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Hydrophobic interaction adsorption of hen egg white proteins albumin, conalbumin, and lysozyme

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Abstract

Hydrophobic adsorption equilibrium data of the hen egg white proteins albumin, conalbumin, and lysozyme were obtained in batch systems, at 25 °C, using the Streamline Phenyl® resin as adsorbent. The influence of three types of salt, NaCl, Na_2SO_4, or $(NH_4)_2SO_4$, and their concentration on the equilibrium data were evaluated. The salt Na_2SO_4 showed the higher interaction with the studied proteins, thus favoring the adsorption of proteins by the adsorbent, even though each type of salt interacted in a distinct manner with each protein. The isotherm models of Langmuir, Langmuir exponential, and Chen and Sun were well fitted to the equilibrium data, with no significant difference being observed at the 5% level of significance. The mass transfer model applied simulated correctly adsorption kinetics of the proteins under the studied conditions.

Keywords: Hydrophobic adsorption; Egg white proteins; Modeling

1. Introduction

Hen egg white is a food ingredient of industrial relevance owing to its complex mixture of proteins. Egg white proteins confer to food products both high nutritional quality and multiple functional properties. These proteins have so high biological values that they are used as a standard for measuring the nutritional quality of other proteins source. The functional properties of egg white proteins such as gelling, foaming, emulsification, thickening, texture modifier, heat setting, and bind adhesion are well divulged in the literature [1–4]. Three egg white proteins of particular functional importance are albumin (ovalbumin or eggalbumin), conalbumin (ovotransferrin), and lysozyme, which represents, respectively, 54 mass%, 12.5 mass%, and 3.5 mass% of the egg white total protein. Albulmin and conalbumin are glycoproteins and therefore could exhibit biological activities in numerous cell functions [5]. Albumin presented coagulation and gelling properties and conalbumin has a broad anti-microbial activity and acts on iron transport. Lysozyme has anti-bacterial properties and is largely used in the food preservation and in the pharmaceutical industry [1,2,5,6].

The potential of individual application of these proteins has instigated the development of new technologies for its isolation aiming to replace or improve traditional separation techniques such as salt precipitation or solvent extraction. These methods presented disadvantages related to the protein denaturation and low level of purity. In view of such difficulties, the adsorption is an alternative unit operation with adequacy to purify hen egg white proteins. In adsorption process certain components of the fluid phase are transferred to the surface of a solid adsorbent in a surface phenomenon. Adsorption by ionic exchange was applied for hen egg white protein purification under laboratory conditions by Awade et al. [3], Croguennec et al. [4], Levison et al. [7], and Vacchier et al. [6].

The fractionating power of the chromatographic adsorption, using either batch systems, or fixed beds and fluidized or expanded beds, makes them an attractive technique for molecular and macromolecular separation and for undesirable contaminating elimination [8]. Adsorption chromatography in batch mode, also called batch adsorption, is a simple process applied to obtain (i) equilibrium and kinetic data of adsorption and desorption processes [9–12] and (ii) model parameters to be used

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in the simulation of chromatography process scale-up, such as expanded bed and simulated moving bed techniques. It can be cited, for example, in the intra-particle diffusivity parameter [13].

The selection of a chromatographic technique to perform a given separation will depend on certain biological and physical-chemical properties of the target compounds, such as net charge (ion exchange chromatography), biospecific characteristics (bio-affinity chromatography), and hydrophobic characteristics (hydrophobic interaction chromatography), among others.

Hydrophobic interaction chromatography (HIC) is based on the reversible interactions of the hydrophobic ligand immobilized at the resin stationary phase with the non-polar regions on the biomolecule surface. Protein adsorption may be increased by increasing the salt content in the mobile phase, while elution is reached by reducing the salt concentration of the eluent [14–16]. Similar to the reverse phase chromatography (RPC), HIC has the advantage of working in more polar and less denaturing protein environments, besides using the hydrophobic characteristics of the proteins. Additionally, it does not require non-polar solvents for protein elution as in the RPC, owing to the weak bond of the biomolecules with the adsorbent. Then the structure and the biological activity of biomolecules could be maintained in the separation processes [17].

HIC is widely used in the downstream processing of proteins as it provides an alternative basis for selectivity compared with ion exchange and other modes of adsorption. Additionally, HIC is an ideal "next step" after precipitation with ammonium sulphate or elution in high salt concentration during ion exchange chromatography [18,19].

Thus, the objective of this work was to evaluate the adsorption of the hen egg white proteins albumin, conalbumin, and lysozyme in batch systems applying a hydrophobic resin at different concentrations and three types of salt at room temperature (25 °C). The analysis of salt-mediated protein adsorption was conducted on the basis of the adsorption data of equilibrium and kinetics. This information leads to a better understanding of the separation process and allows separation efficiency optimization.

2. Experimental

2.1. Material

Albumin (9006-59-1/A-5503), conalbumin (CAS 1391-06-6), and lysozyme (EC 3.2.1.17) were purchased from Sigma chemical company (St. Louis, MO, USA) and the Streamline Phenyl® resin from Amershan Pharmacia Biotech (Uppsala, Sweden). The density and particle size range of the resin were 1.2 g/mL and 100–300 μm, respectively. Ultrapure water (Milli-Q system, Millipore Inc., USA) and chemical reagents of analytical and chromatographic degrees were used in the experiments.

2.2. Batch adsorption experiments

Adsorption equilibrium data for each hen egg white protein albumin, conalbulmin, and lysozyme were determined following the batch adsorption methodology described in the literature [11,20]. Thus, aiming to equilibrate the Streamline Phenyl® resin, 0.2 g of the adsorbent was added to 10 mL of phosphate buffer (20 mM, pH 7.0) containing the evaluated salt (NaCl, Na_2SO_4 or $(NH_4)_2SO_4$) at pre-established quantities. The salt concentrations studied were of 0.0, 0.5, 1.0, 1.5, and 2.0 M for NaCl and of 0.0, 0.3, 0.5, 0.8, and 1.0 M for Na_2SO_4 and $(NH_4)_2SO_4$. After 8 h, the buffer solution was separated from the resin, and to this equilibrated resin was added 10 mL of the protein solution of known concentration.The protein concentration in the solution ranged between 0 and 4 mg/mL.

The mixture was agitated for 8 h [11,20], at 25 °C, using a batch system as proposed by Bonomo et al. [21]. The protein concentration in the supernatant was determined by using a UV–vis spectrophotometer (Varian, Australia) at 280 nm. The quantity of protein adsorbed in the resin was calculated by mass balance.

2.3. Equilibrium data modeling

Three mathematical models described in the literature, based on thermodynamic equilibrium concepts, were used to adjust the equilibrium data:

- Langmuir isotherm model [22]

$$Q = \frac{q_m C_p}{k_d + C_p} \tag{1}$$

where Q is the amount of protein adsorbed per adsorbent mass unit (mg/g); C_p the equilibrium concentration of the protein in the liquid phase (mg/mL); q_m the maximum amount of protein adsorbed in the solid phase (mg/mL resin); and k_d is the dissociation constant that describes the adsorption equilibrium (mg/mL).

- Modified Langmuir exponential model [23]

$$Q = \frac{\lambda b \exp(-kC_s)C_p}{1 + b \exp(-kC_s)C_p} \tag{2}$$

where C_s is the salt concentration in the liquid phase (mol/L) and λ, b, and k are the equation parameters.

- Chen and Sun model [24]

$$\frac{Q}{C_p} = K_{ps} C_s^{\alpha} [\Lambda - (n + \sigma)Q]^n \tag{3}$$

where Λ is the density of the hydrophobic ligand (mol/L) and K_{ps}, n, α, and σ are equation parameters.

2.4. Adsorption kinetics

0.5 g of adsorbent previously equilibrated in phosphate buffer (20 mM, pH 7) containing the salt in the concentration of 1 M for Na_2SO_4 (lysozyme), 0.8 M for Na_2SO_4 (conalbumin), and 0.8 M for $(NH_4)_2SO_4$ (albumin) was used in the experiments for adsorption kinetic determination. For each salt type, the salt concentrations higher than that used in this work led to protein denaturation. Fifty milliliters of a protein solution with concentrations of 0.5 and 1.0 mg/mL were added to the resin. The

adsorption was carried out at 25 °C. Every few minutes, samples of 2 mL were collected for the immediate protein quantification and the samples were then returned to the solution [11]. Thus, the reduction of the protein content in the liquid phase of the system was determined along the time.

2.5. Mass transfer model

The pore diffusion model in batch systems involves the mass balance over the particle and the mass balance in the liquid phase external to the particle [25]. The following assumptions were taken into account for the model formulation:

(1) The adsorption process is isothermic.
(2) The porous adsorbent is spherical and of uniform size.
(3) There is local equilibrium for each component in the pore surface and in the stagnated liquid inside the pores, which may be represented by the Langmuir equation:

$$C_p^s = \frac{q_m C_p}{k_d + C_p} \tag{4}$$

(4) The diffusion and mass transfer coefficients are constant and independent of the component mixture effects.

On the basis of these assumptions, Eqs. (5) and (6) were obtained for each component, respectively, at the extra-particle and intra-particle phases

$$\frac{\partial C_b}{\partial t} + \frac{3V_p k_f}{R_p V_b}(C_b - C_{p,R=R_p}) = 0 \tag{5}$$

$$\frac{\partial C_p^s}{\partial t} + \varepsilon_p \frac{\partial C_p}{\partial t} - \varepsilon_p D_p \left[\frac{1}{R^2}\frac{\partial}{\partial R}\left(R^2 \frac{\partial C_p}{\partial R}\right)\right] = 0 \tag{6}$$

Under the initial and boundary conditions:

$$t = 0 \Rightarrow C_b = C_0 \tag{7}$$

$$t = 0 \Rightarrow C_p = 0 \tag{8}$$

$$R = 0 \Rightarrow \frac{\partial C_p}{\partial R} = 0 \tag{9}$$

$$R = R_p \Rightarrow \frac{\partial C_p}{\partial R} = \frac{k_f}{\varepsilon_p D_p}(C_b - C_{p,R=R_p}) \tag{10}$$

The liquid film mass transfer coefficient for the batch adsorption system, k_f, can be calculated using the following correlation [26]:

$$k_f = \frac{2D_{AB}}{d_p} + 0.31\left(\frac{\mu}{\rho D_{AB}}\right)^{-2/3}\left(\frac{\Delta\rho\mu g}{\rho^2}\right)^{1/3} \tag{11}$$

The diffusion coefficient of proteins in free solution, D_{AB}, was obtained by using the Polson correlation [27], Eq. (12), in which D_{AB} (m^2/s) is a function of the molar mass of each protein.

$$D_{AB} = 2.74 \times 10^{-9}(M_r)^{-1/3} \tag{12}$$

The pore diffusion model for batch adsorption given by Eqs. (5) and (6) with the initial and boundary conditions, Eqs. (7)–(10), was reduced to an algebraic equation system by using the implicit method of finite differences with a step forward. This system of algebraic and differential equations was resolved simultaneously by the Gauss–Seidel method [28]. The pore diffusivity coefficient, D_p, was estimated by fitting experimental data (uptake curve) to the pore diffusion model.

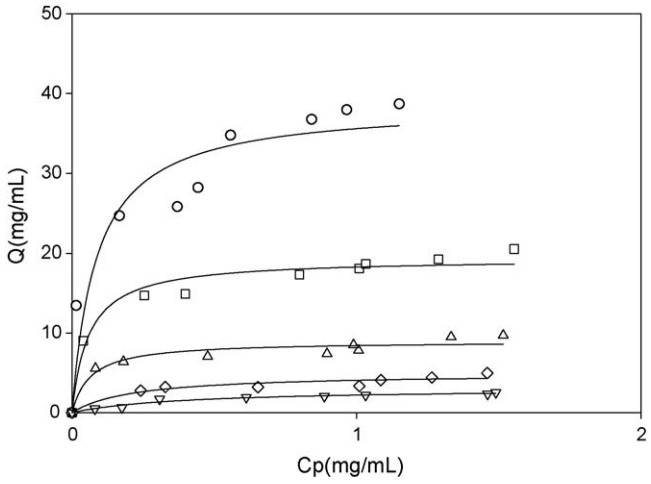

Fig. 1. Adsorption isotherms of conalbumin at different concentrations of $(NH_4)_2SO_4$: (◯) 1.0 M; (□) 0.8 M; (△) 0.5 M; (◇) 0.3 M; (▽) 0.0 M and (—) Langmuir isotherm model.

3. Results and discussion

3.1. Adsorption equilibrium

The adsorption isotherms for conalbumin, lysozyme, and albumin, in different salt types and concentrations, are shown in Figs. 1–9. In all the cases, an increase in protein adsorption was observed on the increase of the salt concentration in the system. Chen and Sun [24] reported a similar result for the salt concentration effect on hydrophobic adsorption equilibrium of bovine serum albumin (BSA). Such behavior may be due to the elevation

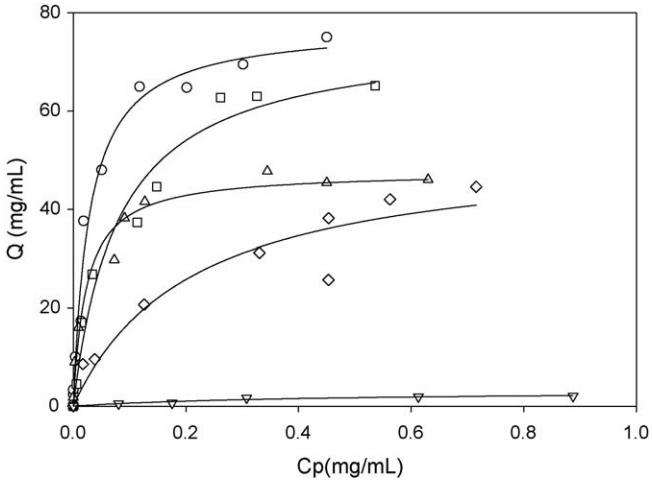

Fig. 2. Adsorption isotherms of conalbumin at different concentrations of NaCl: (◯) 2.0 M; (□) 1.5 M; (△) 1.0 M; (◇) 0.5 M; (▽) 0.0 M and (—) Langmuir isotherm model.

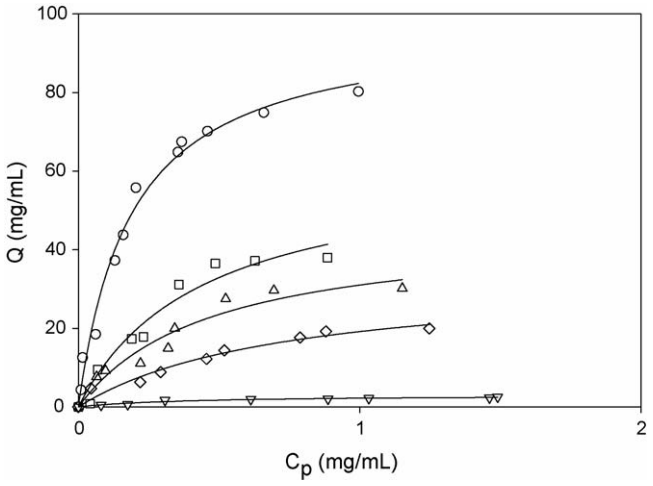

Fig. 3. Adsorption isotherms of conalbumin at different concentrations of Na_2SO_4: (◯) 1.0 M; (□) 0.8 M; (△) 0.5 M; (◇) 0.3 M; (▽) 0.0 M and (—) Langmuir isotherm model.

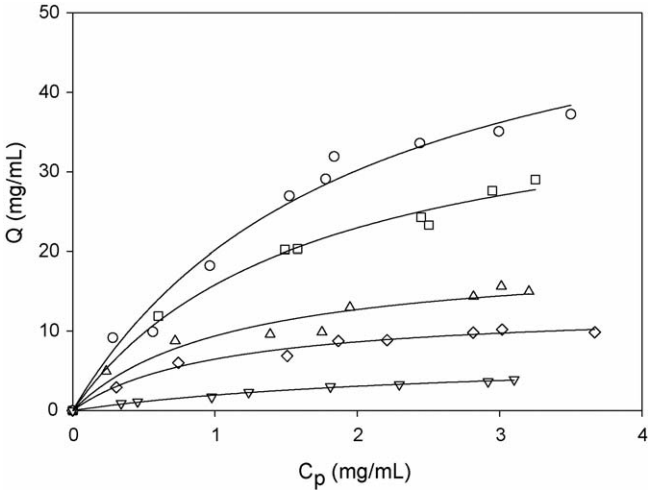

Fig. 4. Adsorption isotherms of lysozyme at different concentrations of NaCl: (◯) 2.0 M; (□) 1.5 M; (△) 1.0 M; (◇) 0.5 M; (▽) 0.0 M and (—) Langmuir isotherm model.

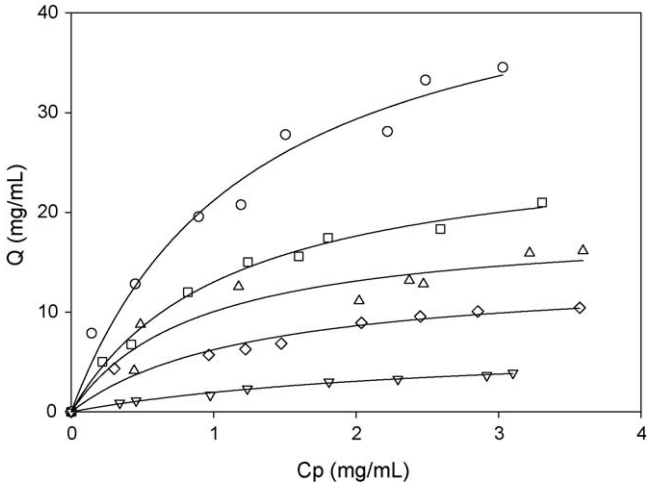

Fig. 5. Adsorption isotherms of lysozyme at different concentrations of $(NH_4)_2SO_4$: (◯) 1.0 M; (□) 0.8 M; (△) 0.5 M; (◇) 0.3 M; (▽) 0.0 M and (—) Langmuir isotherm model.

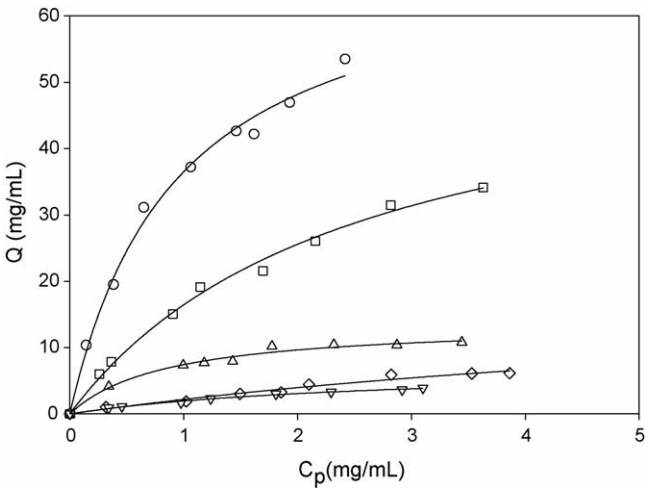

Fig. 6. Adsorption isotherms of lysozyme at different concentrations of Na_2SO_4: (◯) 1.0 M; (□) 0.8 M; (△) 0.5 M; (◇) 0.3 M; (▽) 0.0 M and (—) Langmuir isotherm model.

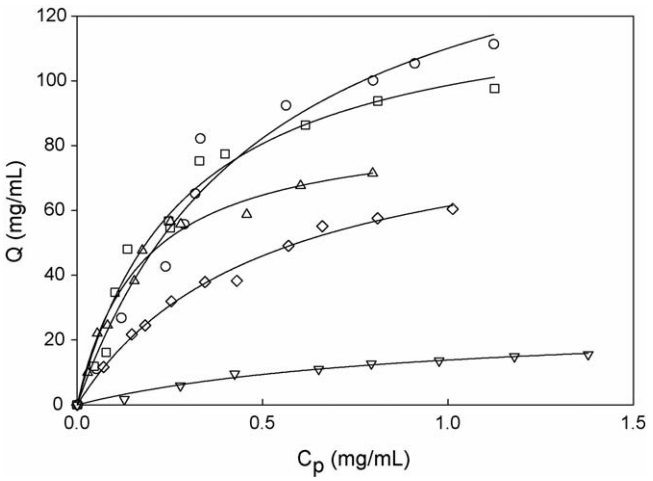

Fig. 7. Adsorption isotherms of albumin at different concentrations of Na_2SO_4: (◯) 1.0 M; (□) 0.8 M; (△) 0.5 M; (◇) 0.3 M; (▽) 0.0 M and (—) Langmuir isotherm model.

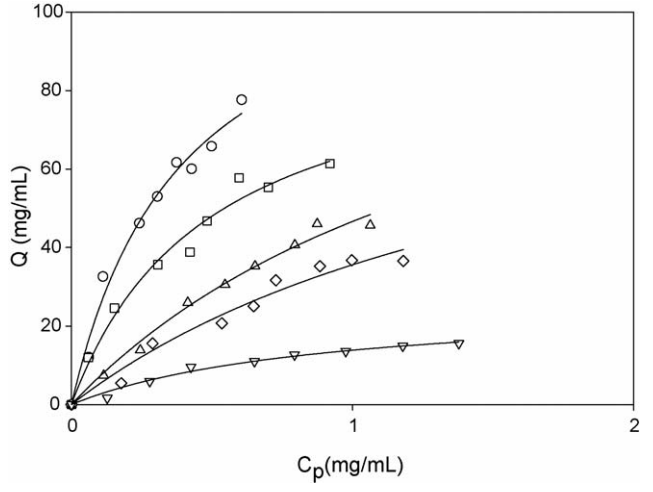

Fig. 8. Adsorption isotherms of albumin at different concentrations of NaCl: (◯) 2.0 M; (□) 1.5 M; (△) 1.0 M; (◇) 0.5 M; (▽) 0.0 M and (—) Langmuir isotherm model.

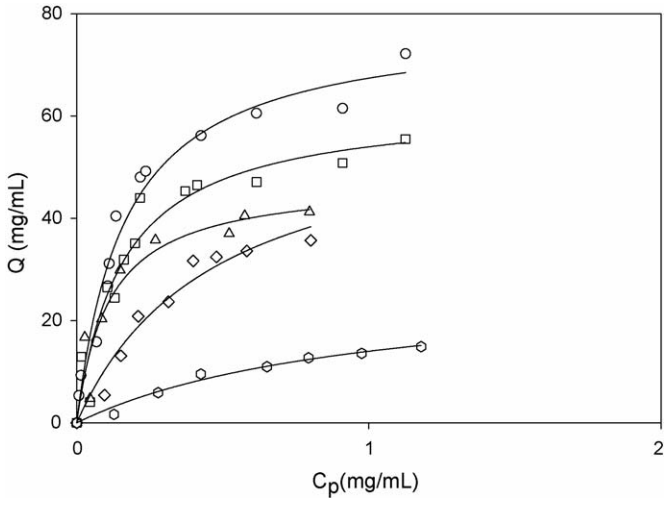

Fig. 9. Adsorption isotherms of albumin at different concentrations of $(NH_4)_2SO_4$: (◯) 1.0 M; (□) 0.8 M; (△) 0.5 M; (◇) 0.3 M; (▽) 0.0 M and (—) Langmuir isotherm model.

of the water–protein surface tension with increased salt content in the solution. A higher amount of salt will lead to a reduction of the number of water molecules around the protein, and, thus, to a greater exposure of the hydrophobic regions of the protein, consequently increasing the bound protein form (protein–ligand) and diminishing protein solubility [29–30]. Such phenomenon is the basis of the solvophobic theory or cavity theory, proposed by Horvath et al. [31]. As the saline concentration should be less than the saturation value, which leads to protein precipitation, most protein separations using HIC are processed at low salt concentrations when the protein has a high density of hydrophobic groups [29]. In the present study, concentrations of Na_2SO_4 and $(NH_4)_2SO_4$ above 1.0 M led to conalbumin and albumin denaturation, rendering difficulties in the quantification and making the process unfeasible.

The type of salt used in hydrophobic adsorption is an important parameter in the optimization of the protein separation process by HIC [32,33]. In Table 1, the behavior of each protein in each type of aqueous saline solution is observed. Na_2SO_4 showed the higher interaction with the three proteins studied, thus favoring protein adsorption by the adsorbent. As the preferential interaction order for each type of salt depends on each protein, the order for lysozyme and conalbumin was $Na_2SO_4 > (NH_4)_2SO_4 > NaCl$ and for albumin was $Na_2SO_4 > NaCl > (NH_4)_2SO_4$. Similar results for other proteins were reported in the literature [24,31,34]. Arakawa and coworker [35–37] presented a hydration model referring the preferential interaction of proteins with salts and the effect of the salt type on the protein solubility. The authors showed that some kind of salts (such as $MgCl_2$), despite increasing the surface tension of water as other salts (such as sodium sulphate, phosphates), do not enhance the protein binding to hydrophobic stationary phases, as much as expected from the surface tension increment. So, they promote the protein solubility. The salts such as sodium sulphate or phosphates are excluded from the immediate domain of the proteins in concentrated solutions, because the proteins are preferentially hydrated.

Table 1
Effect of type of salt on hydrophobic adsorption of hen egg white protein

Protein	Hydrophobicity (cal/res) [42]	q_m (mg/mL of resin)		
		NaCl (1 M)	$(NH_4)_2SO_4$ (1 M)	Na_2SO_4 (1 M)
Conalbumin	1080	47.73	38.64	97.79
Lysozyme	970	19.72	47.76	70.72
Albumin	1110	121.60	78.49	165.99

The major factors responsible for the preferential interaction of proteins with different salts are referred to be the increment of surface tension, pH, and the hydrophobic area of the protein surface [18,32]. In this work, the differentiated behavior is probably due to the hydrophobicity difference, mainly of lysozyme in relation to albumin and conalbumin, (Table 1) and to the difference in the molal surface tension increment of the salts, e.g., $NaCl < (NH_4)_2SO_4 < Na_2SO_4$ as reported by Queiroz et al. [14]. Thus, the salt–protein preferential interaction is an important parameter for understanding salt efficiency in maintaining, or not, the stability of the protein structure, as well as for evaluating the behavior of salt as a promoter of protein salting in or salting out [37].

3.2. Modeling of adsorption equilibrium data

The adsorption equilibrium data were adjusted by three isothermal models represented by Eqs. (1)–(3). Tables 2–4 show the statistical analysis results based on the values of standard deviation (S.D.) and relative sum of the squares of the errors (RSSE), calculated by Eqs. (13) and (14), respectively. RSSE evaluates the quality of the model fitting to the observed data [11].

$$\text{S.D.} = \left[\frac{\sum_{i=1}^{m}(\delta_{\text{exp}} - \delta_{\text{cal}})^2}{m - p}\right]^{1/2} \tag{13}$$

$$\text{RSSE} = \left[\sum_{i=1}^{m}\left(\frac{|(\delta_{\text{exp}})^2 - (\delta_{\text{exp}} - \delta_{\text{cal}})^2|}{\delta_{\text{exp}}^2}\right)\right] \times \frac{100\%}{m} \tag{14}$$

where δ_{exp} and δ_{cal} are the observed and calculated values of the adsorption equilibrium data, respectively; m the number of experimental points; and p is the number of adjusted parameters.

Thus, it can be observed in Tables 2 and 3 that Lagmuir model, Eq. (1), presented the highest RSSE to the lysozyme and conalbumin data. Table 4 shows that modified Lagmuir model, Eq. (2), had the highest RSSE average for albumin. However, the difference between the RSSE averages for the three models studied was not significant at the 5% level probability by the Student's test (t). A similar behavior was described by Chen and Sun [24] when fitting the BSA adsorption equilibrium data using Eqs. (1) and (3). Thus, the Langmuir model comes as a simpler model that expresses a quantitative relation between protein adsorption and salt concentration in mobile phase, and which can be used to simulate protein adsorption equilibrium in a realistic HIC process.

Table 2
Conalbumin adsorption equilibrium data adjustment

	Isothermal models					
	Eq. (1)		Eq. (2)		Eq. (3)	
	S.D. (mg/mL)	RSSE (%)	S.D. (mg/mL)	RSSE (%)	S.D. (mg/mL)	RSSE (%)
NaCl (M)						
0.5	0.05	96.25	0.05	96.25	0.07	92.35
1.0	0.05	97.77	0.04	74.72	0.09	63.89
1.5	0.04	96.09	0.06	97.76	0.10	88.55
2.0	0.05	96.74	0.05	77.39	0.07	77.39
Na_2SO_4 (M)						
0.3	0.01	94.83	0.01	94.83	0.01	94.14
0.5	0.03	96.42	0.03	96.42	0.03	95.16
0.8	0.04	98.68	0.04	98.68	0.03	98.06
1.0	0.04	96.27	0.04	96.27	0.04	94.93
$(NH_4)_2SO_4$ (M)						
0.3	0.01	98.55	0.01	98.55	0.01	95.37
0.5	0.01	99.12	0.01	99.12	0.03	89.83
0.8	0.01	99.31	0.01	99.31	0.05	90.57
1.0	0.05	94.78	0.06	94.98	0.09	87.79

3.3. Kinetic of adsorption

Uptake curves obtained by simulation confirm that the pore diffusion model predicts the correct curve shape of each system. Thus, it was verified a good agreement with the experimental data. The kinetic data of hydrophobic adsorption of albumin, lysozyme, and conalbumin, at concentrations of 0.5 and 1.0 mg/mL, are shown in Figs. 10–12, respectively. The decrease of the protein adsorption was observed on the increase of the protein content in the medium. Chang and Chase [9] and Tong et al. [11] described a similar kinetic adsorption behavior for lysozyme in Streamline DEAE resin and BSA in Streamline SP resin, respectively. The reduction in the protein adsorption could be attributed to the elevation of the solution viscosity. Gundüz [38] reported the viscosity increase of an aqueous BSA solution with increased protein content. Higher viscosity may lead to the agglomeration of protein molecules in the adsorbent particle pores, making difficult the protein diffusion into the resin.

The values of D_p and k_f parameter used in the mass transfer model are shown in Table 5. The values of D_p and k_f for the three studied proteins varied from 1.24×10^{-11} to 1.94×10^{-11} m^2/s and 11.28×10^{-6} to 8.05×10^{-6} m/s, respectively. These results are in the range of D_p and k_f values for macroporous particles reported in the literature [39,20,11,40]. According to Guiochon et al. [41], film thickness and therefore mass transfer coefficient are determined by hydrodynamic conditions and depend on the liquid flow around the particle.

Table 3
Lysozyme adsorption equilibrium data adjustment

	Isothermal models					
	Eq. (1)		Eq. (2)		Eq. (3)	
	S.D. (mg/mL)	RSSE (%)	S.D. (mg/mL)	RSSE (%)	S.D. (mg/mL)	RSSE (%)
NaCl (M)						
0.5	0.03	99.60	0.03	99.60	0.05	98.46
1.0	0.10	97.75	0.09	97.74	0.14	94.32
1.5	0.09	99.79	0.08	99.79	0.13	99.27
2.0	0.15	98.10	0.14	98.10	0.12	98.34
Na_2SO_4 (M)						
0.3	0.02	98.04	0.03	98.04	0.02	97.83
0.5	0.03	99.73	0.03	99.73	0.05	98.69
0.8	0.06	99.68	0.06	99.68	0.10	98.80
1.0	0.12	99.71	0.13	99.71	0.27	98.02
$(NH_4)_2SO_4$ (M)						
0.3	0.05	97.92	0.05	97.92	0.07	96.30
0.5	0.11	94.98	0.12	94.98	0.18	95.91
0.8	0.04	99.59	0.04	99.59	0.11	97.92
1.0	0.13	97.84	0.14	97.83	0.21	95.47

Table 4
Albumin adsorption equilibrium data adjustment

	Isothermal models					
	Eq. (1)		Eq. (2)		Eq. (3)	
	S.D. (mg/mL)	RSSE (%)	S.D. (mg/mL)	RSSE (%)	S.D. (mg/mL)	RSSE (%)
NaCl (M)						
0.5	0.05	94.11	0.06	94.11	0.04	96.98
1.0	0.04	99.42	0.04	99.42	0.02	99.81
1.5	0.06	99.35	0.07	99.35	0.08	98.12
2.0	0.07	97.81	0.08	97.81	0.11	98.67
Na_2SO_4 (M)						
0.3	0.01	88.13	0.19	97.64	0.12	98.94
0.5	0.07	98.92	0.20	96.07	0.05	99.20
0.8	0.03	99.81	0.13	93.22	0.15	97.54
1.0	0.12	93.22	0.16	97.09	0.16	98.92
$(NH_4)_2SO_4$ (M)						
0.3	0.06	89.42	0.06	89.45	0.05	93.60
0.5	0.10	84.94	0.10	97.08	0.15	81.90
0.8	0.10	96.64	0.10	88.58	0.17	87.18
1.0	0.08	95.96	0.08	95.96	0.18	91.29

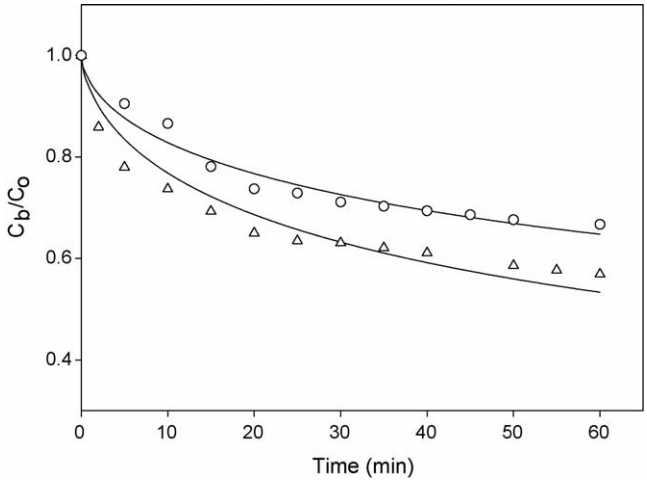

Fig. 10. Albumin adsorption kinetics at concentrations of (△) 0.5; (◯) 1.0 mg/mL and (–) pore diffusion model.

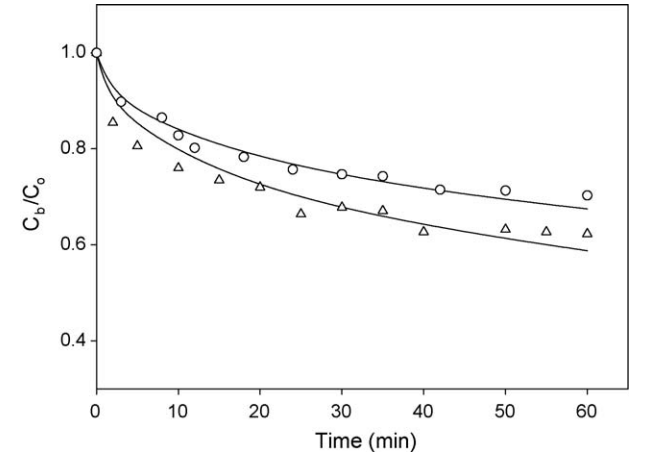

Fig. 11. Lysozyme adsorption kinetics at concentrations of (△) 0.5; (◯) 1.0 mg/mL and (–) pore diffusion model.

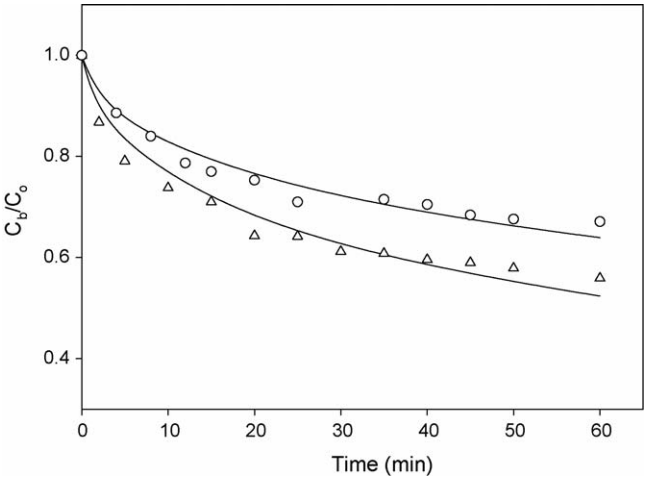

Fig. 12. Conalbumin adsorption kinetics at concentrations of (△) 0.5; (◯) 1.0 mg/mL and (–) pore diffusion model.

Table 5
Mass transfer parameters

Protein	Parameters[a]		
	k_f (m/s)	D_p (m^2/s)	*Bi*
Conalbumin (mg/mL)			
0.5	8.05×10^{-6}	1.56×10^{-11}	51.60
1.0	8.05×10^{-6}	1.24×10^{-11}	64.90
Lisozyme (mg/mL)			
0.5	11.28×10^{-6}	1.47×10^{-11}	76.73
1.0	11.28×10^{-6}	1.40×10^{-11}	80.57
Albumin (mg/mL)			
0.5	9.02×10^{-6}	1.94×10^{-11}	46.49
1.0	9.02×10^{-6}	1.60×10^{-11}	57.50

[a] The particle porosity ($\varepsilon_p = 0.60$).

The Biot number (*Bi*) is used for a macroporous resin to assess the significance of the film mass transfer versus intraparticle mass transfer resistances [20]. The Biot number was calculated by Eq. (15) and the results are provided in Table 5.

$$Bi = \frac{k_f R_p}{D_p} \quad (15)$$

The *Bi* values for lysozyme, albumin, and conalbumin adsorption were larger than 45 indicating that uptake curve was dominated by intraparticle mass transfer resistance although film mass transfer resistances could not be completely neglected in the simulation [10,20].

4. Conclusions

The equilibrium data of hydrophobic adsorption of hen egg white protein albumin, conalbumin, and lysozyme in batch mode, at 25 °C, were affected by the increase in salt concentrations in the systems. Overall, Na_2SO_4 showed the higher interaction with the evaluated proteins, thus favoring its adsorption, although each protein exhibited a differentiated interaction with each salt. The isothermal models of Langmuir, Langmuir exponential, and Chen and Sun were well fitted to the equilibrium data, with no significant differences being observed at the 5% level of significance. The mass transfer model applied simulated correctly adsorption kinetics of the proteins under the studied conditions.

5. Nomenclature

b	parameter of Eq. (2)
C_b	concentration of protein in the extraparticle liquid phase (mg/mL)
C_p	concentration of protein in the liquid phase equilibrium (mg/mL)
C_p	concentration of protein in the intraparticle liquid phase (mg/mL)
C_o	initial concentration of protein in the liquid phase (mg/mL)
C_s	salt concentration (mol/L)
C_p^s	concentration of protein in the solid phase of the particle (mg/mL)
D_p	diffusion coefficient of component (m^2/s)
k	parameter of Eq. (2)
k_d	dissociation constant (mg/mL)
k_f	mass transfer coefficient of the protein (m/s)
K_{ps}	equilíbrium constant
m	number of experimental points
M_r	molar mass of the protein (g/mol)
N	number of bonded sites
p	number of adjusted parameters
q_m	maximum amount of protein adsorbed in the liquid phase (mg/g)
Q	quantity of protein adsorbed per unit of adsorbent mass (mg/g)
R	radial coordinate for the particle
R_p	partícle radius
RSSE	relative sum of the squares of the error (%)
S.D.	standard deviation (mg/mL)
t	time (min)
V_b	liquid volume in the batch suspension (mL)
V_p	adsorbent volume in the batch suspension (mL)

Greek lettersv

α	salt coefficient
δ_{exp}	observed values of the adsorption equilibrium data
δ_{cal}	calculated values from the adsorption equilibrium data
ε_p	particle porosity
λ	parameter of Eq. (2)
σ	steric factor
Λ	ligand density (mol/L)

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