Bi-Langmuir Isotherms' Applicability for Description of Interaction of Ion-Exchange Sorbents with Protein Mixtures

A.A. Demin and **A.T. Melenevsky**

Institute of Macromolecular Compounds, Russian Academy of Science, Bolshoi pr. 31, St. Petersburg, 199004, Russia

Abstract

The sorption of a protein pair on carboxylic cation exchangers with various contents of ionogenic groups is examined. The bi-Langmuir isotherm calculation is carried out from sorption data for single-component systems. It is shown that good agreement of calculated and experimental data is reached only when protein–sorbent interaction weakens because of implementation of the competitive sorption mechanism.

Introduction

Different equilibrium parameters of the components being sorbed provide the basis for chromatographic separation. The greater this difference, the better the component separation (all other factors being the same). The knowledge of sorption isotherm types for individual substances in the multicomponent process is necessary to determine the profile forms of chromatographic zones. Provided only one substance is dissolved in the buffer solution (single-component system), the sorption isotherm of this substance represents a curve on the plane. As for binary systems, the sorption isotherms represent surfaces in three-dimensional space. The construction of such isotherms is a complex experimental task. Thus, attempts to construct isotherms of the multicomponent process as a combination of isotherms for single-component systems are being undertaken.

The elementary theoretical model utilizes the Langmuir equation. It was shown in certain studies that the Langmuir model is not relevant when considering protein sorption processes (1–4). Nevertheless, because of the simplicity of the equations, this model is used when considering multicomponent protein equilibrium conditions. The generalized expression of the sorption isotherm for multicomponent mixture is as follows:

$$m_1 = \frac{a_i C_i}{1 + \sum_{j=1}^{N} b_j C_j} \qquad \text{Eq. 1}$$

where m_i is the concentration of component i in sorbent, and C_i is the concentration of component i in solution.

For sorption from binary solutions, accordingly:

$$m_1 = \frac{a_1 C_1}{1 + b_1 C_1 + b_2 C_2}$$
$$m_2 = \frac{a_2 C_2}{1 + b_1 C_1 + b_2 C_2} \qquad \text{Eq. 2}$$

Bi-Langmuir isotherms were used earlier for the description of protein–sorbent interaction (5–9). The equation analysis shows that an increase in concentration of one substance (one of the proteins, in this case) in the solution results in a decrease in concentration of the other protein in the sorbent phase. The analysis of the multicomponent isotherm equations constructed on the basis of non-Langmuir models (5,11–14) leads to the same conclusion.

Thus, the theory develops an assumption that multicomponent sorption is exclusively a competitive process. At the same time, there are numerous indications that the protein mixture sorption is complicated by synergetic effects. These synergetic effects appear as the ability of the sorbent, saturated previously by another protein, to sorb the protein at least without replacement of previously sorbed protein because of protein multilayer stacking on the surface or aggregation in the sorbent phase.

In our recent studies, the previously mentioned phenomena are observed during the sorption of a protein mixture on high capacity sorbents with high concentrations of the ionogenic groups in the pH range, corresponding to maximal sorption (15–19). The obtained data set allows us to state that synergistic effects during the multicomponent sorption process are caused by the protein's ability to be sorbed irreversibly in the given conditions, depending on the strength of protein–sorbent interaction. The strong protein–sorbent binding first depends on the

* Author to whom correspondence should be addressed: email demin@imc.macro.ru.

number of links of charged macromolecule groups with sorbent-fixed ions predetermines the hysteresis between the ascending and descending branches of isotherm. When all of the ion-exchanger sorption centers are occupied, the binding takes place according to protein–protein interactions.

Weakening of the protein–sorbent interaction by changing the pH value (decrease of dissociation degree of sorbent ionogenic groups or protein recharge) or decreasing the ionogenic group content in the sorbent is responsible for the competitive mechanism of multicomponent sorption (20–22). In this connection, the comparison of calculated and experimental data obtained during the implementation of various mechanisms of protein multicomponent sorption is of specific interest.

Experimental

The study of protein sorption was carried out on cation exchangers synthesized in the laboratory of polyelectrolyte physical chemistry, Institute of Macromolecular Compounds RAS (St. Petersburg, Russia). The first cation exchanger is a copolymer of methacrylic acid and hexahydro-1,3,5-triacryloyltriazine (CMT). The properties of the CMT sorbent are presented in the literature (23).

Carboxylic cation exchangers with various ionogenic group concentrations are the triple copolymers of metacryloylalanine (MAA), hydroxypropylmetacrylamide (HPMA), and ethylendimethacrylamide (EDMA), obtained at various ratios of MAA and HPMA and constant EDMA content (20%). The copolymer synthesis conditions are indicated in the literature (24). The properties of sorbents used in the study are presented in Table I.

During our study of the individual protein sorption, the assessment techniques were applied where protein binding was evaluated by the change in concentration of the protein solution when brought into contact with the sorbent. First, the conditions of maximal protein binding for each sorbent were determined. For this purpose, the protein was dissolved in buffer solutions with an ionic strength of 0.1M at various pH values. These solutions were then brought into contact with the sorbent samples. The solutions were mixed during the time period sufficient for equilibrium to be reached (the time for reaching equilibrium was defined in kinetic experiments). Then, the equilibrium concentration of the protein in the solution was determined, and the protein amount in moles sorbed by 1 L of the sorbent in the swollen state was calculated. The protein concentrations of individual solutions were determined by optical density at 278 nm (wavelength) or from the reaction with Coumassie blue dye (25).

Table I. The Properties of Cation Exchangers

Sorbent	Ionogenic COOH group content (mol. %)	Specific volume in swollen state (mL/g)	Total exchange capacity (mg-equiv/g)
CMT	96	7.1	9.8
MAA–HPMA (1:0)	80	10.1	4.62
MAA–HPMA (1:5)	13.3	7.8	0.92

To construct the sorption isotherms, glass bottles were filled with 10 mL of protein solutions of various concentrations. Then sorbent samples, with their particular water content, were added to the bottles. After equilibrium had been reached, the protein concentration in the solution was determined, and the protein concentration in the sorbent phase was calculated.

During our study of the sorption of bicomponent mixtures, experiments were carried out on the simultaneous sorption of two proteins in static conditions. The binary protein solution with various protein concentrations was brought into contact with the sorbent sample.

In order to determine protein concentrations in mixed solutions, a reversed-phase high-performance liquid chromatography method was used. Chromatography was carried out on a micro-column liquid chromatograph Milichrom (Nauchpribor, Orel, Russia). A stainless steel column (2 × 65 mm) was packed with Lichrosorb C18 (Merck, Darmstadt, Germany). Eluent consumption was 0.1 mL/min, and the wavelength of spectrometric detection was 210 nm. Effective protein separation was obtained by stepped gradient elution with a mixture of acetonitrile (Kriochrom, St. Petersburg, Russia) and 0.3% aqueous phosphoric acid (Sigma, St. Louis, MO) (acetonitrile content was 0%, 10%, 20%, 30%, 40%, and 50%). Protein concentrations were determined from calibration plots of concentration versus peak area. These plots were constructed for protein solutions at concentrations from 0.1 to 1.5 mg/mL. The accuracy of concentration determined by this method was approximately 4%.

When using the bi-Langmuir isotherms, it is understood that the sorbent has equal capacity for all solution components. This supposition is nonrealistic in the case of reviewing multicomponent sorption of proteins with various molecular dimensions. During the sorption process, various proteins occupy various squares on the internal surface of sorbent pores, and this inevitably leads to the various sorbent capacity values for these proteins. In the work of Le Van et al. (26), additional terms were added to the equation, taking into account the different sorbent capacities of each component. Provided that sorption capacity is equal for both components, the obtained equations were reduced in first approximation to Langmuir equations. When two single-component isotherms (for each separate component) differ, the theory gives the numerical definition procedure for isotherms of competing components.

$$m_1 = \frac{\overline{m} b_1 C_1}{1 + b_1 C_1 + b_2 C_2} + \Delta \qquad \text{Eq. 3}$$

$$m_2 = \frac{\overline{m} b_2 C_2}{1 + b_1 C_1 + b_2 C_2} - \Delta \qquad \text{Eq. 4}$$

where

$$\overline{m} = \frac{a_1 C_1 + a_2 C_2}{b_1 C_1 + b_2 C_2}$$

$$\Delta = \left(\frac{a_1}{b_1} + \frac{a_2}{b_2}\right) \frac{b_1 C_1 + b_2 C_2}{(b_1 C_1 + b_2 C_2)^2} \ln(1 + b_1 C_1 + b_2 C_2) \qquad \text{Eq. 5}$$

These equations were used for the calculation of bi-Langmuir isotherms from experimental data obtained for single-component systems.

The values of *a* and *b* coefficients were retrieved from the build-up of single-component isotherms of protein sorption in reversed coordinates:

$$\frac{1}{m} = f\left(\frac{1}{C}\right) \qquad \text{Eq. 6}$$

As a rule, the forms of protein sorption isotherms are far from Langmuir, therefore these calculations gave only the estimated result.

Build-up of bicomponent isotherms requires a great number of experimental points. Therefore, we proceeded with simplification of the task and replaced the isotherm surface by the plane cut at $C_1 = C_2$ for the case of simultaneous sorption of insulin and ribonuclease. During the simultaneous sorption of insulin and ribonuclease from the solution with equal weight concentrations for pH 4.0, the equilibrium protein concentrations have close values. For pH 2.5, the points selected were those with close values of equilibrium concentrations from the large data set on protein sorption with different ratios of initial concentrations. For the case of lysozyme and ribonuclease sorption, the values of individual points corresponding to various isotherm cuts were compared.

Results and Discussion

Figure 1 presents the dependence of CMT cation-exchanger sorption capacity for insulin and ribonuclease on pH values. In the range of pH 4–7 insulin is practically insoluble. For carrying the static experiments on protein sorption from individual and bicomponent solutions, two pH values have been selected: 4.0 (when it is possible to reach maximal CMT capacity for insulin sorption) and 2.5 (when the electrostatic protein interaction with the sorbent weakens).

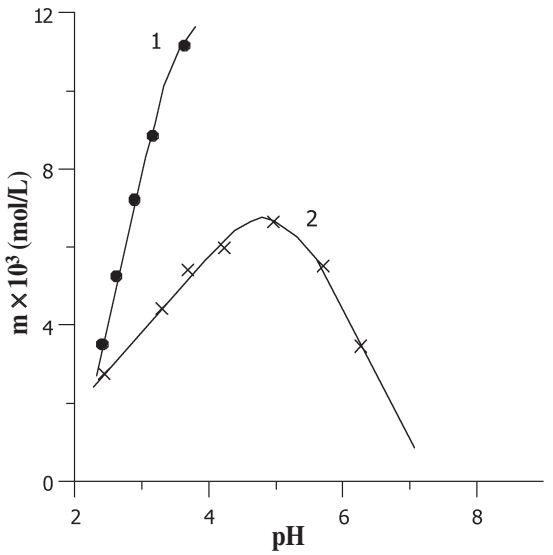

Figure 1. Sorption of insulin (1) and ribonuclease (2) on CMT carboxylic cation exchanger depending on pH of equilibrium solution.

Sorption of the lysozyme–ribonuclease pair by carboxylic cation exchangers on the metacryloylalanine base has been studied at pH 5.8 (i.e., in conditions when sorption of these proteins is close to maximum) (Figure 2).

First, let's consider the process of insulin and ribonuclease sorption on CMT exchanger at pH 4.0 (Figure 3). Under the selected conditions, CMT carboxylic cation-exchanger binds the proteins in considerable amounts. As the molecular sizes of ribonuclease and insulin dimer are close, the component compo-

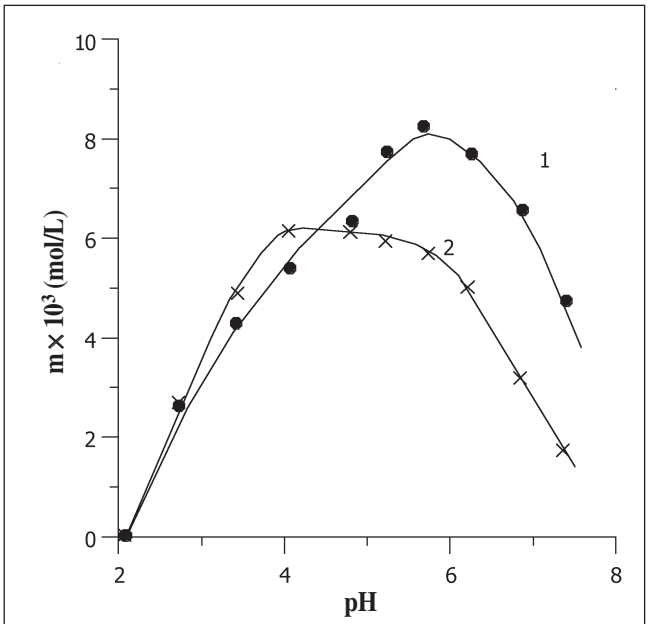

Figure 2. Sorption of lysozyme 1 and ribonuclease 2 on a carboxylic cation exchanger (the MAA and EDMA copolymer), depending on the pH of the equilibrium solution.

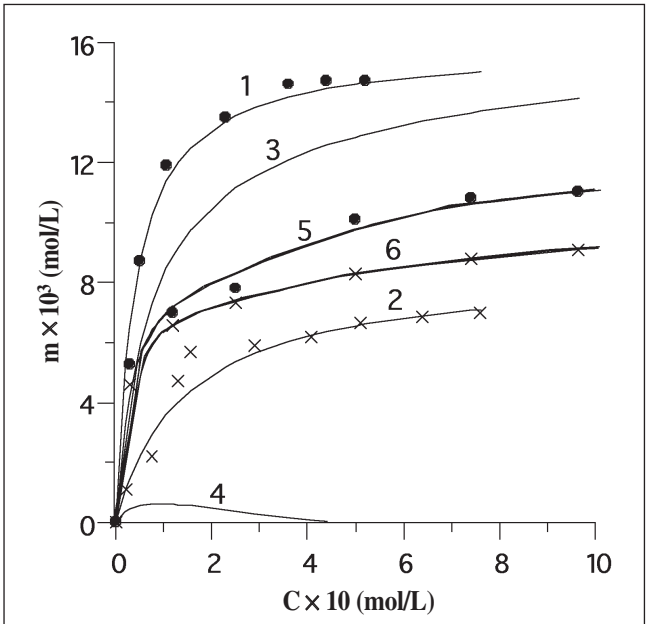

Figure 3. Langmuir isotherm approximation of experimental data on sorption of individual proteins 1 and 2: calculated data for equal concentrations of protein components 3 and 4; experimental data 5 and 6; insulin 1, 3, and 5; and ribonuclease 2, 4, and 6.

sition of the sorbent phase should be determined by the sorption selectivity. From a traditional view, it is impossible to explain the experimentally obtained result, indicating that the selectivity factor of sorption from a binary solution with equal protein concentrations is close to unit (curves 5 and 6 in Figure 3). If the protein binding was determined only by individual protein–sorbent interaction, and there was no cross-influence, the component composition in the sorbent phase should be determined by the ratio of distribution coefficients between the sorbent and the solution obtained for individual proteins. In this case, the sorbent insulin content should exceed the ribonuclease content by a factor of two. In the case of cross-influence, which takes place in the form of competition, as the calculation shows, the ribonuclease sorption should be suppressed by insulin (curves 3 and 4 in Figure 3).

Obviously, the assumption for the absence of binding because of interprotein contacts is not correct. Synergistic sorption mechanism leads to the situation where part of protein molecules have to interact with the sorbent modified by the other protein, and it is possible that selectivity inversion took

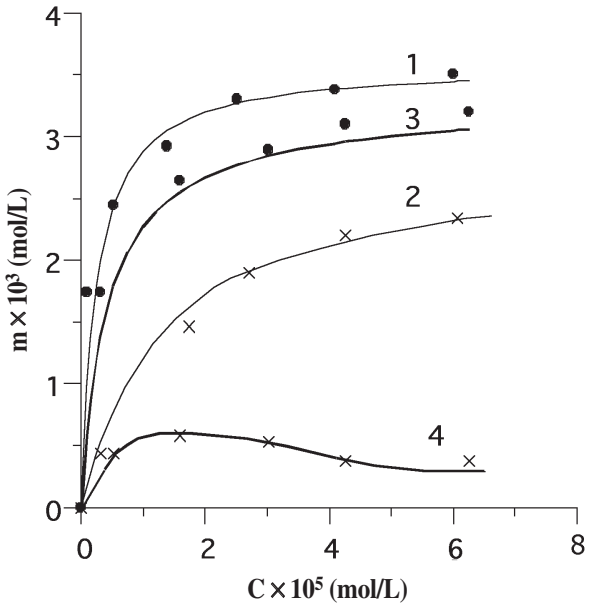

Figure 4. Sorption of insulin 1 and 3 and ribonuclease 2 and 4 on CMT carboxylic cation exchanger at pH 2.5, from individual solutions 1 and 2 and from mixed solutions 3 and 4. Marks on the curves (dots and Xs) are experimental data. Solid line: Langmuir isotherm approximation of experimental data 1 and 2, bi-Langmuir isotherms 3 and 4.

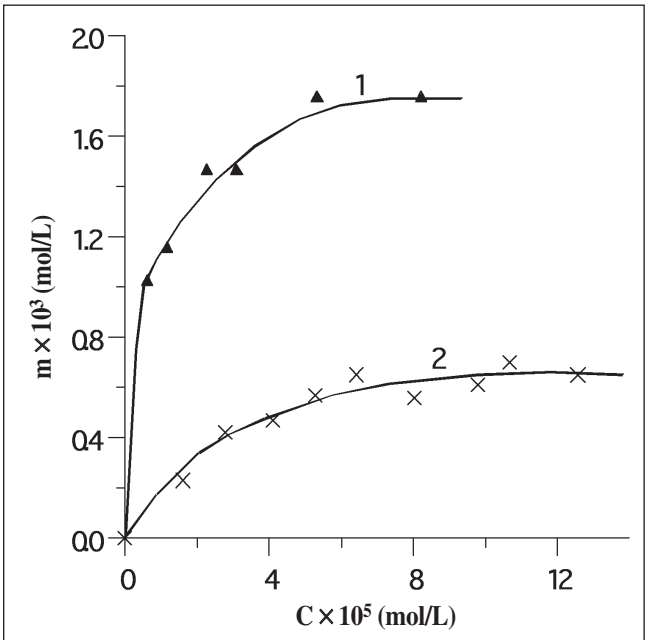

Figure 6. Sorption isotherms of lysozyme 1 and ribonuclease 2 on MMA–HPMA–EDMA copolymers at MMA–HPMA ratio of 1:5.

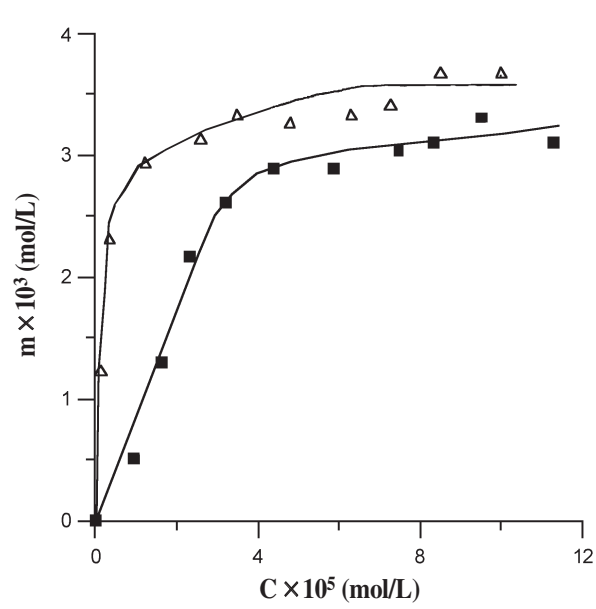

Figure 5. Sorption isotherms of lysozyme 1 and ribonuclease 2 on MMA and EDMA copolymer.

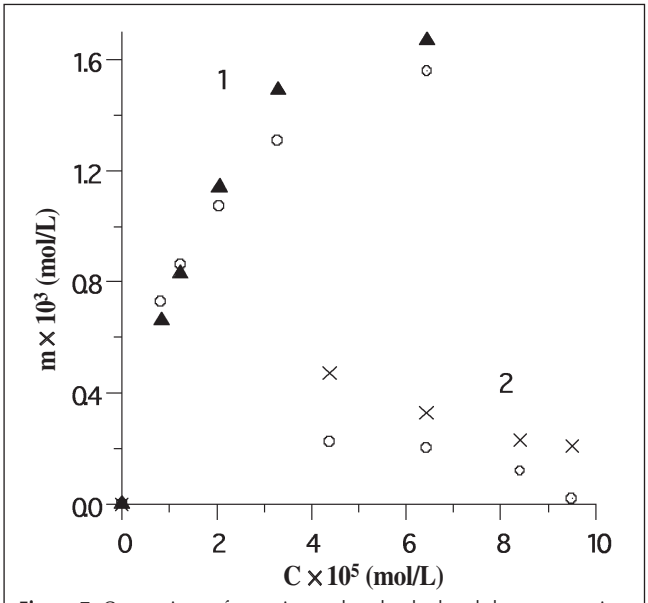

Figure 7. Comparison of experimental and calculated data on sorption of lysozyme 1 and ribonuclease 2 from binary solution. White circles are caculated data.

place. For example, in the initial moment the insulin was more preferably sorbed by empty sorbent, and as the sorbent phase was being filled with proteins, selectivity inversion took place, and ribonuclease was more selectively sorbed during the final process stages (16).

Weakening of the protein–sorbent interaction should result in a competitive effect. It is possible to provide the shift from maximal binding conditions by changing the pH value (i.e., decrease of the degree of ionization for the sorbent groups or decrease the protein charge opposite the sorbent charge). In the insulin–ribonuclease–CMT system, with pH 4.0, the synergistic sorption mechanism is implemented. In contrast, with a shift to pH 2.5, there was a transition to competition (Figure 4). As one can see in Figure 3, during sorption from a mixed solution at pH 2.5, insulin depresses ribonuclease sorption; however, during sorption in conditions close to maximal binding conditions (pH 4.0) in the presence of insulin, the ribonuclease binding increases. The sorption isotherms of individual proteins in this case are quite adequately described by the Langmuir isotherm equation. The evaluation of component composition of the sorbent phase at pH 2.5, with the help of bi-Langmuir isotherms during sorption from mixed solution, gives the result close to that experimentally obtained.

If it is possible to reach transition from synergy to competition by decreasing the degree of ionization for the sorbent ionogenic groups, than a decrease in surface or volume density of the ionogenic groups should result in the same effect. In Figures 5 and 6, the isotherms of lysozyme and ribonuclease sorption on carboxylic cation exchangers with various amounts of ionogenic groups are presented. In both cases, the transition is the result of electrostatic interaction weakening. The decrease in content of sorbent ionogenic groups leads to a transition from synergy to competition (24). In the case of sorption from a mixed solution with equal weight concentrations (total concentration of the solution, 2 mg/mL) on the sorbent with a greater amount of ionogenic groups, the experimentally obtained data for lysozyme and ribonuclease are 4×10^{-3} mol/L and 3.26×10^{-3} mol/L, respectively, and the values of 2.74×10^{-3} mol/L and 0.64×10^{-3} mol/L were obtained by calculation. In the case of competition by sorption on cation exchanger with the diminished content of carboxylic groups, the calculated data reflects, qualitatively, the suppression of ribonuclease sorption by lysozyme (Figure 7), as the concentration of both proteins increases.

Conclusion

The data obtained make it possible to conclude that calculation of multicomponent equilibrium with the help of bi-Langmuir isotherms for implementation of the synergistic sorption mechanism yields results that have nothing to do with reality. In the case of the competitive sorption mechanism, the calculation works very well. Elementary experiments on sequential protein sorption allow determining what sorption mechanism takes place; replacement of one protein by another indicates competition, and the lack of replacement indicates synergy.

Acknowledgments

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