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Research Paper

Protein and Lactose Separation by Modified Ultrafiltration Membrane using Layer by Layer Technique

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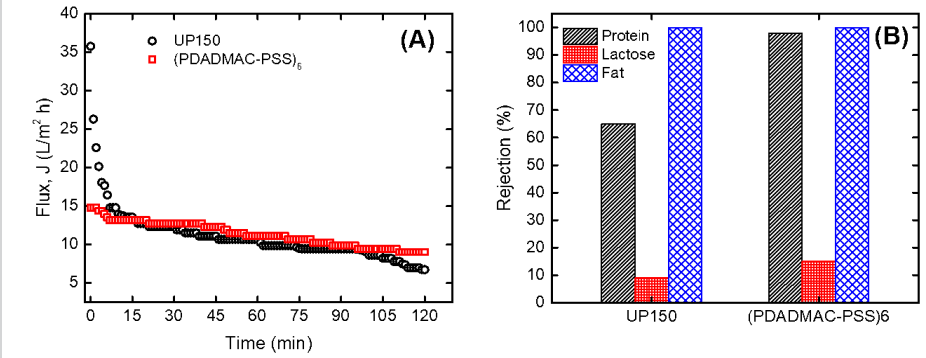
Highlights

- Layer-by-Layer (LbL) self-assembly was used for separation of protein and lactose.
- 10.7% lactose and 100% BSA retention was obtained for model solution with six bilayers.
- 100% fat, 98% protein, and 15% lactose retention was obtained for liquid whey.

Graphical abstract



Abstract

Layer-by-Layer (LbL) is a method which can be used for nanoscale coating and surface functionalization of a material. LbL technique mainly uses the electrostatic attracting between charged materials (polyelectrolytes, nanoparticles, etc.) and an oppositely charged surface. In this study, protein separation (BSA) from lactose solution was carried out using the LbL self-assembly method, which was used to produce a polyelectrolyte (PE) nanofiltration membrane. The impact of number of dual layers of PE and pH of solution on the retention of BSA and lactose was systematically investigated. For separation experiments, the BSA and lactose were used as a model protein and disaccharide sugars, respectively. Maximum retentions of 10.7% lactose and 100% BSA were achieved by the PE nanofiltration membrane with six bilayers at pH 6.5. Moreover, whey was used for the real filtration application, and the retention of fat, protein, and lactose were 100%, 98%, and 15%, respectively. The results showed that the separation of protein and lactose from the mixed solution could be achieved by PE nanofiltration membrane using the LbL method.

1. Introduction

Whey is an important by-product being generated in the dairy industry [1]. About 10 kg of milk can produce 8-9 kg of whey depending on raw material quality [2]. Whey contains several significant nutrients such as lactose, proteins, lipids, salts, and vitamins [3]. Therefore, it should be regarded as a valuable source for obtaining value-added products rather than a pollutant stream. Liquid whey is commonly known as the "pollutant for the environment" since it has high organic contaminants [4]. Therefore, the treatment of the liquid whey to meet the discharge standards is quite challenging by using the conventional physicochemical and biological treatment processes such as anaerobic treatment [5], membrane processes [6], ultrasound treatment [7], Fenton-like application [8], sequential electrochemical methodology [9].

Whey should be considered as a low-cost resource of lactose and protein, and it should be a feedstock for dairy, food, and pharmaceutical industries [10]. Whey protein mainly consists of α-lactalbumin (α-LA), β-lactoglobulin (β-LG), and bovine serum albumin (BSA) [11]. The other important nutrient is lactose, which can be transformed into glucose and galactose [12]. Since lactose and proteins are valuable products, recovering them instead of treating, can be beneficial for the industry while reducing environmental pollution due to the decrease of BOD and COD loading of the treatment plants [13].

The high concentration of lactose is a major problem for people with low lactose tolerance [14]. Thus, the separation of lactose along with the protein from whey is a crucial issue. Membrane processes and spray drying are

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commonly used technologies for whey protein separation [15,16]. Also, an aqueous two-phase extraction (ATPE) system to separate proteins can be used as an alternative method to avoid thermal procedures [17]. Membrane separation technology plays a vital role in processing milk/milk products and the separation/purification of value-added products [18]. Application of ultrafiltration (UF) membrane was tested for milk and whey protein concentration, and nanofiltration (NF) of the ultrafiltration permeate was used for lactose concentration [19]. The protein rejection of UF membranes raised to 92–98% with 30 L/(m^2h) (LMH) permeate flux at 3 bar pressure. Moreover, the lactose concentration was more than 25%, with 40 LMH permeate flux at 20 bar pressure. The results indicated that 0.1–0.3% lactose-containing NF permeate could be recycled, reused, or discharged into sewer [19]. A two-stage tangential flow filtration scheme using 100 and 30 kDa UF membranes in series was used for the purification of both α-LA and β-LG from whey protein isolate [20]. The results showed that the α-LA purification was higher than 10-fold with the 90% yield, but the recovery of β-LG was found difficult than *α*-LA.

The layer-by-layer (LbL) self-assembly has been confirmed as a plane and an advantageous method for building up very thin PE multilayer membranes. Sequential adsorption of PE (polycations or polyanions) is carried out on a charged substrate and weakly bounded polymer chains are removed from the substrate by a rinsing step after each adsorption procedure [21]. PE deposited membranes have been extensively used until now. For example, poly(styrene sulfonate)/poly(diallyl dimethyl ammonium chloride) deposited on porous alumina with 4.5 bilayers was used to recover phosphate from a feed solution including NaH_2PO_4 (1 mM) and NaCl (1 mM). The modified membrane provided 98% phosphate rejection at pH 8.4 [22]. Moreover, poly(styrene sulfonate)/poly(allylamine hydrochloride) NF membrane comprised of six to seven bilayers was used for the separation of neutral and zwitterionic amino acids [23]. Glycine was separated from the blend of glycine, l-alanine, l-serine, l-glutamine, and l-lysine [23]. In another study, 4.5 bilayers of poly(styrene sulfonate)/poly(allylamine hydrochloride) membrane showed 99.4% sucrose rejection with a high flux [24]. A sulfate rejection of 95% was achieved by membranes with 3.5 bilayers of poly(styrene sulfonate)/poly(diallyl dimethyl ammonium chloride) deposited on polyethersulphone support, whereas the selectivity of glucose/raffinose with the value of 100% was obtained with 4.5 bilayers of poly(styrene sulfonate)/poly(allylamine hydrochloride) on the same support [25].

The target of this work is to examine the optimization conditions of protein and lactose separation from both synthetic mixture solution and whey using polyelectrolyte NF membrane. Layer-by-layer (LbL) self-assembly was utilized to produce different pore sized membranes, and the UF150 membrane was chosen as a substrate. The one-step filtration for separating protein and lactose was carried out in this study.

2. Materials and methods

2.1. Chemicals and materials

The substrate, porous flat-sheet polyethersulfone (PES, 150 kDa) membrane, was provided by Microdyn-Nadir. Poly(diallyl dimethyl ammonium chloride) (PDADMAC, 20 wt%) and poly(sodium styrene sulfonate) (PSS, powder), purchased from Sigma-Aldrich, were used as polycation and polyanion, respectively. NaCl solution (0.2 M) was selected to liquefy polyelectrolytes and to wash modified membranes while performing the LbL self-assembly. Aqueous solutions of NaCl (Riedel-de Haën) and lactose (Thermo Scientific™ Oxoid™) were prepared with deionized water obtained by Milli-Q ultrapure water equipment (18.2 MΩ cm). Also, bovine serum albumin (BSA, Merck) was formulated with phosphate-buffered saline solution (pH: 7.4) and used as a feed solution of protein in the membrane experiments. All chemicals used in this study were analytical grade. The chemical structure of the polyelectrolytes and some chemicals are shown in Table 1. The properties of the UP150 kDa membrane are given in Table 2.

Table 1
Chemical structures of the polyelectrolytes and some chemicals (chemical structures of the polymers were obtained from Sigma-Aldrich website).

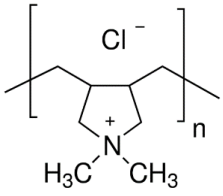
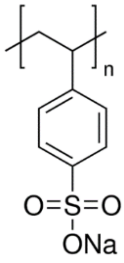
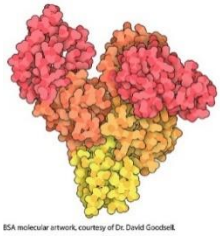
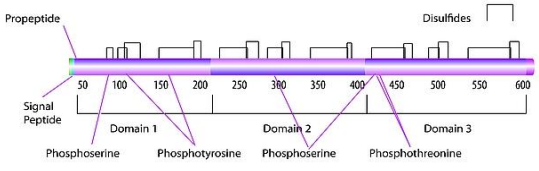
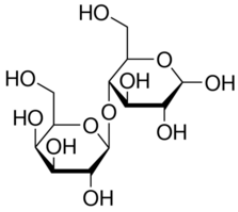
Chemical Name	Chemical Structure	Linear Formula	Average M_w (g/mol)
PDADMAC		$(C_8H_{16}ClN)_n$	200,000-350,000
PSS		$(C_8H_7NaO_3S)_n$	~70,000
Bovine Serum Albumin (BSA)	 		66 kDa
Lactose		$C_{12}H_{22}O_{11}$	342.3

Table 2
UP150 membrane properties.

Membrane	UP150
Material	Polyethersulfone (PES)
Molecular cut-off weight (kDa)	150
Pure water flux (L/m^2.h)	> 200*
pH	0-14
Maximum temperature (°C)	95
Company	Microdyn-Nadir

*Conditions: 0.7 bar, 20°C, stirred cell 700 rpm.

2.2. LbL self-assembly procedure for modification of PES membrane

A detailed explanation of the LbL assembly method, which was also used in this study, was reported by Dizge et al., 2018 [26]. The substrate, PES membrane, was treated with isopropanol solution (25%) for 30 minutes to remove impurity, and then the membrane was washed three times with deionized water. The polyelectrolytes were liquified in NaCl solution (0.2 M) and were mixed overnight to obtain a homogenous solution. Firstly, an aqueous solution of PDADMAC (cationic polyelectrolyte) was treated with the active surface of the UP150 substrate for 10 min. After that, excess cationic polyelectrolyte was removed from the substrate surface using a NaCl solution (0.2 M) for 5 min. Secondly, an aqueous solution of PSS (anionic polyelectrolyte) was treated with the PDADMAC-loaded PES substrate for 10 min. The same rinsing procedure was applied to remove excess anionic polyelectrolyte. After this stage, an initial electrostatically self-gathered bilayer (single-cationic and single-anionic layer) was formed. Multiple bilayers such as 2, 4 and 6 were produced by itareting the stages depicted above. PDADMAC-PSS modified membranes with 2, 4 and 6 bilayers were named as $(PDADMAC\text{-}PSS)_2$, $(PDADMAC\text{-}PSS)_4$, and $(PDADMAC\text{-}PSS)_6$, respectively. The modified membranes were then used for lactose and BSA separation.

2.3. Performance tests of modified membranes for lactose/BSA separation

Membrane filtration experiments were started after the formation of the desired layer by LbL method. Experiments with the dead-end membrane filtration system were first initiated by collecting pure water flux under pressure of 4, 5, 6 bar. The pure water flux was collected for all prepared membranes. After the pure water flux experiments, 100 mg/L lactose/BSA mixture solutions at three different pH's as 3.5, 4.7, and 6.5 were prepared and filtrated under 5 bars constant pressure. Protein and lactose analyzes were performed for the permeate. For lactose and BSA separation experiments, 250 mL of sample was added to the cell, and 80% recovery was obtained. In addition to the modified membranes, a pristine UF150 membrane was also used to compare the separation efficiencies.

The pure water flux was computed according to the Eq. 1 [27]:

$$J = \frac{\Delta V}{A \times \Delta t} \tag{1}$$

where J is permeate flux (L/m^2 h), V is the volume of permeate (L), A is the efficacious membrane area (m^2), t is the penetrating time for deionized water (h).

2.4. Analysis of the samples and membranes

The pH of samples was measured with a multi pH-meter (WTW 3110). Lowry's method was used to estimate the amount of protein in the given sample at the wavelength of 660 nm (GBC, Cintra-20 spectrophotometer) [28], and BSA was utilized for standard. Lactose was analyzed using high-pressure liquid chromatography with Waters IC-Pak™ Ion Exclusion Columns (7.8 × 300 mm, 7µm). Analyses were performed at 0.6 mL/min flow rate and at 50°C for 25 min. H_2SO_4 (5 mM) was preferred as the mobile phase [29]. The content of fat in the liquid whey was measured according to the Gerber method [30]. Duplicate experiments were performed for all membrane filtration. The separation efficiency of lactose and BSA were calculated using the following Eq. 2.

$$Separation\quad efficiency\quad (\%) = \frac{C_i - C_f}{C_i} \times 100 \tag{2}$$

In this Equation, C_i (mg/L) is the lactose and BSA concentration at t=O, and C_f (mg/L) is the lactose and BSA concentration at the end of the time.

SEM cross-sectional images of the pristine and LbL self-assembly membranes were investigated with a scanning electron microscope (Zeiss Supra 55, Germany) in high vacuum mode at 5.00 kV after coating with platinum-palladium sing Ion SputterCoater Quorum Q150R ES to observe polyelectrolyte layer.

In order to estimate the membrane porosity, the gravimetric method was used [31]. The total porosity was calculated using Eq. 3.

$$\varepsilon = \frac{\frac{m_{IPA}}{\rho_{IPA}}}{\frac{m_{IPA}}{\rho_{IPA}} + \frac{m_{polymer}}{\rho_{polymer}}} \tag{3}$$

where m and ρ present mass and density, respectively. IPA stands for 2-propanol, and polymer shows the pristine and modified membranes.

The membrane average pore size was derived according to the Guerout-Elford-Ferry Eq.4.

$$r_m = \sqrt{\frac{(2.9 - 1.75\varepsilon) \times 8 \times \eta \times \mathrm{I} \times Q}{\varepsilon \times A \times \Delta P}} \tag{4}$$

where ε is the total porosity, Q is the volume of deionized water (permeate) expressed in m^3/s, η is the viscosity of deionized water at 25°C (8.9×10^{-4} Pa·s), and ΔP is the operating pressure which is typically one bar.

3. Results and discussion

3.1. Morphologic characteristics of the membranes

The cross-section SEM analyses were conducted to show polyelectrolyte layers on the membrane surface of the pristine and LbL self-assembly modified membranes (Figure 1). The pristine membrane had a dense skin layer compared to modified membranes. Images showed that polyelectrolytes were deposited successfully onto the pristine membrane surface and the thickness of the polyelectrolytes layer raised with increasing of bilayer number. The polyelectrolyte layer of $(PDADMAC\text{-}PSS)_6$ membrane was thicker than $(PDADMAC\text{-}PSS)_2$ and $(PDADMAC\text{-}PSS)_4$ membranes.

The porosity and mean pore size of the pristine and modified membranes are demonstrated in Table 3. It can be clearly observed that the porosity and the modified membranes pore size changed after LbL self-assembly. Membrane porosity increased from 50.5% to 65.4%. However, the average pore size decreased from 86 nm to 17 nm after 6-bilayers.

3.1. Effect of bilayers number on pure water permeability of the membranes

The type of the modified membranes could be predicted with the permeability coefficient. L_p (also called filtration coefficient) can be described as the ability of a porous environment to permeate a solution when a pressure gradient is applied throughout the membrane as a function of dynamic viscosity [32]. Membrane with L_p< 50 L/m^{2}hbar is defined as hyperfiltration, L_p = 50-500 L/m^{2}hbar is clarified as ultrafiltration, and L_p> 500 L/m^{2}hbar is classified in microfiltration membrane according to [33]. The pure water permeability using different LbL modified membranes are depicted in Figure 2A. Calculation of L_p was performed from the slope of graphed of pure water flux versus trans-membrane pressure. It can be clearly observed in Figure 2A that L_p values decreased from 218.6 to 7.3 L/m^{2}hbar when the number of dual layers on the membrane surface raised from 2 to 6. For ΔP=4, 5, 6 bar, the L_p values of pristine PES, $(PDADMAC\text{-}PSS)_2$, $(PDADMAC\text{-}PSS)_4$, and $(PDADMAC\text{-}PSS)_6$ was calculated 493.2, 218.6, 37.9, and 7.3 L/m^{2}hbar, respectively. The higher L_p value indicates that water can easily pass through the membrane. Consequently, $(PDADMAC\text{-}PSS)_2$ can be classified in UF membrane because of considerable flux (L_p = 50-500 L/m^{2}hbar). However, $(PDADMAC\text{-}PSS)_4$ and $(PDADMAC\text{-}PSS)_6$ can be classified in hyperfiltration membrane due to deposition of the polyelectrolytes as a thin film layer on the membrane interface. The thin film layer caused both decreasing the pore size of membrane and increasing the membrane thickness, resulting in lower water flux (L_p< 50 L/m^{2}hbar). As a result, it was prepared in a range of ultrafiltration and hyperfiltration modified membranes.

The membranes were compacted for 1 h before filtration. Pure water flux (PWF) curves versus time are depicted in Figure 2B. PWF was remarkably decreased after LbL self-assembly which can be due to the formation of narrower pore size and thicker skin layer. Furthermore, except (PDADMAC-PSS)$_6$, the slight decrement in flux over time value was caused by the densification of the porous skin layer during compaction by the application of pressure [34]. PWF of (PDADMAC-PSS)$_6$ membrane was considerably decreased (i.e. 9 times the flux of UP150).

3. 2. Impact of solution pH on lactose and protein separation

The impact of solution pH on lactose and protein separation was also investigated using LbL self-assembly. The performance characteristics of the lactose and BSA separation at different pH were compared by assessing the rejection of BSA in the concentrate side and permeation of lactose in the permeate side. Filtration was performed at a fixed pressure of 5 bars. Mix BSA-lactose solution (100 mg/L) was filtrated at different pH (3.5-4.7-6.5) to investigate the impact of pH on membrane separation (Figure 3). Mix solution was filtrated through pristine UP150, (PDADMAC-PSS)$_2$, (PDADMAC-PSS)$_4$, (PDADMAC-PSS)$_6$ membranes. For the 6-bilayer membrane, it was observed that the solution with pH 6.5 decreased the flux decline. However, the pristine UP150 membrane showed serious flux decline. The initial and steady-state flux was the lowest at pH 3.5 for all membranes. However, protein-membrane surface interaction was lower at higher pH (6.5) due to high flux. The reason might be explained by the shifting of the isoelectric point of BSA to the negative side when pH was increased to 6.5. Moreover, electrostatic repulsion force could be decreased by the interaction between negative surface charged of BSA above pH 4.7 and membranes having anionic surface charge due to anionic PSS post layer. Thus, the membrane was protected against protein contamination due to strong anionic surface charge. BSA molecules can stay in the concentrate stream due to strong electrostatic repulsion force and can prevent membrane fouling. Increasing the number of layers applied to the UF membrane decreased the pore size of the membrane (Table 3). The flux of the pristine UP150 membrane declined quite rapidly at the start of the filtration for all pH range compared to modified membranes. This flux decline could be explained by the sieving behaviour and protein adsorption on the UP150 membrane [35]. Although the initial and steady-state values of the flux were very similar for pH 6.5 and 4.7, for pH 3.5, it was very distinct. The study conducted by Mantel et al. showed that UP150 membrane had a negative membrane charge between pH 3 and 9 [36]. The charge of BSA molecules shifted to the positive side at pH 3.5 and adsorbed easily on the negatively charged membrane surface. Higher flux declines were seen at lower pH under such surface charge conditions. The results presented here are similar to previous findings described by Zhu et al. [37]. It was explained that the positive charged BSA and lysozyme adsorbed easily on the negatively charged membrane interface caused by the electrostatic attraction.

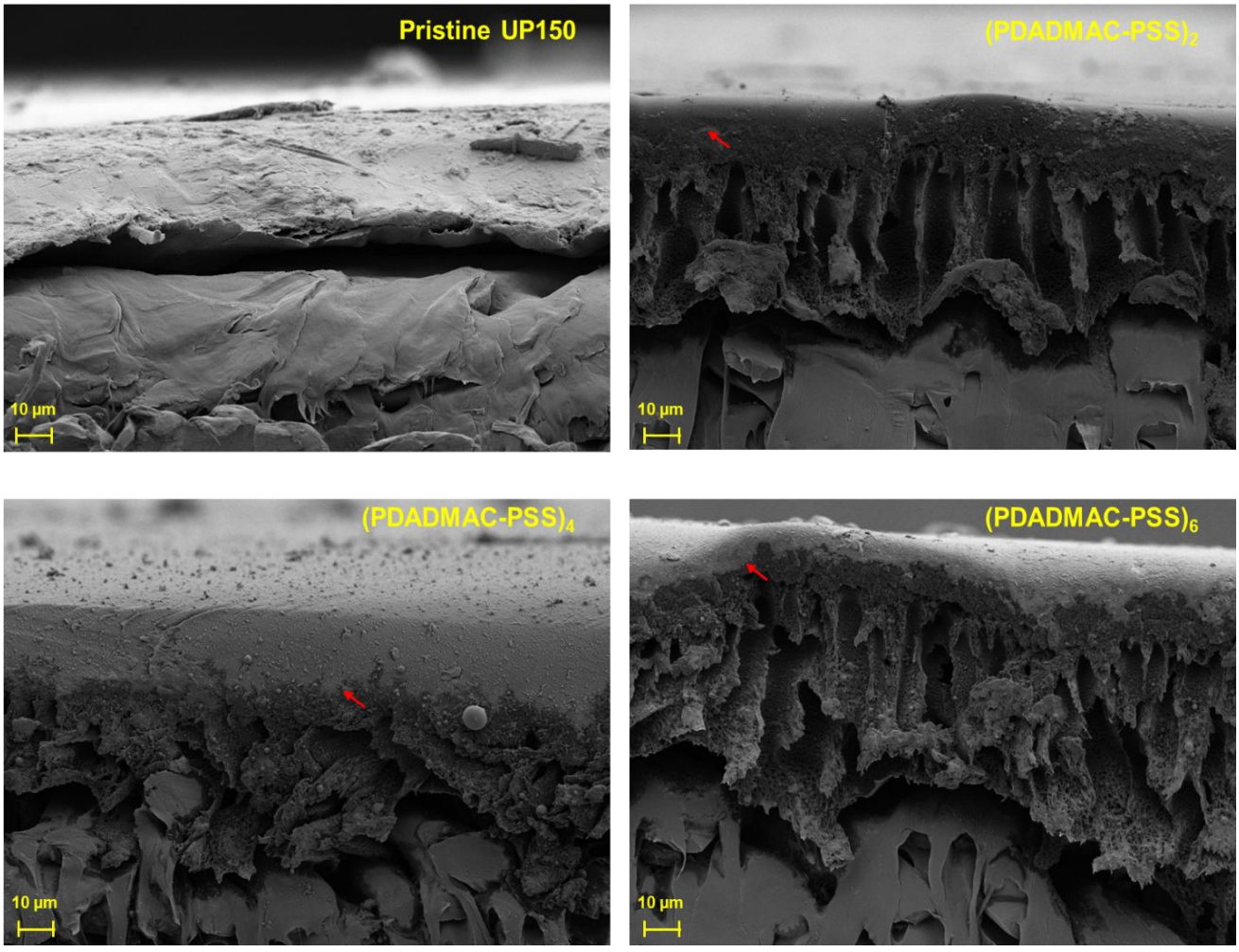


Fig. 1. Cross-sectional SEM images of pristine UP150, (PDADMAC-PSS)$_2$, (PDADMAC-PSS)$_4$, and (PDADMAC-PSS)$_6$ membrane (red arrows indicate polyelectrolyte layer).

Table 3
Total membrane porosity and average pore size of pristine and modified membranes.

Membrane Type	Total Porosity (%)	Average Pore Size (nm)
Pristine UP 150	50.5	86
$(PDADMAC-PSS)_2$	55.2	42
$(PDADMAC-PSS)_4$	60.3	29
$(PDADMAC-PSS)_6$	65.4	17

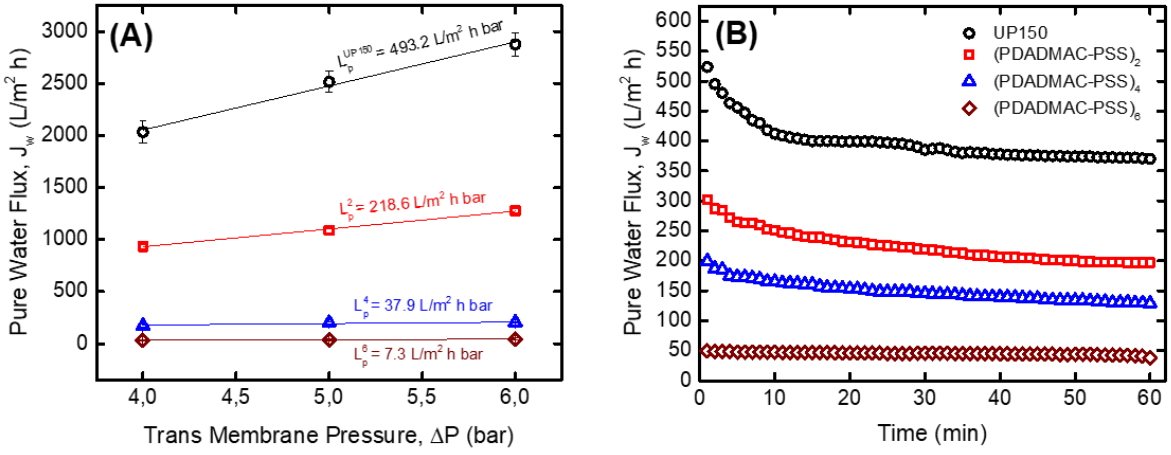


Fig. 2. (A) Pure water permeability coefficient ((L_p) determination and **(B)** Pure water flux for pristine UP150, $(PDADMAC-PSS)_2$, $(PDADMAC-PSS)_4$, $(PDADMAC-PSS)_6$ membranes at 5 bar constant pressure filtration.

Protein and lactose rejection by pristine UP150 and modified membranes were executed, and the results are given in Table 4. Pristine UP150 membrane rejected 10% of protein, and it allowed all of the lactose to pass for the mix solution. The pore size of the pristine UF membrane (150 kDa) was not large enough to reject BSA (66 kDa) and lactose (342 g/mol). A small portion of BSA rejection might be due to protein adsorption of the polymer chains. BSA rejection expanded when the number of bilayers was raised from 2 to 6, whereas BSA was completely rejected by all of the LbL self-assembly modified membranes. However, only 10.7% lactose rejection was measured due to the narrower pore size. It means that 89.3% lactose was permeated from the mix solution to permeate side.

Table 4
Protein and lactose rejections by pristine UP150 and LbL self-assembly modified membranes (BSA concentration: 100 mg/L; lactose concentration: 100 mg/L; pH: 6.5; ΔP: 5 bar; filtration time: 120 min)

Membrane Type	BSA Rejection (%)	Lactose Rejection (%)
Pristine UP 150	10	0
$(PDADMAC-PSS)_2$	100	0
$(PDADMAC-PSS)_4$	100	4.5
$(PDADMAC-PSS)_6$	100	10.7

3. 3. Lactose and protein separation from whey

The separation of the protein and lactose from whey at the industrial level is done conventionally by the membrane separation techniques. Membrane separation is an excellent technique for separating low-concentration molecules from a large amount of whey solution. However, membrane fouling and failure to achieve high separation efficiency are the major problems that need to be solved for membrane processes. Ultrafiltration resultant product liquid whey protein concentrates showed high lipid and lactose content [38]. It was aimed to separate lactose and whey protein in one step using LbL self-assembly modified membranes in this study. The experiments were carried out using $(PDADMAC-PSS)_6$ membrane at constant operating conditions concerning the temperature, solution pH, and pressure. Filtration experiments using whey were carried out for pristine UP150 and $(PDADMAC-PSS)_6$ membranes for 120 min (Figure 4A). The permeate flux decreased rapidly with time for pristine UP150 membrane as a result of the high concentration of the protein and fat. The initial flux decreased from 35.7 to 6.7 LMH for pristine UP150 membrane. However, flux declined from 14.8 to 9.0 LMH for $(PDADMAC-PSS)_6$ membrane. At the beginning of the whey filtration, the flux was higher for pristine UP150 membrane, but within 15 minutes, a layer of gel which decreased the flux was composed on the interface of the membrane. However, strong negative surface charge and narrower pore size of $(PDADMAC-PSS)_6$ membrane provided protection against the gel layer. Figure 4B presents the variation of compositional characteristics between the pristine UP150 and modified membrane permeate. Pristine UP150 and modified membrane completely removed the fat. The protein holding of the membrane was 65% and 98% for pristine UP150 and modified membrane, respectively. The pristine UP150 and modified membranes retained about 9% and 15% of the lactose caused by the gel polarization layer [39]. Conclusively, the separation of lactose and proteins was not effective for pristine UP150 but preferable for $(PDADMAC-PSS)_6$ membrane.

Limsawat and Pruksasri investigated the separation of lactose from milk by ultrafiltration with MWCO of 5 kDa [40]. The impact of some important variables such as pressure and feed flow rate was investigated on lactose and protein rejection. The results showed that the lactose and protein rejection values were approximately 13% and 100%, respectively. A high removal degree of lactose from milk was achieved and was suggested to obtain low lactose milk and milk products [40]. Treatment of whey using the ultrafiltration HFK-131 polyamide membrane was examined to recover and reutilize the proteins. The protein content enhanced 98%, and the permeate

flux was 80 L/m^{2}h at 1.5 bar [41]. Muller et al. focused on concentrating α-LA from acidic whey by the tubular ceramic membranes (150–300 kDa) [42]. Erdem et al. prepared ceramic composite membranes with a pore size of 35 nm. The developed membrane has perfect separation properties for protein and lactose at high protein content (PR ~80%) and with the relatively low lactose retention (LR ~7%) [43]. The UF process for the recovery of component from whey was evaluated by Iltchenco et al. [44]. The concentrated whey obtained by UF resulted in the protein recovery of 55% and 80% for 100 and 10 kDa membranes, respectively.

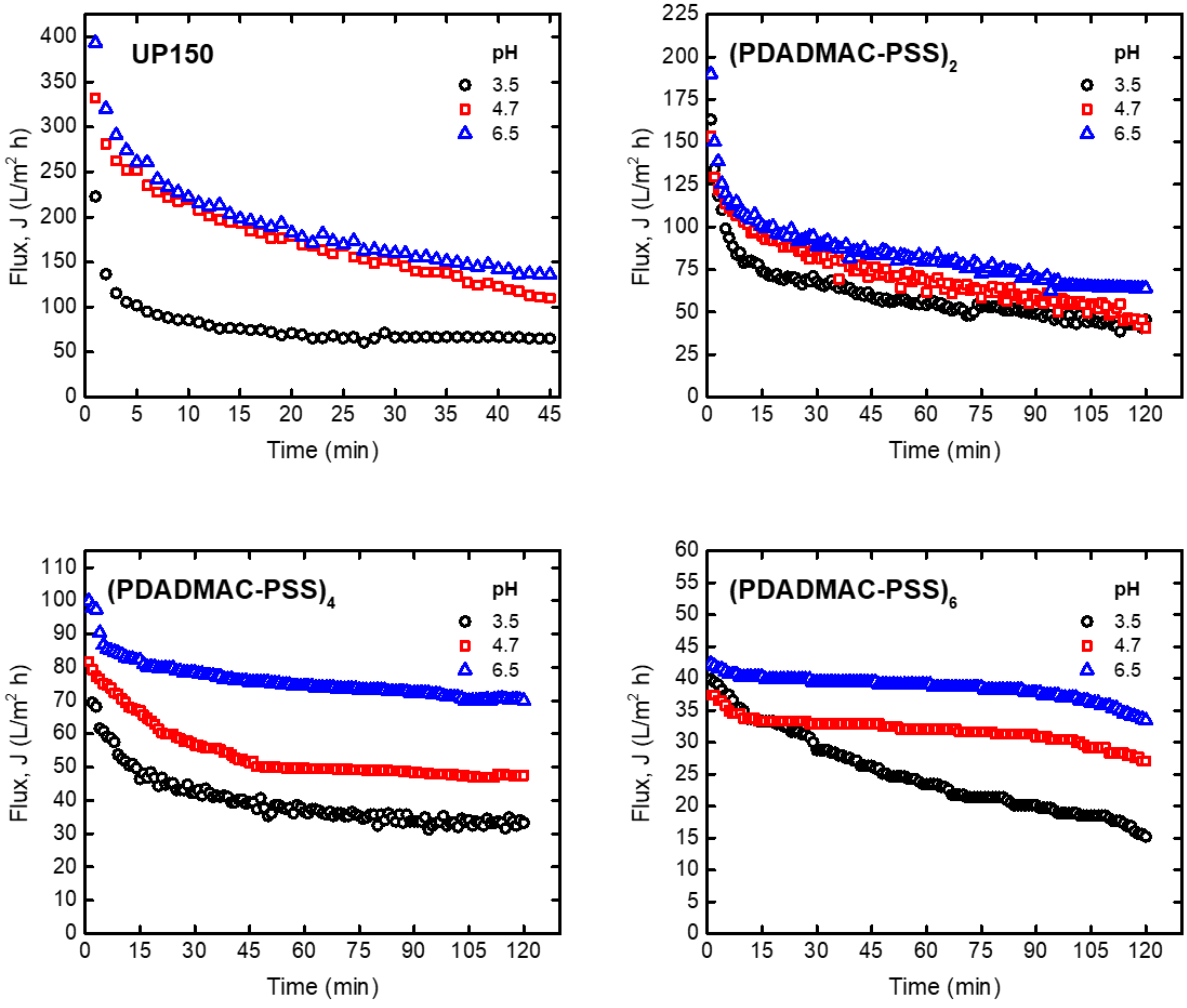


Fig. 3. Protein and lactose filtration flux with pristine UP 150 and LbL self-assembly modified membranes (BSA concentration: 100 mg/L; lactose concentration: 100 mg/L; ΔP: 5 bar).

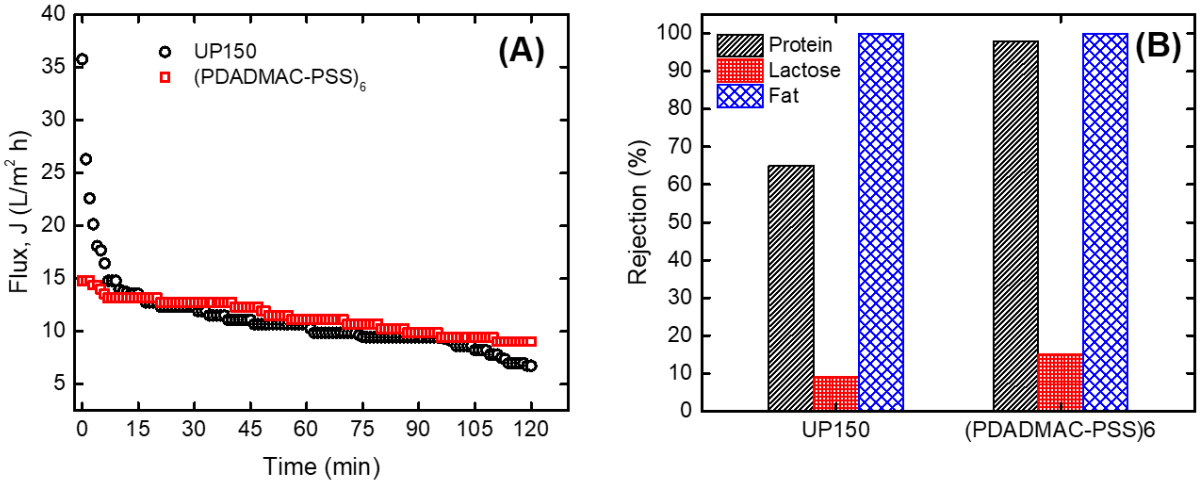


Fig. 4. (A) Whey filtration flux and **(B)** Retention values of the pristine UP150 and (PDADMAC-PSS)$_6$ membranes (Protein concentration: 10.9 g/L; lactose concentration: 55 g/L; fat concentration: 1.1 g/L; solution pH: 5.0; ΔP: 5 bar).

4. Conclusions

The polyelectrolyte modified membrane was developed by layer by layer self-assembly technique to separate protein and lactose from whey. Recovery of lactose-free whey by UF is of great interest to the industry, being an alternative of adding value to the sub-product of the dairy industry. A fat, protein, lactose retention of 100%, 98%, 15% were obtained using PDADMAC-PSS membrane with 6-bilayers. The steady-state permeate flux was close to that of commercial UP150 membrane (9 LMH for TMP at 5 bar). The results of this work showed the possible development of LbL self-assembly membrane for separation of whey components with higher yields. The permeate of $(PDADMAC\text{-}PSS)_6$ membrane can be concentrated by NF membrane and can be combined with crystallization to obtain lactose crystals.

Acknowledgment

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