Solutions to problems for Chapter 6

6.1 The results of the calculations are tabulated below.

Protein	A_{280} (stock)	A_{205} (stock)	Concentration (mg mL^{-1})		
			Based on A_{205}	Based on A_{205} and A_{280}	Based on A_{280}
BSA	1.22	54.8	1.77	1.85	1.85
Lysozyme	5.39	72.6	2.34	2.02	2.05
Trypsin	2.59	53.8	1.74	1.64	1.65
Ovalbumin	1.32	57.3	1.85	1.93	1.91

From this we can see that the method based on A_{205} alone using eqn. 6.4 gives values that can differ significantly from the 'true' concentrations based on A_{280} measurements. This is particularly true for lysozyme with an unusually high Trp content which contributes to the A_{205} values. Use of A_{280} in conjunction with A_{205} (according to eqn. 6.5) gives values that are very close to the 'true' concentrations.

6.3 1) Plot log (molecular mass) vs distance travelled and then use this plot to estimate the molecular masses of the two proteins enclosed in the circle (~25 and ~27.5 kDa) and the protein band indicated by the arrow (~60 kDa).

2) The larger membrane protein has a mass of 27.5k Da, whereas the smaller membrane protein has a mass of 25 kDa. We can estimate what this difference in mass corresponds to terms of numbers of amino acids, since the average mass of amino acids in proteins is 110 Da (see Chapter 2, section 2.1). Thus the difference between the two proteins (2.5 kDa) is equivalent to 2 500/110 Da amino acids, i.e. 22.7 amino acids. A peptide of this length may represent a targeting (signal) peptide, which is later removed to give the mature integral membrane protein.

3) As the proteins indicated by the arrow have identical amino acid sequences but different pI values, they must differ in their post-translational modification e.g. they may represent different phosphorylated forms of the same protein.

6.5 From Chapter 4, section 4.3.1, $K_d = 1/K_a = 1.1 \times 10^{-3}$ M. $-\Delta G^0 = RT \ln K_d$; so putting $K_d = 1.10 \times 10^{-3}$ (M), $\Delta G^0 = 16\,870$ J mol^{-1}. Since $\Delta G^0 = \Delta H^0 - T\Delta S^0$ (Chapter 4, section 4.1), $\Delta S^0 = (25\,000 - 16\,870)/298 = 27.3$ J K^{-1} mol^{-1}. (Note ΔH^0 for the dissociation reaction = +25 kJ mol^{-1}).