DIRECT AND INVERSE MODELING OF ENZYMES ADSORPTION KINETICS IN MACRO-POROUS ADSORBENTS

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ABSTRACT

The estimation of adsorption parameters for chromatographic systems is a very important step for column characterization used in the design of continuous separation processes by adsorption phenomenon as simulated moving beds (SMB). The turbulent hydrodynamics aspects of batch procedures, which minimize the diffusion effects, become the kinetic modeling an interesting tool for the process modeling that is used for the determination of the primordial parameters that will be considered in the equipment design. The implemented irreversible kinetic model, which is both depended on solute and site concentrations, showed to be very effective in the experimental correlation of two different enzymes adsorption systems: adsorption of Inulinases and b-Galactosidase using two different adsorbent, the CM-Sepharose CL-6B and Accell plus QMA, respectively. The implementation of a new error analysis methodology for continuous systems associated with the inverse problem approach was successful in determining the kinetic parameters with a high accuracy. The success of the implemented technique of error analysis with an inverse approach become viable for application to another continuous systems, especially in chromatography process, in which experimental error determination is difficult and the domain error is high. The experimental error treatment for chromatography adsorption processes must be considered as these systems generally work with very low concentration.

1. NOMENCLATURE

k_i = Kinetic constant
r_i = Kinetic rate
V = Tank volume
n_j = Moles number of compound j
C_t = Total site concentration
C_s = Vacant site concentration
C_{As} = Occupied site concentration
C_A = Solute concentration
C_{A0} = Initial solute concentration
C_{eq} = Equilibrium solute concentration
σ = Standard deviation
ϕ = Maximum deviation
N = Total number of experimental points
x_i = Experimental value
$\bar{x}$ = Mean value
Q = Residue function

2. INTRODUCTION

The separation techniques of biomolecules, such as enzymes and proteins, through perfusion chromatography techniques, is dependent upon the selection of material supports with pores and distribution that minimize the diffusion effects inside the solid particles and maximize the mass transfer mechanism by convection [1], as well as the superficial area of interaction. Therefore, there is a tendency to work with flow regimes that reduce the separation time, leading to the dominance of adsorption kinetic factors.

The agitated batch process of enzymes adsorption is an important method used for equilibrium parameters estimation, which are applied in the processes modeling such as chromatography and simulated moving bed (SMB) separation. The hydrodynamics aspects of these processes become the kinetic modeling an interesting tool for the process

modeling in obtaining parameters that will be incorporated in the equipment design.

The application of an inverse problem methodology for chromatographic systems is a new promising area as the solution of inverse problems has several relevant applications such as in the engineering field [2]. Some publications can be found such as Vasconcellos et al. [2,3,4], Lugon et al. [5], Denisov [6], Felinger at al. [7], and Folly et al. [8]. In the work of Vasconcellos et al. [2] the inverse methodology by a cost function of square residues was applied in the mass transfer parameters estimation of protein adsorption (Bovine serum albumin-BSA). The optimization method utilized is the same applied by Folly et al. [8], the Levenberg-Marquardt. The method in both applications had successful in determining the parameters of non-linear equations.

The importance of adsorption kinetic phenomenology can be found incorporated in some different ways, such as in the processes modeled by the equilibrium dispersive theory, which considers immediate equilibrium between solid and liquid phase, in which there is equivalence between mass transfer and kinetic phenomenology. Generally, these models assume solute adsorption rates that consider either variations in the equilibrium isotherms of Langmuir [2,9] or linear driving forces models-LDF [10,11,12]. The first one is limited as kinetic constants correspond to equilibrium conditions, when the kinetic rate is low. The last one corresponds to empirical models that are restrict to some chromatographic conditions. Therefore, the development and application of new kinetic models are encouraged to contributions that can improve the process modeling of liquid chromatography.

Contributions in the application of adsorption kinetic models for liquid phase can be encountered through some following publications: Chase [13], Sarkar and Chattoraj [14], Hamadi et al. [15,16], Otero et al. [17], Gulen et al. [18] and Aroguz [19]. An important contribution come from the work of Chase [13], which implemented semi-analytical expressions to model adsorption in agitated tank and chromatographic column. He utilized the kinetic concepts to model the adsorption process as a reversible system with a rate global order of 2(two). In a general point of view, such as the above publications, with the Chase model exception [13], the kinetic models corresponds to simplified or empiric expressions. The advantage of utilizing the concepts of kinetic theory to develop new models is that the stoichiometric and order, related to the compounds in the adsorption system considered, can be varied and analyzed independently, leading to a better comprehension of the evolved kinetic phenomenology.
The experimental uncertainly analysis is not a establish technique utilized by the scientific community, especially in liquid chromatography area, as can be noted from the literature [20,21,22]. The importance of such treatment is obvious, mainly on chromatography systems, in which the matrix and low concentration has serious implications in the acceptance criteria of the applied method [21]. As example, we have the work of Gritti and Guiochon, [22], which showed the importance of the systematic elimination of parts of the data points for reaching betters model correlations.

In this work a new-implemented kinetic model is applied in the modeling of two different protein adsorption systems: adsorption of Inulinases [23] and β-Galactosidase [24] using two different adsorbents with macro-pores distribution, which is a characteristic of perfusion chromatography. The adsorbents utilized were CM-Sepharose CL-6B e Accell Plus QMA, respectively. The model validation through experimental data is done with the application of a new error analysis methodology for continuous systems that is associated to an inverse problem approach by square residues. This technique contributed to best correlations making possible the kinetic parameters estimation with high accuracy.

3. APPLIED METHODOLOGY

3.1 Formulation of the direct modeling

The agitated adsorption techniques to measure adsorption parameters of proteins are modeled with the following expression for batch processes.

$$r_j = \frac{1}{V}\frac{dn_j}{dt} \tag{1}$$

in which r_j, that corresponds to the adsorption rate of solute *j*, is proportional to the moles number variation, being the tank volume (V) constant.

The adsorption stoichiometry is represented in Fig. 1. It is related to an adsorption irreversible model. This adsorption mechanism depends both on the solute concentration (liquid phase) and the active surface concentration (solid phase).

Fig. 1- Adsorption Stoichiometric representation

The representation given in Fig. 1 shows that 1(one) mol of solute A adsorbs in 1(one) mol of active site (s). The kinetic modeling in terms of consumption rate of solute j (r_j) is written in the following form.

$$(-r_j) = k_i C_j{}^{n}.C_{j.s}{}^{m} \tag{2}$$

where k_i, C_j and C_s represents the kinetic constant, the concentration of solute j in the liquid phase and the concentration of sites in the solid phase, respectively. For a first-order elementary adsorption, the exponents n and m are equal to 1, which corresponds to an overall rate of second-order. The irreversible adsorption is an adequate hypothesis, since in the experimental studies [23,24] the desorption procedures are necessary to return the original adsorbent properties, without solute traces. This is done with elution and washing steps.

With the considerations just described, Eq. (2) can be solved analytically through expression (1), applying a balance in the number of mols in the active sites, i.e.

$$C_t = C_s + C_{A.s} \tag{3}$$

in which C_t corresponds to the maximum sites concentration, that is the summation of vacant sites (C_s) and occupied sites ($C_{A.s}$) concentration. Another important balance is due to the solute *j,* in which the initial concentration (C_{A0}) is the sum of the free solute in the solution (C_A) and the solute adsorbed in the solid phase ($C_{A.s}$), i.e.

$$C_{A0} = C_A + C_{A.s} \tag{4}$$

The Combination of Eqs.(1-4) leads to.

$$\int \frac{dC_A}{C_A(a + C_A)} = \int -k_i dt \tag{5}$$

in which $a = C_t - C_{A0}$. Performing the integrations in Eq.(5) results.

$$\ln\left(\frac{C_A}{a + C_A}\right) = -a.k_i.t + c \tag{6}$$

Using the following initial condition: for t=0, C_A=C_{A0} and C_t=C_s. We obtain the final expression for the irreversible kinetic model with adsorption overall rate of second-order (IKM2), which relates the solute concentration (A) with C_t, C_{A0} and k_i, in the time domain.

$$\frac{C_A}{a+C_A} = \left(\frac{C_{A0}}{a+C_A}\right)e^{-ak_i.t} \text{ or } C_A = \frac{aC_{A0}}{(a+C_{A0})e^{-ak_i.t} - C_{A0}} \tag{7}$$

Note that the implemented IKM2 expression come from the moles balance following the moles relation shown in Fig. 1, which can be calculated independently of the volume of each phase system. The parameter *a* in the IKM2 expression (Eq. 7) can be substituted by the term $-C_{eq}$, becoming the model dependent on the liquid phase parameters.

The correlations and comparisons with the kinetic model implemented in this work (IKM2) were realized through the simulated results and experimental data from both Silva [23] and Pereira [24]. The results of other models presented in these works were also used for comparison purposes.

3.2 Inverse Methodology with Error Analysis Approach

The acquisition and use of experimental data must be accompanied by a careful error treatment, especially in time-dependent continuous systems related to chromatographic processes. The adsorption techniques are subject to experimental errors as any ones, but a special analysis must be considered, once these techniques work in general with very low concentrations, which can lead to experimental results in the error domain. The correlation results between these experiments and the models may not be satisfactory, leading to significant errors in the estimated values for the unknown parameters. This has implications in the process design.

One important statistical parameter assumed in the analysis is the standard deviation (σ),

$$\sigma = \sqrt{\frac{1}{N-1}\sum_{i=1}^{N}(x_i - \bar{x})^2} \tag{8}$$

where N, x_i and $\bar{x}$ corresponds to the total number of experimental data, the experimental value and the mean value, respectively,. The mean value ($\bar{x}$) is assumed to be equal to the value obtained from the kinetic model (IKM2) in each specific time. For each specific time we have an experimental value (x_i) and the respective mean value ($\bar{x}$), which is determined by the kinetic model. The deviation (ϕ) corresponds to the difference between the experimental data and the mean value from the model.

In order to obtain estimates for the unknown parameters we formulate the inverse problem implicitly, as an optimization problem in which we look for the minimum value of the cost function given by the summation of the squared residues between experimental and calculated values of the solute concentration.

$$Q = \sum_{n=1}^{N}(C_{\exp_n} - C_{cal_n})^2 \tag{9}$$

4. RESULTS AND DISCUSSIONS

Two different adsorption phenomenological processes, described in Pereira [24] and Silva [23], are considered in the present work and used to demonstrate the potential of the analytical adsorption model associated with the numerical technique for the inverse problem solution.

The correlation of the model results with the experimental data from Silva [23] is presented in Fig. 2, in which three different initial concentrations are considered. The term UA corresponds to enzymatic activity unit.

From Fig. 2 it can be observed an excellent fit of the kinetic model (IKM2) results and experimental data, being more precise than the results obtained with the Chase model [23]. Besides the good agreement of the model results with real experimental data, it must observed that the computational effort is very small because of the analytical solution given by Eq.(7) for the direct problem solution. The IKM2 model described in the present work requires only two parameters (C_{A0} and C_t or C_{eq}) to obtain the rate kinetic constant (k_i) whereas the Chase model [13] needs five parameters ($C_{A0,}$ v, V, C_t and k_d) to calculate k_1 and k_{-1}, the adsorption and desorption kinetic parameters, respectively. The k_d parameter comes from the estimation of Langmuir model with experimental equilibrium data, which has an implicit error. This error will be addressed later.

The mathematical expression obtained for the IKM2 solution allows the numerical treatment of the adsorption process with a simple inverse methodology, with the minimization of the cost functional represented by Eq. (9). It makes possible to study other important system aspects such as the error experimental analysis.

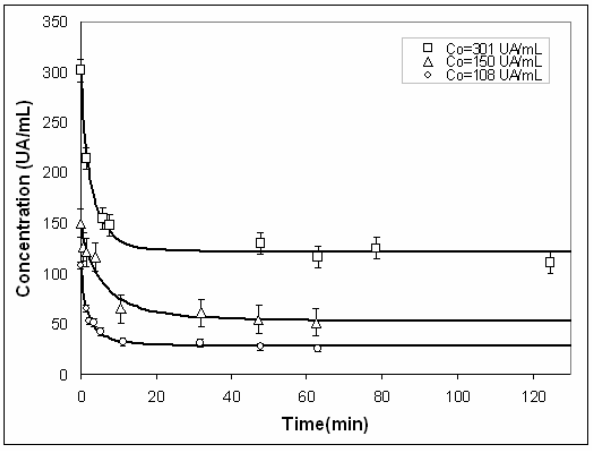

Fig. 2- Correlation between experimental data (points) and calculated values (solid lines) including the maximum deviation for each experimental condition. Experimental data from Silva, [23].

The simulation was realized in two conditions. The first condition assumed that equilibrium concentration (C_{eq}) corresponds to the final concentration (C_f) of enzyme in the system. The equilibrium concentration is an important parameter in the adsorption process once it determines the sites concentration (solid phase concentration) from a balance with the initial concentration of enzyme. In the second condition the equilibrium concentration was varied in the domain between –10 to 10% of the final concentration, allowing an optimization with a better fit of the model with experimental data, being confirmed by a significant reduction in the squared residues cost function (Q). The kinetic constants (k_i), cost function (Q) and maximum deviation between experimental and calculated values (ϕ) are presented in Table 1. The units of Q and ϕ in the text correspond to $(UA/mL)^2$ and UA/mL, and $(mg/mL)^2$ and mg/mL, for the statistical analysis of the experimental results of Silva [23] and Pereira [24], respectively.

Table 1- Estimated parameters and error analysis applying IKM2 model. Experimental data from Silva, [23].

Co (UA/mL)	$C_{eq}=C_f$			$C_{eq}=\beta\%C_f$		
	k_i (mL/UA.min)	Q	ϕ	k_i (mL/UA.min)	Q	ϕ
301 [a]	$1{,}333.10^{-3}$	743,2	18,9	$1{,}542.10^{-3}$	297,9	10,5
150 [b]	$1{,}379.10^{-3}$	423,8	13,5	$1{,}436.10^{-3}$	417,6	13,8
108 [c]	$5{,}534.10^{-3}$	68,2	5,2	$5{,}979.10^{-3}$	47,00	3,7

In a, β=9,4; In b, β=3,3; In, β=9,8;

As can be observed from Table 1, there is a significant reduction in the residues and maximum deviation with the optimization of C_{eq} according to a C_f range (-10 to 10% of the C_f values). The optimum values of C_{eq} (β% of C_f) are in the range studied (less 10%) with the intermediate concentration (C_o=150 UA/mL) having the smallest one (β=3,3%). Only this case (C_o=150 UA/mL) showed a small increase in ϕ with a reduction in residue Q.

In Fig. 2 the maximum deviation (ϕ), considering the optimization for the equilibrium concentration (C_{eq}), is incorporated in experimental points for each curve with the respective size of ϕ.

The Table 2 presents the standard deviation (σ) with corresponding confidence interval (CI). The confidence interval (CI) corresponds to the number of experimental points with deviation size inside the range of $\pm\sigma$ (standard deviation) divided by the total number of experimental points in the condition studied. These calculations were obtained for the optimized condition of Table 1. As can be seen from Table 2, the small value of σ corresponds to C_0 equal to 108 UA/mL, in which has a considerable CI value. For the

higher concentration, although this one presents a considerable value for σ, it is compensated with a high CI. In the intermediate concentration the considerable σ values is not compensated with CI, which is relatively smaller than the other cases. These observations can be seen through Fig. 2, which shows clearly that the intermediate concentration case has a great dispersion of experimental points, with higher values of ϕ.

Table 2- Standard deviation (σ) and confidence interval (CI) values . Experimental data from Silva, [23].

C_o (UA/mL)	σ	CI(%)
301	7,05	71,43
150	8,34	57,14
108	2,59	62,5

The elimination of the data point with maximum deviation (third one) for the intermediate concentration leads to an improvement in the statistical parameters as can be seen in Table 3. There was a significant reduction in the standard deviation (σ) with an increase in the confidence interval (CI%). These results are coherent with the new kinetic parameter obtained (it is greater than the previous one), following the trend of an increase in the rate of adsorption with a decrease in the initial concentration. The rate is directly proportional to the adsorption kinetic constant (k_i). The kinetic parameters from simulated results by Silva [23], applying the Chase model [13], do not follow this tendency.

This observation can be inferred from Langmuir model. In small solute concentration the variation (derivate) or adsorption rate is great (high k_i value), decreasing with the increase in the solute concentration, which has an asymptotic behavior.

Table 3- Standard deviation (σ) and confidence interval (CI) values. Experimental data from Silva, [23].

C_o (UA/mL)	σ	CI (%)	k_i (mL/UA.min)
301	7,05	71,43	$1,542.10^{-3}$
150*	4,05	66,67	$2,121.10^{-3}$
108	2,59	62,5	$5,979.10^{-3}$

* Recalculated value

In Figs. 3, 4 and 5 is shown the good agreement of the results obtained with IKM2 and the experimental data of Pereira [24], applying the square residues inverse methodology with the error analysis approach. The experimental data corresponds to the total protein concentration results.

As can be seen from Fig. 3, the adjustment of experimental points through the implemented model (IKM2) is greatly satisfactory in both initial concentration cases. The simulation results show a better correlation if compared to the results obtained with Chase model as well as with the rigorous model of Pereira [24]. These models were used in the work of Pereira, being related to initial concentrations of 0,076 and 0,148 mg/mL, respectively. The rigorous model showed a poor adjustment in long times.

The rigorous model of Pereira [24] assumed both mass balance in the adsorbent and liquid phase. The approach is based in the equilibrium dispersive theory that considers immediate equilibrium between solid and liquid phase, in which there is equivalence between mass transfer and the kinetic phenomenology. In the work of Pereira the adsorption rate is related to the variation in the Langmuir isotherm model. It corresponds to kinetic constants in the equilibrium, when the kinetic rate is low. It must also be pointed out that the parameters from the equilibrium isotherm correlation can be associated with a significant error degree.

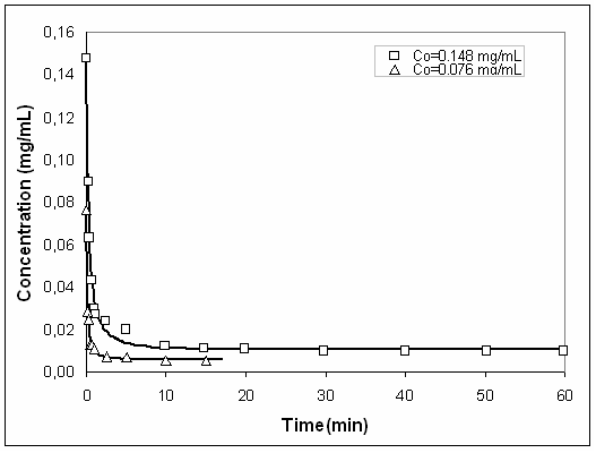

Fig. 3- Correlation between IKM2 model (solid lines) and experimental data from Pereira [24].

The statistical error analysis from experimental results of Pereira [24] is presented through the Tables 4 and 5. Again, the C_{eq} optimization (Table 4) in the range of –10 to 10% of C_f led to a reduction in the residues (Q). The small increase in the maximum deviation (ϕ) is compensated by the reduction in the residues that contribute to a best model fit. The increase of kinetic constants with decrease in concentration is observed.

Table 4- Estimated parameters and error analysis applying IKM model .
Experimental data from Pereira, [24].

Co (mg/mL)	$C_{eq}=C_f$			$C_{eq}=\beta\%C_f$		
	k_i (mL/mg.min)	$Q.10^4$	$\phi.10^3$	k_i (mL/mg.min)	$Q.10^4$	$\phi.10^3$
0,148 [a]	27,9842	2,35	8,859	28,4045	2,30	9,073
0,076 [b]	130,0007	0,16	2,650	132,3528	0,15	2,703

In a, β=8,2; In b, β=6,9;

A better analysis is made by the standard deviation (σ) and confidence interval (CI) of Table 5. The optimized original result, without asterisk, yielded a not satisfactory confidence interval (CI) value for initial concentration of 0,148 mg/mL, having a large value of the standard deviation (σ). The initial concentration of 0,076 mg/mL showed a great value of CI with low value of σ which is very interesting. The high and low dispersion by C_o equals to 0,148 and 0,076 mg/mL, respectively, can be observed in the Fig. 4.

Table 5- Estimated parameters and error analysis applying IKM model
Experimental data from Pereira, [24].

C_o (mg/mL)	σ	CI (%)	k_i (mL/mg.min)
0,148	$9{,}0733.10^{-3}$	57,14	28,4045
0,148*	$3{,}0905.10^{-3}$	84,61	31,6118
0,076	$1{,}3921.10^{-3}$	77,78	132,3528

* Recalculated value

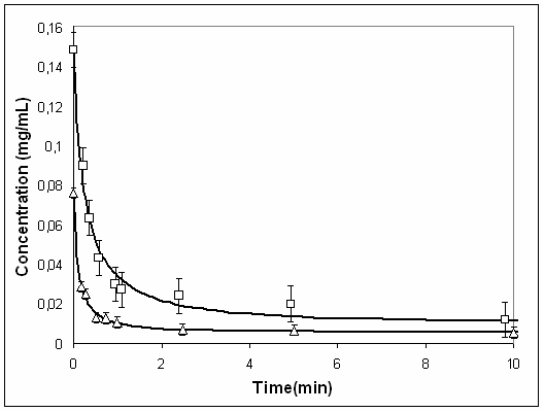

Fig. 4- Correlation between IKM2 model (solid lines) and experimental data from Pereira [24] with error analysis- Original condition.

The elimination of the experimental data point with maximum deviation (first one) from the high dispersion condition (Co=0,148 mg/mL) led to best results in terms of statistical parameters as seen in Table 5, in the concentration case with asterisk. The results can be compared with Fig. 5 that presents the reduction in the dispersion.

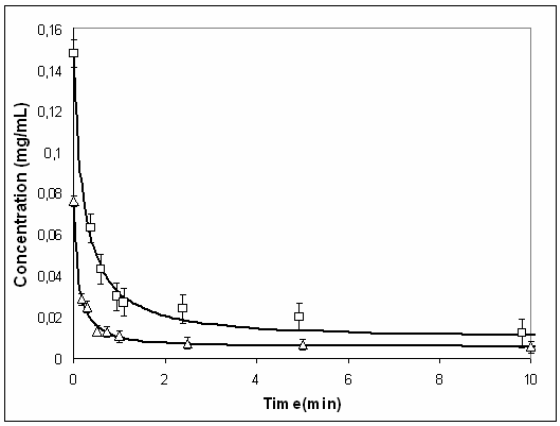

Fig. 5- Correlation between IKM2 model (solid lines) and experimental data from Pereira [24] with error analysis-Improved condition.

5. CONCLUSIONS

The analytical kinetic model developed (IKM2) has proved to be satisfactory due to a number of aspects. Firstly, it provided better agreements with experimental data when compared to other models, such as the semi-analytical Chase model [13] and the equilibrium dispersive model of Pereira [24]. Other relevant aspects are related to the necessity of a small number of parameters in the model (compared to Chase model, [13]) and the straightforward procedure obtaining the solution. These aspects permitted the implementation of a new error analysis methodology for continuous system such as chromatography adsorption processes. It turned out to be very useful when associated to the square residues inverse modeling. The continuous error methodology applied to IKM2 makes possible the kinetic parameters (k_i) estimation with higher accuracy, as confirmed by the standard deviation (σ) and confidence intervals values (CI).

The experimental error treatment for chromatography adsorption processes must be considered as these systems generally work with very low concentration. The model correlation procedures must be analyzed carefully by statistical parameters in order to avoid erroneous estimation.

The consideration of an acceptable error domain for the equilibrium concentration (C_{eq}) provided good results by a reduction in the cost minimization function, which let to a better experimental correlation with an increase in the accuracy of the parameters estimated. The continuous error analysis with square residues inverse approach showed to be a successful tool in the estimation of kinetic parameters of the model developed (IKM2). This technique can be applied to other continuous systems, especially in chromatography process, in which experimental error determination is difficult and the domain error is high.

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