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Evaluation of a Commercially Available Ion Exchange Membrane for Separation of Proteins from a Whey Protein Mixture: Modeling and Experimental Results

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Abstract

Separation processes based on ion exchange principle either in chromatography or membrane-based systems have been demonstrated to be a potentially selective process for proteins. This method takes the advantages of electrostatic interaction between the charges of the protein molecules and the exchangeable charge groups on the solid/membrane supports. Efforts are being devoted to develop separations based on ion exchange membranes that combines this added advantage to simpler membrane diffusional processes resulting in faster recovery of bioproducts. In this article, a commercially available ion exchange membrane was evaluated to selectively separate major whey proteins, α-Lactalbumin (α-La), β-Lactoglobulin (β-Lg) and Bovine Serum Albumin (BSA). The experiments were performed with protein solutions using a membrane unit, Sartobind R Anion Exchanger-D75. The membrane showed good capacity for adsorption: 1.71 mg/cm^2 (for α-La), 4.3 mg/cm^2 (for β-Lg) and 0.43 mg/cm^2 (for BSA), respectively. The membrane showed high selectivity towards (α-La) compared to BSA and a pure protein fraction (97% w/w) can be obtained. The adsorption data is modeled with Langmuir isotherm and the parameters give good fit between the model predictions and experimental breakthrough curves. The above-mentioned results are useful considering the fact that these were obtained with a commercially available membrane unit in a single stage. This process when optimized conditions are determined and upgraded has the potential to selectively separate a desired component from a multicomponent mixture with other proteins.

Keywords: Ion Exchange Membrane; Protein Adsorption; Separation; Whey Proteins

Introduction

The opportunities for the commercial extraction of biomolecules from food, dairy and pharmaceutical waste streams have increased in last two decades. One example of a potential field for bio-product recovery is the extraction of proteins from milk because of its large content of whey. Whey is a liquid mixture (a co-product from cheese and casein manufacturing) [1] and important source of bioactive molecules (proteins) with characteristics desirable in many food and pharmaceutical products [2]. They are present at low concentrations as multi-components in their native mixture (milk) [3], therefore, separation and purification into individual protein would be more useful and would offer superior functionality, greater stability and biological activities [4]. Whey proteins (from bovine source) constitute about 18-20% of the total milk proteins with concentrations of 0.7-1.0 g/L. The major components of whey proteins are: α-lactalbumin (α-La), β-lactoglobulin (β-Lg) and bovine serum albumin (BSA) [2]. There are also minor components with high pharmaceutical values although they are present at very low concentrations. The main characteristics of the main proteins are listed in Table 1 [5-7]. The unique nutritional, therapeutic, and functional characteristics of the individual whey proteins are largely unrealized because in the mixed form (as in whey) they can undergo interactions between them and can be degraded during processing. Therefore, considerable efforts are being devoted to their separation and purification in the individual (purified) protein form or their rich fractions without much damage to their well-characterized functional and biological properties. Several techniques have been developed to enable the separation of individual whey proteins, such as precipitation [8,9], membrane-based

techniques [7,10-12], adsorption [13,14] and chromatography [6,15]. Precipitation is not considered cost-effective and it creates additional separation problems [16]. Membrane techniques based on ultrafiltration have disadvantages: are prone to denaturation of proteins, other components such as lactose is not fully removed, and difficult to obtain high purity due to the relatively similar physicochemical properties of the whey components. Adsorption gives moderate efficiency and has the drawback to create secondary separation problems [17]. Within last decades' chromatographic separation techniques have been applied because they can deliver high-purity products, relatively easy to develop, and can readily be scaled from the laboratory scale to the desired production level [18]. However, chromatography with packed-bed configuration could become limiting where high throughputs are required [19].

Separating proteins on the basis of their isoelectric points gives two distinct groups, namely the major whey proteins: β-Lg, BSA and α-La, which are negatively charged at the pH of rennet whey (pH 6.2 to 6.4); and minor whey proteins: lactoferrin and lactoperoxidase that carry a positive net charge at the pH of whey [14]. These distinct properties offer the possibility of selectively separating the groups using ion exchange membranes or particles. Separations based on ion exchange allow the electrostatic interactions between the surface charges on the membrane or particles and the charges on the specific protein under that conditions [20]. Adequate buffering may be required to protect the native multidimensional structure [16]. **Ion exchange** membranes has been examined extensively in the last decade as the most frequently used chromatographic technique for the separation and purification of proteins. The reasons for the success of ion exchange in this regard are its widespread applicability, high resolving power (high selectivity), high capacity, and the simplicity and controllability of the method [21]. In this work a commercially available ion-exchange membrane has been studied for their adsorption performance from single and binary component feed solutions. The aim was to demonstrate the capacity of the system for producing a purified protein or a fraction in one of the major proteins, α-La, β-Lg and BSA, from feed concentrations similar to native whey. The equilibrium behavior and the adsorption kinetics were determined by applying mathematical models on the experimental data of single and binary components.

Protein	Concentration mg/ml	Molecular weight g/mol	Isoelectric kDa	Functions point
β-Lg	2.4-4.1	18.3	5.2-5.4	Binding, synthesis, anti-cancer, antihypertensive
α-La	0.7-1.8	14.2	4.3-5.1	Absorption, apoptosis of
BSA	0.3	66	4.7-4.9	Transport of ligands
Immunoglobulins	0.3-0.6	50-1,000	5.8-7.3	Protection from infections
Lactoferrin	0.02-0.4	7.7-7.8	7.8-8.0	Anti-microbial
Lactoperoxide	0.02	7.8	9.2-9.9	Anti-microbial

Table 1: Physical characteristics of individual whey proteins.

Materials and Methods

Theoretical Background

Equilibrium Adsorption of Single-Component Systems: Langmuir isotherm in the following form was used to correlate the equilibrium adsorption of proteins [22]. It is expressed as:

$$q^* = \frac{q_m C^*}{C^* + K_d} \quad (1)$$

Where q^* is the adsorbed protein concentration on absorbent/membrane at equilibrium, q_m is the maximum protein binding capacity of the ion exchanger, C^* is the soluble protein concentration at equilibrium, and K_d is the dissociation or desorption constant for the protein-adsorption interaction. This is based on the adsorption of protein with the ion exchange support with the following assumptions: (a) It is completely reversible, and the chemical's interaction with the adsorption site causes no alteration in its solution properties or solution state, (b) The protein molecules bind to sites in a one-to-one fashion, and they bind only to sites ("Specific" binding between the molecules and the surface). (c) All binding site offer equal capacity and is defined by the equilibrium adsorption capacity [23].

Equilibrium Adsorption of Two-Component System: For mixtures of binary protein components two types of model were examined, the non-competitive Langmuir model and the totally competitive Langmuir model.

Non-competitive Langmuir model: This model assumes that the adsorption sites for the two proteins are mutually independent, that is the adsorption of one type of protein to the ion-exchanger in no way affects the adsorption of the other species and there is therefore no competition between the proteins for the adsorption sites. For this type, the adsorption characteristics of the protein in the binary mixture would be same as that in single-component systems [14,24]:

$$q_1^* = \frac{q_{m1}C_1^*}{C_1^* + K_{d1}} \text{ and } q_2^* = \frac{q_{m2}C_2^*}{C_2^* + K_{d2}} \quad (3)$$

Where the subscripts 1 and 2 represent the protein 1 and 2, respectively.

Totally competitive Langmuir model: This assumes that there is total competition between proteins for adsorption to the ion-exchanger. The model involves a fractional occupancy of the adsorption capacity for each protein species and uses Langmuir parameters derived from single-component systems. The adsorption equations at equilibrium are [14,24,25].

$$q_1^* = \frac{q_{m1}C_1^*}{C_1^* + K_{d1} + C_2^*\frac{K_{d1}}{K_{d2}}} \quad (4)$$

$$q_2^* = \frac{q_{m2}C_2^*}{C_2^* + K_{d2} + C_1^*\frac{K_{d2}}{K_{d1}}} \quad (5)$$

The above two equations are solved simultaneously with the mass balance equations:

$$VC_1 = VC_1^* + vq_1^* \quad (6)$$

$$VC_2 = VC_2^* + vq_2^* \quad (7)$$

Where v is the settled volume of the ion exchanger, V the volume of liquid external to the ion exchanger, and C_1 and C_2 are the initial concentrations of the two proteins. This set of equations (4-7), for a particular set of initial conditions V, v, C_1 and C_2 can be solved by using the values of K_{d1}, K_{d2}, q_{m1}, and q_{m2} determined in single-component adsorption isotherm measurements for values of C_1^*, C_2^*, q_1^*, and q_2^* [14].

Kinetic rate constant model: The model takes an empirical approach to the adsorption process and assumes that all the rate limiting processes can be represented by kinetic rate constants. In such an approach, the rate of mass transfer of protein to the adsorbent is assumed to be described by [26]:

$$\frac{dq}{dt} = k_1 C(q_m - q) - k_{-1}q \quad (10)$$

$$V\frac{dC}{dt} = -v\frac{dq}{dt} \quad (11)$$

Where k_1 and k_{-1} are the adsorption and desorption rate constants respectively. For batch uptake adsorption, the protein concentration in solution at time t could be obtained by solving equations (10) and (11) numerically as following [14]:

$$C = C_i - \frac{v}{V}\frac{(b+a)\left(1-\exp\left\{-\frac{2av}{V}k_1 t\right\}\right)}{\left(\frac{b+a}{b-a}\right)-\exp\left\{-\frac{2av}{V}k_1 t\right\}} \quad (12)$$

Where

$$a^2 = b^2 - \left(\frac{C_i V}{v}\right)q_m \quad (13)$$

$$b = \frac{1}{2}\left(\frac{C_i V}{v} + q_m + \frac{K_d V}{v}\right) \quad (14)$$

Equation (12) is the solution of the rate model predicted on the kinetic form of the Langmuir isotherm, from which the concentration-time profile for a given batch adsorption system can be calculated. When experimental conditions are specified (C_i, v, and V), Equation (12) can be fitted to the batch concentration-time data, in order to identify the three adjustable parameters (K_1, K_d, and q_m) which appear in a nonlinear fashion within the equation. A nonlinear regression method can be used to facilities this [14]. Polymath software 6.1was used to fit the experimental data within a nonlinear regression with specific adjustable parameters.

The proteins were purchased from various sources – Bovine Serum Albumin (BSA) from GIBCO, (α-Lg (90% pure) and α-La (85% pure) were purchased from Sigma Chemical Company, elution – sodium chloride was purchased from Reidel-de Haen, regeneration – sodium hydroxide was purchased from Fisher Scientific, storage solution – 99.8% ethanol was purchased from Reidel-de Haen and diluted to 20% in 1mol/l potassium chloride with trace bacteriostatic agents. The reagents used for buffer solutions (anhydrous sodium acetate and Tris-HCl) were purchased from Fluka. Figure 1 shows the schematic of the experimental set-up using the membrane.

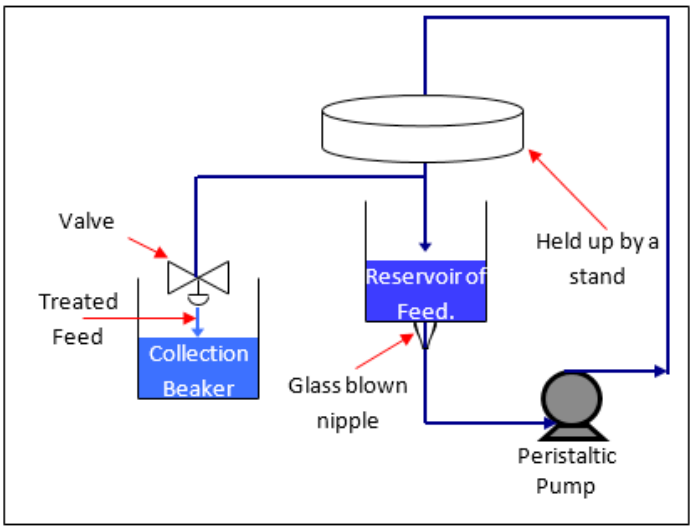

Figure 1: Diagram of the experiment set-up.

The proteins of interest were β-Lg, α-La and BSA. Therefore, to simulate a whey sample, these were in concentrations of 3.216mg/ml, 1.284mg/ml and 0.318 mg/ml respectively, as can be seen from Table 2. The solution containing the "target" protein was circulated using a peristaltic pump (Masterflex 7521-35) through the membrane at a flow rate of 15 ml/min from a reser-

voir with a solution volume of 100 ml. The samples were taken at regular intervals and the concentrations were measured by using a spectrophotometer (Lambda 35- UV/VIS spectrophometer, Perkin Elmer). For measurements of pH, a pH meter - Cyberscan Ion 510 was used.

	Concentration, mg/ml
Lactose	50
Beta Lactoglobulin (β-Lg)	3.216
Alpha Lactalbumin (α-La)	1.284
Protease-peptones	0.642
Immunoglobulin	0.5
Bovine Serum Albumin (BSA)	0.318
Ash	6
Non-Protein-Nitrogen	2
Fat	0.5
Sodium Chloride	trace
Water	1000

Table 2: Composition and concentration of a typical whey sample.

Results and Discussion

Single-Component Systems

The time-concentration profiles for the proteins (non-dimensional solution concentration) in the reservoir for all three in the pure form: α-La, β-Lg and BSA, are shown in Figure 2 (a–c), at their natural pHs. It shows that the faster rate of separation (i.e. faster adsorption on the ion-exchange membrane) from the aqueous solution occurs in the first 20-35 minutes. This is because the adsorption sites of the membrane are completely empty at the initial stage. As the sites get saturated with time the rate of depletion continues at a lower rate for an additional 30 minutes. Finally, in the last 20 minutes the concentration in the membrane levels off because the membrane has become saturated. This progressive reduction in the uptake of the protein is due to the decrease in the available sites and the available concentration difference between the solution and the adsorbed phase. The maximum capacity of this membrane is about 128.4 mg (q_m=33.8mg/ml of membrane) of α-La. In the case of β-Lg the time for faster uptake is smaller, i.e. the first 10-15 minutes, followed by similar progressive decline. After this time the uptake rate is lower with a smaller rate for an additional 20 minutes. Finally, after 40 minutes the maximum uptake is attained. Compared to the other protein (Figure 2a for α-La), the rate of depletion or the rate of adsorption is longer, i.e. about 30 minutes, and finally it attained a higher value, about 30% higher.

This could be explained by the fact that the size of β-Lg is similar to that of α-La, but the initial concentration of β-Lg is much higher and may allow faster diffusion to reach the site. Finally, the membrane absorbed 98% of the β-Lg protein with a saturation capacity of at least 322mg (q_m = 84.73 mg/ml). The concentration profile of BSA follows rather a constant rate of uptake until about 50 minutes. The adsorption capacity of BSA was lowest ca. 8.4 mg/ml of the membrane support. This could be because of the smallest concentration in the feed solution compared to the other proteins. α-La is considered as the most important protein as it is rarely found in high purity. Therefore, separation of α-La from other proteins such as BSA has been studied in the literature [1,2] and will be conducted as a binary feed using the same ion-exchange membrane.

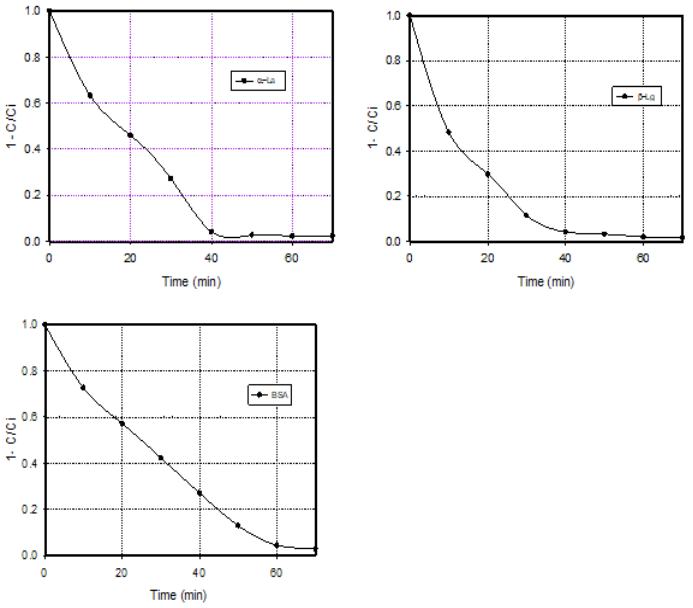

Figure 2: Single-component adsorbed protein concentration profiles for the adsorption of **(a)** α-La, **(b)** β-Lg and **(c)** BSA onto a 3.8-ml column of (Sartobind® Anion Exchanger Unit-D75) at pH 5 and at a flow rate of 15 ml/min. The feed concentrations were of 1.284 mg/ml (α-La), 3.22 mg/ml (β-Lg), and 0.32 mg/ml (BSA), respectively.

The performance of the membrane was tested for its robustness to any variation in protein concentration as happens in a dairy plant. The protein chosen was BSA and a small variation of its concentrations (0.26 – 0.4 mg/ml) was examined for the adsorption. The results of this change in initial protein concentration on the uptake is shown in Figure 3.

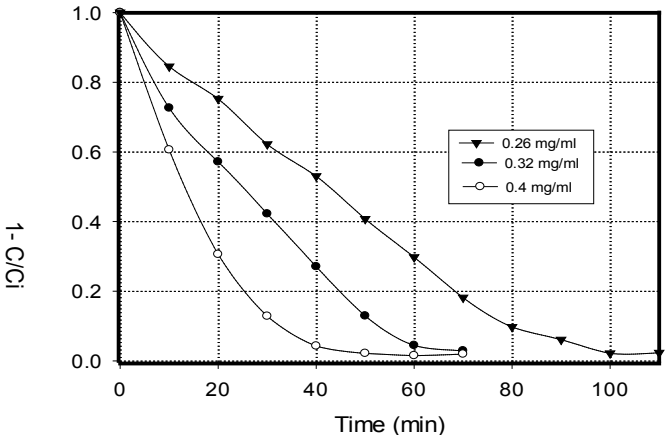

Figure 3: Concentration profiles for various concentration of BSA. Initial volume of protein solution: 100ml at concentration (0.26, 0.32 and 0.4 mg/ml) and at a flow rate of 15ml/min through the ion-exchange membrane.

These results imply that the membrane can handle this concentration change and a near complete recovery was achieved at different completion time. The concentration profiles changed with the variation of the initial BSA concentration. As the initial concentration increased the rate of depletion (i.e., the rate of recovery) increased, i.e. shorter time required for recovery from higher concentration. This could be explained by the fact the membrane has a capacity to adsorb more in this concentration range and at a higher available protein to each exchange site the protein absorption was faster. Conversely, a lower initial solution concentration results in a decrease in the rate of depletion (i.e. rate of recovery). Although the studied concentration range was not large (Table 3), the data was used to determine the equilibrium isotherm. Langmuir isotherm gave a good correlation (Figure 4) and the parameters were determined using least squares linear regression by taking the inverse of Equation (1), to be in the form of Equation (14). From intercept, the values of q_m and K_d were found to be 29.3 mg/ml and 0.0176 mg/ml, respectively. This suggests that the maximum capacity of the ion-exchange membrane was not attained because of the small feed concentration of BSA.

$$\frac{1}{q} = \left(\frac{K_d}{q_m} * \frac{1}{C}\right) + \frac{1}{q_m} \qquad (14)$$

C_i (mg/ml)	C_e (mg/ml)	$V_{S\ (ml)}$	$V_{M\ (ml)}$	q_e (mg/ml) = $V_S(C_i-C_e)/V_M$
0.4	0.0092	100	3.8	10.29
0.32	0.0061	100	3.8	8.26
0.26	0.005	100	3.8	6.71

Table 3: Values of the equilibrium isotherm parameters for BSA protein at different concentrations.

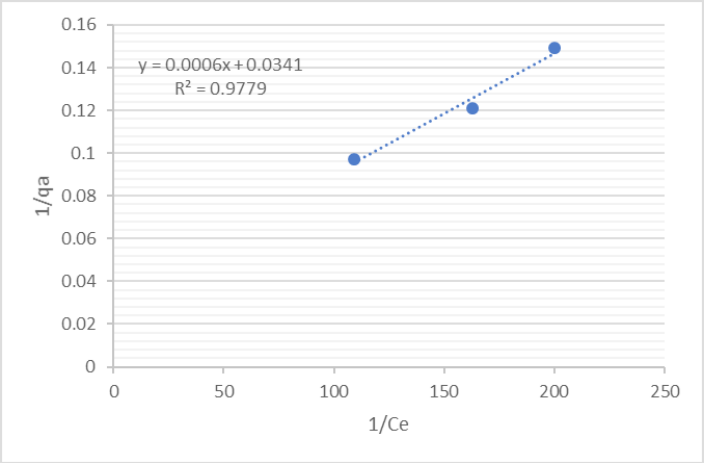

Figure 4: Least squares linear regression for BSA adsorption profile.

The second method uses Equation (12) by fitting the batch C_{-q} data, which appear in a nonlinear fashion within the equation. A nonlinear regression method can be used to compare the results. The results are shown in Figure 5. A nonlinear regression model was fitted with q_m=29.3 mg/ml and K_d=0.017 0.0176 mg/ml.

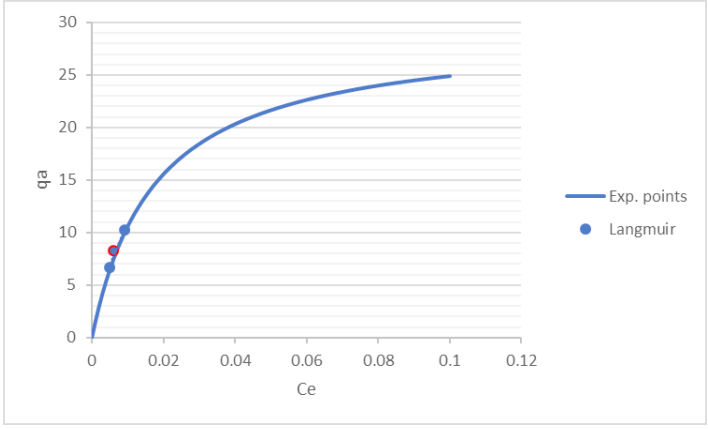

Figure 5: Equilibrium isotherm for BSA (▪ experimental values) and Langmuir type expression (solid line) with q_m=29.3 mg/ml[3] and K_d=0.0176.

By comparing these experimental data with other systems, Chu and Hashim [27] and El-Sayed and Chase [14], it was found that the full recovery of BSA was within 80 min, while in other system [27], the recovery of protein didn't reach 50% even after 170 min, as shown in Figure 6. By comparing the β-Lg concentration profile for the experimental data of Sayed system [14], as

shown in Figure 7, it was found that there is a difference in the half time. The experimental data [14] gave $t_{1/2}$ about 4 min, while $t_{1/2}$ for these data was about 10 min. Figure 8 shows the α-La concentration profile (the experimental data in this report) and when compared with El Syed system [14], a difference in the half time is observed. El Sayed system gave $t_{1/2}$ about 2 min , while $t_{1/2}$ for this experimental data is about 16 min. These differences could be due to membrane type, flow rate, initial concentration and V/ v ratio in addition to the difference in pH, where q_m will increase with decreasing pH as listed in Table 4, until it reaches the maximum binding capacity at pH=3.7. Tables 5,6 list the experimental conditions and results for this work (referred to as Collins data) along with the other systems.

Figure 6: Comparison of concentration profiles for BSA. (•) – Collins data and (0)- Chu & Hashim [27] data.

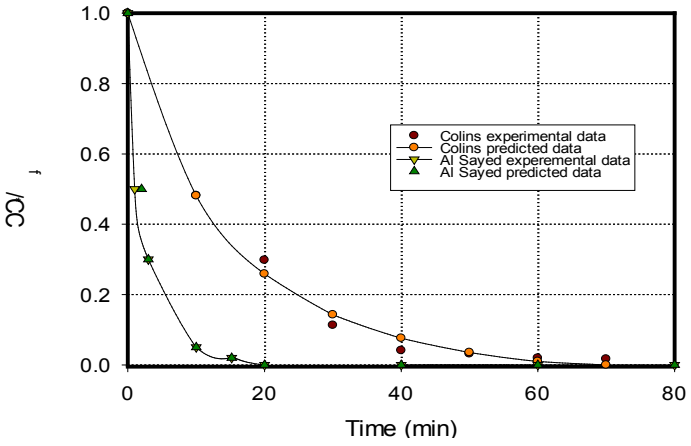

Figure 7: Comparison of concentration profiles for β–L_g, both the experimental and predicted data.

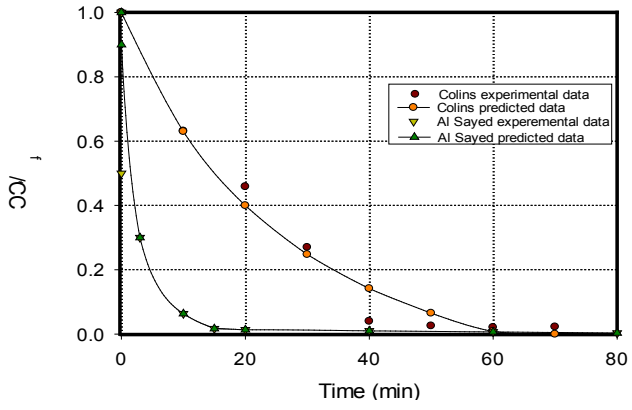

Figure 8: Comparison of concentration profiles for α-La, both the experimental and predicted data.

	This work	[27]
C_i, mg/ml	0.32	1.55
V, ml	100	50
V, ml	3.8	0.25
q_m, mg/ml	11.842	121.923
K_d, mg/ml	0.199	0.01311
Membrane type	Sartobind® Anion Exchanger Unit-D75	Fractogel EMD SO3 650
Flow rate, ml/min	15	-
Run time	70	167
pH	5	5

Table 4: Comparison of the experimental conditions with Chu & Hashim [27].

	This work	[14]
C_i(mg/ml)	3.22	3
V(ml)	100	14
v (ml)	3.8	1
q_m(mg/ml)	136.07	79
K_d(mg/ml)	0.00671	0.719
Membrane Type	Sartobind® Anion Exchanger Unit-D75	SP Espharose FF
Flow Rate(ml/min)	15	1
Run time(min)	70	80
PH	5	5

Table 5: Experimental conditions of El Sayed System (β-LG).

	This work	[14]
C_i(mg/ml)	1.284	1.5
V(ml)	100	14
v (ml)	3.8	1
q_m(mg/ml)	24.23	95.9
K_d(mg/ml)	0.10034	0.088
Membrane type	Sartobind® Anion Exchanger Unit-D75	SP Espharose FF
Flow rate(ml/min)	15	1
Run time(min)	70	80
pH	5	3.9

Table 6: Experimental conditions for α-La.

Protein Adsorption from A Binary Feed

In reality, a dairy plant will not be recovering a protein from a single component solution. It will be recovering a single protein from whey that contains many components. For the separation from real sample, the selectivity is important. The membrane needs to absorb the protein of interest and leave the other components in the solution. In this experiment the protein of interest is Alpha Lactalbumin, α-La. The flow rate used in the recovery process from the binary mixture was also 15ml/min and the results are shown in Figure 9. It can be observed that both curves display a similar profile as seen in their individual component tests. However, the membrane adsorbed 97% (125.6mg) of the α-La and only 31% (9.8mg) of BSA. Clearly the membrane has a higher selectivity for a-Lac over BSA. This is because α-La was present in a much higher concentration and is also smaller in size. Furthermore, after eluting with 40ml of 0.1mol/l of salt solution 123.5mg (98% of the absorbed α-La and 3mg (30% of the absorbed BSA) of BSA was desorbed from the membrane. If the eluted solution were dried it would contain salt (232mg), BSA (3mg) and a-Lac (123.5mg). This is a purity of 34% (weight basis) for α-La. However, if the salt is removed (possibly with reverse osmosis) a purity of 97% (weight basis) of α-La would be achieved. It must be kept in mind that drying and reverse osmosis on solutions containing protein is very difficult, as the protein needs to be kept at low temperatures.

Using equation. (12) the batch concentration-time data can be fitted, which appear in a nonlinear fashion within the equation. A nonlinear regression method was used to facilitate this. As can be seen from Figure 10, the α-La profiles are steeper than the BSA ones. This could be due to the fact that the BSA concentration is lower than that of α-La, and although likely to be bound less strongly to the adsorbent (as it has higher K_d than α-la), the path of α-La through the column should be more tortuous owing to its accessibility to finer pores as it is a smaller protein. By using equations (12), (13) and (14) and considering α-La as component 1 and BSA as component 2, the values calculated were: q_1^*=33.05mg/ml and q_2^*=2.581mg/ml for noncompetitive Langmuir model, while q_1^*=22.518 mg/ml and q_2^*=1.706mg/ml for competitive Langmuir model.

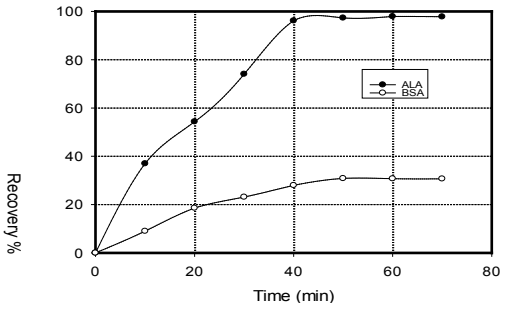

Figure 9: Recovery profile for two-component mixture of BSA and α-La. Initial protein solution volume of 100ml at a concentration of 0.32mg/ml and 1.284mg/ml, respectively and at a flow rate of 15ml/min.

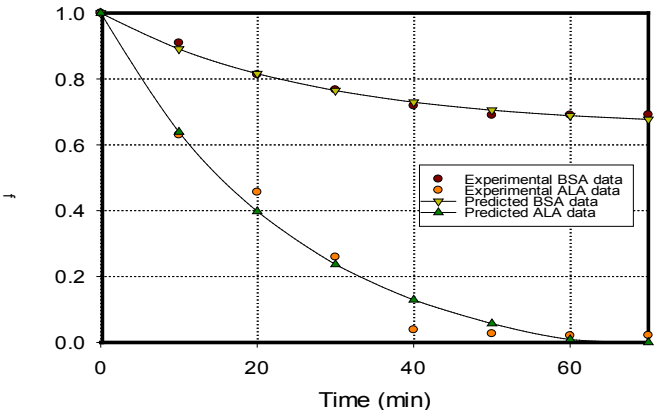

Figure 10: Predicted and experimental concentration profiles for two-component adsorption of BSA and α-La onto a 3.8-ml packed-bed of (Sartobind® Anion Exchanger Unit-D75) at pH 5, feed concentration of 0.32, 1.284 mg/ml respectively flow rate of 15 ml/min.

Conclusions

The adsorptive separation of proteins α-La, β-Lg and BSA was studied at pH 5 using an ion-exchange membrane unit (Sartobind® Anion Exchanger Unit-D75). The experiments were performed using pure α-La, β-Lg and BSA as single component solution, in a 3.8-ml column and at a linear velocity of 15ml/min. The recovery rate of α-La was 2 times higher than that of β-Lg, probably due to its lower concentration, as well as its smaller size (half that of β-Lg). The α-La profiles are steeper than the BSA ones, this could be due to the fact that the BSA concentration is lower than that of α-La although they are of similar size. The time required for full recovery of BSA was about 80 minutes, while in the literature the recovery of the same protein didn't reach 50% even after 170 minutes. This is a very good outcome of this ion-exchange system and this could be due to membrane type, flow rate, initial concentration and the ratio of feed to the adsorption system. Analysis of the two-component experiments of α-La and BSA (their pure binary mixture) suggest that it is possible to selectively adsorb and separate these two proteins. The percentage of recovery of α-La (the valuable of these two) can be as high as 98% compared to BSA. Hence this method with commercially available ion-exchange membrane can be considered as a potential alternative to recover proteins from a real whey mixture after further optimization of separation conditions.

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