The Effect of Electrostatic Properties in Binary Protein Ultrafiltration

Yiheng Wang, Victor G. J. Rodgers
Department of Chemical and Biochemical Engineering
The University of Iowa
4133 Seamens Center
Iowa City, IA 52242

Introduction

During ultrafiltration, electrostatic properties of the membrane systems have significant effects on species partitioning, and hence on separation performances. This work investigated the effect of electrostatic properties in binary protein ultrafiltration systems. In this work, bovine serum albumin (BSA) was separated from either hen egg lysozyme (HEL), cytochrome-c (Cyt-C) or α-lactalbumin (α-LA), using 30,000-MWCO polyestersulfone (PES, negatively charged) and composite regenerated cellulose (CRC, relatively neutral charged) membranes. Table 1 is a brief summary of the properties of the proteins used in this study. HEL and Cyt-C were selected because of their similarity in size and electrostatic properties while α-LA was selected because of its significant difference in isoelectric point (pI). All proteins were obtained from Sigma Company (St. Louis, MO) and the membranes were obtained from Millipore Company (Bedford, MA). HEL, Cyt-C and α-LA will be referred to as the 15 kDa protein below. Experiments were carried out at three different pH values, pH 4.7, pH 7 and pH 10, in order to study the effects of system pH in ultrafiltrations. Table 1 is a brief summary of the properties of the proteins used in this study.

Table 1. The properties of the proteins

Proteins	MW (kD)	pI
Bovine serum albumin (abbr. BSA)	67	4.7
Hen egg lysozyme (abbr. HEL)	14	11.0
Bovine α-lactalbumin (abbr. α-LA)	15	5.2
Cytochrome-c (abbr. Cyt-C)	15	10.5

Experiment method

The protein concentration of the binary protein mixture solutions were 0.05%(15 kDa protein)/0.05%(BSA). All solutions were made by dissolving measured mass of protein powder into 0.15 M NaCl buffer solution. Sodium azide at a total concentration of 0.02% was also added to protein solution as a preservative. The pH value of the solution was adjusted by HCl or NaOH solution. The solution pH was tested using a pH meter (Model 720A plus, Thermo Orion, Beverly, MA). After preparation, all binary protein solutions were pre-filtered by 0.22 micron membrane to eliminate large aggregates. Before use, membranes were cut from membrane sheet stock, and soaked in deionized water for 1 hour. All experiments were performed in a tangential-flow ultrafiltration system.

Experiments were run in diafiltration mode, with an operating pressure for all experiments of 96 kPa. During experiment, first the initial hydraulic permeability was tested by running corresponding protein-free buffer solution through the membrane. Then the protein were placed into the 500 mL tank and separation experiments were run at cross-flow flow rates 10 ml/min. Permeate flux rates were recorded at various time intervals, and small samples were collected at various times from both the permeate streamline and the retentate. The protein concentrations of the samples were determined later by spectrophotometer. The experiments were stopped after 2 hours. Next, the hydraulic permeabilities of the used membrane were measured. Afterwards the membranes were removed from the ultrafiltration system and a sample of the fouled membranes were cut and then placed in device to test the apparent zeta-potential of the membrane.

Results and discussion

In order to study the membrane charge properties, the apparent zeta-potential, ζ_a, of new CRC and PES membranes were tested at various pH values using the method described by Burns and Zydney [1], and the results were plotted in Figure1. The apparent zeta-potential of new PES membranes shows a linear dependence on the system pH value. It can also been seen that the PES membranes are generally negatively charged at pH higher than 2, which can be considered as the pI of the PES membranes. Although slightly negatively charged at all pH values studied, the CRC membranes are relatively neutral compared to the PES membranes, and do not show as strong dependence on the system pH values as the PES membranes do.

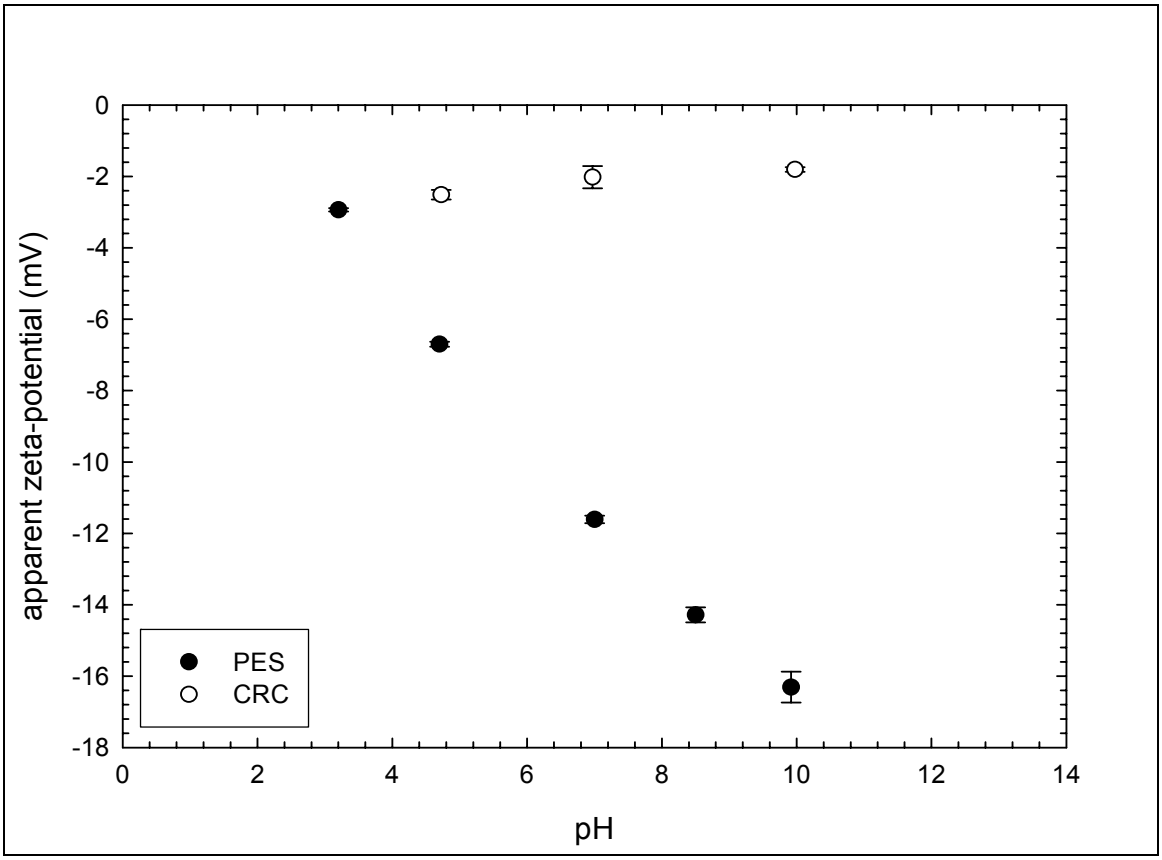

Figure 1. Apparent zeta-potential of new CRC and PES membranes.

After the binary protein ultrafiltration experiments, the apparent zeta-potential of the fouled membranes were also tested and the results were plotted in Figure 2. In Figure 2, the open symbols represent data of the fouled CRC membranes and the filled symbols represent data of the fouled PES membranes. The three different binary protein systems, BSA/α-LA, BSA/HEL and BSA/Cyt-C were abbreviated as B/A, B/H and B/C. It can be seen that the surface charge of the fouled CRC membranes is very similar to that of the new membranes, and it is not significantly effected by the system pH value or the different binary protein systems. However, the surface charge of the fouled PES membranes is effected by both pH value and protein systems. Compared to the new membranes, the pI point of the fouled PES membranes from all three binary protein systems have more or less shifted: in BSA/α-LA system, the pI is around 5.5, in BSA/HEL system, the pI is around 6.8, and in BSA/Cyt-C system, the pI is around 7.8. This is probably due to protein adsorption onto the membranes and the fouled membranes start to show charge properties similar to the adsorbed proteins [2].

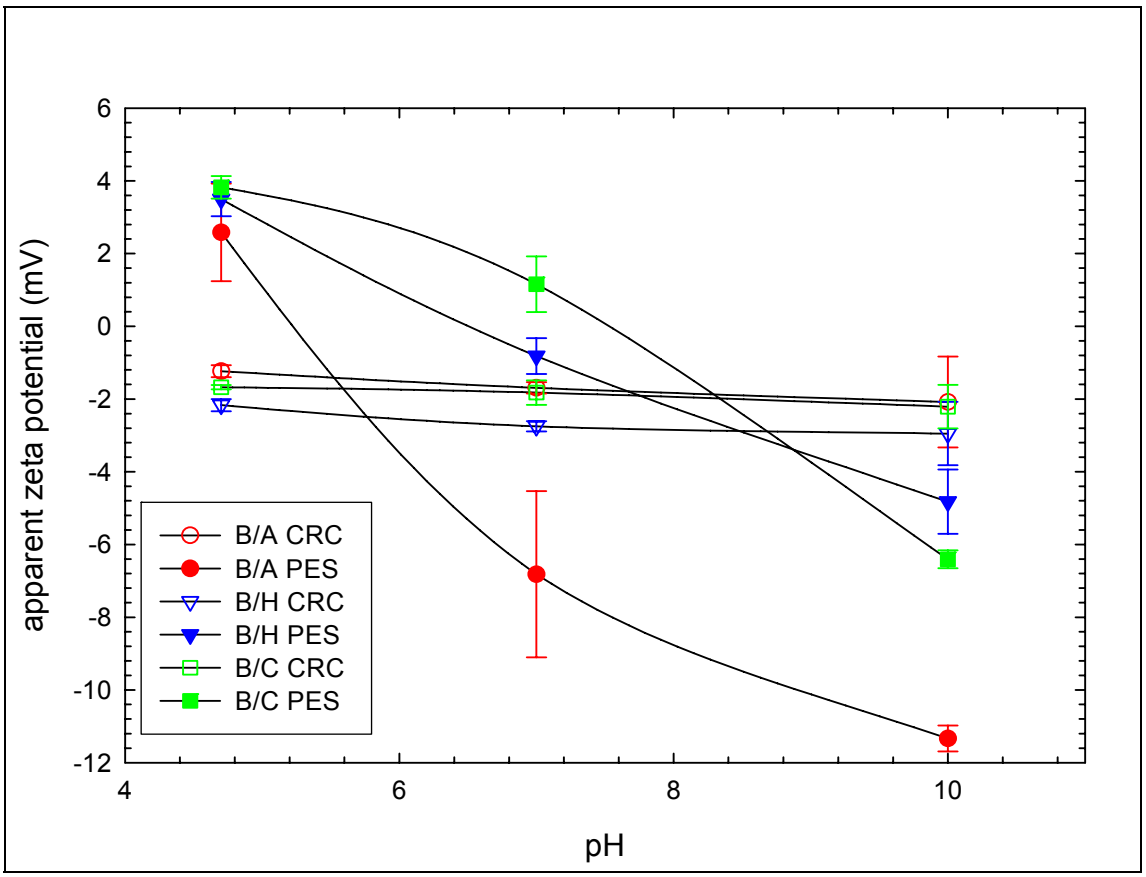

Figure 2. Apparent zeta-potential of fouled membranes.

The observed sieving coefficients of 15kDa proteins from experiments of BSA/HEL and BSA/Cyt-C systems at different pH values using CRC membrane were plotted in Figure 3. It can be seen that at all pH values, the sieving of Cyt-C in the BSA/Cyt-C system is higher than the sieving of HEL in the BSA/HEL system, even though HEL and Cyt-C are similar in size and charge properties. However, there is no obvious dependence on system pH value. The observed sieving coefficients of 15kDa proteins from experiments of BSA/HEL and BSA/Cyt-C systems at different pH values using PES membrane were plotted in Figure 4. The effect of system pH can be observed from this figure, as sieving of 15kDa proteins at pH 4.7 are notably

lower than at pH 7 or pH10, in both binary protein systems. Also, at pH 7 and pH 10, the sieving of Cyt-C in the BSA/Cyt-C system is higher than the sieving of HEL in the BSA/HEL system. Similar phenomena are observed from the selectivity data (defined as the ratio of the observed sieving coefficient of 15 kDa protein and the observed sieving coefficient of BSA), and the selectivity data from experiments at different pH values using CRC and PES membranes were plotted in Figure 5 and Figure 6 respectively.

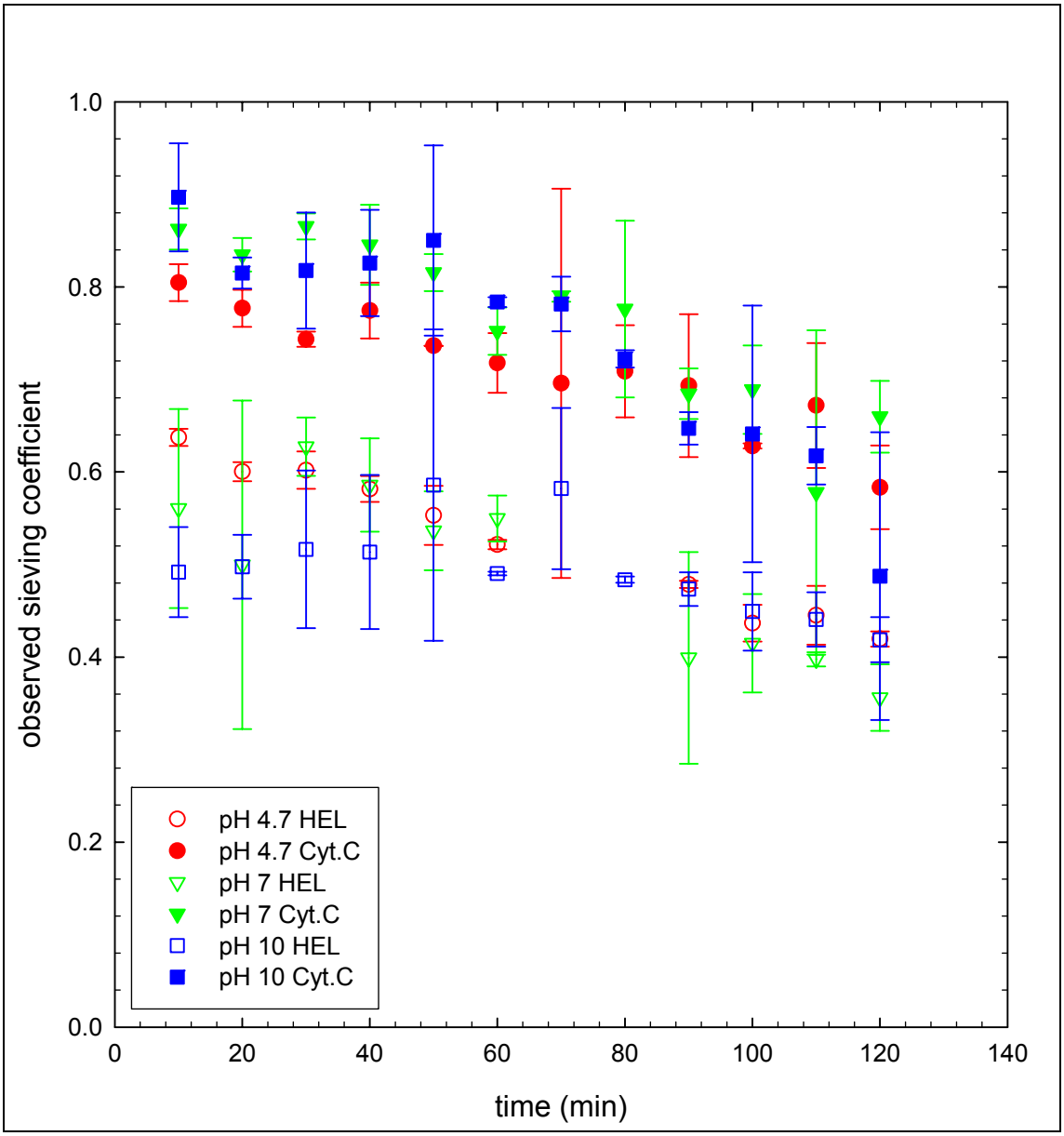

Figure 3. Observed sieving coefficient of the 15 kDa proteins (CRC membrane).

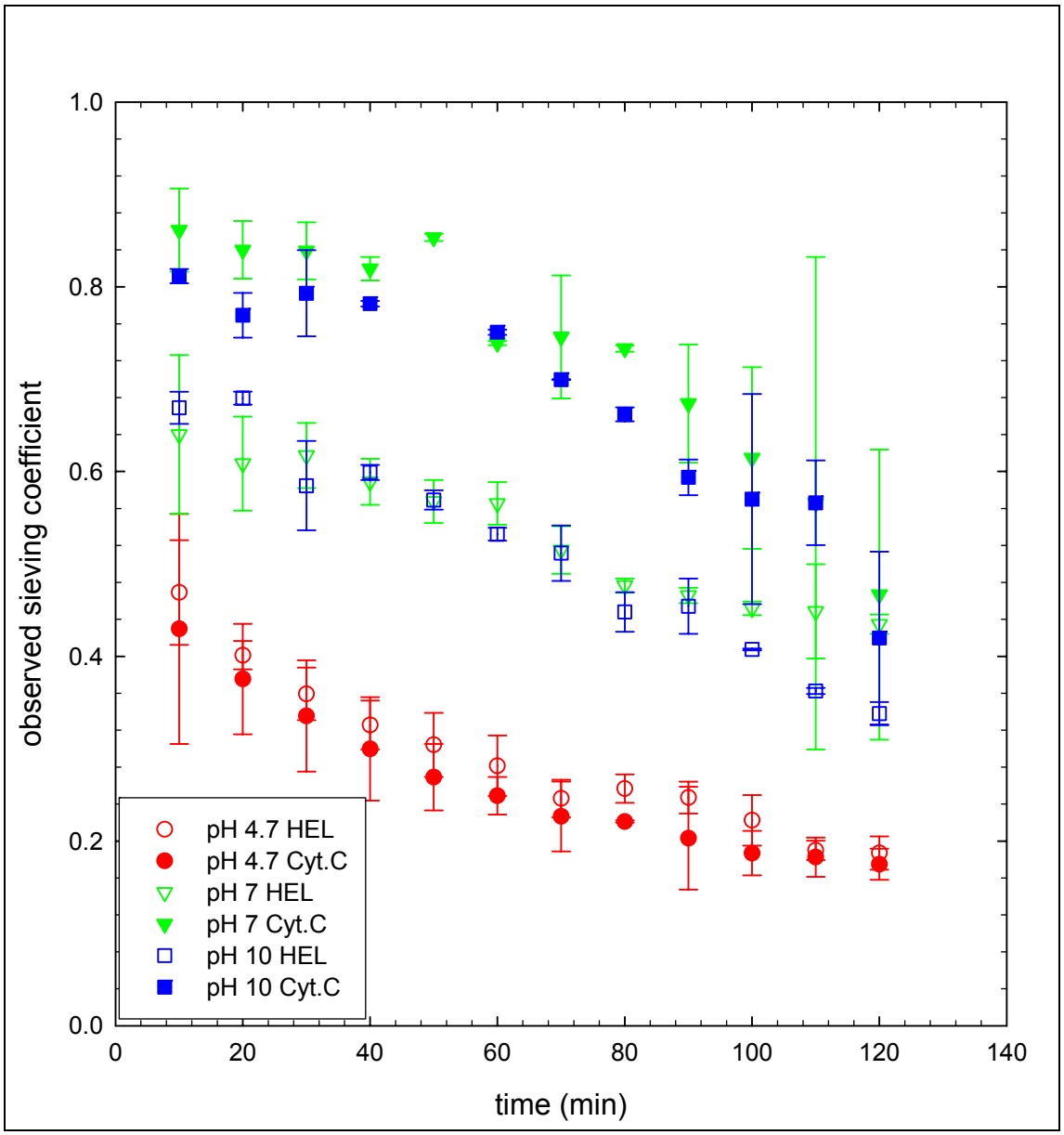

Figure 4. Observed sieving coefficient of the 15 kDa proteins (PES membrane).

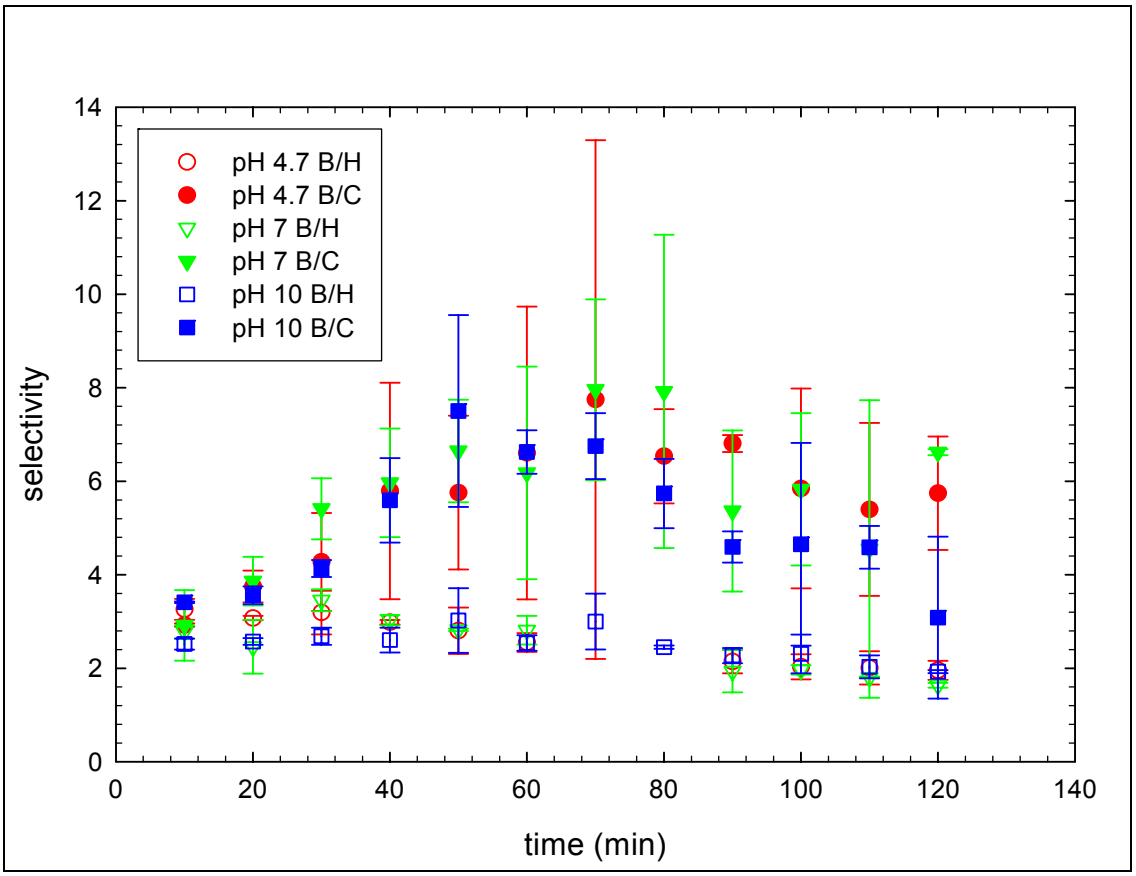

Figure 5. Selectivity (15 kDa protein/BSA, CRC membrane).

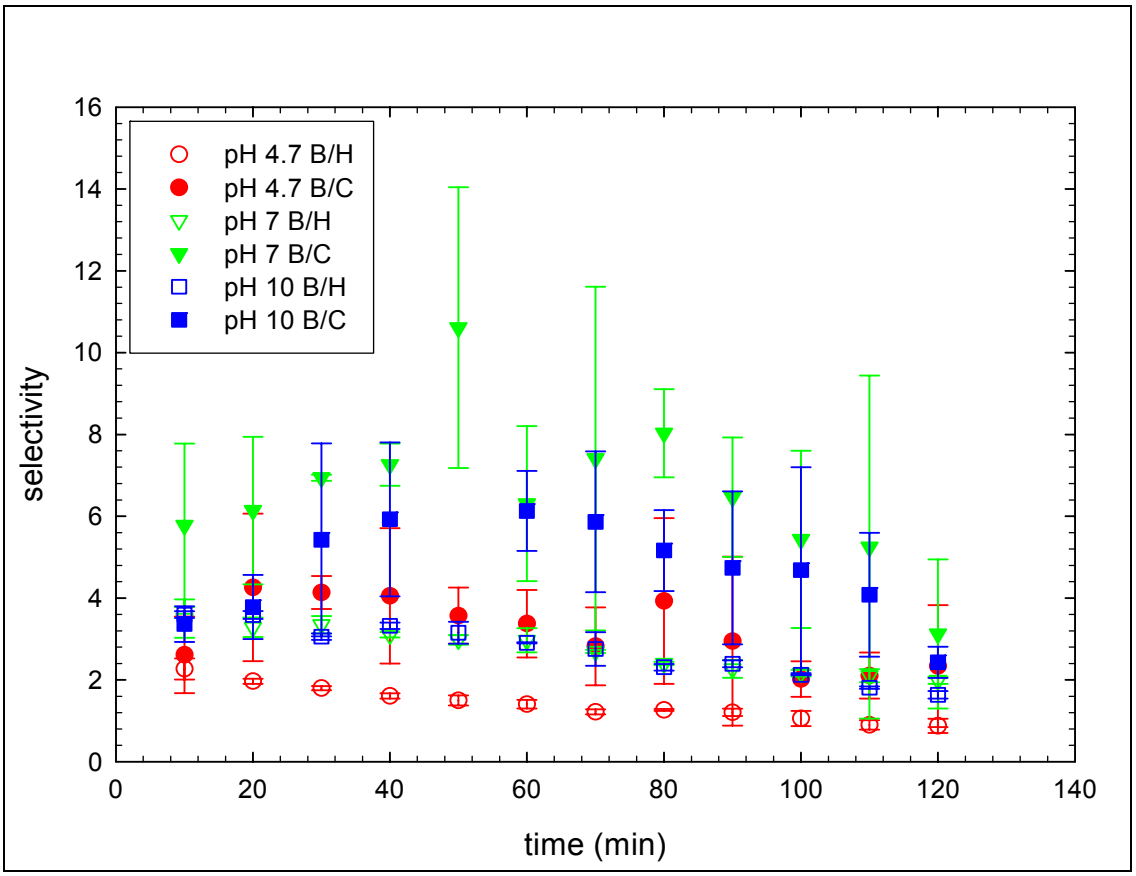

Figure 6. Selectivity (15 kDa protein/BSA, PES membrane).

Conclusion

The pH values of ultrafiltration systems have notable effects on ultrafiltration processes using PES membranes; however do not effect those processes using CRC membranes much. Although HEL and Cyt-C are similar in size and charge properties, the BSA/Cyt-C system shows better separation performances (higher sieving of 15 kDa and higher selectivity) than those of the BSA/HEL systems. The study of BSA/α-LA system is still on-going.

Reference

1. Burns DB, Zydney AL. Buffer effects on the zeta potential of ultrafiltration membranes, Journal of Membrane Science, 172, 2000, 39-48.
2. Causserand C, Nyström M, Aimar P. Study of streaming potentials of clean and fouled ultrafiltration membranes, Journal of Membrane Science, 88, 1994, 211-222,