtained by the Lowry method as previously described by described by Yap et al. [22].

## 3. Model formulation

The mathematical model for the 503 antigen adsorption process in an expanded bed column was developed based on the following considerations:

**Table 1**
Experimental and predicted efficiencies for the adsorption of the 503 antigen into the Streamline chelating resin in the expanded bed.

| Run | $H_o$ (cm) | $Vz$ (cm s$^{-1}$) | $C_o$ (mg mL$^{-1}$) | Process efficiency (%) | | Column efficiency (%) | |
|---|---|---|---|---|---|---|---|
| | | | | Exp. | Pred. | Exp. | Pred. |
| 1 | 5.0 | $41.7 \times 10^{-4}$ | 0.357 | 71.5 | 86.2 | 62.1 | – |
| 2 | 4.0 | $55.5 \times 10^{-4}$ | 0.334 | 84.4 | 87.5 | 66.4 | 57.8 |
| 3 | 8.0 | $55.5 \times 10^{-4}$ | 0.304 | 87.2 | 86.0 | 75.2 | 65.0 |
| 4 | 5.0 | $69.4 \times 10^{-4}$ | 0.104 | 78.9 | 87.6 | 73.7 | 64.8 |
| 5[a] | 8.0 | $41.7 \times 10^{-4}$ | 0.248 | 88.8 | 87.1 | 81.9 | 64.1 |

Exp.: experimental; Pred.: predicted; $H_o$: initial settled bed; $Vz$: superficial liquid velocity; $C_o$: 503 antigen concentration.
[a] Validation.

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