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Formation of Molecularly Imprinted Polymer Matrices using

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Formation of Molecularly Imprinted Polymer Matrices using Poly(Acrylonitrile-co-Acrylic Acid) For Chiral Separations with Consideration of Peptoid Incorporation

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Abstract

This study serves as a proof of concept that a poly(acrylonitrile-*co*-acrylic acid) may be used to form an chiral selective polymer membrane for discrimination of L-tryptophan from racemic tryptophan. Due to the unavailability of necessary equipment, a polymer membrane was not tested, rather, two experiments were run in conjunction; (1) A membrane of L-tryptophan imprinted P(AN-*co*-AA) was cast and tested on a scanning electron microscope to determine the membrane density, and (2) Imprinted polymer was ground and tested for absorption of L-Tryptophan by finding circular dichroism and concentration for different samples. It was found that it is feasible that P(AN-*co*-AA) may be used to make a dense membrane for this application. Spectrophotometer results indicate that the concentration of tryptophan in aqueous DL-tryptophan significantly decreases after it has been in the presence of the imprinted polymer matrix. Circular dichroism indicates that significant spectroscopic changes occur when a racemic mixture of tryptophan is held in the presence of an imprinted polymer matrix. These results reveal that it is highly feasible that the polymer exhibits a high selectivity for L-Tryptophan, which decrease with each use of the polymer. This study also discusses the potential benefits of incorporating a peptoid cross linker into the imprinted polymer matrix.

Introduction and Background

Chirality, a property of some organic molecules is of ever-increasing importance in many industries today. The term "chiral" refers to a substance that may take on more than one spatial conformation of atoms, thus affecting its chemical properties and interactions with biological systems. Two molecules that are the same in every aspect other than their chirality are called enantiomers. In the synthesis of chiral molecules, it is often impossible or difficult to restrict synthesis to only one enantiomer, resulting in a product of a racemic mix. A racemic mix is defined as an equimolar mix of enantiomers. Because enantiomers share physical properties (melting point, boiling point, solubility), traditional methods of separation prove to be ineffective.

One particular example of why enantiomeric separation is crucial was apparent in the 1960s, when the drug Thalidomide was introduced. Thalidomide was prescribed to relieve nausea for pregnant women. While the L-enantiomer demonstrated the desired effects, the D-enantiomer caused birth defects in unborn babies.[1] Since then, enantiomerically pure drugs have come to be a huge part of the pharmaceutical industry, representing approximately $150 billion in 2002[1]. There are four existing methods of chiral separation; crystallization resolution, kinetic resolution, chromatographic separation, and membrane based separation[1]. In addition to being one of the only methods that has the potential to be effective on an industrial scale, membrane based separation is one of the least expensive alternatives[1]. One major problem with membrane based separations is that once a membrane is formed, it has a short lifetime, and may only be useful once or twice

before it loses its selectivity. Because a high value product is required to cast the molecularly imprinted membrane, it is apparent that a long lasting membrane with high selectivity is necessary in order for this process to be economically savvy.

Methods and Materials

Poly(acrylonitrile-*co*-acrylic acid), shown in Figure 1, was selected as the major matrix component in this study because it exhibits three distinct charactistics: (i) Solubility in an inexpensive, readily available solvent, (ii) Insolublity in water, (iii) Intermolecular bonding capabilities. The former two properties are necessary for the casting and use of membranes, while the latter is key to membrane functionality. While this study only tests the polymer matrix on an absorption basis, use in membranes is the ultimate goal. Poly(acrylonitrile-*co*-acylic acid) was polymerized according to the procedure outlined by Kobayashi.[2] According to the literature, the average molecular weight of a polymer chain is 77,000, making it a reasonable size for this application.

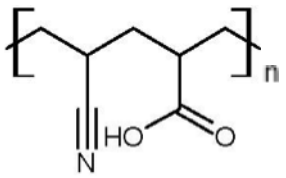


Figure 1. Structural Repeat Unit of Poly(acrylonitrile-co-acrylic acid)

Intermolecular bonding capabilities of the polymer are necessary to this study on the most fundamental level; they allow the polymer form in a matrix around a template molecule, while still allowing the template molecule to be easily washed out of the matrix. The mechanism by which the polymer matrix forms around the template molecule is discussed further later in this section

In order to attempt to form a dense membrane to perform the diffusion limited separation required to separate racemic molecules with high selectivity, several different variations of membrane casting techniques were employed.

Immersion precipitation[5] of 5% polymer in DMSO solution with 5% template molecule relative to the polymer was precipitated in 10 °C water to enable slow coagulation. This set of parameters and techniques was found to be the preferred method. Immersion precipitation takes place when a polymer in solution is immersed in an anti-solvent, moving the polymer back to the solid phase in the desired formation (membrane). A membrane that displayed the characteristic transparency of a dense membrane was tested on a scanning electron microscope. The SEM image shown in Figure 2 reveals a relatively consistent topography for the membrane structure.

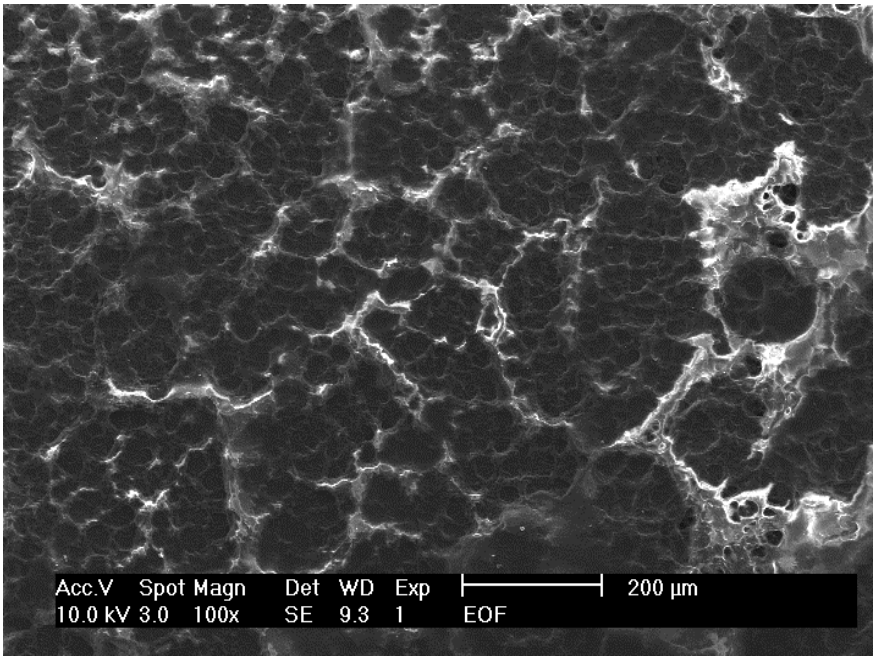
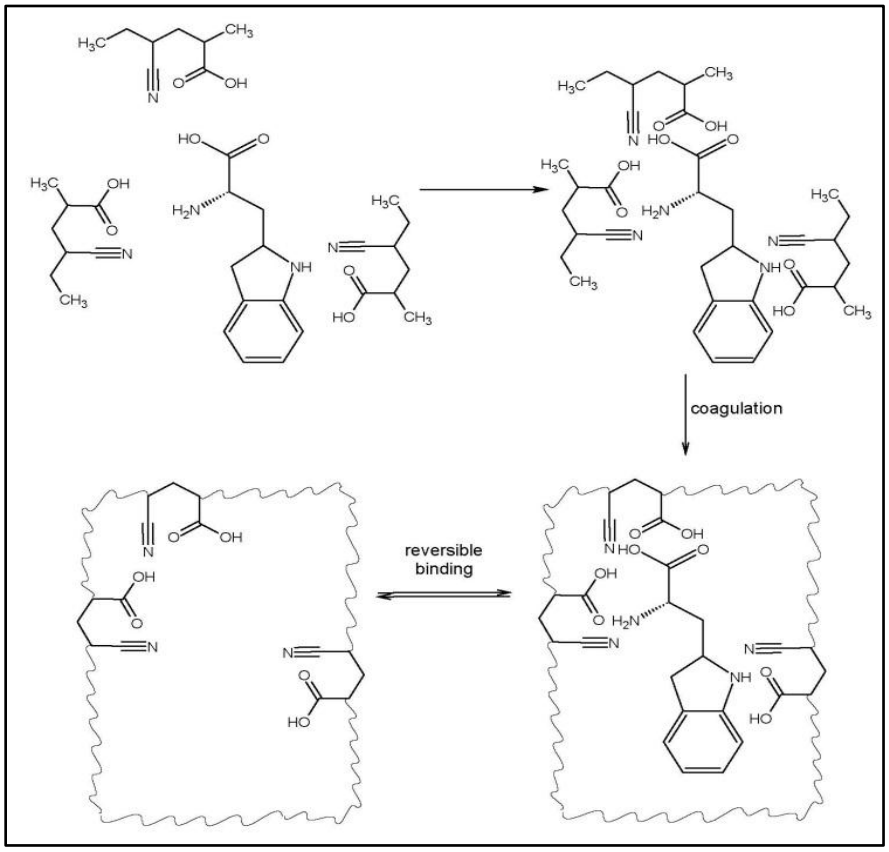


Figure 2. SEM Image of Membrane

Due to the non-conductive nature of the membrane, magnification to the scale necessary to determine density was impossible. It is clear that this membrane does not have large pores (micron scale), further imaging is required to determine whether it is a dense membrane.

Because an apparatus that supplies sufficient back-pressure to test a dense membrane was not available, a different method of testing the polymer matrices absorption and selectivity was employed. A quantity of polymer was dissolved in DMSO at a temperature of 50 °C, along with 5% of L-tryptophan by weight. In solution, the template molecule loosely bonds to the polymer chain via intermolecular forces. When it is taken out of solution, the polymer remains attached to the template molecule. Repeated washing of the polymer matrix with water frees most of the template molecule, leaving a space for the template in the polymer matrix. A schematic of the molecular imprinting process is shown in Scheme 1.



Scheme 1. Schematic of Imprinted Polymer Matrix Formation

After an imprinted polymer matrix was created, the template molecule was washed out, and the polymer finely ground. The ground polymer was then washed to remove the template molecule. Next, the polymer was immersed in a racemic solution of DL-Tryptophan in deionized water for two hours. The same polymer was used for three trials, washing the polymer with water sufficiently in between each trial in order to remove absorbed tryptophan. Circular dichroism was performed on each of the samples, and is discussed in depth in the next section.

As discussed previously, polymer matrices of this type tend to lose selectivity with each pass. Peptoids, N-substituted glycines, are a very stable species, as is demonstrated in Figure 3. The use of peptoids as cross linkers in the polymer matrix has potential to greatly increase the stability of the polymer matrix. Though this study did not successfully synthesize and incorporate a peptoid into the polymer matrix as a cross-linker, it is the logical next step in the process.

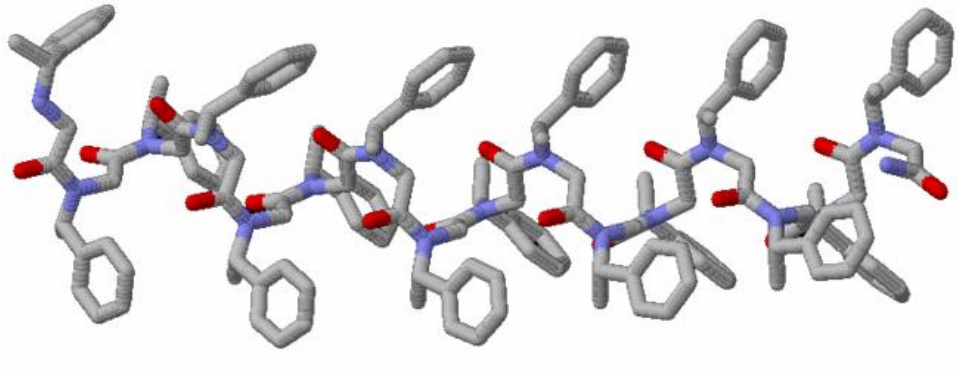


Figure 3. Robust Peptoid Structure

Two peptoids have been designed that may serve as cross-linkers in the polymer matrix that will enhance the matrix rigidity and lifetime. In order accomplish this goal, a peptoid must be; (i) stable, and (ii) achiral. Peptoid 1 and

Peptoid 2, shown in Figures 4 and 5, are aimed at fulfilling the criteria to different degrees. Peptoid A contains a ratio of 4:1 aromatic groups to allyl side chains. The aromatic groups are in place for molecular stability, and the allyl groups to allow cross-linking with polymer. Peptoid B shows a ratio of 3:2 aromatic groups to allyl groups, allowing for more cross-linking at the sacrifice of stability, and possibly solubility.

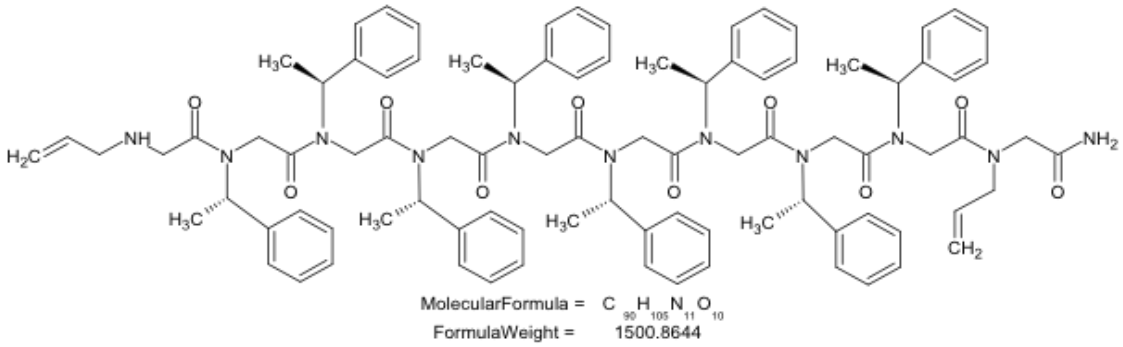


Figure 4. Peptoid Cross Linker Design 1

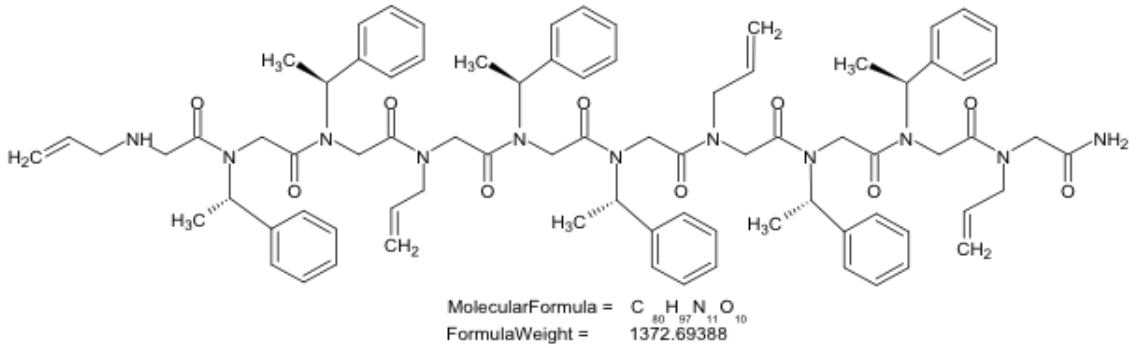
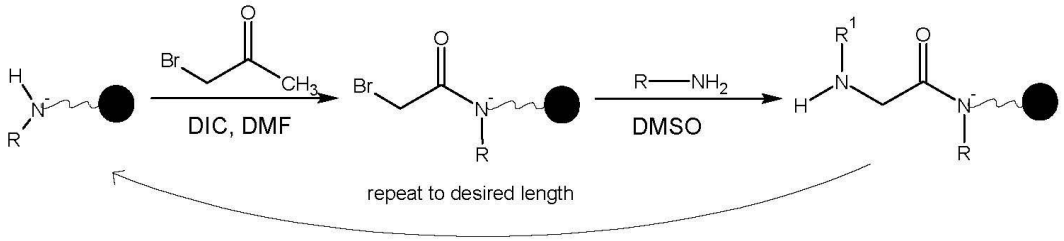


Figure 5. Peptoid Cross-Linker Design 2

An attempt of the synthesis of peptoid 1 was made using a solid phase protocol shown in Scheme 2[3]. First, bromoacetylation occurs on a rink amide resin, then the bromine is displaced by nitrogen in an S_N2 reaction. This reaction is carried out until the peptoid has reached the desired length.



Scheme 2. Solid Phase Peptoid Synthesis Protocol[3]

Matrix assisted laser desorption/ionization (MALDI) was performed on the crude peptoid sample after synthesis. The results are discussed further in the next section.

Results and Discussion

MALDI was performed on the crude peptoid sample to confirm the presence of Peptoid 1. The expected molecular weight of 1500.86 was not present in the sample, as shown in Figure 6. No further attempt to synthesize the peptoid was made.

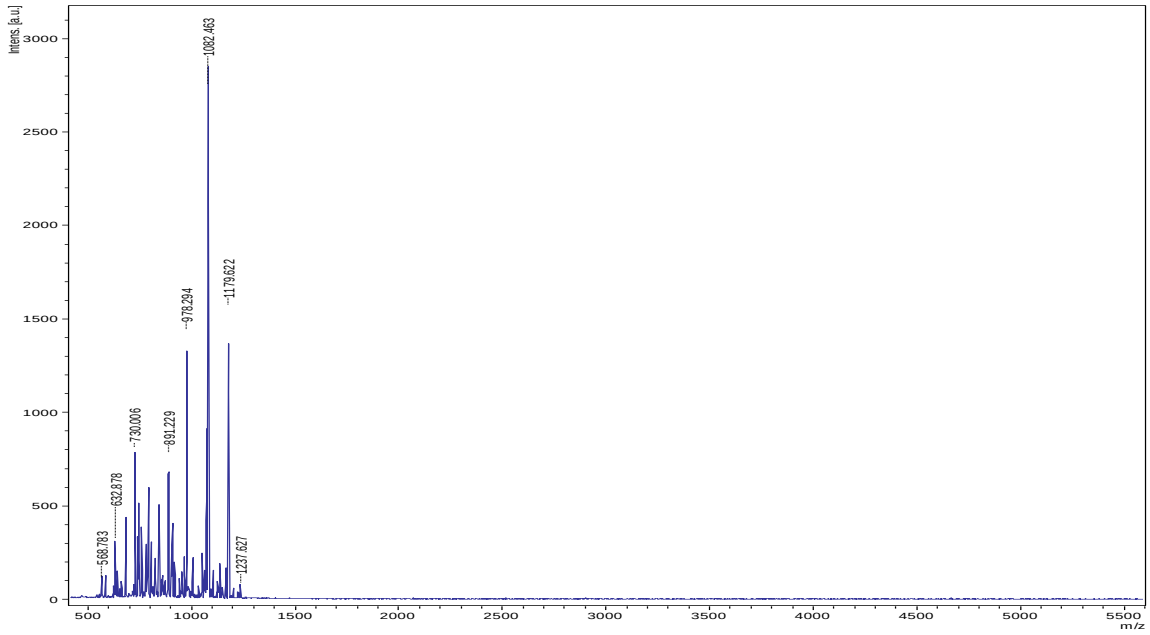


Figure 6. MALDI Results from Peptoid 1 Synthesis

A spectrophotometer was used to measure the concentration of each sample that was tested with the imprinted polymer matrix., as well as the racemic mix. The concentration for the pure racemic solution is, as expected, the highest; it was not subjected to any filter. Racemic mixture that was soaked with the new imprinted polymer had a significantly lower tryptophan concentration, presumably because the polymer absorbed a significant amount of L-tryptophan. The racemic mix that was subjected to the once-used polymer had a higher concentration than the previous, but lower than the pure racemic mix. This is conceivably because the

polymer lost some of its ability to absorb L-tryptophan. Curiously, the last sample, which was subjected to the twice-used polymer, displays the smallest concentration of dissolved tryptophan. This may be due to the fact that the polymer has lost much of its selectivity and is beginning to absorb D-Tryptophan, as well as its enantiomer. Table 1 displays the concentration data for each sample.

Table 1. Spectrophotometer Concentration Data from Tryptophan Samples

Sample	Concentration (mg/mL)
Racemic Mix	0.900
First Pass	0.592
Second Pass	0.647
Third Pass	0.590

Circular dichroism is defined as the difference between the absorption of left and right circularly polarized light.[4] Circular dichroism was performed for each of the samples, and data in the wavelength range of 240-300 nm is displayed, although tryptophan absorbs light at the 280 nm wavelength[6]. Figure 7 displays the composite CD results for each of the four samples tested.

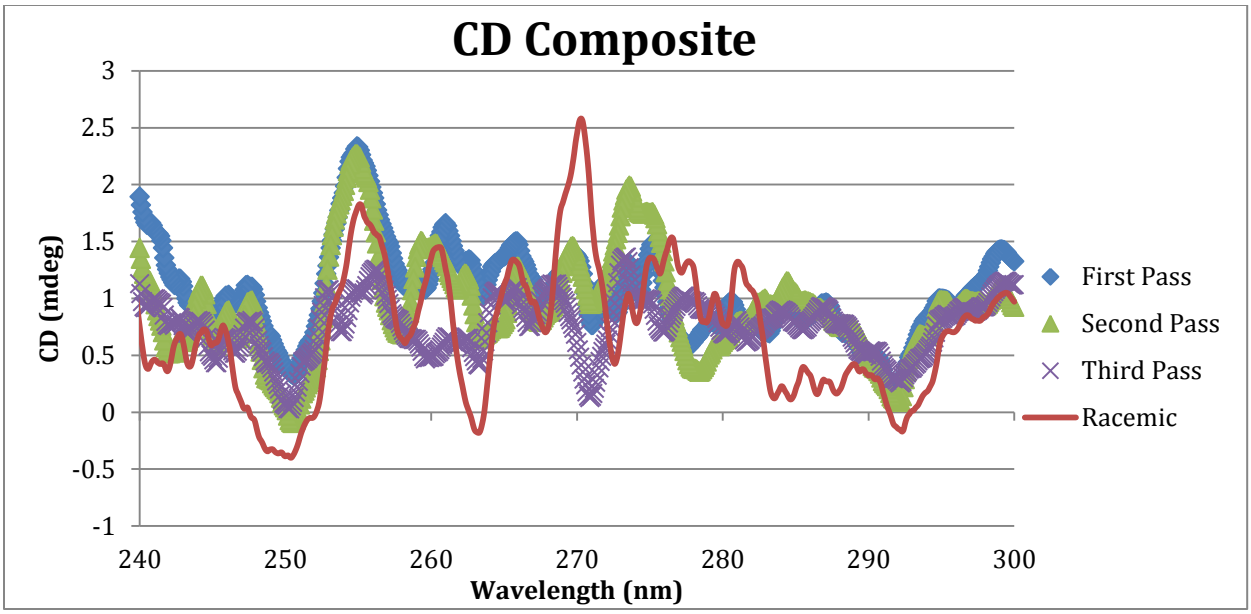


Figure 7. Composite Results of Circular Dichroism for all samples

From Figure 7, one can see that there are three distinct regions in which there is a noticeable difference in the shape of the racemic curve and the sample curves . The three regions mentioned fall in the ranges of; 260-266 nm, 267-272 nm, and 282-290 nm. Upon closer inspection, the former two peaks actually go in the same direction for all samples, but are more pronounced in the racemic sample. The latter region, however, actually shows that the peaks from the samples are inverted versions of the peaks from the racemic mix. This is an area of interest because tryptophan typically absorbs in the 280-300 nm range. Theoretically, a baseline correction is not necessary for CD, because a racemic mix should give a flat line (optical enantiomers should yield mirror spectra). In reality, the best method is to subtract the racemic mix from the unknown samples, according to Berova[4]. Figure 8 is a composite of all three samples that have been corrected for the solvent (water) and the racemic mix over the wavelength range 282-290 nm. The wavelength range

was chosen to match the wavelength range that is suspected to be representative of the difference in stereochemistry of L and D-Tryptophan.

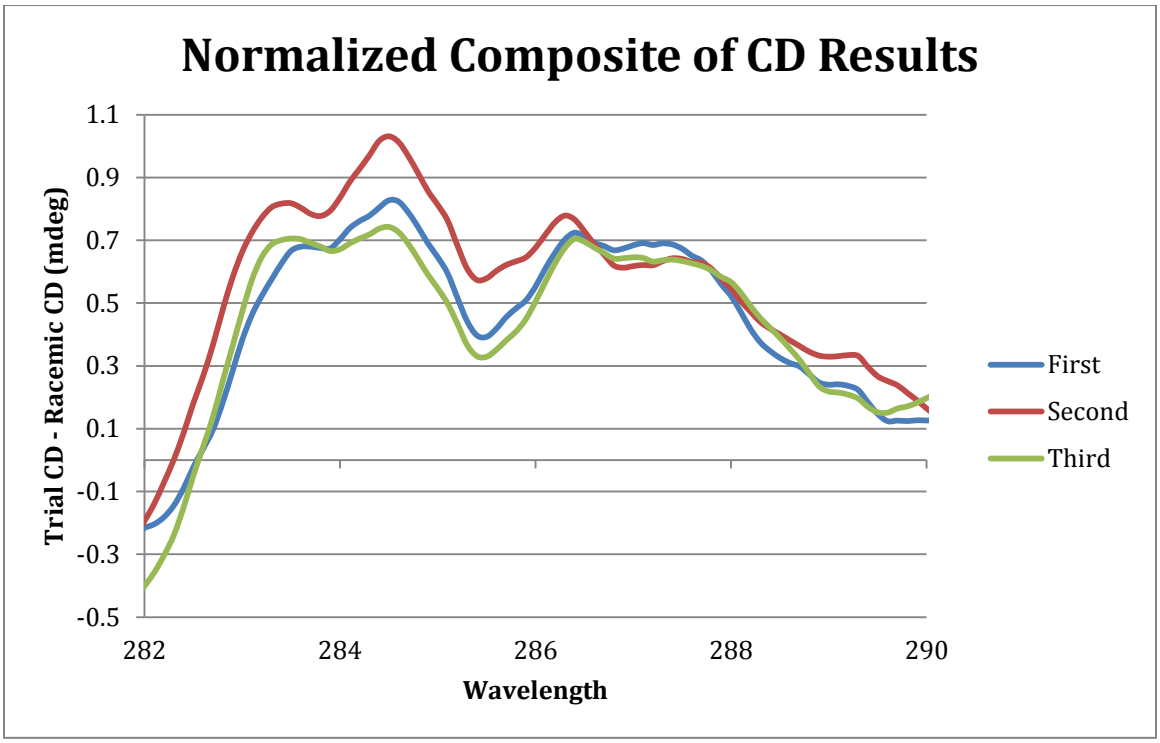


Figure 8. Normalized Composite of Circular Dichroism Results

According to Berova, often the best way to analyze CD results is by visual comparison of the spectra[4]. While there is no clear difference in the magnitude of the spectra in Figure 13, the numerical average of each point along the spectra was calculated so as to give an estimate of the relative ratios of D to L-tryptophan remaining in the samples. This is possible because L-tryptophan absorbs light that produces a negative output, a higher value for CD difference indicates a higher amount of D-Tryptophan in the mixture, relative to the L-tryptophan. Table 2 displays the numerical average of the three samples over the wavelength range of 282-290 nm.

Table 2. Average CD deviation from racemic over wavelength range 282-290 nm

Trial	Average Difference in CD
First	0.455792667
Second	0.563429753
Third	0.438913704

Although the data in Table 2 does not follow a defined trend, it is impossible to know whether it is anomalous without further tests.

L and D- Tryptophan are shown in Figure 9, the chromophore producing each enantiomer's characteristic signal is boxed. Although this particular chromophore was not found in literature, it is likely that this chromophore is responsible for the difference in the signal found from 282-290 nm.

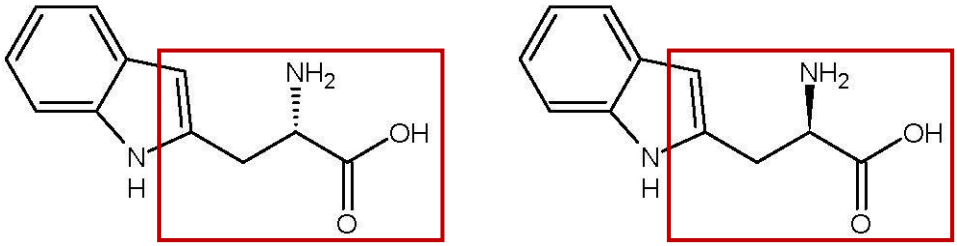


Figure 9. L and D-Tryptophan

Conclusion

Promising results regarding the future of this topic were found in this study. While there is no definitive evidence of dense membrane formation, it is likely that this may be easily accomplished. Furthermore, substantial evidence of the P(AN-*co*-AA) matrix' ability for molecular selectivity was found. Future work should include confirmation and expansion of the results found in this study, as well as the incorporation of peptoids into polymer matrix.

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References

1. Xie, Rui, Chu, Liang-Yin, Deng, Jin-Gen, *Membranes and membrane processes for chiral resolution.* Chemical Society Reviews, 2008. 37(6): p. 1243-1263.
2. Kobayashi, Takaomi, Hong Ying Wang, and Nobuyuki Fujii. "Molecular Imprinting of Theophylline in Acrylonitrile-acrylic Acid Copolymer Membrane." *Chemistry Letters* (1995): 927-28.
3. Zuckermann, Ronald N., Janice M. Kerr, Stephen B. H. Kent, and Walter H. Moos. "Efficient Method for the Preparation of Peptoids [oligo(N-substituted Glycines)] by Submonomer Solid-phase Synthesis." *Journal of the American Chemical Society* 114.26 (1992): 10646-0647.
4. Berova, Nina, Lorenzo Di Bari, and Gennaro Pescitelli. "Application of Electronic Circular Dichroism in Configurational and Conformational Analysis of Organic Compounds." *ChemInform* 38.33 (2007).
5. Mulder, Marcel. "Phase Inversion Membranes." *Encyclopedia of Separation Science* (2000): 3331-346.
6. Kamel, Mostafa. "Handbook of Ultraviolet and Visible Absorption Spectra of Organic Compounds 1967." *Scribd*. Web. 24 Apr. 2012. <http://www.scribd.com/doc/39141355/Handbook-of-Ultraviolet-and-Visible-Absorption-Spectra-of-Organic-Compounds-1967>.
7. Ramakrishna, Seeram, Zuwei Ma, and Takeshi Matsuura. *Polymer Membranes in Biotechnology: Preparation, Functionalization and Application*. London: Imperial College, 2011. 1-37. Web. 12 Apr. 2012. <http://www.icpress.co.uk/etextbook/p636/p636_chap01.pdf>.