# Investigation of Interactions between β-blocker Drugs and Polymers via NMR Diffusion Experiments

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## Abstract

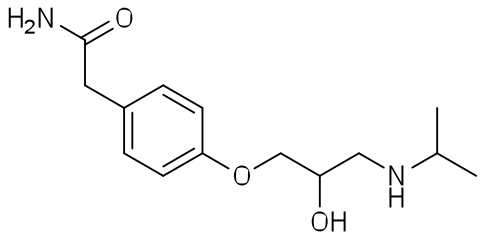
NMR spectroscopy was used to study the binding of β-blockers to chiral polymers. In order to effectively separate these mixtures, the chiral interactions need to be well understood. Chiral polymers were used to study binding constants and free energy values for each analyte. The Propranolol had the strongest interaction with each polymer while Atenolol had the weakest. Correlations were seen between NMR association constants and chromatographic retention times as well as between the NMR free energies of binding and chiral selectivities. It was also concluded the polymer forms a chiral pocket with which the analytes interact.


## Introduction

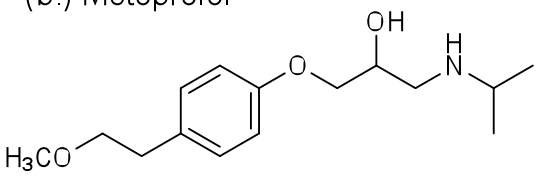
Beta-adrenergic blocking agents, more commonly referred to as β-blockers, have an interesting chiral structure. These analyte structures efficiently inhibit the effects of adrenaline in the body. Beta-blockers are most commonly known for their uses in preventing heart attacks by decreasing the contracting force of the heart muscle which results in increased blood flow and decreased blood pressure. These compounds have differing ring structures which allow them to be used in medicine to specifically treat glaucoma, arrhythmias, and pectoral angina as well as prevent migraines.[1] The β-blockers used in this study are shown in Figure 1.

Figure 1: β-blocker structures

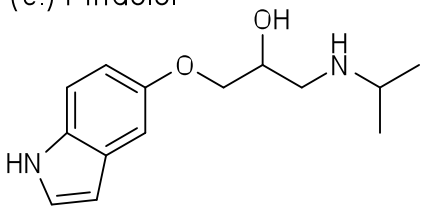
(a.) Atenolol



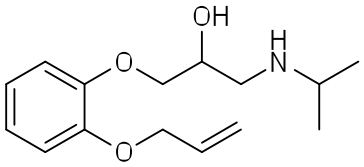
(b.) Metoprolol



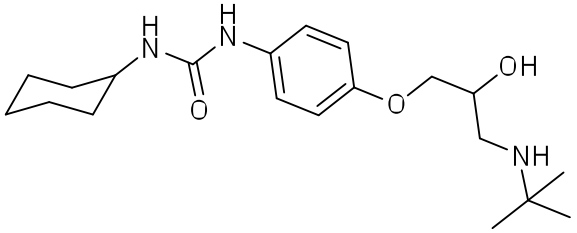
(c.) Pindolol



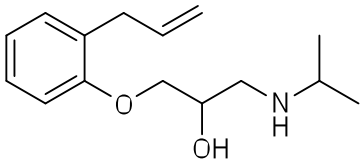
(d.) Oxyprenolol



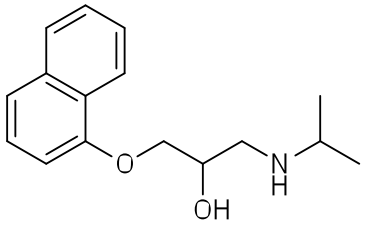
(e.) Talinolol



(f.) Alprenolol

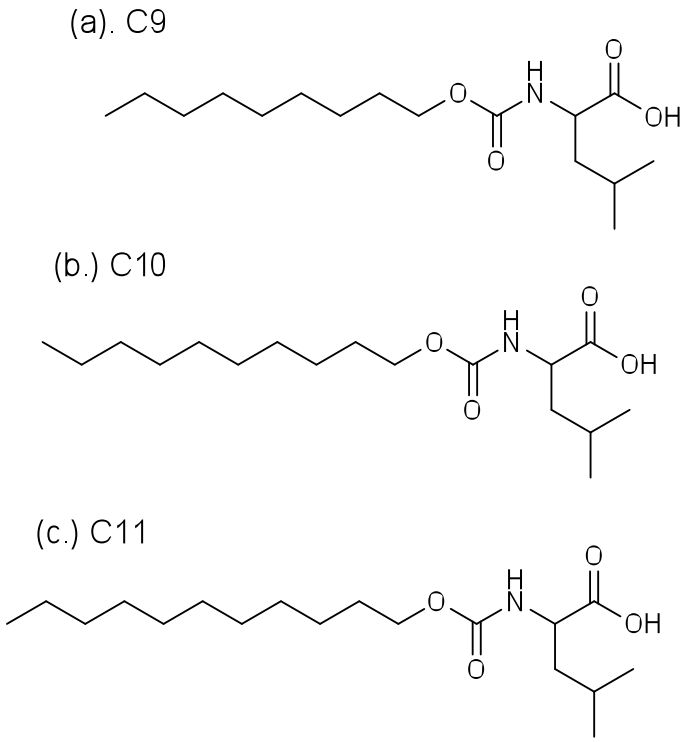


(g.) Propranolol



In this study, we used NMR spectroscopy to study the association of these β-blockers with the chiral molecular micelles. The monomer units for each molecular micelle are shown in Figure 2.

Figure 2: Chiral Molecular Micelle Structures




This study was undertaken because Rizvi, et. al used chiral chromatography in the presence of the molecular micelles in Figure 2 to simultaneously separate enantiomers of all 7 β-blockers in Figure 1.

Their study showed the following:

- The β-blockers eluted in the order Atenolol, Metoprolol, Pindolol, Oxyprenolol, Talinolol, Alprenolol, and Propranolol for the C9, C10 and C11 polymers.
- The chiral selectivity, α, for Atenolol was lowest for the C9 polymer and was larger for C10. The C10 and C11 values were very similar.

In this research NMR spectroscopy was used to study the thermodynamics that govern the binding of the β-blockers to the three polymers in Figure 2. The association between polymer and β-blocker can be represented with the equation with a binding constant given by:

$$K = \frac{[complex]}{[B - bloc\,\mathrm{ker}]\cdot[MM]}$$

The K values can then be used to calculate the free energy of binding, ΔG, with the equation; where R is the gas constant and T is the Kelvin temperature.

$$\Delta G = -RT^* \ln (K)$$

Finally, for Atenolol, ΔG values were measured for the (S) and (R) enantiomers and the quantity Δ (ΔG) was calculated with the following equation:

$$\Delta G = |\Delta G(S) - \Delta G(R)|$$

By comparing our NMR K and ΔG values to those in the Rizvi, et. al paper we hoped to answer the following questions.

- Do the analytes separate based upon differences in their free energies of bonding to each polymer? In other words, does Atenolol, which elutes first, have the least negative ΔG and Propranolol have the most negative ΔG?
- Is there a scaling relationship between the ΔG of binding and the chromatographic retention time?
- For Atenolol, does Δ (ΔG) scale with the α values from the chromatography.

Also, we used two dimensional NMR experiments to study the conformation of the surfactant chain.

These specific surfactant polymers have a chiral carbon center and a hydrocarbon tail of varying carbon length. Surfactants are used in industry as well as domestically. Pharmaceutical applications are but one of their many uses in which the binding constants of enantiomers and molecules with similar structures are studied.

Experimental Procedure

The buffer solution used in all experiments had a pH of 8.8 and was composed of 50mM $NH_4Cl$ and NaOD. The molecular weight of $NH_4Cl$ is 53.49g/mol. This molecular weight was used to determine that a mass of 0.067 grams was required to create 25mL of the buffer. The $NH_4Cl$ was weighed and mixed in a beaker with a 20mL volume of $D_2O$. The pH of the solution was raised with NaOD until it reached 8.8; the buffer solution was diluted to 25mL in a volumetric flask and inverted to complete the buffer solution.

NMR diffusion analyses were conducted with Talinolol and three different polymers. The three polymers studied were Sodium N-noneoxy carbonyl-L-leucinate (C9), N-deceoxy carbonyl-L-leucinate (C10), and N-undecenoxy carbonyl-L-leucinate (C11). The Talinolol samples were prepared using the same procedure. A total of 1.0mg of the analyte (1.5mg for the C11 polymer) and 10mg of polymer were weighed out and added to a small sample vial. One milliliter of the buffer solution was added to the sample vial. The solution was sonicated and vortexed until the sample was mostly dissolved. The Talinolol was hydrophobic and would not fully dissolve into solution. Each solution was transferred via pipet into its respective

labeled NMR tube and capped. After proton spectra had been collected, diffusion experiments were run on each sample. The percent gradients used for the diffusion experiments were as follows: 5, 7, 9, 11, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 40, 45, 50, and 60. Thereafter, the single polymer and two analyte aromatic peak intensities were recorded and input into a Microsoft Excel document. Each diffusion experiment consisted of three trials of 19 experiments a piece, a total of 57 experiments for each diffusion experiment as a whole. The recorded data was graphed for both polymer and analyte with $((26753*\%G*0.004)^2)*(0.0250-0.004/3)$ on the x- axes and the natural log of the peak intensities (sum of aromatic peaks for the analyte graphs) on the y- axes. The variable G represents the percent gradient values listed above. These graphs generated information: $D_{free}$, $D_{obs}$, and $D_b$ values through the slope of the equation of the lines made by the decay of the analyte and polymer. These values are identified as the diffusion coefficient of the β-blocker analyte in free solution, the analyte diffusion coefficient in the polymer solution and the polymer diffusion coefficient, respectively.

This information was fitted into a Microsoft Excel template and used to calculate $f_{bound}$ and K values.

$$D_{obs} = f_b \cdot D_b + (1 - f_b) \cdot D_{free}$$

$$K = \frac{f_b}{(1 - f_b) \cdot [MM]}$$

The K values were used to calculate free energy (ΔG) values using the free energy equation ΔG = -RTlnK. Errors associated with ΔG were also calculated for each ΔG value found and graphed.

The same procedure was done for (R)-Atenolol, (S)-Atenolol, Pindolol, Oxyprenolol and Alprenolol however, a total of 2.0mg of analyte and 12mg of polymer were used as with previous trials of the research. These repeated experiments were conducted only in the polymer(s) whose data needed to be reanalyzed.

The free diffusion experiment for the Talinolol was conducted with 1mg of Talinolol sonicated and vortexed with 1mL of the buffer. The percent gradients used for this experiment were 3, 5, 7, 9, 11, 12, 14, 16, 18, 20, 22, and 24. In total, three trials of 12 gradients were run to give a total of 36 diffusion data sets. The same information was graphed in a Microsoft Excel file as mentioned above.

NOESY and TOCSY experiments were conducted on samples with the use of a slightly different buffer so that the nitrogen in the polymers would be protonated. This buffer was also at a pH of 8.8 and made of 50mM $NH_4Cl$ and NaOD. The molecular weight of the $NH_4Cl$ was

used to calculate that a mass of 0.027g was needed for the creation of 10mL of buffer solution. The buffer was made with 90% water and 10%$D_2O$. Each NOESY and TOCSY sample consisted of 10mg of polymer and 1mL of the buffer which was vortexed until all polymer entered solution. Each spectrum was analyzed, each peak was identified, and the spectra collectively determined these were reasonable and accurate interactions between the hydrogens in the molecules.

The addition of the amine proton in the TOCSY and NOESY Spectra displayed an extra interaction between spin systems. The proton allowed the molecule to be drawn in 3D with the Spartan Program and assisted in visualization of a chiral pocket.

However, this additional interaction was not informative enough to declare that the chiral pocket did exist. NOESY experiments were run on the polymers using (S)-BNP to test the hypothesis that the surfactant polymers were making a chiral pocket. Ten milligrams of polymer and 2mg of (S)-BNP were sonicated and vortexed until fully dissolved in 1mL of the buffer previously mentioned for use with the diffusion experiments.

In order to determine that the (S)-BNP was the correct enantiomer to use to study the chiral pocket, binding constants were studied for the (S)-BNP and the (R)-BNP via diffusion experiments. The samples for these experiments were composed of 10mg of polymer and 2mg of either the (S)-BNP or the (R)-BNP analyte. The buffer used was the $D_2O$ buffer which was used for previous diffusion experiments.

The results in tables 1-4 show that (R)-BNP sometimes binds more strongly to the polymer. T-tests were conducted on the binding constants for the C9 polymer and were determined to be statistically the same. Therefore, additional NOESY experiments did not need to be conducted for the C9 polymer and the (R)-BNP in order to conclude the polymer forms a chiral pocket. NOESY spectra were collected with the polymers and these spectra were used to produce binding maps.

## Results and Discussion

The NOESY experiments on all 3 polymer compounds showed the same interactions. Some were stronger and some were weaker however, the interactions labeled with the arrows in Figure 3 were the only two interactions that suggested the polymer formed a chiral pocket. The interactions were not enough evidence that the polymer was forming a chiral pocket for the analytes to interact with.

Figure 3: NOESY Interactions of the surfactant structure

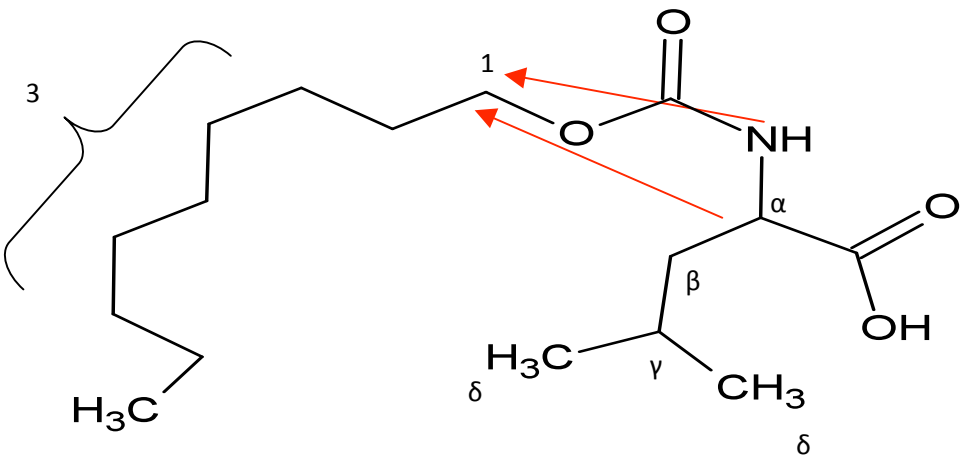



Table I: Binding Constants of 1', 1'-Binapthyl-2', 2'-diyl hydrogen phosphate (BNP) with the Three Polymers

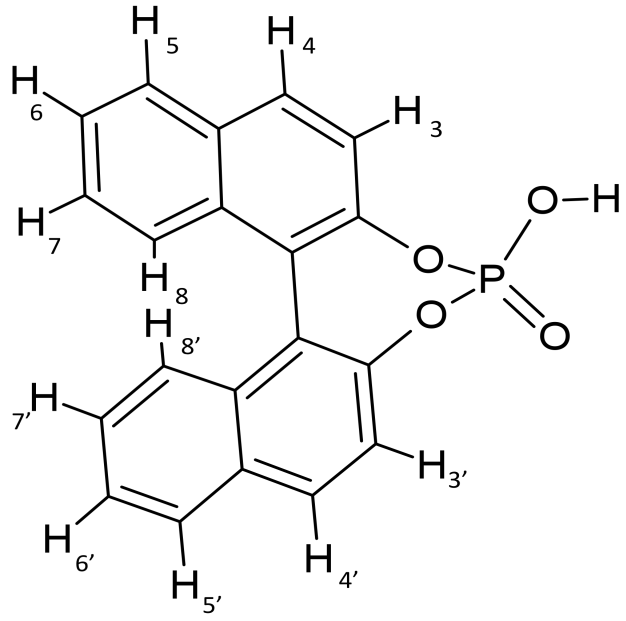
| Binding Constants K ($M^{-1}$) | | |
|---|---|---|
| C9 | | |
| | (S)-BNP | (R)-BNP |
| | 62 ± 2 | 64 ± 3 |
| C10 | | |
| | 82 ± 3 | 75 ± 3 |
| C11 | | |
| | 63 ± 3 | 58 ± 2 |

The binding constants of the polymers to the different enantiomers of BNP indicate which binding map data to use in order to determine the formation of a chiral pocket. In each case (S)-BNP was used in the binding studies. (S)-BNP had a larger binding constant than (R)-BNP with the C10 and C11 polymers. The binding constants for (R)-BNP and (S)-BNP to the C9 polymer were not statistically different.

Binding Maps for (S)-BNP interacting with the Leucine $H_\alpha$ and hydrocarbon chain protons are shown in Table II. Proton labels for BNP are shown in Figure 4.

| Leucine Alpha Proton | | | | | | |
|---|---|---|---|---|---|---|
| | H3 (H3') | H4 (H4') | H5 (H5') | H6 (H6') | H7 (H7') | H8 (H8') |
| C9 | 57.28% | 88.79% | 62.23% | 56.52% | 69.04% | 100.00% |
| C10 | 80.70% | 83.70% | 84.10% | 85.80% | 94.61% | 100.00% |
| C11 | 72.12% | 91.84% | 65.07% | 84.32% | 66.39% | 100.00% |
| Hydrocarbon Chain | | | | | | |
| | H3 (H3') | H4 (H4') | H5 (H5') | H6 (H6') | H7 (H7') | H8 (H8') |
| C9 | 28.09% | 37.04% | 100.00% | 96.04% | 75.50% | 56.48% |
| C10 | 39.05% | 49.53% | 99.93% | 100.00% | 96.30% | 68.30% |
| C11 | 36.10% | 46.45% | 100.00% | 96.88% | 87.87% | 64.23% |

Figure 4:




The tables above and their corresponding figures suggest that the polymer structures do create chiral pockets in which the β- blocker analytes insert into. This conclusion is proven

because there are two areas of binding preference. One end of the molecule, namely BNP protons $H_3$, $H_4$ and $H_8$, selectively binds more strongly with the polar head group while the other end, containing $H_5$, $H_6$ and $H_7$, selectively binds with the nonpolar hydrocarbon tail. Note that in all three binding maps, percentages are larger for $H_3$, $H_4$ and $H_8$ in maps to the Leucine $H_\alpha$. Conversely, percentages are larger for the $H_5$, $H_6$ and $H_7$ protons in maps to the hydrocarbon chain. Previous studies on other surfactants with BNP have been conclusive in the determination that these interactions indicate the surfactant forms a conformation like that shown in Figure 3.[2] This folded polymer structure must occur in order to interact with both ends of the molecule in the NMR data analysis.

The diffusion coefficients for both polymer and analytes in all mixtures studied are shown in Table III:

| C9 | | | |
|---|---|---|---|
| Analyte | $D_{free}$x $10^{10}$ ($m^2s^{-1}$) | $D_{bound}$ x $10^{10}$ ($m^2s^{-1}$) | $D_{obs}$ x $10^{10}$ ($m^2s^{-1}$) |
| (S)-Atenolol | 5.54 ± 0.03 | 1.02 ± 0.01 | 3.49 ± 0.06 |
| (R)-Atenolol | 5.54 ± 0.03 | 1.03 ± 0.02 | 3.36 ± 0.02 |
| (±)- Metoprolol | 5.22 ± 0.12 | 0.94 ± 0.04 | 2.01± 0.03 |
| (±)-Pindolol | 5.34 ± 0.04 | 1.74 ± 0.06 | 9.869 ± 0.008 |
| (±)-Oxyprenolol | | | |
| (±)-Talinolol | 4.06 ± 0.03 | 1.05 ± 0.04 | 0.97 ± 0.003 |
| (±)-Alprenolol | | | |
| (S)-Propranolol | 5.59 ± 0.04 | 0.84 ± 0.01 | 0.85 ± 0.01 |
| (R)-Propranolol | 1.0 ± 0.04 | 1.00 ± 0.01 | 1.03 ± 0.06 |
| | | | |
| C10 | | | |
| Analyte | $D_{free}$x $10^{10}$ ($m^2s^{-1}$) | $D_{bound}$ x $10^{10}$ ($m^2s^{-1}$) | $D_{obs}$ x $10^{10}$ ($m^2s^{-1}$) |
| (S)-Atenolol | 5.54 ± 0.03 | 0.93 ± 0.03 | 3.55 ± 0.02 |
| (R)-Atenolol | 5.54 ± 0.03 | 0.86 ± 0.02 | 3.17 ± 0.01 |
| (±)- Metoprolol | 5.22 ± 0.12 | 0.92 ± 0.04 | 2.19 ± 0.03 |
| (±)-Pindolol | 5.34 ± 0.04 | 1.62 ± 0.01 | 0.90 ± 0.01 |
| (±)-Oxyprenolol | | | |
| (±)-Talinolol | 4.05 ± 0.04 | 1.02 ± 0.06 | 0.88 ± 0.01 |
| (±)-Alprenolol | 5.54 ± 0.10 | 0.79 ± 0.05 | 0.65 ± 0.05 |
| (S)-Propranolol | 5.59 ± 0.04 | 0.80 ± 0.01 | 0.86 ± 0.05 |
| (R)-Propranolol | 5.59 ± 0.04 | N/A | N/A |
| | | | |
| C11 | | | |
| Analyte | $D_{free}$x $10^{10}$ ($m^2s^{-1}$) | $D_{bound}$ x $10^{10}$ ($m^2s^{-1}$) | $D_{obs}$ x $10^{10}$ ($m^2s^{-1}$) |
| (S)-Atenolol | 5.53 ± 0.03 | 2.91 ± 0.03 | 0.93 ± 0.002 |
| (R)-Atenolol | 5.53 ± 0.03 | 2.52 ± 0.04 | 0.86 ± 0.0007 |
| (±)- Metoprolol | 5.22 ± 0.12 | 0.88 ± 0.01 | 1.91 ± 0.01 |
| (±)-Pindolol | 5.34 ± 0.04 | 1.50 ± 0.11 | 0.93 ± 0.03 |
| (±)-Oxyprenolol | 5.54 ± 0.10 | 1.16 ± 0.06 | 0.77 ± 0.005 |
| (±)-Talinolol | 4.05 ± 0.04 | 1.01 ± 0.01 | 0.87 ± 0.01 |
| (±)-Alprenolol | 5.54 ± 0.10 | 0.75 ± 0.01 | 0.68 ± 0.01 |
| (S)-Propranolol | 5.59 ± 0.04 | 0.79 ± 0.03 | 0.80 ± 0.03 |
| (R)-Propranolol | 5.59 ± 0.04 | 0.79 ± 0.02 | 5.59 ± 0.04 |

Values calculated from diffusion experiments were inserted into the equation below and used to calculate the value of the fraction of analyte bound to the polymer in free solution.

$$D_{obs} = f_b \cdot D_b + (1 - f_b) \cdot D_{free}$$

From the calculated fraction bound value, the binding constant, K, was calculated via a modified formula and then used to calculate free energy values as well with the Gibs free energy equation.

$$K = \frac{f_b}{(1 - f_b) \cdot [MM]} \qquad \Delta G = -RT^* \ln (K)$$

Fraction bound, binding constants and free energy values are shown in Table IV:

| C9 | | | |
|---|---|---|---|
| Analyte | $F_{bound}$ | K ($M^{-1}$) | ΔG (KJ/mol) |
| (S&R)-Atenolol | 0.469 ± 0.013** | 22.8 ± 0.9 | 7746.8 ± 305.8 |
| (±)- Metoprolol | 0.749 ± 0.033 | 76.9 ± 4.9 | 10758.9 ± 685.5 |
| (±)-Pindolol | 0.8263 ± 0.0187 | 142.9 ± 5.5 | 12294.1 ± 473.2 |
| (±)-Oxyprenolol | | 465 ± 21 | 15217.3 ± 687.2 |
| (±)-Talinolol | 0.9724 ± 0.0202 | 1059 ± 37.81 | 17256.5 ± 619.2 |
| (±)-Alprenolol | | 1538 ± 77 | 18181.0 ± 910.2 |
| (S&R)-Propranolol | 0.987 ± 0.018** | 6350 ± 150 | 21694.1 ± 512.5 |
| | | | |
| C10 | | | |
| Analyte | $F_{bound}$ | K ($M^{-1}$) | ΔG (KJ/mol) |
| (S&R)-Atenolol | 0.480 ± 0.010** | 23.1 ± 0.7 | 7779.2 ± 235.8 |
| (±)- Metoprolol | 0.705 ± 0.032 | 61.7 ± 4.0 | 10213.3 ± 662.1 |
| (±)-Pindolol | 0.8364 ± 0.0122 | 161.3 ± 5.02 | 12594.2 ± 390.4 |
| (±)-Oxyprenolol | | 428 ± 23 | 15011.9 ± 806.7 |
| (±)-Talinolol | 0.9577 ± 0.0253 | 715.3 ± 31.35 | 16284.3 ± 714.8 |
| (±)-Alprenolol | 0.9714 ± 0.0031 | 1073 ± 54 | 17289.0 ± 518.0 |
| (S&R)-Propranolol | 0.988 ± 0.015** | 2210 ± 60 | 19079.2 ± 518.0 |
| | | | |
| C11 | | | |
| Analyte | $F_{bound}$ | K ($M^{-1}$) | ΔG (KJ/mol) |
| (S&R)-Atenolol | 0.566 ± 0.012** | 43.3 ± 1.4 | 9333.0 ± 302.1 |
| (±)- Metoprolol | 0.761 ± 0.032 | 82.2 ± 4.9 | 10924.0 ± 651.2 |
| (±)-Pindolol | 0.8715 ± 0.0280 | 186.4 ± 11.5 | 12952.5 ± 799.1 |
| (±)-Oxyprenolol | 0.9182 ± 0.0326 | 308.5 ± 16.8 | 14200.8 ± 772.9 |
| (±)-Talinolol | 0.9569 ± 0.0165 | 611.3 ± 20.8 | 15895.1 ± 540.8 |
| (±)-Alprenolol | 0.9781 ± 0.0030 | 2100 ± 107.6 | 18952.7 ± 971.1 |
| (S&R)-Propranolol | 0.997 ± 0.013** | 8250 ± 250 | 22342.7 ± 677.1 |

**Average values have been taken between enantiomers.

The binding constants for the C9 polymer were t-tested. All binding constant terms are significantly different from each other at a 90% confidence interval. It is not until the 99.9% confidence interval is examined that the binding constants for Talinolol and Alprenolol become statistically the same.

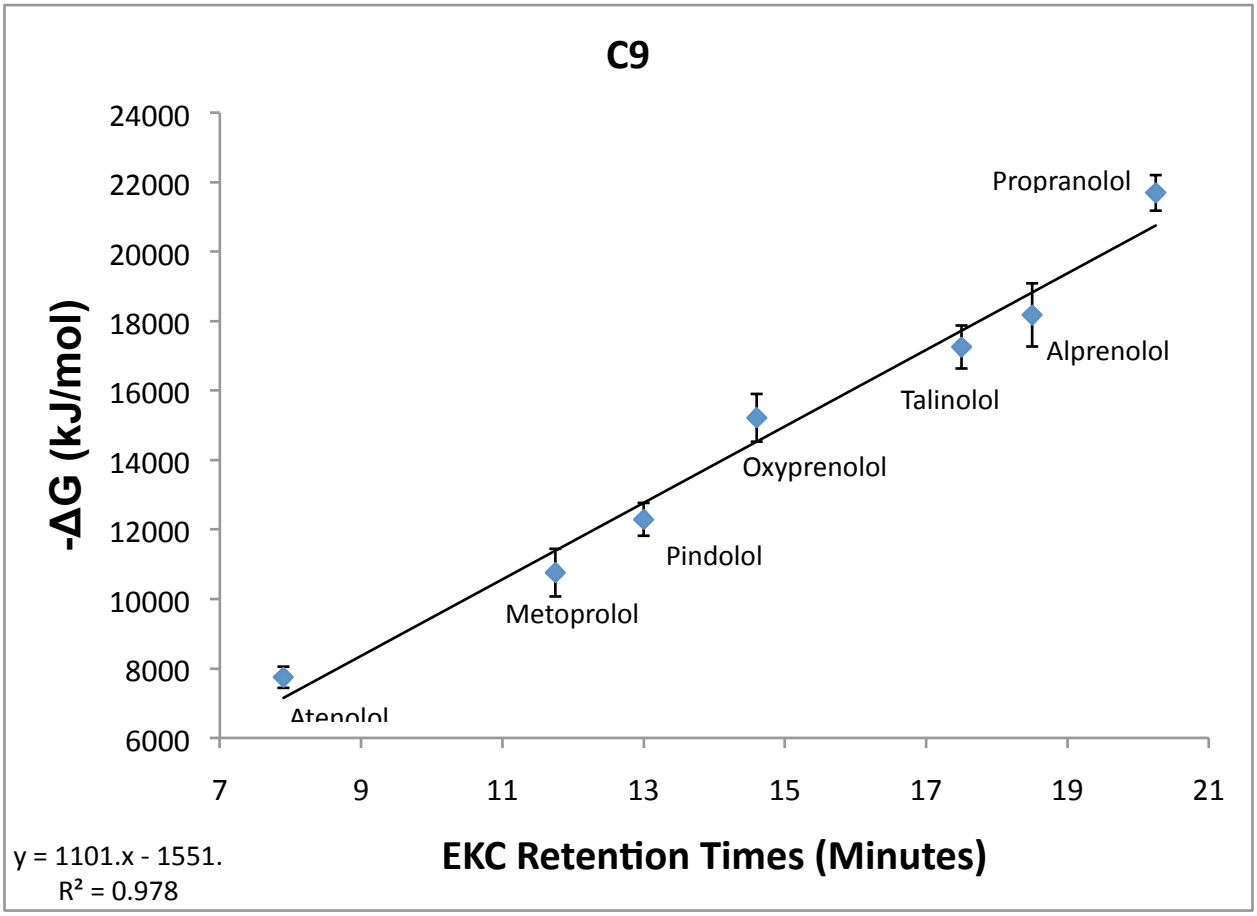
T-tests were also performed on the C10 binding constants. All binding constants at the 90% confidence interval are statistically different from one another. At the 99.9% confidence

interval, the only values that are statistically the same are Oxyprenolol and Talinolol, and Talinolol and Alprenolol. The majority of the binding constants test to be statistically different at the 99.9% confidence interval.

Finally, when the t-tests were performed on the binding constants for the β-blockers and C11, all binding constants test to be significantly different at a 90% confidence interval. The Pindolol and Oxyprenolol test to have binding constants that are statistically the same at a 99.9% confidence interval. These are not immediately apparent in the retention time vs. ΔG graph.

Free energy values of binding for each of the β-blockers were graphed versus electrokinetic chromatography retention times, in minutes. The retention times were from the results of previous researchers from Georgia State University.[3] Figure 5 shows a graph of –ΔG vs. retention time for the C9 polymer.

Figure 5: C9 Correlations between EKC Retention Times and Free Energy Values

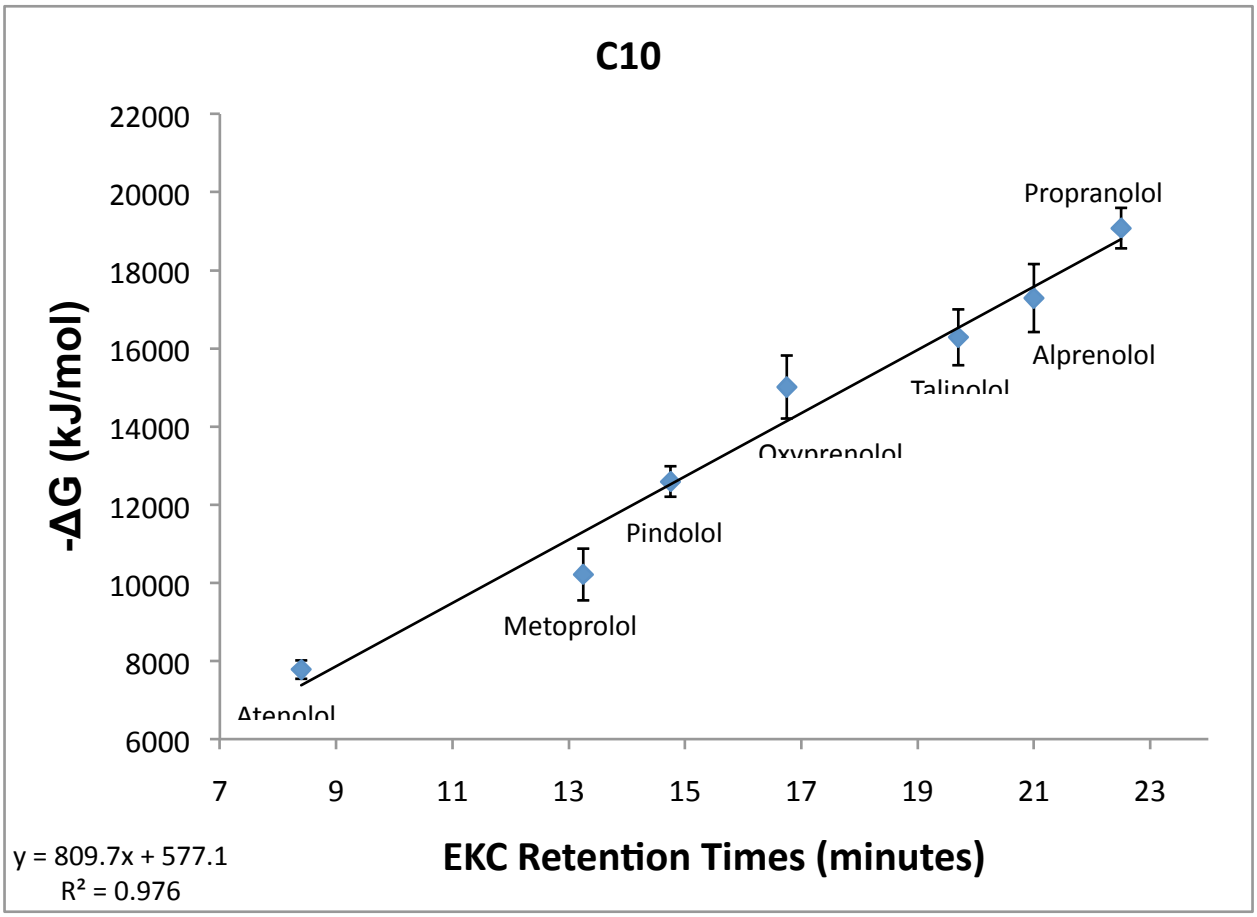



The C9 results shown in Figure 5 show that the β-blockers separated in the Rizvi, et. al study based upon differences in their free energies of binding to the polymer. For example,

Figure 5 shows that Atenolol had the least negative ΔG followed by Metoprolol, Pindolol, Oxyprenolol, Talinolol, Alprenolol and finally Propranolol. This is exactly the order in which the β-blockers eluted in the Rizvi, et. al study with Atenolol eluting first followed by Metoprolol, Pindolol, Oxyprenolol, Talinolol, Alprenolol and finally Propranolol. The strong relationship between elution order and free energy of binding is also illustrated by the relatively linear relationship between –ΔG and retention time. When these values were plotted and a linear regression line was performed, an $R^2$ value of 0.978 was obtained.

Some β-blockers had similar free energy of binding values so t-tests were done to determine which ΔG values were statistically different. All β-blockers tested to have ΔG values that are statistically different from one another except Talinolol and Alprenolol. At the 90% confidence interval, Talinolol and Alprenolol have free energy values that test to be the same. Upon a more critical examination at the 95% confidence interval, Oxyprenolol and Talinolol have ΔG values that are statistically the same as well as Metoprolol and Pindolol.

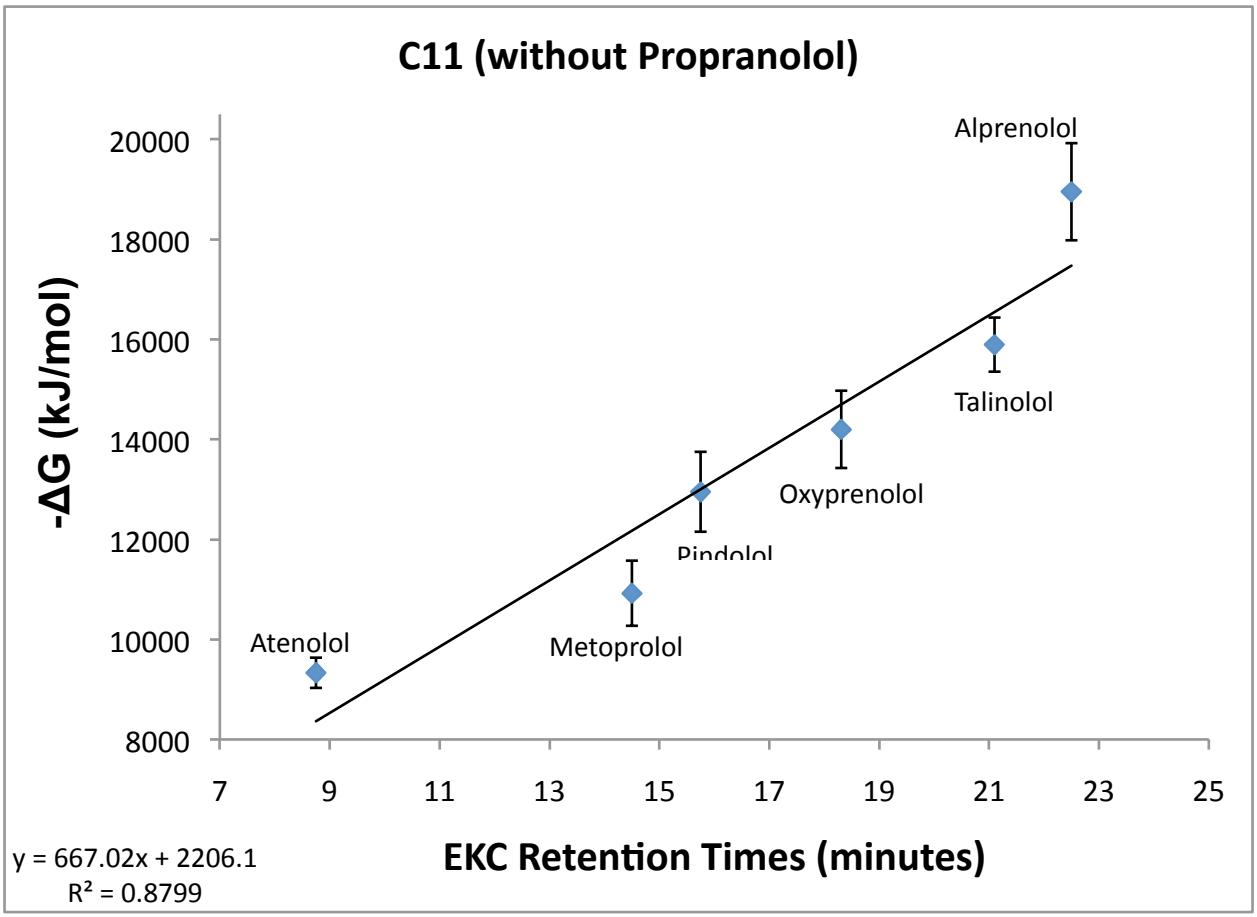
Figure 6: C10 Correlations between EKC Retention Times and Free Energy Values



Plots of each β-blocker's free energy of binding vs. retention time are plotted in Figure 6 for the C10 polymer. Again the plot confirms that the β-blockers elute in chromatography based upon differences in the free energies of binding because the ΔG values rank in the exact same order as the elution order. A linear relationship between free energy of binding and retention time was observed for the C10 polymer as well with an $R^2$ value of 0.976. This $R^2$ value is similar to the value of 0.978 obtained for C9. It is also interesting to note that the slope of the ΔG vs. retention time graph for C10 is less than the slope for the C9 graph.

Error bars graphed on the C10 retention time vs. ΔG graphs determined a need for closer examination of the free energy data. At the 90% confidence interval, the β-blocker ΔG values for Oxyprenolol and Talinolol and for Talinolol and Alprenolol test to be statistically the same. Talinolol and Alprenolol are hydrophobic analytes and are difficult to dissolve in solution and often have a tendency to crash out of the solution. This effect contributes to less accurate ΔG and K values. At the more critical 95% confidence interval, the free energy values for the Alprenolol and the Propranolol test to be statistically the same as well as those for Oxyprenolol and Alprenolol. This result can be explained with the amount of error associated with the moderately hydrophobic Oxyprenolol and the hydrophobic Alprenolol and Propranolol. Each of these analytes have a larger error bar associated with their free energy value. These similarities and differences are visibly apparent on the graphs for the C10 polymer.

Figure 7: C11 Correlations between EKC Retention Times and Free Energy Values




*Propranolol was removed from the correlation due to polymer aggregation and inaccurate diffusion coefficients resulting in binding constants

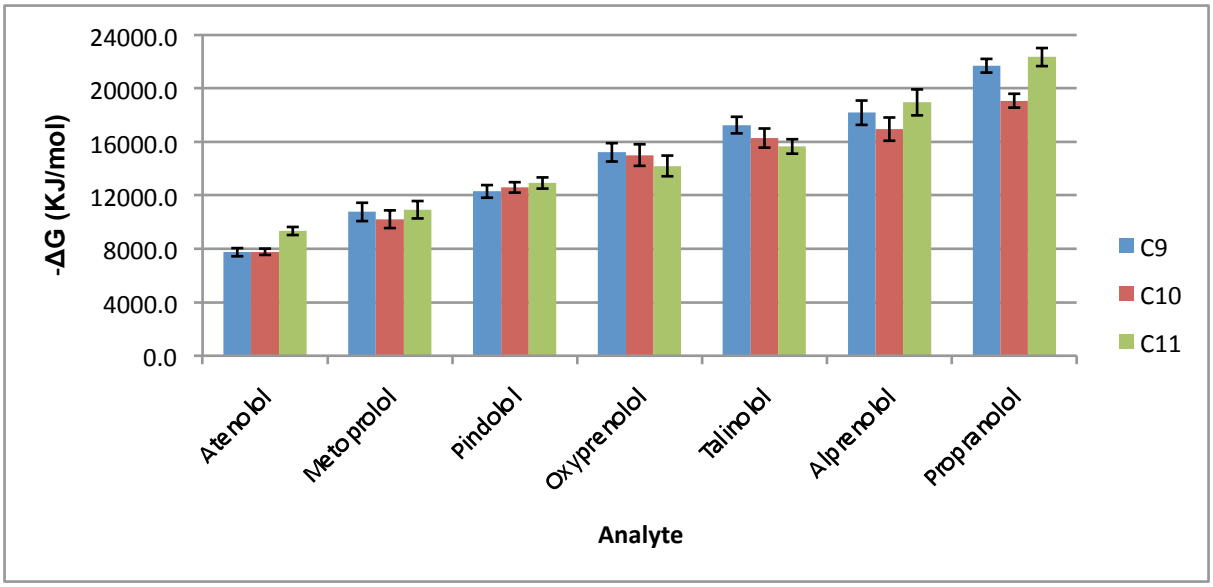
Figure 7 shows free energy of binding for each β-blocker to the C11 polymer. Again the ΔG values agree with the elution order reported by Rizvi, et. al. However, here the correlation between the ΔG value and retention time is much weaker than with the C9 and C10 polymers. An $R^2$ value of only 0.880 was obtained for the plot with the C11 data. Furthermore, the slope of the ΔG vs. retention time graph was found to be the smallest for the C11 data set.

Upon a critical examination of the C11 retention time vs. ΔG graphs at a 90% confidence interval, the free energy value for Pindolol is statistically the same as that of Oxyprenolol. The free energy values for Oxyprenolol and Talinolol also test to be statistically the same. The Pindolol exhibited hydrophobic characteristics and fell out of solution over time. This may be a reason for the large amount of error associated with it and why this β-blocker tests to be statistically the same as the Oxyprenolol. In fact, the error on the Pindolol is so large, it tests to

be statistically the same as the Metoprolol at the 95% confidence interval. The same problem occurred with the Talinolol and may be why the Oxyprenolol and Talinolol free energy values overlap and test to be statistically the same as well. Although the error on the Talinolol is not very large in comparison to that of the Pindolol, the values for the Talinolol read very low in comparison to the other β-blocker's loose correlation. Oxyprenolol also has a large error associated with it. This may be due to the fact that Oxyprenolol is also moderately hydrophobic. Overall, the large error bars and difficulty carrying out the experiments with C11 make it difficult to draw definitive conclusions about the relationship between free energy of binding and retention time.

To summarize, the free energy graphs previously shown (Figures 5-7) demonstrate a correlation between EKC retention times and free energy values of each β-blocker and polymer mixture. As the polymer gains additional carbons in the hydrophobic hydrocarbon tail, the correlation between the retention time in minutes and the free energy value becomes less tight as indicated by the $R^2$ value on each graph (found in the lower left hand corner).

Figure 8: Free Energy Value Comparison Between Polymers and β-blockers

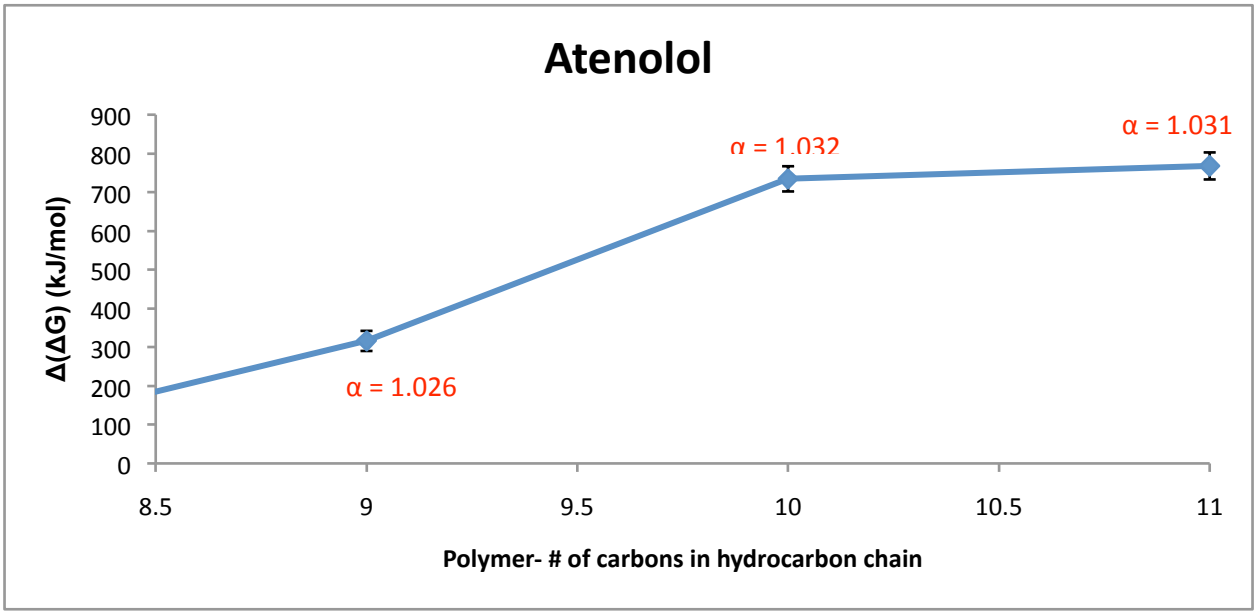



The magnitude of the ΔG values for the β-blockers rank in the same order when interacting with each polymer. The effect is shown in Figure 8. However, there does not appear to be a trend in the way the hydrophilic and hydrophobic analytes interact with polymers of different hydrocarbon chain length. The Atenolol and Pindolol demonstrate more negative ΔG value as the methylene groups are added. Oxyprenolol and Talinolol however decreased in binding affinity when the hydrocarbon tails got longer. Finally, Metoprolol,

Alprenolol, and Propranolol show a decrease in negative free energy values from the C9 to C10 polymer but then a large increase in negative free energy from the C10 to C11 polymer.

Figure 9 shows the Δ (ΔG) values for Atenolol plotted against the number of carbons in the polymer's hydrocarbon chain. Alpha values determined by Rizvi, et. al appear above each point.

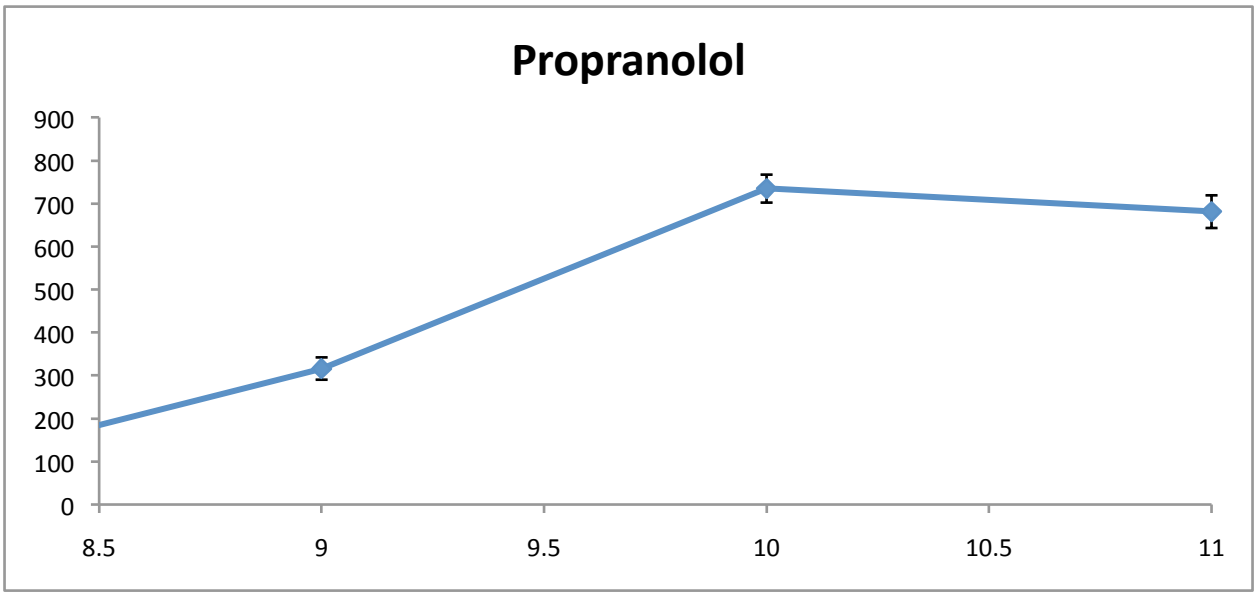
Figure 9: Atenolol free energy value comparison




The Δ (ΔG) values for the Atenolol analyte T test to be statistically different between the C9 and C10 polymer as well as between the C9 and C11 polymer. The C10 and C11 polymers have free energy values that T test to be statistically the same. These results are consistent with those found for alpha values for the Atenolol analyte in the by Rizvi, et. al. Note that the α value for C9 is lower than C10 and C11 and the C10 and C11 values are virtually the same. This result confirms that in the separations of Atenolol analytes separate based upon their free energies of binding to the polymer.

The same trend can be found in the Δ (ΔG) values for the Propranolol analyte. Note that in Figure 9, the C9 α value is smaller than the C10 or C11 and the C10 and C11 values are almost equal. Alpha values for Propranolol were not published in the Rizvi, et. al paper, but the chromatography results presented clearly show that chiral resolution is highest with C9 and much lower with the C10 and C11. Again these results show that the analyte enantiomers separate based upon differences in the free energy of binding with a large α value resulting from a large Δ (ΔG).

Figure 10: Propranolol free energy comparison




Conclusion

The surfactant head group of the polymer was determined to form a chiral pocket due to the binding maps constructed from NOESY experiments. The binding maps show that one side of the BNP molecule preferentially interacts with the Leucine alpha proton of the polymer headgroups while the other side of the BNP molecule binds more strongly with the hydrocarbon tail. These results were similar to those of previous studies and indicated the formation of a chiral pocket into which the (S)-BNP and other analytes inserted.[4] Diffusion experiments, binding constants, fraction bound values and free energy values supported the chromatography elution order. The order of elution of the β-blockers from the Rizvi, et. al study was accurately represented by the strength with which the analytes bound to the polymer structures. The polymer, because it is so large, has a large diffusion coefficient and moves slowly through the matrix. The stronger the analyte binds to the polymer, the slower the complex will travel through the matrix. When these molecules move more slowly relative to each other based on preferential binding constants, an elution order can be determined. It was hypothesized that the weaker the binding to the polymer, the quicker an analyte will elute. Atenolol has the smallest K value and the least negative free energy while Propranolol has the largest K value and the most negative free energy value. Atenolol elutes first; Propranolol elutes last. All β-blockers between the Atenolol and Propranolol bind to the polymer in their order of elution. The binding constants indicate that Atenolol, Metoprolol, Pindolol, Oxyprenolol, Talinolol, Alprenolol and Propranolol elute in that order based upon that hypothesis. The order based off the binding constants and free energies is depicted in the EKC

retention time graph from the chromatography paper. This order is also supported by the fact that Propranolol is the most nonpolar of the studied analytes. The β-blockers bound more strongly to the C9 polymer than the C10 polymer in most cases (not including the Atenolol and the Pindolol). The results also show a strong correlation between the Atenolol α values and the Δ (ΔG) values from NMR. When Δ (ΔG) is large, α is also large and chiral resolution is high.

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