Comments on the Answers to Exercise, 20, 21
Chem 8157 – Spring 2008

Every group did manage to report a Kd value. The values are summarized below.

Question 8. The only issue of concern is the determination of the lowest and highest Kd that could be experimentally measured for a similar system (question 8).

1. Perhaps, the most satisfactory answer (without actually doing the experiment with the new system, would come from assuming that the **same concentration of ligand are used.** Then, determine the extreme variations that could be measured.

For example, assign the minimum separation in the CE peaks to the highest concentration of AT. Indeed, you then need to assume that other AT concentrations have even smaller undetectable peak separations. At the other extreme, you may consider that the lowest concentration causes a peak shift that it is barely within the migration time window of the electropherogram because the shift is very large. You would then need to assume that other peaks would be out of this range.

2. The other possibility is to base your estimates on the errors associated with Kd. Please note that the Kd comes from the slope in a Scatchard plot or from the error in the 'e' coefficient in the binding isotherm. Consider then, for the Scatchard plot for example, that one can detect a slope that is as small as 3x Std. Dev. above a slope of zero. The other extreme comes from considering the the slope is infinity. One then need to consider the smallest measurable value of the x-axis and the highest measurable value on the y-axis.

Similar arguments would hold when using the 'e' coefficient to estimate the limits.

Keep in mind that these are only estimates!

Why are the values so different?
Data analysis – the quality of the data limits their resolution; a large error comes from the actual measurements. This factor is particularly important for the Gel, AT experiments. It does not matter whether an x-reciprocal plot or a NLR plot is used.

The technique – CE determinations provide migration time data with better precision than the gel data.

Heparin heterogeneity – It appears that the CE and the Gel reports used different types of heparin.

Group	Method	Fitting	Kd Value (nM)	Lowest Kd	Highest Kd
1	Gel, AT	Scatchard	7.62±0.01	4.8	16210
2	Gel, AT	NLR	1.7±0.4	1.3	2.1
3	**CE, AT**	**Scatchard**	**(20±10)x1000**	**11,000**	**100,000**

4	CE, AT	NLR	(32±13)X1000	39,000	
5	Gel, bFGF	Scatchard	1.3		
Literature	Gel, AT	Scatchard	16, 12	11	100
Literature	CE, AT	NLR	(20±60x1000		
Literature	Gel, bFGF	Scatchard	2	0.3	35