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Kinetic modeling of biomolecular capture processes involved in molecular recognition reaction. Validation by the affinity chromatography technique.

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Highlights

- Efficiency of immunoassays design could be improved with a predictive model.
- Fixed bed column based on affinity chromatography has been built.
- A kinetic model of antigen/antibody interaction processes has been developed.
- Parameters of the model have been estimated from experimental breakthrough curves.

1. Introduction

Immunoassay technique is one of the most efficient and reliable tools for *in vitro* diagnostics. It mainly relies on the specific recognition between an antigen and an antibody [1]. When designing immunoassays, sensitivity, specificity and time-to-result can be optimized with the choice of a dedicated ligand to specifically capture the analyte of interest as well as the solid support (material and design) or the flow-rate of the liquid which contains the molecules to be detected. These choices have commonly been made empirically, that is time and money consuming. Using an adequate experimental set-up and a predictive model could improve the efficiency of immunoassays design and will allow having a good representation of antibody/antigen interactions via a kinetic model. This kinetic model of antibody/antigen interactions could be useful to simplify and speed-up the identification of the best immunoassays conditions or to improve the immunoassays currently available. This approach is supported by the fact that the characterization of antigen/antibody interactions kinetics plays a major role for the selection of the best molecules for immunoassays [2 – 3].

The global aim of this work is to improve the performance level of immunoassay diagnostic tests in a rational and effective way (compared to the fastidious empirical approaches used today) by better understanding and predicting the complex molecular interactions that occur in the different steps of a diagnostic immunoassay. An experimental tool based on a fixed bed column is proposed in order to build a predictive model of antigen/antibody interaction processes. These experimental/modeling tools will be used i) to refine our knowledge of the mechanisms involved in the capture of antigens by antibodies immobilized on surfaces and ii) to identify the critical parameters of the diagnostic systems.

2. Methods

The experimental tool, a fixed bed column, is based on affinity chromatography in order to study physical and chemical phenomena involved in molecular recognition processes between antigens and antibodies. Antibodies are immobilized on a particle bed in a column while antigens are injected at the inlet of the column.

The fluid flow is characterized through Residence Time Distribution (RTD) measurements with sodium chloride as a tracer. In parallel, a kinetic model of the experimental system that takes into account mass transfer processes has been developed and implemented in Matlab and solved using finite-difference method. Parameters of the model are estimated by minimizing the difference between experimental and simulated data.

3. Results and discussion

Antigens are injected as a step input at the inlet of the column at a constant flow rate (1mL/min) and at a constant concentration from t=0 and during 35000 seconds. Antigen concentration is measured at the outlet

of the chromatography column (see blue circles in figure 1). According to RTD experiments, the mean residence time of a non-retained solute in the chromatography column is less than 30 seconds. In our experiment, after 35000 seconds, the outlet antigen concentration barely reaches the inlet antigen concentration, showing that antigens are retained in the column, captured by the antibodies.

The response to an injection of antigens during 35000s has been simulated using the column model (see red line in figure 1). Four parameters have been adjusted to minimize the difference between experimental and simulated data with nonlinear least-squares fitting method: intrinsic antibody/antigen association rate constant, intrinsic antibody/antigen dissociation rate constant, mass transport coefficient and immobilized antibody concentration on the particle bed.

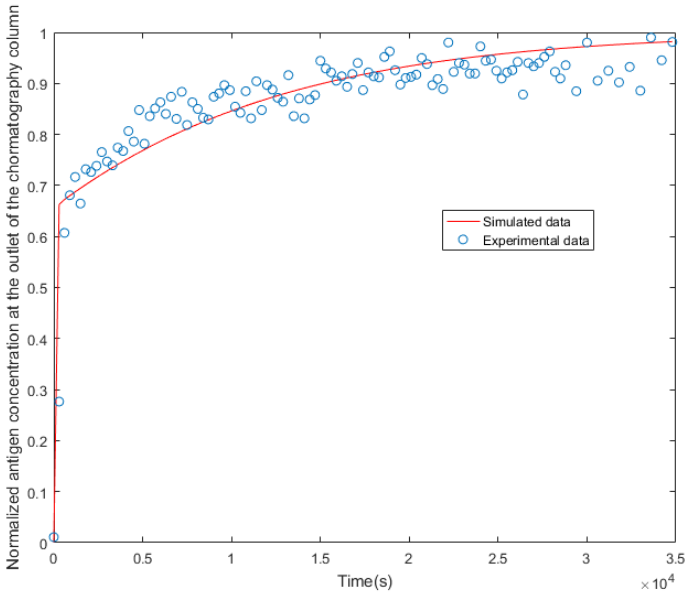

Figure 1. Simulated and experimental normalized analyte concentration at the outlet of the chromatography fixed bed column. Response to a step injection. Outlet concentration is normalized with respect to the inlet concentration.

The predictive value of the developed model and of the estimated parameters should now be validated under different operating conditions (other flow rates or other inlet concentrations for example). The next step is to adapt the model to an *in vitro* diagnostic system.

4. Conclusions

This work is part of a collaboration between the bioMérieux company and the LAGEP (Chemical Engineering and Process Control Laboratory) of Claude Bernard Lyon 1 university. It is an opportunity to make the link between the deductive approaches of physics-based modeling and the empirical knowledge of biology/biochemistry settings, with a clear potential impact in the field of diagnostics and, ultimately, public health.

References

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Keywords
immunoassays; kinetic modeling; affinity chromatography; parameters estimation.