Adsorption of Major Whey Proteins on Commercially Available Ion Exchange Membrane: Modeling and Experimental Results

Md M. Hossain, H. Collins, and A.F. Mohammed

Abstract—Ion exchange membrane adsorption was studied 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 commercially available membrane unit, Sartobind R Anion Exchanger-D75. The membrane showed good capacity for adsorption: 1.71 mg/cm² (for α-La), 4.3 mg/cm² (for β-Lg) and 0.43 mg/cm² (for BSA), respectively. The membrane showed high selectivity towards (α-La) compared to BSA and a pure product (97% w/w) was obtained after desorption and drying. The adsorption process parameters - the saturation capacity, and the dissociation constant, have been estimated by fitting the experimental data with a nonlinear regression method. These values give a good fit between the predicted and experimental breakthrough curves. The experimental data for a binary system of BSA and α-La was also obtained and the parameter values that best fit the data were also determined.

Keywords—Adsorption, ion exchange, membrane, protein.

I. INTRODUCTION

The opportunities for the commercial extraction of biomolecules from food, dairy and pharmaceutical waste streams are increasing. One example of a potential field for bioproduct recovery is the extraction of proteins from milk because large amounts of whey, approx. 150 million tons are annually produced. Whey is a liquid mixture (a co-product from cheese and casein manufacturing), an important source of bioactive molecules (proteins) with characteristics desirable in food and pharmaceutical products. They are present at low concentrations as multi-components in their native mixture (milk), therefore, separation and purification into individual protein would be more useful and would offer superior functionality, greater stability and biological activities. 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) [1]. There are also minor components with high pharmaceutical values although they are present at very low concentrations shown in Table I [2, 3, 4, 5].

Several techniques have been developed to enable the separation of individual whey proteins, such as precipitation [6, 7], membrane-based techniques [5, 8] adsorption [9, 10] and chromatography [2 - 4, 11, 12]. These methods are effective but have disadvantages, precipitation is not considered cost-effective, membrane techniques cause denaturation of proteins, and the adsorption process creates secondary problems. Chromatographic separation techniques have been applied because they can deliver high-purity products, relatively easy to develop, and can readily be scaled up. However, chromatography with packed-bed configuration could become limiting where high throughputs are required.

Ion exchange membranes have been examined extensively in the last decade as the most frequently used chromatographic technique for the separation and purification of proteins [11-14]. They provide high resolving power (high selectivity), high capacity, and simple operating procedure.

In this research a commercially available ion-exchange membrane has been studied for their adsorption performance from single and binary component feed solutions. This was to demonstrate the capacity of the system for producing a purified protein or a fraction with enrichment of one of the major proteins: α-lactalbumin (α-La), β-lactoglobulin (β-Lg) and Bovine Serum albumin (BSA), from feed similar to native

<table>
<thead>
<tr>
<th>Protein</th>
<th>Concentration (g/L)</th>
<th>Mol. Wt. (MW, kDa)</th>
<th>Isoelectric point (Iep)</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Lg</td>
<td>2.4-4.1</td>
<td>18.3</td>
<td>5.2 - 5.4</td>
<td>Food emulsions</td>
</tr>
<tr>
<td>α-La</td>
<td>0.7-1.8</td>
<td>14.2</td>
<td>4.2 - 4.5</td>
<td>Infant food</td>
</tr>
<tr>
<td>BSA</td>
<td>0.3</td>
<td>66.2</td>
<td>4.7 - 4.9</td>
<td>Therapeautic</td>
</tr>
<tr>
<td>Iglobulins</td>
<td>0.3-0.6</td>
<td>153- 163</td>
<td>5.5 - 7.3</td>
<td>Antioxidant</td>
</tr>
<tr>
<td>Lactoferrin</td>
<td>0.02-0.1</td>
<td>7.7-7.8</td>
<td>7.8-8.0</td>
<td>Anti-infective</td>
</tr>
<tr>
<td>Lactoperoxide</td>
<td>0.01-0.03</td>
<td>7.8</td>
<td>9.2-9.6</td>
<td>Anti-microbial</td>
</tr>
</tbody>
</table>

Md M. Hossain is with the United Arab Emirates University, Al Ain, P.O. Box 15551, UAE (corresponding author) phone: +971-3-7135315; fax: +971-3-7134996; e-mail: mmonwar@uaeu.ac.ae.

H. Collins, was an undergraduate student at the Department of Chemical & Materials Engineering, University of Auckland, Auckland City, New Zealand.

A. F. Mohammed is a postgraduate research assistant at the United Arab Emirates University, Al Ain, UAE, e-mail: A.Fares@uaeu.ac.ae.
whey. The equilibrium behavior and the adsorption kinetics were determined by applying models on the experimental data.

II. MATHEMATICAL MODELS

A. Single-component systems (Langmuir isotherm)

\[ q^* = \frac{q_m C^*}{C^* + K_d} \]  

(1)

where \( q^* \) is the adsorbed protein concentration 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.

B. Adsorption kinetics

1. Kinetic rate constant model

In this approach, the rate limiting processes are represented by kinetic rate constants, and the rate of mass transfer to the adsorbent is described by

\[ \frac{dq}{dt} = k_1 C (q_m - q) - k_1 q \]  

(2)

\[ V \frac{dC}{dt} = -v \frac{dq}{dt} \]  

(3)

Where \( C \) is the protein concentration, \( V \) and \( v \) are the volumes of the feed solution and the ion-exchange membranes, and \( k_1 \) and \( k_1^{-1} \) 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 (2) and (3) numerically. The alternative solution of these equations would be

\[ C = C_0 - \frac{v}{V} \left( b + a \right) \frac{1 - \exp\left\{ -\frac{2aV}{b} k_1^{-1} t \right\}}{b - a} - \exp\left\{ -\frac{2aV}{b} k_1^{-1} t \right\} \]  

(4)

Where

\[ a^2 = b^2 - \left( \frac{C_0 V}{v} \right) q_m \]  

(5)

\[ b = \frac{1}{2} \left( \frac{C_0 V}{v} + q_m + K_d V \right) \]  

(6)

III. MATERIALS AND METHODS

The proteins were purchased from various sources – Bovine Serum Albumin (BSA) from GIBCO, \( \beta \)-Lg (90% pure) and \( \alpha \)-La (85% pure) from Sigma Chemical Company (USA). For elution – sodium chloride (Reidel-de Haen), for regeneration – sodium hydroxide (Fisher Scientific) and for storage solution – 99.8% ethanol (Reidel-de Haen), diluted to 20% in 1mol/l potassium chloride with trace bacteriostatic agents were used.

R-eagents used for buffer solutions (anhydrous sodium acetate and Tris-HCl) were from Fluka. HCl acid, phosphate and citric acid were from BDH Chemicals, England.

A. Experiments with ion-exchange membranes

The intended application is to apply the method to separate the proteins: \( \beta \)-Lg, \( \alpha \)-La and BSA from a whey. Therefore, the concentrations of these proteins were similar to those in sweet whey: 3.216mg/ml (\( \beta \)-Lg), 1.284 mg/ml (\( \alpha \)-La) and 0.318 mg/ml (BSA), respectively. 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 reservoir volume of 100 ml (Fig. 1). The samples were taken at regular intervals and the concentrations were measured by using a spectrophotometer (Lambda 35-UV/VIS spectrophotometer, Perkin Elmer). For measurements of pH, a pH meter –Cyberscan Ion 510 was used.

IV. RESULTS AND DISCUSSION

A. Recovery profile for pure BSA, (\( \square \)Lg) and (\( \square \)La)

From the recovery-time data it is observed that recovery is faster for the first 30-40 minutes. This is expected because initially the adsorption sites of the membrane is completely empty. After this time the adsorption trend continues at a lower rate for an additional 30 minutes. Finally in the last 20 minutes the rate of recovery levels off. The rate of recovery progressively diminishes as the available sites are decreased as they are filled with the protein already absorbed and finally completely saturated with them. The maximum capacity of this membrane was approx. 0.32mg (\( q_m =32.8 \)mg/ml) for BSA, 322 mg (\( q_m =84.73 \)mg/ml) for \( \alpha \)-La and 128.4mg.(\( q_m =33.78 \)mg/ml) for \( \beta \)-Lg, respectively.

From recovery data the breakthrough curves for single-components of \( \alpha \)-La, \( \beta \)-Lg and BSA were determined and is shown in Fig. 2. The recovery rate for \( \beta \)-Lg is the fastest, followed by \( \alpha \)-La and BSA being the slowest. Eventually it was possible to recover 99% of these proteins.
The results obtained by fitting the batch concentration-time data with those of the model predictions using Polymath (a nonlinear regression program, 2006) are shown in Table II.

### Table II

VALUES OF THE EQUILIBRIUM ISOTHERM PARAMETERS

<table>
<thead>
<tr>
<th>Protein</th>
<th>a</th>
<th>b</th>
<th>qm(mg/ml)</th>
<th>kd(mg/ml)</th>
<th>k1(ml mg⁻¹min⁻¹)</th>
<th>R²</th>
<th>R² (qm mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Lg</td>
<td>25.6</td>
<td>110.4</td>
<td>136.07</td>
<td>0.0067</td>
<td>0.035</td>
<td>0.99</td>
<td>84.73</td>
</tr>
<tr>
<td>α-La</td>
<td>10.05</td>
<td>30.33</td>
<td>24.23</td>
<td>0.1003</td>
<td>0.027</td>
<td>0.98</td>
<td>33.78</td>
</tr>
<tr>
<td>BSA</td>
<td>3.34</td>
<td>10.02</td>
<td>10.58</td>
<td>0.0391</td>
<td>0.035</td>
<td>0.99</td>
<td>11.84</td>
</tr>
</tbody>
</table>

As evident from the coefficient of correlation the agreement between the experimental value and those calculated one is excellent. The concentration profiles attain the final adsorption value faster than those reported in the literature for ion-exchange resin adsorption [15].

It is observed that most full recovery of BSA within 80 min (our data). The adsorption data at various initial concentrations were plotted and the best fit line is shown in Fig. 3. It is shown that the data follows the predictions of Langmuir adsorption isotherm.

The results (both the experimental and the theoretical) obtained for β-lg are compared with those in the literature obtained on a similar system (Al Sayed, 2010). The experimental conditions of these systems are listed in Table III. The calculated results and those of the experiments are compared in Fig. 4.

### Table III

COMPARISON WITH THE LITERATURE DATA

<table>
<thead>
<tr>
<th></th>
<th>This work</th>
<th>Al Sayed (2010)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ci(mg/ml)</td>
<td>3.22</td>
<td>3</td>
</tr>
<tr>
<td>V(ml)</td>
<td>100</td>
<td>14</td>
</tr>
<tr>
<td>υ (ml)</td>
<td>3.8</td>
<td>1</td>
</tr>
<tr>
<td>qm(mg/ml)</td>
<td>136.07</td>
<td>79</td>
</tr>
<tr>
<td>Kd(mg/ml)</td>
<td>0.00671</td>
<td>.719</td>
</tr>
<tr>
<td>Membrane Type</td>
<td>Sartobind® Anion Exchanger Unit-D75</td>
<td>SP Espharo se FF</td>
</tr>
<tr>
<td>Membrane Type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flow Rate(ml/min)</td>
<td>15</td>
<td>1</td>
</tr>
<tr>
<td>Run time(min)</td>
<td>70</td>
<td>80</td>
</tr>
<tr>
<td>pH</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

It is observed that there is a difference between the concentration profiles. However, they attain the same values in about 60 mins showing the adsorption of all the proteins. The variation can be attributed to the differences in the membrane type, flow rate, initial concentration and V/υ ratio.

### B. A Binary mixture of BSA and α-Lactalbumin

The results of adsorption behavior of the feed containing BSA (0.32 mg/ml) and α-lactalbumin (1.24 mg/l) is shown in Fig. 5. It can be observed that the membrane absorbed 97% (125.6mg) of the α-La and only 31% (9.8mg) of BSA. Clearly the membrane has a higher selectivity for α-La over BSA. This could be the fact that α-La is present at a higher concentration (as in real whey solution) and is also it is smaller in size. Furthermore, after eluting with 40ml of 0.1mol/l of salt solution 123.5mg (98% of the absorbed) of α-La and 3mg (30% of the absorbed) of BSA was desorbed from the membrane. If the eluted solution were dried it would contain salt (232mg), BSA (3mg) and α-La (123.5mg). This is a purity of 34% (weight basis) for α-La. However, if the salt was removed (possible with reverse osmosis) a purity of 97% (weight basis) α-La would be achieved.
Using eq. (4-6) and fit the batch concentration-time data, which appear in a nonlinear fashion within the equation. A nonlinear regression method has been used to fit the data. The results are shown in Table IV.

### Table IV

<table>
<thead>
<tr>
<th>Parameter Values of the Proteins α-LA and β-Lg.</th>
<th>a</th>
<th>b</th>
<th>q (mg/ml)</th>
<th>k (1/ml·mg·min⁻¹)</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-LA</td>
<td>4.39</td>
<td>41.2</td>
<td>9.6</td>
<td>0.12</td>
<td>0.98</td>
</tr>
<tr>
<td>β-Lg</td>
<td>14.11</td>
<td>17.04</td>
<td>10.8</td>
<td>0.035</td>
<td>0.98</td>
</tr>
</tbody>
</table>

As can be seen from Figure 7, the α-La concentration attains the initial value with a faster rate than that of BSA, its outlet concentration of reached approx. 27% of the initial value. This could be due to (i) the α-LA concentration is about 4 times greater, (ii) the size of α-LA molecules smaller (faster diffusion) and (iii) stronger adsorption and less desorption as it shows lower values of higher Kd than BSA.

### V. Conclusions

The ion-exchange adsorption of the major proteins in milk: α-LA, β-LG and BSA was studied at their natural pH using ion-exchange membrane unit (Sartobind® Anion Exchanger Unit-D75). The remarks are:

- The α-LA profiles are steeper than the BSA ones. This could be due to the fact that the α-LA is smaller molecule and its concentration is greater than that of BSA, which is larger in size.
- The time required for full recovery of BSA was approx. 80 min, which is much less than the time reported (approx. 170 minutes) was reported in the literature to recover approximately 50% protein.
- The ion-exchange membrane is capable of recovering proteins from the binary mixture with high purity, which can be used to formulate concentrated protein fractions.

### Acknowledgement

This work was financially supported by the Emirates Foundation (Abu Dhabi, UAE) Grant No. #2010/088, in the Science and Engineering Program. The authors also acknowledge the support from the UAE University.

### References


