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Hydrophobic interaction adsorption of whey proteins: Effect of temperature and salt concentration and thermodynamic analysis

Renata C.F. Bonomo [a], Luis A. Minim [b,*], Jane S.R. Coimbra [b], Rafael C.I. Fontan [a], Luis H. Mendes da Silva [b], Valéria P.R. Minim [b]

[a] *Universidade Estadual do Sudoeste da Bahia, Engineering Process Laboratory, 47500-000 Itapetinga, BA, Brazil*
[b] *Universidade Federal de Viçosa, Department of Food Technology, Process Separation Laboratory, Av. P. H. Rolfs, s/n Campus, 36570-000, Viçosa, MG, Brazil*

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Abstract

The adsorptive behavior of bovine serum albumin (BSA) and β-lactoglobulin (β-lg) on hydrophobic adsorbent was studied at four temperatures and different salt concentrations. The Langmuir model was fitted by experimental equilibrium data showing that an increase in temperature and salt concentration results in an increase on the capacity factor of both proteins. A thermodynamic analysis coupled with isotherm measurements showed that salt concentration and temperature affected the enthalpic and entropic behavior of the adsorption process of both proteins, mainly to the β-lg. The fast variation in the *Z* value for temperature over than 303.1 K suggest a great conformational change occurring in the β-lg structure, which almost duplicated the maximum adsorption capacity of this protein.

Keywords: Bovine serum albumin; β-Lactoglobulin; Thermodynamic parameters; Isotherm; Hydrophobic interaction

1. Introduction

Hydrophobic interaction chromatography (HIC) is a methodology commonly used in the purification of biomolecules [1]. This technology is based on the hydrophobic interaction between hydrophobic ligands and non-polar regions on the surface of biomolecules [2,3]. It is a powerful adsorptive separation technique because of the fast separations achieved with little product degradation, low solvent requirements and very good purification levels [4].

The mechanism of hydrophobic interactions between solutes has been studied because of its importance in protein precipitation by salting-out [5]. It is well known that the type of salt and salt concentration greatly influences the hydrophobic interactions between proteins with hydrophobic media and HIC processes being often carried out by gradient elution with decreasing salt concentrations [6,7].

Temperature is another factor affecting HIC performance. Increasing temperature enhances protein retention and decreasing temperature generally promotes protein elution [8]. Chen et al. [9] showed that the exposed hydrophobic regions of the protein increased with temperature, resulting in the binding mechanism changing from adsorption to partition in some cases. To study the interaction between proteins and hydrophobic solid surfaces, researchers have traditionally developed thermodynamic analyses based on the van't Hoff dependencies [10]. Generally, the classical linear van't Hoff equation has been used to calculate the thermodynamic parameters in experiments performed in a narrow temperature range. Since heat capacity, enthalpy changes and entropy changes are expected to be invariable, the enthalpy and entropy of the interaction can be obtained by linear plotting from the logarithm of the equilibrium constant with inversed temperature [5]. When the heat capacity changes with temperature, the non-classical van't Hoff equations are used to obtain a proper analysis. Enthalpy and entropy changes at different temperatures can be obtained, being important to estimate a significant sub process in the adsorption procedure [5–11].

* Corresponding author. Tel.: +55 3138991617; fax: +55 3138992208.
E-mail address: lminim@ufv.br (L.A. Minim).

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Over the years a variety of HIC sorbents have been developed to fulfill the needs of different purifications. Ligand type and size have a great impact on the property of HIC. Besides, porous matrix and density can affect greatly mass transfer parameters and binding capacity for large scale purifications. In this article we describe the adsorption behavior of the cheese whey proteins bovine serum albumin (BSA) and β-lactoglobulin (β-lg) at different salt concentrations and temperatures. A hydrophobic adsorbent Streamline Phenyl® was used which has proper characteristics for use in large scale expanded bed columns. The thermodynamic parameters of HIC data from non-linear van't Hoff equations were also determined. This study will support new developments on whey proteins fractionation.

2. Theory

2.1. Determination of single-component isotherms by frontal analysis

The most convenient and fast methods for our purpose are the frontal analysis (FA), elution by characteristic point (ECP) and pulse methods [12]. Among the methods used for determination of single-component isotherm, the frontal analysis is the most accurate [12,13]. The adsorbed amount Q_{i+1} is given by:

$$Q_{i+1} = Q_i + \frac{(C_{i+1} - C_i)(V_{F,i+1} - V_0)}{V_a} \quad (1)$$

where Q_i and Q_{i+1} are the amounts of adsorbed component by volume of adsorbent after the ith and the $(i+1)$th step, in equilibrium with the concentrations C_i and C_{i+1}, respectively. $V_{F,i+1}$, is the retention volume at the inflection point of the $(i+1)$th breakthrough curve, V_0 is the column void volume, and V_a is the volume of the adsorbent in the column.

2.1.1. The linear isotherm

This isotherm, which relates the stationary phase concentration, Q, with the fluid concentration, C, is written as:

$$Q = aC = k' \frac{\varepsilon}{1-\varepsilon} C = \frac{k'}{\varphi} C \quad (2)$$

where a is the slope of the isotherm (Henry's adsorption constant). k' is the retention factor, $k' = (t_R - t_0)/t_R$, t_R is the retention time, t_0 is the dead time, φ is the phase ratio, $\varphi = (1-\varepsilon)/\varepsilon$, and ε is the total porosity of the column [12–14].

2.1.2. The Langmuir isotherm

Langmuir proposed this model for adsorption in a gas–solid system in 1916. It was assumed a constant adsorption heat and finite number of surface adsorption sites. By using these assumptions, maximum adsorption corresponds to a saturated monolayer of solute molecules on the adsorbent surface [15,14], written as:

$$Q = q_s \frac{bC}{1+bC} \quad (3)$$

In this model, q_s is the monolayer saturation capacity of the adsorbent and b is the equilibrium constant of adsorption.

2.2. Calculation of the thermodynamic parameters

For a better understanding of the influences of system temperature and hydrophobicity of the adsorbent and composition of the mobile phase in the selectivity of HIC, it is essential to quantify the mechanisms that establish equilibrium characteristics, such as capacity and selectivity [1]. Thus, Geng et al. [16] proposed the stoichiometric displacement retention model for the HIC of proteins. This model assumes that a rational mechanism for adsorption in a liquid–solid system based on the stoichiometric displacement for solute adsorption can be used, no matter how different are the interactions between adsorbent and solute or solvent molecules, or how heterogeneous is the distribution of these active sites [1,16]. When applied to linear chromatography, the model is reduced to:

$$\ln k' = \ln I - Z \ln[H_2O] \quad (4)$$

where

$$I = K(L_d)^{n'} \varphi \quad (5)$$

and Z is a characteristic constant related to protein conformation, when salt concentration, ligand and temperature are fixed. The intercept of this equation, $\ln I$, contains a number of constants related to the affinity of a protein to the HIC resin. K is the equilibrium constant, L_d corresponds to the concentration of hydrated ligand in salt solution, n' is the number of ligand interactions with protein molecule and φ, the phase ratio in the column.

Wu et al. [17] used a plot of $\ln k'$ versus the water concentration ($\%B$, volume fraction) to characterize protein adsorption in HIC. The authors demonstrated that the slope of the plot $[\partial(\ln k')/\partial(\ln \%B)]$ is a sensitive measure of protein conformation, which is related to the contact area of the adsorbed protein on the surface. The Z value can then be obtained taking the derivative $\partial(\ln k')/\partial(\ln \%B)$. Thus, there is no fundamental difference between the Z values obtained by Geng et al. and Wu et al. [16,17].

All of the models discussed above are applicable only to linear chromatography. For the overloaded region, the commonly used approach is to characterize the behavior with isotherm measurements and calculation of the thermodynamic parameters. The thermodynamic treatment of adsorption effectively began with the Gibbs equation, which provides a convenient definition of the interfacial region [14]. Besides free energy, enthalpy and entropy are other important parameters for the study of the adsorption process.

The linear and the non-linear van't Hoff equations are used for the calculation of these parameters. The former equation shows the dependence of k' (capacity ratio) on temperature, and is defined as:

$$\ln k' = -\frac{\Delta H^\circ}{RT} + \frac{\Delta S^\circ}{R} + \Phi \quad (6)$$

where ΔH° and ΔS° are the standard changes in enthalpy and entropy, respectively, associated with the transfer of the solute from the mobile to the stationary phase, R is the gas constant, Φ is a system constant depending on the phase ratio in the column and T is the temperature. This equation assumes that the phase ratio does not change significantly due to temperature variation and ΔH° includes contributions due to changes in φ [18]. The value of ΔH° is calculated from the slope of the plot of $\ln k'$ versus $1/T$. According to Jacobson et al. [14] the constant k' is related to the equilibrium constant for the sorption process in the domain of Henry's law, K, by

$$k' = K\varphi \tag{7}$$

where φ represents the phase ratio and $K = a$. The parameter a is the Langmuir isotherm slope at low solute concentration ($a = q_s b$) [14].

The parameters calculated from this equation are averaged for the entire range of T. To calculate these parameters at a fixed temperature, the non-linear van't Hoff [19] equation is used. This equation, proposed by Horváth and Vailaya [20], has a refinement that corrects the variation of ΔC_P° (heat capacity) with temperature, resulting in the following equation:

$$\ln\ k' = a_1 + \frac{a_2}{T} + \frac{a_3}{T^2} + \cdots + \ln\ \Phi \tag{8}$$

where a_1, a_2, and a_3 are parameters of Eq. (8).

The derivative of the Eqs. (8) and (6) in function of $1/T$ is

$$\left(\frac{\mathrm{d}\ \ln\ k'}{\mathrm{d}(1/T)}\right) = a_2 + 2\frac{a_3}{T} + \cdots + \ln\ \Phi \tag{9}$$

According to Levine [21] and Boysen et al. [10] the ΔG° can be calculated by

$$\Delta G^\circ = -RT\ \ln\ K \tag{10}$$

According to Gerstner et al. [22] for adsorption process, K is given by

$$K = \frac{k'}{\varphi} \tag{11}$$

Thus, the change in entropy is calculated from the Gibbs-Helmholtz relationship, given by

$$\Delta G^\circ = \Delta H^\circ - T\,\Delta S^\circ \tag{12}$$

3. Experimental

3.1. Materials

BSA and β-lg were purchased from Sigma (St. Louis, MO, USA). BSA is a globular ellipsoid protein, with a molar mass of 69 kDa, and isoelectric point (p*I*) of 4.7. The β-lg has a molar mass of 32 kDa, when in dimer form, and isoelectric point of 5.2 [23]. The adsorbent used was Streamline Phenyl, packed in a column HR 5/5, purchased from Amershan Pharmacia Biotech (Uppsala, Sweden). Sodium phosphate (monobasic), sodium phosphate (dibasic) and sodium sulfate were of analytical grade (VETEC, Brazil).

3.2. Apparatus

Frontal chromatography was carried out using an Äkta Purifier System (MOD 10X) (Amershan Pharmacia Biotech, Sweden) with a UV detector fixed at 280 nm at a flow rate of 2.0 mL/min. The equipment was controlled using the software Unicorn v.1.0 (Amersham Biosciences). The system temperature was controlled by immersion of the column in a thermostatic bath with a precision of ±0.1 K (Quimis, Brazil).

3.3. Procedures

Frontal analysis method was used to obtain the equilibrium data. The column, packed with 1.0 mL of adsorbent, was initially equilibrated with 50 column volumes (CV) of the carrier buffer (20 mM phosphate, pH 7,0) containing various concentrations of sodium sulfate (50, 300, 600 and 900 mM) at different temperatures (283.1, 293.1, 303.1 and 313.1 K). In order to verify the effect of temperature and salt concentration on the isotherm parameters and thermodynamic properties of adsorption, a 4×4 factorial design was applied to develop the experiments. Aqueous solutions containing the protein (BSA or β-lg) in concentrations of 0.25, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0 mg mL^{-1} were fed to the column, until the completion of the breakthrough curve. After finishing each experiment, the column was regenerated using 30 CV of a phosphate buffer (20 mM, pH 7.0). Langmuir isotherm was adjusted to the equilibrium data using non-linear regression and the dependence of the isotherm parameters on the temperature and salt concentration were obtained through polynomials regression. The statistical package SAS [24] was used to perform all statistical analysis.

4. Results and discussion

4.1. Effect of salt concentration and temperature on the adsorptive equilibrium

Adsorption experiments were carried out with four concentrations of sodium sulfate at the following temperatures: 283.1, 293.1, 303.1 and 313.1 K. Tables 1 and 2 show the values of the equilibrium concentration in the solution and the solid phase for all the conditions studied for BSA and β-lg, respectively. The isotherms for both proteins measured at 313.1 K are shown in Fig. 1. In any studied conditions, the maximum protein adsorbed was higher for BSA than for β-lg, except when the temperature reached 313.1 K.

As shown in Fig. 1, the amount of the proteins bounded on the adsorbents increases as the concentration of sodium sulfate increases for both proteins. This behavior is in agreement with the results of Chen et al. and Arakawa [25,26] for the interaction of protein on hydrophobic octyl-Sepharose and polysaccharide adsorbent using ammonium sulfate buffer. Many theories were used in attempt to explain the stronger hydrophobic interaction at higher salt concentrations in hydrophobic interaction systems. According to Lin et al. [3], the bound water prevents protein molecules from binding to the hydrophobic ligands on the adsorbent surface. However, in the presence of salt, the protein will

Table 1
Experimental values of Q (mg mL^{-1}) for BSA at different adsorption conditions

C (mg mL^{-1})	C_s (M)				C_s (M)			
	0.05	0.30	0.60	0.90	0.05	0.30	0.60	0.90
	T = 283.1 K				T = 293.1 K			
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.25	0.57	0.69	1.82	9.51	0.65	0.67	0.97	1.32
0.50	2.89	3.46	7.27	16.21	3.23	3.52	7.09	10.78
1.00	7.53	9.74	17.52	29.88	8.80	9.61	17.41	25.12
1.50	13.08	15.34	24.49	37.27	14.26	16.10	23.20	34.47
2.00	17.66	20.12	29.31	41.13	20.29	21.72	29.35	42.77
3.00	26.89	27.74	37.17	48.55	30.54	30.43	40.29	56.88
4.00	36.13	37.10	45.57	58.06	40.24	42.37	52.27	72.13
6.00	52.28	53.23	57.82	72.81	55.90	61.45	70.45	87.02
8.00	60.46	65.53	68.23	87.14	68.73	74.29	87.63	97.59
	T = 303.1 K				T = 313.1 K			
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.25	0.62	0.79	0.93	5.15	0.62	0.75	4.81	6.14
0.50	3.56	4.88	6.61	16.57	3.79	4.74	16.48	17.05
1.00	10.01	13.30	16.74	37.42	11.10	13.7	33.90	37.32
1.50	16.01	22.99	21.06	47.24	17.45	20.01	39.41	54.87
2.00	21.44	29.89	29.52	52.5	24.81	28.47	46.73	61.41
3.00	32.02	38.33	46.22	65.36	36.62	39.55	61.07	73.12
4.00	43.01	49.44	62.45	76.20	48.13	52.22	72.70	84.60
6.00	60.96	66.35	84.45	93.18	66.70	74.16	91.40	102.20
8.00	74.97	77.39	99.43	106.53	79.57	86.63	102.50	115.61

be dehydrated due to the hydration effect of salt molecules surrounding the protein. Thus, the hydrophobic zones of the protein will be gradually naked with increasing salt concentration, strengthening the hydrophobic interactions between protein and adsorbent surface [5]. The isotherms show that the influence of salt concentration on adsorption is more significant for β-lg, mainly at 313.1 K, where the amount adsorbed in 0.9 M is about twice the amount adsorbed in 0.6 M.

Increasing temperature will proportionally increase the amount of adsorbed protein in all salt concentrations. It is noted an appreciable increase in the adsorption of β-lg in salt concentration higher than 0.6 M. Xie et al. and Goheen and Gib-

Table 2
Experimental values of Q (mg mL^{-1}) for β-lg at different adsorption conditions

C (mg mL^{-1})	C_s (M)				C_s (M)			
	0.05	0.30	0.60	0.90	0.05	0.30	0.60	0.90
	T = 283.1 K				T = 298.1 K			
0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.25	0.70	0.75	0.78	1.18	0.71	0.73	0.82	2.05
0.50	3.06	3.47	4.27	8.96	3.17	3.49	4.63	13.24
1.00	8.29	9.26	10.83	18.52	8.52	9.29	12.52	28.45
1.50	13.35	14.91	16.33	25.56	13.95	13.98	19.55	40.64
2.00	17.70	19.77	21.79	32.19	18.52	19.84	25.61	48.66
3.00	25.86	28.65	31.91	43.88	27.39	29.16	36.20	63.65
4.00	32.75	36.77	40.84	55.12	34.95	37.85	46.57	76.96
6.00	42.48	47.63	52.82	68.39	47.18	49.32	61.06	94.04
8.00	42.48	47.63	52.82	68.39	47.18	49.32	61.06	94.04
	T = 303.1 K				T = 313.1 K			
0.00	0.00	0.00	0.00	0.00	0.05	0.30	0.60	0.90
0.25	0.72	0.78	0.93	2.05	0.00	0.00	0.00	0.00
0.50	3.37	3.56	5.43	13.24	0.72	0.84	1.10	15.61
1.00	9.13	10.01	13.87	28.45	3.61	4.39	6.04	32.64
1.50	14.63	16.64	21.43	40.64	9.59	11.42	14.31	40.68
2.00	19.51	22.55	28.27	48.66	15.80	18.61	21.67	57.00
3.00	29.13	32.80	40.59	63.65	22.91	25.23	28.58	71.71
4.00	37.69	41.87	51.64	76.96	33.45	36.44	41.22	93.83
6.00	49.31	54.35	69.14	94.04	42.98	47.86	54.26	107.43
8.00	49.31	54.35	69.14	94.04	55.99	62.50	72.43	124.27

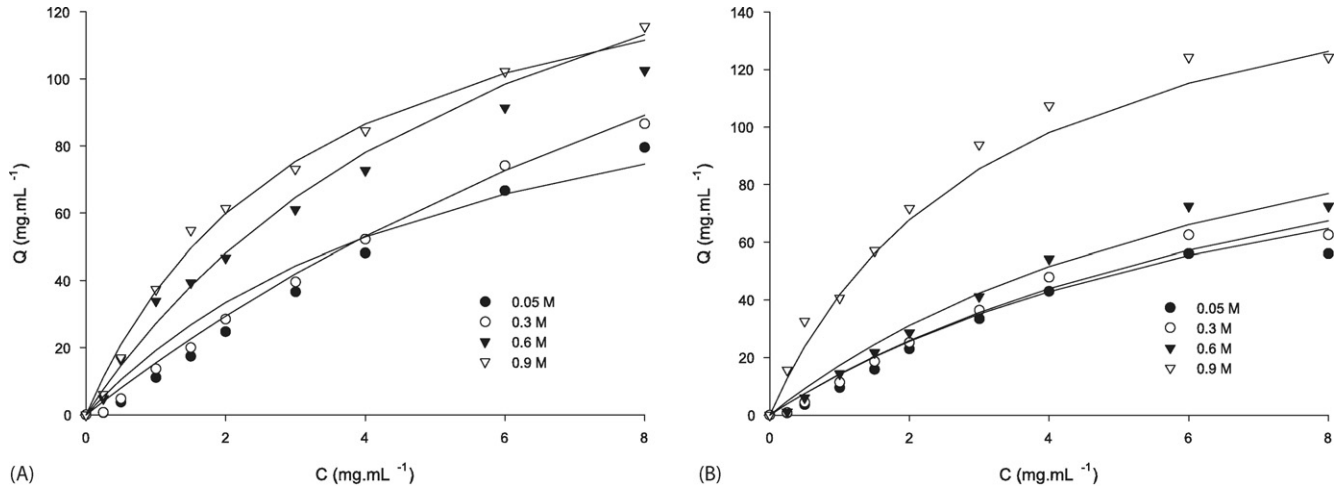

Fig. 1. Isotherms of BSA and β-lg at different Na_2SO_4 concentrations at 313.1 K: (A) BSA and (B) β-lg. Prediction is given by solid lines.

bins [27,28] suggest that the reduced solution polarity and the decreased stability of the protein structure increase the exposure of the hydrophobic residue of the inner protein core to aqueous solution at higher temperature. Fang et al. [29] have also reached a similar conclusion by showing that hydrophobic interactions contribute significantly for the interaction of horse heart cytochrome *C* with the cation exchanger at the highest temperatures.

The structure of folded protein can be partially damaged at high temperatures, exposing the inner hydrophobic core. Also, the increased hydrophobic interaction between proteins and hydrophobic ligands may induce irreversible thermal inactivation [30]. In spite of, the β-lg was considered a "hard" protein, which is more stable due to the presence of the disulfate bonds, the results suggest that this protein possibly undergoes a higher change in its structure. This fact suggest that in temperatures near 313.1 K and high salt concentration the β-lg structure is strongly changed, possibly due to alterations in disultate bonds, so that its adsorption capacity increases significantly at this temperature.

The Langmuir isotherm model (Eq. (3)) was fitted to the experimental data, Tables 1 and 2, using the NLIN procedure of the SAS® package [24], and the model parameters are shown in Tables 3 and 4. The determination coefficients was larger than 0.97 in all cases. It demonstrated that the Langmuir model have a good adjustment.

The dependence of the parameters q_s and b on the temperature and salt concentration was determined by regression analysis (Eqs. (13) and (14) for BSA and β-lg, respectively). The results showed a good adjustment ($R^2 > 0.90$ in all cases) and all the parameters were significant.

$$q_s = -24357.8 - 263.9C_s + 165.4T - 0.27T^2,$$
$$b = 20.24 - 0.05C_s - 0.14T + 0.0023T^2 + 0.32C_s^2 \quad (13)$$

$$q_s = -327.9 - 48.5C_s + 1.5T,$$
$$b = 0.13 - 1.25C_s + 0.41C_s^2 + 0.0034TC_s \quad (14)$$

4.2. Thermodynamic analysis

The retention factor k' was determined according to Eq. (7) and with the adjusted parameter of the Langmuir isotherm model. As shown in Fig. 2, k' increases in a non-linear way as the temperature is increased and this behavior is more evident as salt concentration increases. The same results were presented by Dias-Cabral et al. [1] for BSA adsorption on PPG-Sepharose using as modulator ammonium sulfate and sodium sulfate. According to Wu et al. [17], the non-linearity is in part due to changes in protein conformation, which results in an increase in the conformational entropy at higher temperature.

Table 3
Adjusted parameters of the Langmuir isotherm model for BSA, at different temperatures and salt concentrations

T (K)	q_s (mg mL^{-1}) C_s (M)				b (mL mg^{-1}) C_s (M)			
	0.05	0.30	0.60	0.90	0.05	0.30	0.60	0.90
283.1	304.50	320.05	123.33	121.66	0.03	0.03	0.15	0.26
293.1	354.38	370.95	261.71	175.48	0.03	0.02	0.06	0.16
303.1	398.77	381.58	194.94	152.21	0.02	0.08	0.04	0.26
313.1	329.26	325.26	161.31	162.83	0.04	0.04	0.21	0.28

Table 4
Adjusted parameters of the Langmuir isotherm model for β-lg, at different temperatures and salt concentrations

T (K)	q_s (mg mL^{-1}) C_s (M)				b (mL mg^{-1}) C_s (M)			
	0.05	0.30	0.60	0.90	0.05	0.30	0.60	0.90
283.1	91.08	102.91	113.19	121.13	0.12	0.12	0.12	0.18
293.1	110.97	112.65	130.54	151.07	0.1	0.11	0.12	0.24
303.1	112.56	119.50	152.56	151.07	0.11	0.12	0.11	0.24
313.1	128.76	142.36	166.03	175.76	0.11	0.11	0.11	0.35

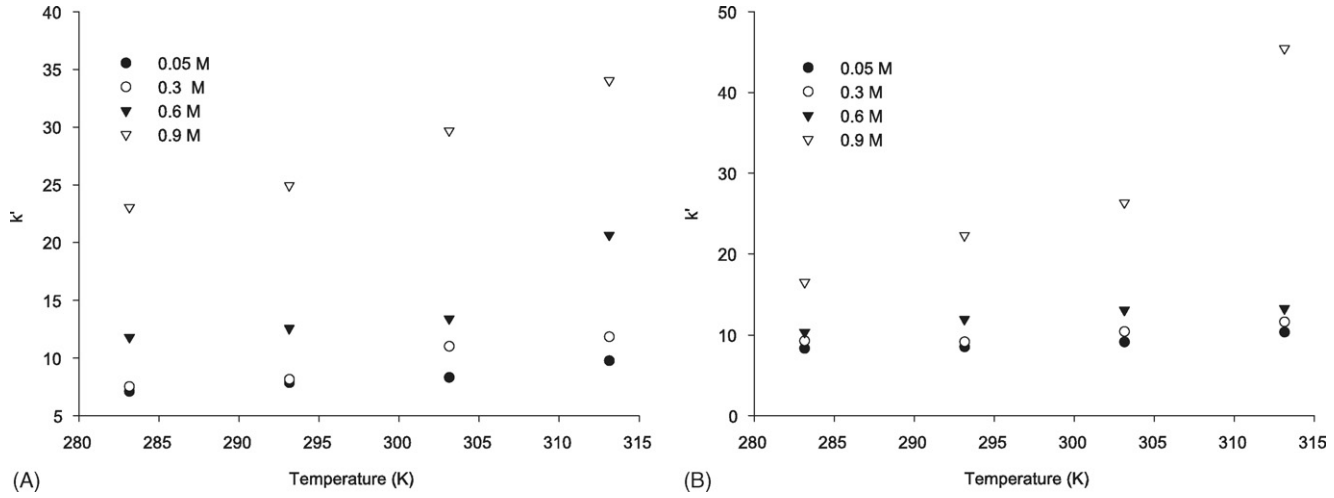

Fig. 2. k' vs. temperature for protein adsorption on Streamline Phenyl at various salt concentrations: (A) BSA and (B) β-lg.

The Z value, which is a measure of protein conformation, is obtained through Eq. (4). Fig. 3 shows the Z values as a function of temperature. For both proteins, Z value increases with temperature and there is a sharp change at approximately 303.1 K. According to Dias-Cabral et al. and Wu et al. [1,17], this behavior can indicate a changes in the conformational structure of the proteins. The results obtained suggest that the conformational change was higher for β-lg and its adsorbed concentration exceeded the value of BSA. The Z values found in this work are smaller than that found by Dias-Cabral et al. [1] for BSA on PPG-Sepharose using Na_2SO_4 as modulator, which is in accordance to the fact that different ligands promote different changes in the conformational structure of proteins.

Retention data obtained for BSA and β-lg on Streamline Phenyl as a function of temperature are shown in Fig. 4, and a non-linear relationship is observed. The results obtained by Esquibel-King et al. [19], presented the same relationship for adsorption of BSA on an epoxy-$(CH_2)_4$ Shepharose support and using $(NH_4)_2SO_4$ at four concentrations. Dias-Cabral et al. [1] adjusted the Eq. (8) up to the quadratic term ($1/T^2$), for retention data obtained for BSA on PPG-Sepharose at pH 7.0 and at different concentrations of Na_2SO_4 and $(NH_4)_2SO_4$, with good results.

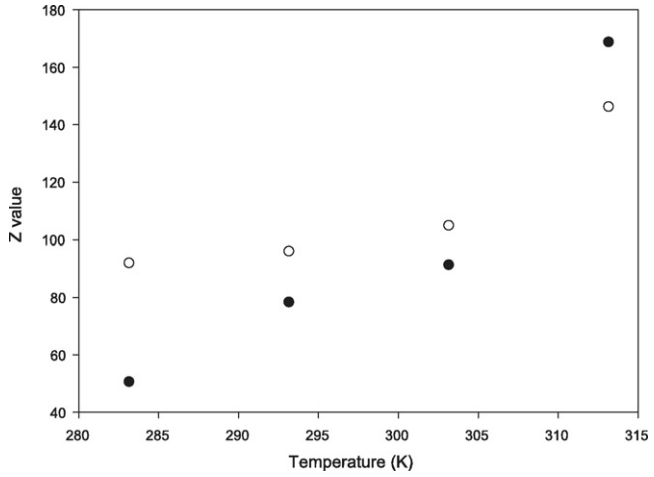

Fig. 3. Z values vs. temperature: (◯) BSA and (●) β-lg.

The solid curves in the figures represent the predicted values using three terms of Eq. (8). In all cases higher values of the determination coefficients ($R^2 > 0.989$) were obtained. This fact was observed by Boysen et al. [10] for the adsorptive behavior of the polypeptides with immobilized lipophilic compounds and by Purcell et al. [31] for the adsorptive behavior of the hormonal polypeptides β-endorphin, glucagons and bovine insulin with immobilized *n*-butyl and *n*-octadecyl groups at different temperatures. The adjusted parameters of the van't Hoff equation obtained are shown in Table 5.

The thermodynamic quantities for the retention of BSA and β-lg on Streamline Phenyl were determined from the data of Table 5 and using the Eqs. (9), (10) and (12). The results for both proteins are shown in Figs. 5–7. It is observed that the adsorption process of both proteins is entropically driven as temperature is increased. From the results, it can be observed that the influence of salt concentration at higher temperature is larger for β-lg than BSA. The values of ΔH° and ΔS° of β-lg varied from 11.54 kJ mol^{-1} to 38.98 kJ mol^{-1} and 58.97 J mol^{-1} K^{-1} to 158.90 J mol^{-1} K^{-1}, respectively, at temperature of 313.1 K as the salt concentration was increased.

It was observed that the increase on enthalpy and entropy was higher at 0.9 M. This fact can be associated to a conformational

Table 5
Adjusted parameters of the van't Hoff equation

Protein	C_s (M)	Parameters (Eq. (8))		
		a_1	a_2	a_3
BSA	0.05	20.55	−10098.15	1369193.32
	0.3	52.64	−28763.95	4086296.06
	0.6	56.70	−31079.89	4453156.58
	0.9	24.80	−11608.18	1549193.88
β-lg	0.05	29.29	−15498.52	2209278.75
	0.3	34.42	−18418.40	2633061.20
	0.6	−20.67	14550.06	−2274806.80
	0.9	74.74	−39752.62	5490080.20

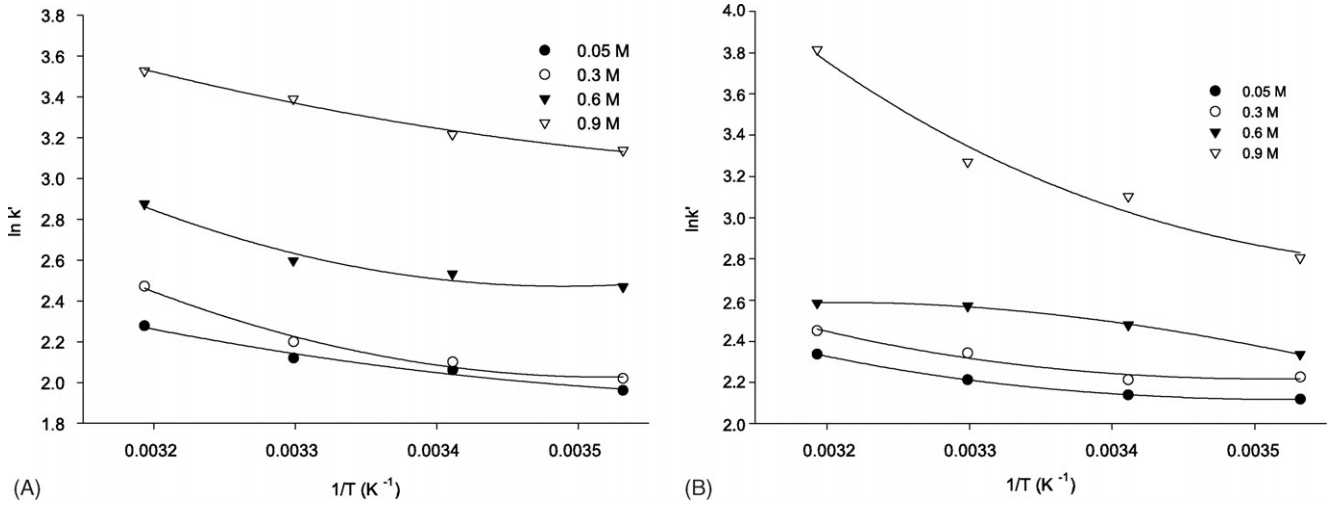

Fig. 4. van't Hoff plots for the retention of BSA and β-lg on Streamline Phenyl at different concentrations of Na_2SO_4: (A) BSA and (B) β-lg.

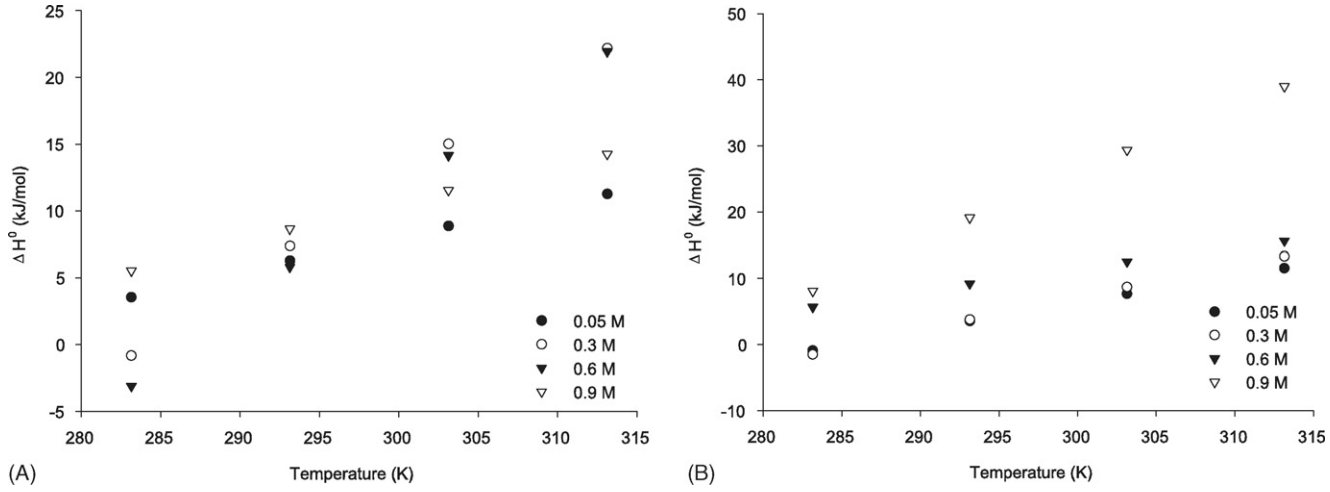

Fig. 5. Enthalpy change of the proteins interacting with Streamline Phenyl at different concentrations of Na_2SO_4: (A) BSA and (B) β-lg.

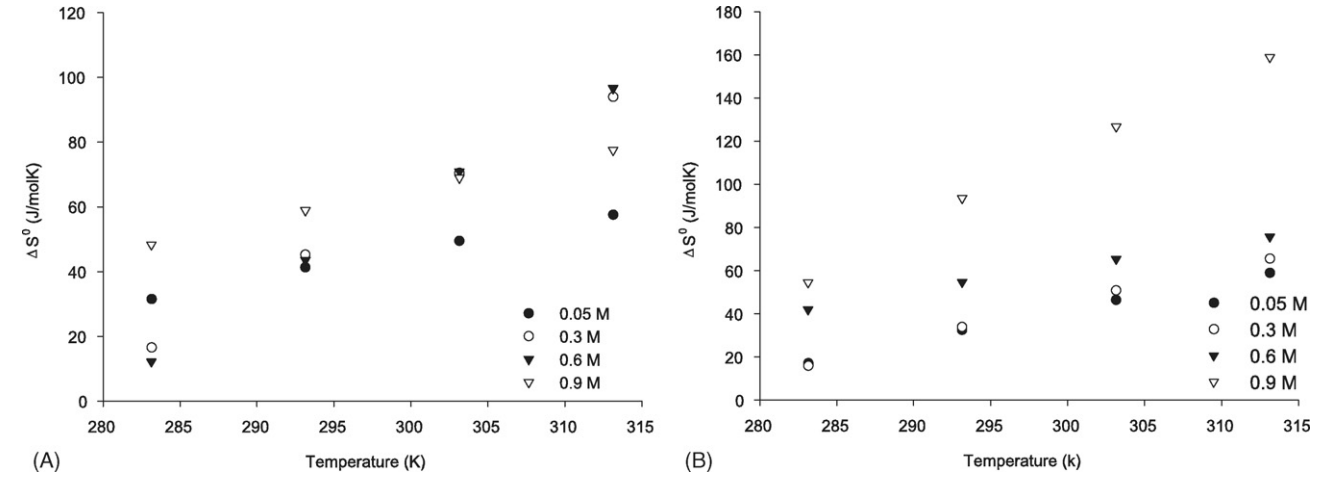

Fig. 6. Entropy change of the proteins interacting with Streamline Phenyl at different concentrations of Na_2SO_4: (A) BSA and (B) β-lg.

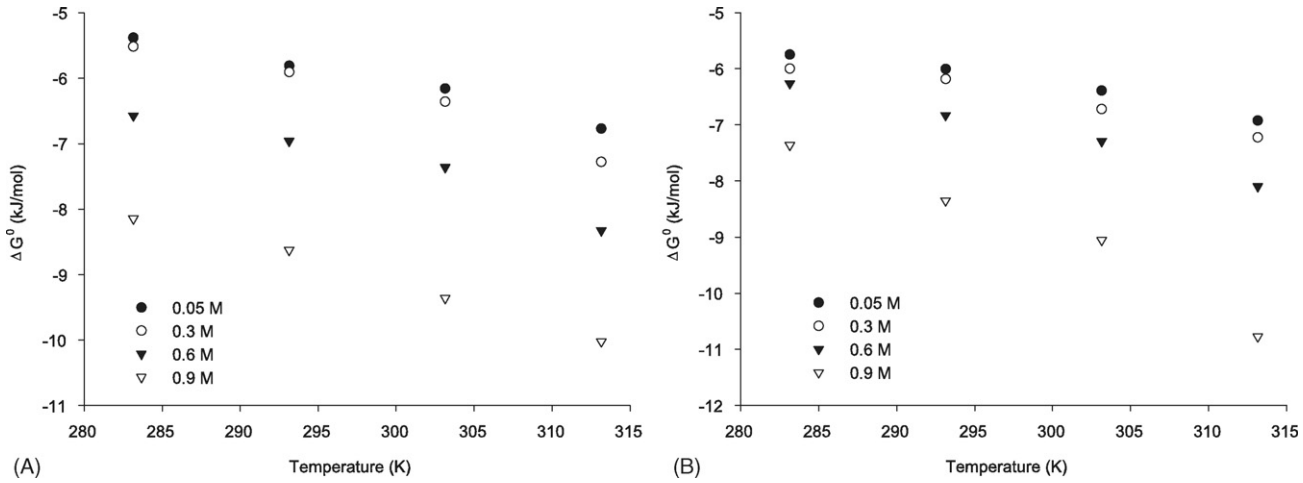

Fig. 7. Free energy change of the proteins interacting with Streamline Phenyl at different concentrations of Na_2SO_4: (A) BSA and (B) β-lg.

change in protein structure, i.e., at high salt concentration, the protein has its hydration capacity decreased and its hydrophobicity increased, allowing a larger interaction with the adsorbent. Besides, the results indicated that the ΔG° value for β-lg is more negative than for BSA. According to Dias-Cabral et al. [1], endothermic values are observed at high salt concentrations due to stronger hydrophobic interactions.

5. Conclusions

In this study we investigated the binding characteristics between proteins and hydrophobic adsorbents. The results showed that the effect of salt concentration and temperature was more significant to β-lg than BSA. The analysis of the Z values showed that there were conformational changes for both proteins and these were more significant to β-lg. The thermodynamic values presented here showed that such process is entropically driven and is favorable for both proteins. In all cases, the values of ΔH° and ΔS° increased with the increase of salt concentration and there is a linear dependence of ΔH° and ΔS° on temperature, for all salt concentrations, for both proteins. It can also be observed that the influence of salt concentration at higher temperature is larger for β-lg than BSA and the protein β-lg presented a great conformational change for temperatures above 303.1 K and salt concentration of 0.9 M. The Gibbs free energy decreases with temperature, for all the cases and the results indicated that the ΔG° value for β-lg is more negative than for BSA.

Nomenclature

a	Henry's constant of adsorption
a_1, a_2, a_3	parameters of Eq. (8)
b	adsorption equilibrium constant
$[\%B]$	concentration of water (volume fraction)
C	protein concentration
C_s	salt concentration
ΔC_P°	heat capacity
ΔG°	Gibbs free energy change
ΔH°	enthalpy change
I	characteristic constant related to the affinity of a protein for the HIC sorbent
k'	retention factor
K	equilibrium constant
L_d	concentration of hydrated ligands in salt solution
n'	number of ligand interactions with a protein molecule
q_s	saturation capacity
Q	amount of compound adsorbed
R	universal gas constant
ΔS°	entropy change
t_0	dead time
t_R	retention time
T	temperature
V_0	column void volume
V_a	volume of adsorbent in the column
$V_{F,i+1}$	retention volume at the inflection point
Z	number of moles of water displaced per mole of protein adsorbed on the bonded phase surface

Greek letters

ε	total porosity of the column
Φ	system constant depending on the phase ratio in the column
φ	phase ratio

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