The aim of this work was to determine multicomponent adsorption isotherms of the α-lactoalbumin (α-la) and β-lactoglobulin (β-lg) present in cheese whey by ion exchange, using the shake-flask method. The experiments were carried out in 2.0mL eppendorf tubes containing a strong anionic resin equilibrated with different concentrations of whey protein solutions. Non-competitive and competitive models were evaluated to predict the experimental data. The non-competitive isotherm of Langmuir fitted the experimental equilibrium data very well, despite its simplicity. The values for \( q_m \) and \( k_d \) were, respectively, 83.9mg/g and 0.57mg/mL for α-la and 252.1mg/g and 0.35mg/mL for β-lg. It was observed that α-la had a lower adsorption capacity and a higher dissociation constant, probably due to its lower concentration and greater hydrophobicity, compared to β-lg.
1. INTRODUCTION

Preparative and process-scale high-performance liquid chromatography (HPLC) has assumed an increasingly important role in the production of highly purified substances. Protein adsorption at solid-liquid interfaces has been reported since the beginning of the last century, when it was demonstrated that several inorganic powders adsorbed to horse serum in solution (HUANG; HORVÁTH, 1987). The biotechnological industry has been using ion exchange chromatography as one of the main techniques for protein concentration and purification in approximately 75% of preparative processes (CHANG; CHASE, 1996).

Since HPLC is a relatively expensive process and provides very high resolution, an adequate understanding of its dynamics is essential to develop project and equipment operations. Process modeling is a powerful tool in the development of the chromatographic process, helping the optimization of the HPLC steps. A key element to accurately model the chromatographic process is the data describing the equilibrium adsorption of each component in the system constituting the feed material and the mobile phase. To predict column performance, it is essential to measure or estimate the adsorption isotherms for the components under interest. Many studies have been published on pure protein adsorption on several adsorbents (SKIDMORE; CHASE, 1990; JAMES, 1994). However few studies have been reported using more realistic multicomponent systems (SKIDMORE; CHASE, 1990; LISEC et al., 2001), such as α-lactoalbumin (α-la) and β-lactoglobulin (β-lg), present in cheese whey.

Whey is a cheese-manufacturing by-product often used to produce whey protein concentrate powders for food applications. During recent years new processes for producing high quality whey products have been developed. The commonest methods used so far enable one to obtain products, such as whey powder and whey concentrates, which generally have a protein content of ca 10-15%. More sophisticated methods, such as ultrafiltration, coupled to adsorption on ion exchange beds, permit one to obtain protein mixtures, which are practically pure and in their native form. This permits the use of whey proteins to obtain high protein content products and products in which the proteins maintained their functional properties intact.

β-lg is the major whey protein in bovine milk (2-4) g/L, with a molar mass of 18.4 kDa and 162 amino acid residues. It is the major allergenic component of milk, particularly in infancy. A selective removal of β-lg could thus permit the use of the remaining proteins for the production of dietetic hypoallergenic food formulas of high additional value. α-la is an albumin consisting of 123 amino acid residues with a molar mass of 14.2 kDa. Its concentration in cheese whey is (0.6-1.7) g/L. This protein has a high nutritional value and functional properties that allow for its use in food formulations, substituting some other high cost ingredients (WIT, 1998).

In general, the measurement of adsorption isotherms is conducted by the shake-flask method, which is the most widely used technique. Although this technique is time-consuming and usually requires a relatively large amount of material, no special equipment is necessary. Therefore, our study aimed at measuring the adsorption isotherms of α-la and β-lg, adsorbed onto an anionic resin, by the shake-flask method. Two models based on non-competitive and competitive adsorptions were used to fit the experimental results.

Non-competitive adsorption model: This model assumes that the adsorption sites of both proteins are mutually independent, i.e., the adsorption of one type of protein onto the ion exchanger does not affect the adsorption of the other species present; therefore there is no competition among the proteins for the adsorption sites. If this is true, the adsorption characteristics of each protein will be the same as if the other proteins were not present, i.e.

\[
q^* = \frac{q_{mi} \cdot C^*_{i}}{k_{dij} + C^*_i}
\]  

where \( i \) is a specified component in a multi-component system, \( q^* \) is the adsorbed component by the adsorbing unit at equilibrium; \( q_{mi} \) is the maximum adsorbed component by the adsorbing unit at equilibrium; \( C^*_i \) is the equilibrium concentration of the component and \( k_{dij} \) is the dissociation constant.

Competitive adsorption model: Another approach to the analysis of multi-component adsorption is to assume that the adsorption of a component is affected by the presence of the others. The model, after some arrangement, can be expressed by equation 2. Although the Gibbs-Duhem equation is not satisfied by this model (GUIOCHON et al., 1994), it is usually used, due to its simplicity.

\[
q^*_i = \frac{q_{mi} \cdot C^*_{i}}{1 + \sum_{j=1}^{n} [k_{dij} \cdot C^*_j]}
\]

where \( n \) is the number of components.

2. EXPERIMENTAL

Whey protein isolate (WPI) containing α-la and β-lg was used throughout the experiments, buffered with Tris-HCl (laboratory-grade) 0.05 M, pH 7.6. The resin Accel Plus QMA, an anionic adsorbent with a quaternary ammonium group, was purchased from Waters.

Protein Analysis: The proteins α-la and β-lg were measured simultaneously by reverse phase high-performance liquid chromatography (RP-HPLC). All the solutions and samples were filtered through a 0.2μm cellulose acetate membrane (Duranpor). 20μL samples were applied to a reverse phase column (CLC-ODSM/C-18 – 25 cm - SHIMADZU - Japan), equilibrated with a 0.15 M sodium chloride solution, pH 2.5.
(solution A) and acetonitrile (solution B). The column was eluted with a gradient formed by solutions A and B, at a flow-rate of 1.0mL/min. The gradient was programmed as follows:

→ 100 % A, 0 % B to 64 % A, 36 % B in 3 minutes
→ 64 % A, 36 % B to 55 % A, 45 % B in 18 minutes
→ 55 % A, 45 % B to 55 % A, 45 % B in 2 minutes
→ 100 % A, 0 % B to 100 % A, 0 % B in 10 minutes

Each sample was analyzed in 33 minutes and detection was at 210nm with a diode array detector, the column being maintained at 40°C. Peak integration was performed by the SCL-10AVP chromatography controller unit (SHIMADZU - Japan). The concentrations of each protein were then determined from their peak areas by reference to the calibration data.

**Static equilibrium adsorption studies:** A number of experiments were conducted in 2mL eppendorf tubes using aliquots of Accel Plus QMA equilibrated with different mixtures of whey solutions containing α-la and β-lg. The tubes were prepared by weighing 50mg of adsorbent, washed and conditioned with Tris/HCL 0.05 M, pH 7.6. Aliquots of 500µL of protein solutions with different concentrations of α-la and β-lg were then added to each tube. The tubes were gently shaken for 3h at 25°C to allow for the establishment of equilibrium then centrifuged (7000g, 20min) and the supernatant assayed by HPLC. All the experiments were replicated. The quantity of each adsorbed protein was calculated by mass balance.

**Isotherm determination:** A non-linear regression was performed to adjust the non-competitive and competitive models to the equilibrium data obtained, using the successive quadratic programming algorithm. A computational program in FORTRAN language was developed for the model parameter computation.

### 3. RESULTS

Adsorption isotherm measurements are taken to determine the parameters $q_m$ and $k_d$ which characterize the systems studied in terms of maximum adsorbent capacity and the velocity with which equilibrium is achieved. According to DRAEGER; CHASE (1990), the Langmuir model gave a fairly accurate prediction of the protein adsorption results obtained by the shake-flask method.

Figures 1 and 2 show the results of the equilibrium adsorption experiments. Each point for a α-la pair with a corresponding β-lg point resulted from a single experiment. The experimental data were compared to the results predicted by the two models.

The competitive model (Figure 1) gave a fairly accurate prediction of the β-lg adsorption results, but the prediction for α-la was poor, underestimating the adsorbed protein. When the non-competitive model was used (Figure 2), both adsorbed proteins were predicted satisfactorily. SKIDMORE; CHASE (1990), studying the multicomponent adsorption of bovine serum albumin (BSA) and lysozyme, found different behavior where competitive adsorption was demonstrated. However, their work showed that neither of the tested models correctly predicted the adsorbed proteins.

According to HUANG; HORWÁTH (1987), the non-Langmuir behavior of multicomponent systems is probably the result of various phenomena, including molecular solute-solvent interactions, hydrophobic interactions and conformational changes. The results obtained in this work suggest that the phenomena mentioned above are not present or have a fairly limited influence on the system.

The results obtained for $q_m$ and $k_d$ were, respectively, 83.9mg/g and 0.57mg/mL for α-la and 252.1mg/g and 0.35mg/mL for β-lg. Dissociation constant ($k_d$) values below 1.0, as obtained for both proteins, show that the adsorption phenomenon was favorable and that equilibrium was achieved rapidly. The dissociation constant, $k_d$, is a measure of the
strength of the interaction between the protein and the ion exchanger. The results showed a smaller $k_d$ value for $\beta$-lg than for $\alpha$-la. This is probably due to the greater hydrophobicity of $\alpha$-la conferred by the presence of tryptophan, a non-polar amino acid. This fact, associated with a higher concentration of $\beta$-lg, should lead to a greater occupancy of the adsorption sites of the adsorbent, resulting in a greater $q_m$ value.

4. CONCLUSIONS

In this study, two models of multicomponent protein adsorption were considered. The competitive model gave a fairly accurate prediction while the non-competitive model fitted the equilibrium data for the proteins $\alpha$-la and $\beta$-lg present in a WPI solution very well. The results showed that the Accel Plus QMA resin has a high adsorption capacity for both proteins studied but that the adsorption capacity for $\beta$-lg was greater than for $\alpha$-la.

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