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KINETICS AND ADSORPTION ISOTHERM OF C-PHYCOCYANIN FROM *Spirulina platensis* ON ION-EXCHANGE RESINS

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Abstract - C-phycocyanin is a natural blue dye extracted from *Spirulina platensis*, which has many applications in the food and pharmaceutical industries. In this paper the effect of pH and temperature on the adsorption of C-phycocyanin onto two different ion exchange resins (Streamline DEAE and Streamline Q XL) for expanded bed adsorption chromatography was investigated. Moreover, the kinetics and adsorption isotherm were evaluated. The equilibrium for the Q XL matrix was reached after 60 min, while for DEAE it was only reached after 140 min. C-phycocyanin showed the highest partition coefficient at pH 7.5 for both resins at 25 °C. The C-phycocyanin adsorption isotherm was very well represented by the Langmuir, Freundlich and Langmuir-Freundlich models, where the estimated values for Q_m and K_d obtained by the Langmuir isotherm were, respectively, 33.92 mg.mL^{-1} and 0.123 mg.mL^{-1} for DEAE, and 28.12 mg.mL^{-1} and 0.082 mg.mL^{-1} for the Q XL matrix. A negative cooperativity was observed for C-phycocyanin binding when the Q XL matrix was used, while the cooperativity was purely independent using the DEAE matrix.
Keywords: Adsorption; C-phycocyanin; Freundlich isotherm; Kinetic parameters; Langmuir isotherm.

INTRODUCTION

The phycobiliproteins are photosynthetic pigments present in cyanobacteria, responsible for about 50% of the light captured by these microorganisms (Santiago-Santos *et al*., 2004). One of the most interesting phycobiliproteins for the food (Yoshida *et al*., 1996) and cosmetic (Cohen, 1986) industries is C-phycocyanin, its applicability being based on the fact that it can be used as a natural blue dye in these sectors as a substitute for synthetic dyes, which are generally toxic or otherwise unsafe (Moraes *et al*., 2011b; Minkova *et al*., 2003; Arad and Yaron, 1992).

Of the various existing sources of this natural dye, the cyanobacterium *Spirulina platensis* deserves special attention, since C-phycocyanin may constitute up to 20% of the protein fraction (Vonshak, 1997). C-phycocyanin is formed by two dissimilar α and β protein subunits of 17,000 and 19,500 Da, respectively, with one bilin chromophore attached to the α subunit (a84) and two to the β subunit (b84, b155) (Turner *et al*., 1997). Patel *et al*. (2005) found that the molecular mass of this biomolecule extracted from *Spirulina* sp. was 112 kDa.

In addition to its potential for use in food and cosmetics, studies have shown that C-phycocyanin has antioxidant (Bhat and Madyasatha, 2001), anti-inflammatory and antitumor properties (Reddy *et al*., 2003), as well as acting as an immune system stimulator, increasing the total number of leukocytes, whose

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main function is to maintain the health of body organs and protect against cancer and ulcers (Madhyastha *et al*., 2006). Furthermore, this protein has been reported to induce apoptosis in some carcinoma cells (Roy *et al*., 2007) and human chronic myeloid leukemia cells (Subhashini, 2004).

The phytotherapeutic property presented by this product requires a high purity level, achieved through various purification techniques, which present an elevated cost. One of the processes applied in protein purification is based on chromatographic techniques such as expanded bed ion exchange chromatography (EBIEC). Adsorption in expanded beds enables proteins to be recovered directly from disrupted cell preparations, without the need for prior removal of the suspended solids, which would normally result in blockage of the packed beds. The implementation of this technique greatly reduced the complexity of downstream processing by eliminating certain centrifugation, dialysis, precipitation, two-phase aqueous system and gel filtration steps, resulting in cost reductions and simplification of the purification and recovery process of the bioproduct (Chase, 1994).

However there are some factors that are critical to the success of the product, including the correct choice of adsorbent, together with a careful design of the apparatus in which the separation is performed.

The EBIEC makes use of adsorbents such as ion exchange resins, which adsorb proteins as a result of ionic interactions between charged groups on the protein surface and ionic groups with opposite charges on the ion exchanger (Chase, 1994). The pH is able to promote changes in the ionic strength while the temperature affects the spatial structure of the protein, which is why these are important parameters in the study of the adsorption kinetics of an interesting compound.

In an adsorption study, it is essential to determine the model which describes the adsorption isotherm in question (Dotto *et al*., 2013). Adsorption isotherms are curves that describe the adsorption of a solute by solids at a constant temperature. An adsorption isotherm shows the amount of solute adsorbed by the adsorbent surface as a function of the solute equilibrium concentration. The most commonly found isotherms for the adsorption of bioproducts are the Freundlich, Langmuir, Langmuir-Freundlich and linear models. The technique used to create the adsorption data is, in principle, quite simple, since a known quantity of solute is added to a system containing a known amount of adsorbent. It is assumed that the difference between the amount added and that remaining in solution was adsorbed onto the adsorbent surface (Alleoni *et al*., 1998).

The selection of the C-phycocyanin adsorption conditions is an important step for further purification using EBIEC techniques, since increases in the adsorption of the compound by the ion exchanger lead to reductions in process costs due to decreased losses and the use of reduced volumes of the target product and resin.

There is a lack of studies related to the behavior of C-phycocyanin during its adsorption onto ion exchange resins in an expanded bed. Silveira *et al*. (2008) studied the effects of pH and temperature on the partition coefficient, and determined the adsorption isotherm under the best conditions evaluated for the adsorption of C-phycocyanin onto the ion exchange resin Q-Sepharose FF, a widely used and highly successful resin applied in fixed beds. Adsorption in an expanded bed resin of Streamline DEAE was studied by Bermejo *et al*. (2006), Ramos *et al*. (2010), Ramos *et al*. (2011) and Bermejo and Ramos (2012), obtaining the adsorption isotherms for crude C-phycocyanin and/or a purified extract to obtain the parameter Q_m. In these studies the kinetic behavior was not evaluated and the pH and temperature were not optimized for better adsorption.

According to the aspects mentioned above, and considering the importance of an analysis of the equilibrium data in the development of equations that can be used for the design of adsorption models and the choice of the best adsorbent, the aim of this study was to investigate the effects of the process parameters – temperature and pH – on the adsorption of C-phycocyanin onto the ion exchange resins Streamline Q XL and Streamline DEAE for expanded beds, as well as verifying the typical equilibrium conditions for the adsorption of C-phycocyanin onto ion exchange resins.

MATERIAL AND METHODS

Preparation of the Crude Extract and Quantification

The cyanobacterium *Spirulina platensis* was grown and maintained in outdoor photo-bioreactors under uncontrolled conditions in the south of Brazil. During these cultivations, the water was supplemented with 20% Zarrouk synthetic medium (Costa *et al*., 2000). At the end of cultivation, the biomass was recovered by filtration, and then dried, frozen and milled to a particle diameter of between 0.106 and 0.125mm (Moraes *et al*., 2010).

The extraction of C-phycocyanin from the biomass was carried out according to Moraes *et al*.

(2010), who used water as the solvent extractor at a rate of 0.16 g: 1 mL (powdered biomass: solvent extractor). The C-phycocyanin concentration (*C–FC,* Equation (1)) was calculated as described by Bennett and Bogorad (1973) with little modification in the optical densities.

$$C - FC = \frac{(OD_{620} - 0.474\ OD_{652})}{5.34} \quad (1)$$

Adsorbents

Two suitable adsorbents for use in expanded beds were studied for the adsorption of C-phycocyanin. These adsorbents consisted of an agarose macroporous matrix with a quartz core. One of the adsorbents, Streamline DEAE® (GE Healthcare) is a weak anion exchanger, the functional group being -O-CH_2CH_2-$N^+(C_2H_5)_2H$. The other adsorbent, Streamline Q XL® (GE Healthcare) is a strong anion exchanger, with a quaternary ammonium as the functional group. The working pH range of both exchangers is between 2 and 13 (Amersham Biosciences, 2002). These resins can promote different degrees (2-3-fold) of bed expansion.

Effect of pH on the Partition Coefficient

The tests were carried out in a thermostatic mixture reactor at 25 °C, with mechanical agitation for 200 min as shown in Figure 1.

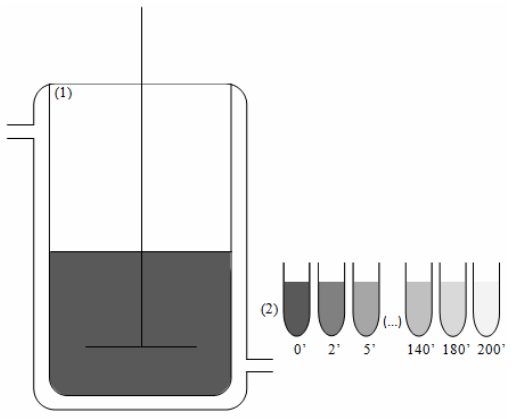

Figure 1: Schematic mixture reactor used for the adsorption of C-phycocyanin onto the ion exchange resins, where (1) is a temperature controlled mixture reactor and (2) non-adsorbed samples of C-phycocyanin taken at determined time intervals.

In this system, 30 mL of C-phycocyanin extract with a fixed initial C-phycocyanin concentration was added to 3 mL of ion exchange resin (Streamline Q XL or Streamline DEAE) pre-equilibrated in 0.025M sodium phosphate buffer at the pH of the study. The pH of the C-phycocyanin extract was adjusted to the following pH values (5.0, 5.5, 6.0, 6.5, 7.0 and 7.5), according to the range where the C-phycocyanin was considered stable (Silva *et al*., 2009). A blank was prepared for each test, in which the C-phycocyanin at the pH under study was added to the system without the ion exchange resin. Samples were taken at pre-determined time intervals (0, 2, 5, 7, 10, 15, 20, 25, 30, 40, 60, 80, 100, 140, 180 and 200 min), calculating the C-phycocyanin concentration according to Equation (1). The experiments were carried out in duplicate.

Based on the initial and final concentrations of free C-phycocyanin (non-adsorbed) in the liquid phase (C^*), the equilibrium concentration of C-phycocyanin adsorbed onto the solid phase (q^*) can be calculated, thus obtaining the partition coefficient (f), which indicates the protein fraction adsorbed at equilibrium. The partition coefficient was calculated according to Harsha and Furusaki (1994) (Equation (2)):

$$f = \frac{q^*}{C^*} \quad (2)$$

Effect of Temperature on the Partition Coefficient

After established the equilibrium time and the better C-phycocyanin adsorption pH (fixed at 7.5), the effect of temperature on the partition coefficients was studied (5, 15 and 25 °C) in mixture reactors with orbital agitation (200 rpm). In this system, 30 mL of C-phycocyanin extract with a fixed initial C-phycocyanin concentration was added to 3 mL of ion exchange resin (Streamline Q XL or Streamline DEAE) pre-equilibrated in 0.025M sodium phosphate buffer at pH 7.5. A blank was prepared for each test, in which the C-phycocyanin at the temperature under study was added to the system without the ion exchange resin. Samples were taken at 0 and 200 min. The C-phycocyanin concentrations and the partition coefficients were calculated as shown in Equations (1) and (2), respectively. The experiments were carried out in triplicate.

Adsorption Isotherms

The experiments used to determine the adsorption isotherm at equilibrium were carried out in a batch system with orbital agitation containing pre-equilibrated ion exchange resin (Streamline Q XL or Streamline DEAE) and the C-phycocyanin extract

with different initial concentrations at pH 7.5 and 25 °C and incubated at 200 rpm for 200 min. After this period the samples were collected to determine the C-phycocyanin concentration. The experiments were carried out in triplicate.

Adsorption isotherms can be described in many mathematical forms, some of which are based on a simplified physical model of adsorption and desorption, whereas others are purely empirical and intended to correlate the experimental data. Various isotherm equations, such as the Langmuir, Freundlich and Langmuir-Freundlich models, have been used to describe the equilibrium characteristics of adsorption. If the adsorption of C-phycocyanin follows the Langmuir model, the adsorption process can be expressed as:

$$q^* = \frac{Q_m \cdot C^*}{K_d + C^*} \tag{3}$$

where Q_m represents the maximum binding capacity of the resin (mg.mL^{-1}) and K_d is the Langmuir equilibrium constant (mg.mL^{-1}), which is the effective dissociation constant representing the ratio between desorption and adsorption. The proposed model is based on an ideal surface and predicts that the adsorption surface is energetically ideal (Barboza *et al*., 2002).

On the other hand, if the adsorption of C-phycocyanin follows the Freundlich model, the isotherm can be described as follows:

$$q^* = K_f \cdot \left(C^*\right)^{1/n} \tag{4}$$

where K_f and n are the Freundlich physical constants related to the adsorption capacity and adsorption intensity of the adsorbent, respectively.

Langmuir-Freundlich model (Eq. 5) proposed by Sips (1948), is a combined form of Langmuir and Freundlich models and has three parameters: Q_m is the maximum adsorption capacity of the monolayer; K_{dLF} is the apparent dissociation constant; and n_{LF} is the Langmuir-Freundlich coefficient which indicates the presence or absence of cooperativity.

$$q^* = \frac{Q_m (C^*)^{n_{LF}}}{K_{dLF} + (C^*)^{n_{LF}}} \tag{5}$$

The Freundlich, Langmuir and Langmuir-Freundlich models applied using a non-linear regression method were used to fit the experimental equilibrium data, and the coefficient of determination (R^2) used to analyze the fit of each model. The parameters for the models were estimated by the non-linear regression method from the model expressions.

RESULTS AND DISCUSSION

Effects of pH on the Partition Coefficient

The partition coefficient correlates the amount of target compound that migrates from one phase to another. In the present case, the partition coefficient correlated the amount of C-phycocyanin present in the solid phase, or adsorbed onto the resin, with the amount of the same bioproduct free in the solution at equilibrium.

Figure 2 shows the variation in the C-phycocyanin concentration in the liquid phase at the different pH values during the time period studied. It can be seen that equilibrium was reached after 60 minutes for all the pH values studied using the Streamline Q XL resin (Figure 2b), whereas with the Streamline DEAE resin (Figure 2a), equilibrium was only reached after 140 minutes. The assays carried out without the addition of any resin showed there was no C-phycocyanin denaturation during the time period studied at any of the working pH values. The time necessary to reach equilibrium for the adsorption of C-phycocyanin onto the Streamline DEAE resin at the different pH values was very similar to that found by Silveira *et al*. (2008), when equilibrium was achieved after 150 min for the adsorption of C-phycocyanin onto the Q Sepharose Fast Flow resin used for the fixed bed.

Figure 3 shows the behavior of the partition coefficient with the variation in pH for adsorption of C-phycocyanin onto the Streamline DEAE and Streamline Q XL resins. The lowest partition coefficient values were obtained at the lowest pH value studied, that is, at pH 5. The structure of C-phycocyanin includes a protein part, and hence the net surface charge of the biomolecule can vary according to the surrounding pH value. When above its pI (isoelectric point) the biomolecule will be negatively charged. On the other hand, when the pH is below its pI the biomolecule will be positively charged. The isoelectric point of C-phycocyanin is around 4.6 to 5.2 (Santiago-Santos *et al*., 2004; Abalde *et al*., 1998), and thus the extract used in this study was negatively charged and the adsorbents were anionic. Hence, when C-phycocyanin made contact with the adsorbent, the buffer-adsorbent bond formed was weaker than the C-phycocyanin-adsorbent bond, resulting in an adsorption process. The higher the pH value, the greater the net negative charge on the biomolecule, resulting in higher partition coefficients, as shown in Figure 3.

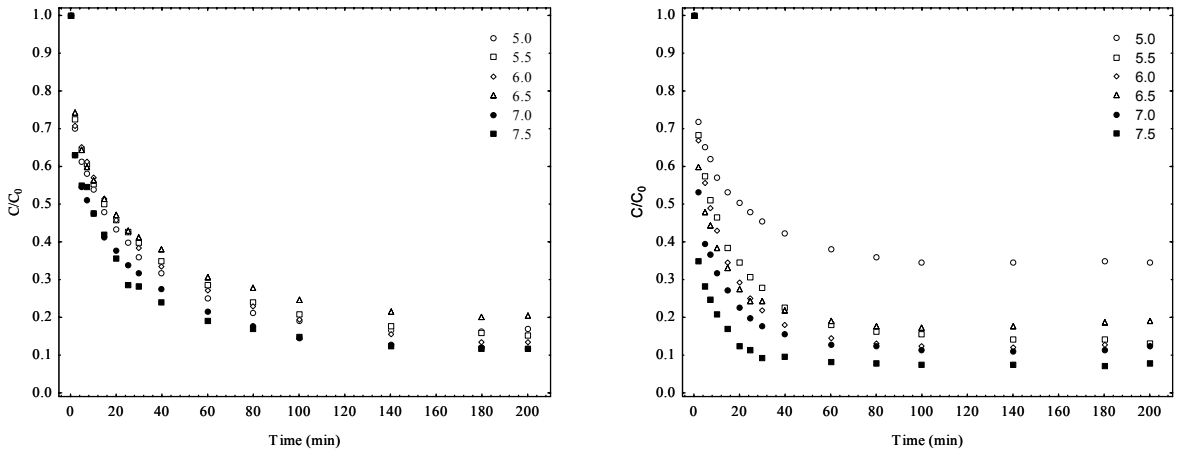

Figure 2: Adsorption kinetics of C-phycocyanin at different pH values for the (a) Streamline DEAE ion exchange resin with average errors of 0.001 for pH 5.0; 0.002 for pH 5.5; 0.001 for pH 6.0; 0.011 for pH 6.5; 0.003 for pH 7.0 and 0.009 for pH 7.5 and (b) Streamline Q XL ion exchange resin with average errors of 0.004 for pH 5.0; 0.002 for pH 5.5; 0.001 for pH 6.0; 0.003 for pH 6.5; 0.005 for pH 7.0 and 7.5.

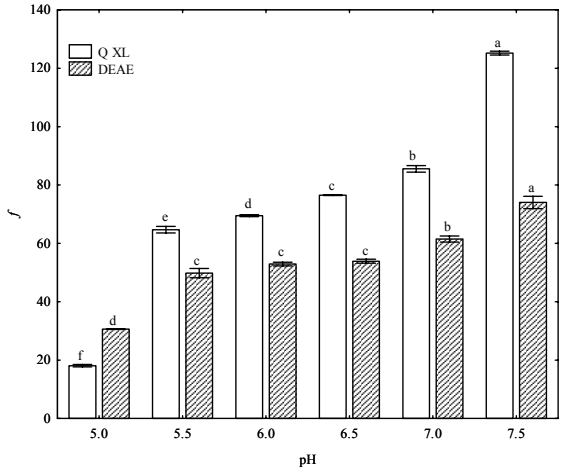

Figure 3: Behavior of the partition coefficients (*f*) with average error for the adsorption of C-phycocyanin onto the resins Streamline DEAE and Streamline Q XL at different pH values. Equal letters for the same resin do not differ statistically at the significance level of 5% (Tukey's Test).

The variation in the partition coefficients on the Streamline DEAE resin was modest as compared to that on the Streamline Q XL resin, showing that the pH had only a slight influence on adsorption onto this resin. In the study carried out by Silveira *et al*. (2008), the influence of pH on the partition coefficient was also measured at pH 8.0 and 9.0 and, although the partition coefficient was slightly higher at pH 8.0 than at pH 7.5, the authors reported that C-phycocyanin was not very stable at pH 8.0. It is of limited interest to carry out adsorption studies where there is a loss of biological activity of the protein. Silva *et al*. (2009) reported that the concentration of C-phycocyanin decreased to 70% of its initial value at pH 8.0 at room temperature and, for this reason, pH values above 7.5 were not included in the present study. The largest partition coefficients were obtained at pH 7.5 for both resins and therefore this pH was chosen for further work.

Effect of Temperature on the Partition Coefficient

The partition coefficients found in the temperature range evaluated are shown in Table 1.

The lowest C-phycocyanin adsorption occurred at the lowest temperature (5 °C) for both resins, while the C-phycocyanin adsorptions at 15° and 25 °C for both resins were equal. Thus, the temperature of 25 °C was

chosen for the following experiments with both adsorbents, since the choice of this temperature would reduce the costs of cooling during purification on a larger scale in an expanded bed column. Moreover, according to Sarada *et al*. (1999) and Silva *et al*. (2009), C-phycocyanin is stable at this temperature and pH.

Table 1: Partition coefficients with average error at the different temperatures for the different adsorbents.

Temperature (°C)	Adsorbents	
	Streamline Q XL	Streamline DEAE
5	150.6 ± 7.3^{b}	39.5 ± 5.3^{b}
15	180.1 ± 5.6^{a}	69.3 ± 2.9^{a}
25	$165.2 \pm 6.2^{a,b}$	76.8 ± 3.7^{a}

Equal letters in the same column do not differ statistically at the significance level of 5% (Tukey's Test).

Adsorption Isotherm

An analysis of the equilibrium data is important to develop an equation that can be used for design purposes. In this study, three classical non-linear adsorption models, Langmuir, Freundlich and Langmuir-Freundlich, were employed to describe the C-phycocyanin adsorption equilibrium.

The Langmuir model refers to monolayer sorption onto surfaces containing a finite number of identical sites, which means that the binding sites of the adsorbents are identical, and the target protein molecule only binds to the binding sites (Rozie *et al*., 1991). Other assumptions are also made for the Langmuir model, i.e., reversible reaction and no interaction between adsorbed species (Nie *et al*., 2007). For this reason the Langmuir model presumes homogeneous adsorption.

The Freundlich equation is an empirical relationship, whereby it is assumed that the adsorption energy of a protein when binding to a site on an adsorbent depends on whether or not the adjacent sites are already occupied.

The Langmuir-Freundlich isotherm is a simple generalization of both isotherms (Sips, 1948). By analogy with protein–multiple ligand interactions, it has been suggested that this isotherm serves to model adsorption cooperativity. As the equation has three fitting terms, it is much better for approximating adsorption of a heterogeneous nature and explaining adsorption cooperativity. For purely independent, noninteracting sites, the value of n_{LF}=1. When n_{LF}>1, positive cooperativity is suggested, while when 0< n_{LF}<1 negative cooperativity in the binding process is indicated. The value of n_{LF} can thus be employed as an empirical coefficient, representing the type and extent of cooperativity present in the binding interaction (Sharma and Agarwal, 2001).

Figure 4 shows the fits to the Langmuir, Freundlich and Langmuir-Freundlich models of the C-phycocyanin adsorption isotherms obtained on the Streamline DEAE and Streamline Q XL resins.

Table 2 shows the Langmuir, Freundlich and Langmuir-Freundlich adsorption constants, with their respective determination coefficients, obtained for the C-phycocyanin isotherms at pH 7.5 and ambient temperature (25 °C), when adsorbed onto the Streamline DEAE and Streamline Q XL resins.

Analyzing the Langmuir model, K_d is the dissociation coefficient of the solute–adsorbent complex, which represents the affinity between the solute and the adsorbents (Lan *et al*., 2001). Evaluating the Langmuir isotherm constants (Table 2) one can conclude that the Q XL matrix shows more affinity for the adsorption of C-phycocyanin than the DEAE matrix. The value of Q_m for the Q XL adsorbent (28.12 ± 0.10 mg.mL^{-1}) was lower than that for the DEAE adsorbent (33.92 ± 0.27 mg.mL^{-1}), which probably occurred because other negatively charged compounds linked first to the functional group before C-phycocyanin.

Silveira *et al*. (2008) studied the adsorption of the C-phycocyanin from *Spirulina platensis* onto the ion-exchange resin Q Sepharose Fast Flow in a fixed bed and obtained values for Q_m and K_d of 22.67 mg.mL^{-1} and 0.031 mg.mL^{-1}, respectively. The values for Q_m of the same support and target product – Streamline DEAE and C-phycocyanin, respectively – for different cyanobacteria are variable, and values for Q_m of 0.8 mg.mL^{-1} (Bermejo *et al*., 2006), 1.6 mg.mL^{-1} (Bermejo and Ramos, 2012), 1.74 mg.mL^{-1} (Ramos *et al*., 2010) and 5.2 mg.mL^{-1} (Ramos *et al*., 2011) can be found in the literature. Thus the Streamline DEAE resin showed maximum adsorption 7 times greater than the maximum value mentioned above, reflecting the importance of studying the adsorption conditions better, for instance the influence of pH and temperature. Furthermore, in the present study, an extract with a higher initial concentration of C-phycocyanin was used, which leads to an increase in the adsorption capacity of the dye onto the resin. This is due to the increase in the driving force of the concentration gradient, caused by the increase in the initial dye concentration (Chiou and Li, 2002).

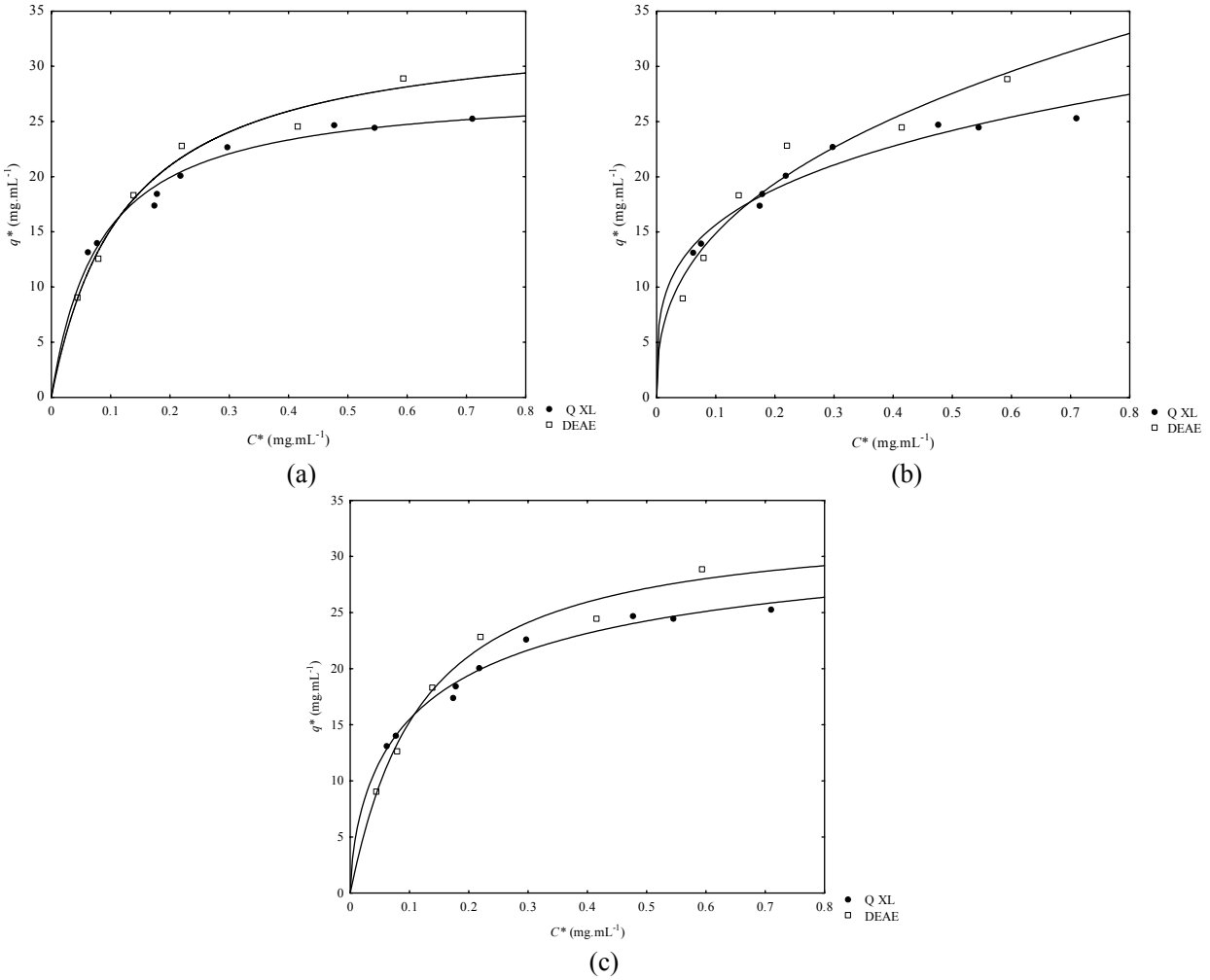

Figure 4: Equilibrium adsorption isotherms according to the (a) Langmuir equation; (b) Freundlich equation and (c) Langmuir-Freundlich equation for the adsorption of C-phycocyanin onto the Streamline DEAE and Q XL matrixes.

Table 2: Comparison between the Langmuir, Freundlich and Langmuir-Freundlich isotherm parameters for the adsorption of C-phycocyanin onto the Streamline DEAE and Streamline Q XL resins.

Model	Parameters	Streamline DEAE	Streamline Q XL
Langmuir	Q_m (mg.mL^{-1})	33.92 ± 0.27	28.12 ± 0.10
	K_d (mg.mL^{-1})	0.123 ± 0.003	0.082 ± 0.001
	R^2	0.983	0.963
Freundlich	K_f	35.94 ± 0.32	29.18 ± 0.11
	n	2.61 ± 0.05	3.69 ± 0.03
	R^2	0.947	0.959
Langmuir – Freundlich	Q_m (mg.mL^{-1})	33.08 ± 3.04	34.75 ± 0.40
	K_{dLF} (mg.mL^{-1})	0.106 ± 0.005	0.275 ± 0.010
	n_{LF}	1.04 ± 0.04	0.66 ± 0.01
	R^2	0.983	0.977

The values for the Freundlich constants showed a relatively easy uptake of C-phycocyanin onto the resins with high adsorption capacity. In particular (Table 2), the value for *n*, which is related to the distribution of the bound molecules on the adsorbent surface, was greater than unity, indicating that C-phycocyanin was favorably adsorbed under the experimental conditions examined for both resins.

For the Langmuir-Freundlich isotherm, the Q_m values were 33.08 ± 3.04 mg.mL^{-1} and 34.75 ± 0.40 mg.mL^{-1} for DEAE and Q XL resins, respectively. For Q XL, the Q_m value was greater than that obtained when using the Langmuir model (28.12 ± 0.10 mg.mL^{-1}). Thus, both resins show similar capacity for binding C-phycocyanin. Another important parameter of this model is n_{LF}, which represents the cooperativity. For Q XL there was negative cooperativity (0.66 ± 0.01) while for DEAE adsorption was purely independent (1.04 ± 0.04). The negative cooperativity implies heterogeneous adsorption due to the negative lateral interaction between adsorbed C-phycocyanin molecules where the adsorption of one C-phycocyanin molecule disfavors the adsorption of other C-phycocyanin molecules. Cooperativity depends on the nature of the macromolecule and the multiple functional groups, which usually produce multiple interactions (Bresolin *et al*., 2010; Sharma and Agarwal, 2001).

The adsorption isotherms obtained for the uptake of C-phycocyanin by Streamline Q XL and Streamline DEAE, were found to follow the predictions made by the Freundlich, Langmuir and Langmuir-Freundlich models to a satisfactory extent within the concentration range studied. This observation implies that both monolayer bio-sorption and heterogeneous surface conditions may co-exist under the experimental conditions applied. Hence, the overall adsorption of C-phycocyanin onto the ion exchange resin is a complex process, involving more than one mechanism, such as ion exchange, surface complexation and electrostatic attraction.

The determination coefficients for the Langmuir, Freundlich and Langmuir-Freudlich models for the non-linear regression, were R^2>0.94 (Table 2), thus characterizing the adsorption behavior as non-linear, as explained by the models. It is well known that the Freundlich adsorption isotherm gives an excellent representation of many data sets for moderate concentrations, while the Langmuir equation is better for low concentrations (Redlich and Peterson, 1959). In addition, Nie *et al*. (2007) reported that one limitation of the Freundlich model is that the amount of adsorbed solute increases indefinitely with the solute concentration in the solution.

In the literature some authors, i.e., Silveira *et al*. (2008), Bermejo *et al*. (2006) and Ramos *et al*. (2010), expressed their results for the adsorption of C-phycocyanin with this model, using the Langmuir model more for design purposes. The Langmuir model was also used for the adsorption isotherms carried out on several types of ion exchange resins for different proteins of interest, such as the adsorption of clavulanic acid onto Amberlite IRA (Barboza *et al*., 2002) or of amyloglucosidase onto DEAE cellulose (Manera *et al*., 2008). Bresolin *et al*. (2011) used the Langmuir model for negative chromatography by agarose gels with immobilized amine-based ligands (poly-L-lysine) for adsorbed human serum albumin and the Langmuir-Freundlich model for describe the isotherm adsorption of immunoglobulin G onto the same adsorbent.

No studies were found that determined the isotherms on a Q XL matrix, except for one that studied the use of this matrix for the adsorption of C-phycocyanin by EBIEC from a crude extract in the presence of cells (Moraes *et al*., 2011a).

The appropriate choice between the two resins (Streamline DEAE or Streamline Q XL) for the adsorption of C-phycocyanin using expanded bed chromatography will depend on the equilibrium time required, the adsorbent selectivity, the cost and re-use of the matrix and other factors that must be evaluated. In this instance, the next steps of this work will evaluate the re-use of these resins.

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NOMENCLATURE

C – FC	C-phycocyanin concentration	mg.mL^{-1}
C*	concentration of free C-phycocyanin (non-adsorbed) in the liquid phase	
EBIEC	expanded bed ion exchange chromatography	
f	partition coefficient	
K_d	Langmuir equilibrium constant	mg.mL^{-1}
K_f	Freundlich physical constant related to the adsorption capacity	

n	adsorption intensity of the adsorbent	
OD_{620}	optical densities at 620 nm	
OD_{652}	optical densities at 652 nm	
q*	equilibrium concentration of the C-phycocyanin adsorbed onto the solid phase	
Q_m	maximum binding capacity of the resin	$mg.mL^{-1}$
K_{dFL}	Langmuir-Freundlich apparent dissociation constant	
n_{LF}	Langmuir-Freundlich coefficient	

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