# Novel Charged Ultrafiltration Membranes for Protein Separations

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## Introduction

A number of recent reports [1-3] have demonstrated that the performance of ultrafiltration membranes can be enhanced by exploiting electrostatic interactions between charged proteins and charged membranes. These charged membranes have the potential to dramatically improve commercial-scale bioseparation processes including protein concentration, buffer exchange, and protein fractionation [4-6].

The performance of charged ultrafiltration membranes depends on the electrical charge on the proteins and membranes pores, both of which are functions of the solution pH and ionic strength. Several researchers [4, 7-12] have investigated the effects of solution environment and surface charge density on protein and solvent transport through charged membranes.

Although there have been no significant studies of the effects of different coupling chemistries, spacer arm length, or the nature of the functional group on the performance of ultrafiltration membranes, there is an extensive literature on these parameters in the field of chromatography. An optimal spacer arm ensures that the functional groups are placed a suitable distance from the surface of the solid support and are easily accessible to the macromolecules. Results from a variety of studies in ion exchange and dye-affinity chromatography suggest that the ideal spacer arm has [13]: (1) proper length (at least three atoms); (2) no active center that could cause nonspecific adsorption; and (3) at least two functional groups, one to react with the base support and one to serve as the chromatographic ligand.

Results with membrane adsorbers have also demonstrated the importance of the spacer arm to the overall performance characteristics. For example, Suen et al. [16] showed that attaching 1,4-diaminobutane as a spacer arm to the crosslinking molecule (ethylene glycol diglycidyl ether) provided higher dye ligand density and lysozyme adsorption capacity compared to immobilized metal affinity membranes produced using only the ethylene glycol diglycidyl ether. Tsai et al. [17] found that polyvinylidene fluoride affinity membranes with 1,8-diaminooctane as the spacer arm had the highest adsorption capacity for lysozyme, with the capacity decreasing for both shorter and longer spacers. The authors hypothesized that this

spacer arm had the optimal length in terms of functional group accessibility and number of binding sites.

These studies of chromatographic systems provide useful insights into the effects of the ligand attachment on protein binding, but it is impossible to use these results to understand the behavior of membrane systems in which the performance is determined by electrostatic exclusion effects instead of binding interactions. The objective of this work was to examine the effects of spacer arm length on the behavior of electrically charged ultrafiltration membranes.

## Materials and Methods

Composite regenerated cellulose (CRC) membranes with nominal molecular weight cut offs of 30 and 100 kD were provided by Millipore (Bedford, MA). The pore size distributions and the charge characteristics for the charge-modified membranes were determined from dextran sieving measurements and streaming potential measurement as described earlier[12]. All experiments were performed with 25 mm diameter membrane disks cut from a large flat sheet of membrane (roll stock) provided by Millipore Corporation (Bedford, MA). The CRC membranes disks were stored overnight in a 0.1 M NaOH solution prior to surface modification.

### *Membrane Preparation using Diamines*

Epichlorohydrin (EPI) and a series of diamines with different alkyl chain length (1,2-diaminoethane, 1,4-diaminobutane, 1,6-diaminohexane, 1,8-diaminooctane, and 1,10-diaminodecane) were purchased from Sigma. The reaction scheme used to couple epichlorohydrin (EPI) and the diamines to the hydroxyl groups on the cellulose membrane is shown in Figure 1. The basic approach was adapted from the work by Liu et al.[18], but the specific process conditions (e.g., temperature and NaOH concentration) were modified to avoid degradation of the cellulose membrane. All reactions were carried out in a 25 mL capped plastic jar agitated at approximately 150 rpm. The clean membrane disk was immersed in a solution containing 10 mL of 0.1 M NaOH and 5 mL of EPI, and the resulting reaction mixture was incubated at $45^0$ C for 2 hours. The membrane was then carefully removed, rinsed with deionized (DI) water, and then immersed in 20 mL of a 1 M diamine solution with the pH adjusted to 11.2 by addition of a small amount of 1 M HCl. The reaction was allowed to progress at $45^0$ C for 12 hours, with the membrane then removed from the solution and rinsed thoroughly in DI water for a minimum of 60 min.

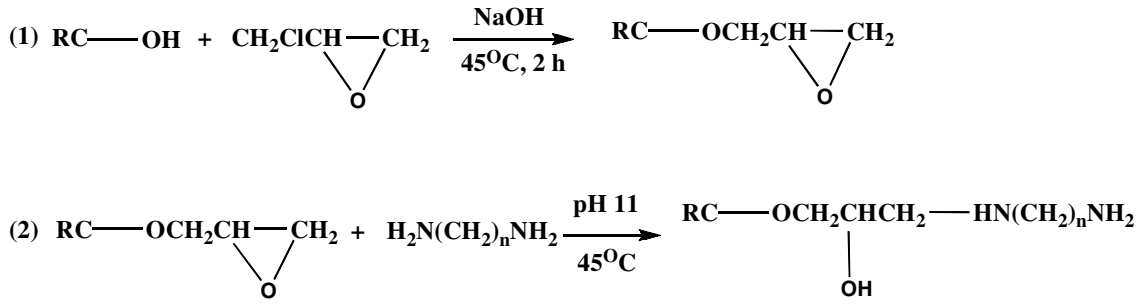


**Figure 1:** Schematic of reactions used to couple EPI and different diamines to the composite regenerated cellulose membranes

The diamines possess a secondary amine group that is located relatively close to the membrane surface and a primary amine group at the end of the alkyl chain (furthest from the membrane). The location of the terminal amino group was controlled by selection of the alkyl chain length in the original diamine. Membranes were constructed with diamines having between 2 and 10 carbon atoms, corresponding to alkyl chain lengths of approximately 0.15 to 1.35 nm.

***Protein Ultrafiltration***

All ultrafiltration experiments were performed in a 25 mm diameter Amicon stirred cell connected to a peristaltic pump (Rainin Instrument Corp.) on the filtrate line. Cytochrome c solutions were prepared by dissolving cytochrome c powder in a Bis-Tris buffered KCl solution with the resulting solution prefiltered through a 0.22 μm μ-Star nylon filter (8010, Costar Corporation) prior to use. The membrane permeability was evaluated using a buffered saline solution at the same pH and ionic strength that was used for the protein filtration. The stirring speed was set at 600 rpm and the filtrate flux was maintained at 25 $L/m^2/h$ using a peristaltic pump connected directly to the filtrate exit port. The protein concentrations in the filtrate and bulk samples were determined spectrophotometrically from the absorbance at 410 nm, which is the natural absorbance peak for cytochrome c.

## Results and Discussion

A series of charge-modified membranes with different spacer arm length was generated by activation of the base cellulose membrane using epichlorohydrin (EPI) followed by reaction with different diamines. The apparent zeta potential for the membranes with n = 4, 6, and 8 were very similar, with values ranging from 7.5 to 7.9 mV (Table 1). The apparent zeta potential for the membrane with n = 10 was significantly larger ($\zeta_{app}$ = 9.5 mV). This increase in membrane

charge is likely due at least in part to a shift in the $pK_a$ values of the charge groups associated with electrostatic interactions between the two amine groups in each functional ligand.

**Table 1:** Apparent zeta potential and pore size characteristics for charge-modified membranes with different spacer arm length

| **Spacer Arm Length (n)** | **Native Membrane (kD)** | **Mean Pore Size, $\bar{r}$ (nm)** | **Coeff. of Variation ($\sigma/\bar{r}$)** | **Apparent Zeta Potential (mV) at pH 7** |
|---|---|---|---|---|
| 2 | 30 | 2.9 | 0.21 | 6.0 $\pm$ 0.1 |
| 4 | 30 | 3.1 | 0.19 | 7.9 $\pm$ 0.4 |
| 6 | 30 | 3.1 | 0.21 | 7.5 $\pm$ 0.3 |
| 8 | 30 | 2.9 | 0.19 | 7.8 $\pm$ 0.3 |
| 10 | 30 | --- | --- | 9.5 $\pm$ 0.5 |
| 4 | 100 | 5.2 | 0.31 | 10.2 $\pm$ 0.6 |
| 8 | 100 | 5 | 0.30 | 10.2 $\pm$ 0.9 |

Dextran sieving data were obtained at a flux of 25 $L/m^2/hr$, with the results used to estimate the membrane pore size distribution. The best fit values of the mean and standard deviation of the log-normal distribution (Table 1) were determined by minimizing the sum of the squared residuals between the measured and calculated values of the sieving coefficients[12]. In contrast to the apparent zeta potential data, the best fit values of the mean pore size of the charge-modified 30 kD membranes were all approximately 3 nm, irrespective of the length of the spacer arm used to generate the charged membrane. This behavior was also consistent with the permeability measurements, which were also independent of the spacer arm length.

***Protein Ultrafiltration***

Figure 2 shows the observed sieving coefficients for cytochrome c at pH 7 as an explicit function of the solution ionic strength for the charge-modified membranes with different spacer arm lengths. The observed sieving coefficients decrease with decreasing ionic strength

due to the increase in electrostatic exclusion of the positively-charged protein from the positively-charged membranes. The sieving coefficient for the membrane made with the 1,2-diaaminoethane (n=2) decreased by slightly more than an order of magnitude as the ionic strength was reduced from 1 to 0.005 M. A much greater reduction was seen with the membranes having longer spacer arms, with the sieving coefficients for the membranes with n = 4, 6, and 8 decreasing by approximately two orders of magnitude. This behavior is very consistent with the results for the apparent zeta potential and counter-electroosmosis, both of which showed a significant increase in effective charge as the chain length was increased from 2 to 4. The results for the membrane with n = 10 showed an even greater reduction in cytochrome c transmission, with the sieving coefficient in the 0.005 M solution dropping to 0.001.

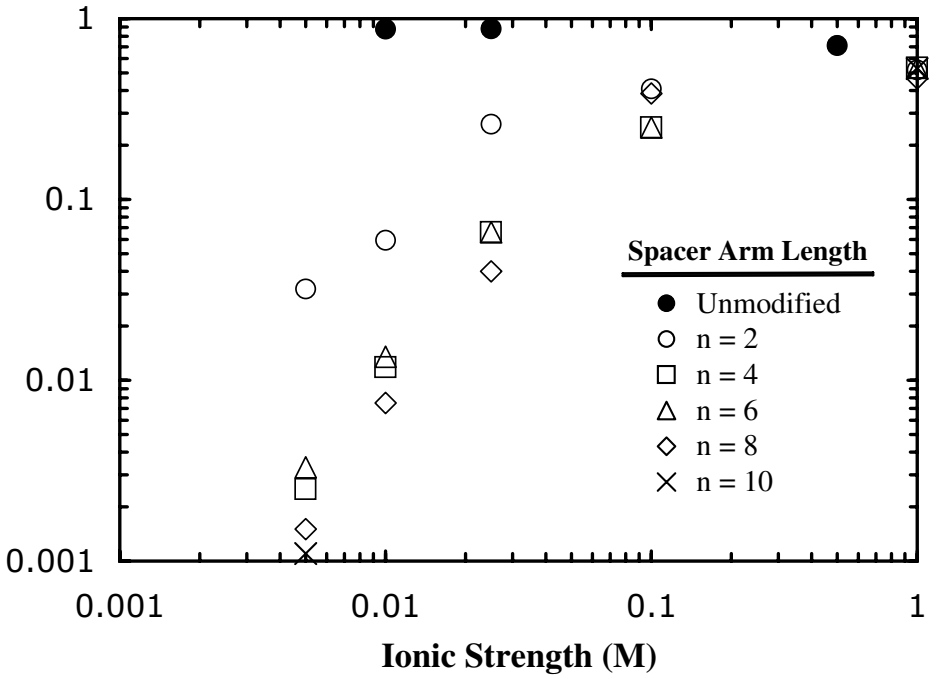


**Figure 2:** Observed sieving coefficients for cytochrome c as a function of solution ionic strength through a series of charge-modified CRC 30 kD membranes possessing different spacer arms

Figure 3 shows a Robeson type analysis[21] for charge-modified membranes with different spacer arms. The solid curve represents the theoretically predicted trade-off between the permeability and selectivity for cytochrome c through an uncharged membrane and was developed using equations given by Mehta and Zydney[21]. Calculations were performed by varying the mean pore size (keeping $\sigma/\bar{r}$ and $\varepsilon/\delta_m$ fixed at values of 0.2 and 0.35 $\mu m^{-1}$, respectively), with the

permeability and selectivity evaluated over a range of $\bar{r}$. The filled squares represent experimental data for cytochrome c sieving through the charge-modified membranes in the 0.5 M ionic strength solution where electrostatic interactions are negligible, while the open symbols represent data obtained in the 5 mM ionic strength solution. The results at high ionic strength fall along the expected trade-off curve. In contrast, the results for the charged membranes at low ionic strength fall well above and to the right of the expected upper bound.

The dashed curve in Figure 3 represents the theoretical trade-off for a positively charged membrane with a charge density of $5.5\times10^{-3}$ $C/m^2$, with this value determined from the apparent zeta potential of the 100-kD membranes made with the diamines. The theoretical values of the permeability and selectivity were evaluated at 5 mM ionic strength by varying the mean pore size while keeping $\sigma/\bar{r}$ fixed at 0.3 (Table 1). The open triangles at permeabilities near 15 LMH/Psi represent data with surface modified 30 kD membranes while the open triangles with permeabilities from 60-80 LMH/Psi represents data for 100-kD membranes. The experimental data for the 100-kD membranes with diamines lie fairly close to the predicted trade-off while the 30-kD membranes with diamines lie well below the predicted trade-off, which could in part be due to the lower apparent zeta potential compared to those of the 100-kD membranes made with the same ligands (Table 1).

Also shown in Figure 3 are data obtained with charge-modified membranes having quaternary ammonium (strong base) groups (having a very small spacer arm) as discussed previously by Mehta and Zydney[12]. The results for these membranes fall close to the predicted trade-off curve for the charged membranes, with behavior similar to the results seen using the 100 kD membranes but lying well above the results for the 30 kD membranes. For example, at a hydraulic permeability of 15 LMH/Psi, the selectivity for the membrane with attached quaternary ammonium groups was nearly 3-fold higher than that for the diamine membrane having a spacer arm length of 12 carbon atoms and it was 1500-fold larger than the selectivity of the neutral membrane. The superior ultrafiltration performance obtained with membranes having quaternary ammonium groups is most likely due to their strong basic nature which provides a higher charge density than membranes made with either primary or secondary amines. The performance characteristics of the 30 kD membranes with the diamine modification were better with longer spacer arms, with the selectivity increasing from 80 to 2000 as n goes from 2 to 10 with essentially no change in the permeability. This increase in performance is again consistent with the increase in net charge arising from the reduction in the electrostatic interactions between the two amine groups in the diamine.

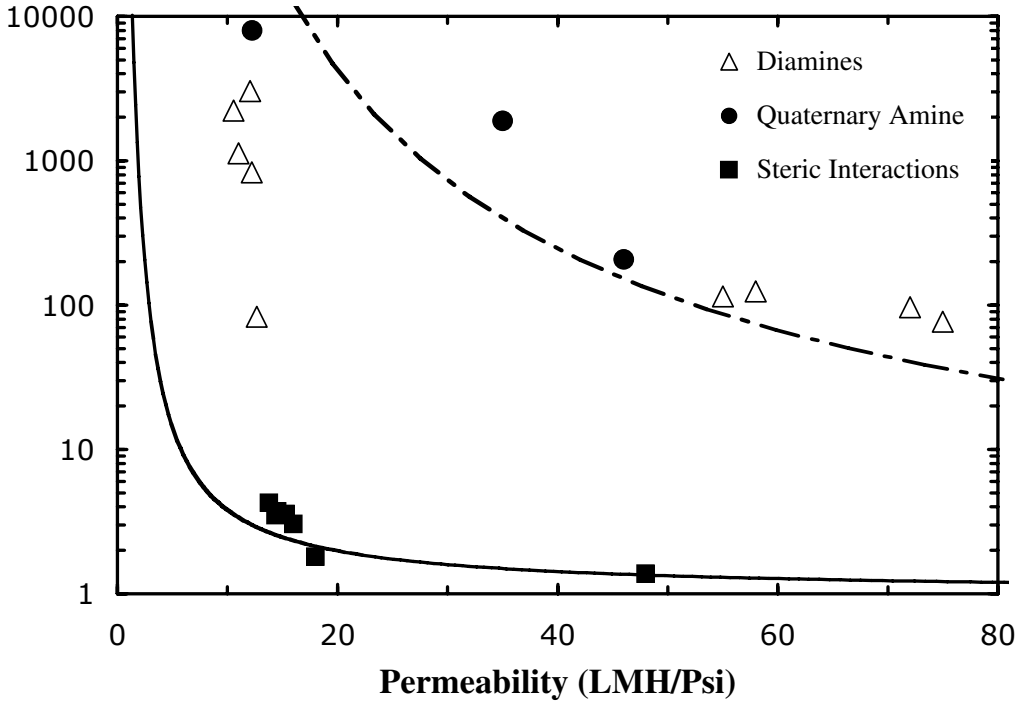


**Figure 3:** Permeability-Selectivity analysis for membranes developed using different surface modification chemistries. The solid curve represents the predicted trade-off between the permeability and selectivity for cytochrome c through an uncharged membrane while the dashed curve represents the predicted trade-off for membranes with a charge density of $5.5\times10^{-3}$ C/m$^2$.

## Conclusions

The experimental data obtained in this study provide the first ever analysis of the effects of different physical and chemical properties of charged ligands on the performance characteristics of ultrafiltration membranes. Charged ultrafiltration membranes were developed to examine the effects of different spacer arm length. In each case data were obtained for both the hydraulic permeability and the sieving coefficient of cytochrome c, which are the critical performance characteristics of these membranes during protein ultrafiltration.

The results for membranes having different spacer arm lengths

clearly showed that the observed sieving coefficients decreased with increasing spacer arm length. For example, the observed sieving coefficient of cytochrome c for the membrane made with the 1,2-diaaminoethane (n=2) was approximately two orders of magnitude higher than a similar membrane made using 1,10-diaminodecane (n=10) with essentially no change in the permeability at high ionic strength. This behavior was very consistent with the results for both the apparent zeta potential and the magnitude of counter-electroosmosis, both of which showed a significant increase as the chain length was increased from 2 to 10. These results clearly demonstrate the potential for optimizing membrane performance by controlling the detailed properties of the charged ligands used to generate novel ultrafiltration membranes for protein separations.

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