1) (15%) The partial specific volume ($\bar{v}$) of a DNA molecule was measured and found to be 0.50 ml/g in 150 mM NaCl. After dialyzing the molecule against a low salt buffer (1 mM NaCl), the $\bar{v}$ increased to 0.55 ml/g. Explain why the molecule has an increased $\bar{v}$ in low salt – what is happening to the molecule in low salt?

At high salt, the PO4- groups are complexed with Na+ ions that neutralize the negative charges. When the molecule is bound to Na+ ions, which are dense metal, the overall density of the particle increases, because metal is more dense than DNA. The opposite occurs when DNA is put into a low salt environment and some of the Na+ ions are stripped from the DNA. In that case, there is a negative charge on the DNA molecule and water molecules will reorient themselves to align their dipoles with the negative charges. The interaction between water and the DNA is strong enough to make it appear that the water is co-migrating with the DNA molecule. Water is less dense than DNA or Na+, so replacing Na+ with water molecules decreases the density of the overall particle, and since $\bar{v}$ is the inverse of density, the $\bar{v}$ goes up.

2) (15%) In going from high salt to low salt, would the anhydrous DNA molecular weight increase, decrease or stay the same? Explain your answer.

Since the DNA is unchanged, the DNA molecular weight would stay the same. The sedimenting particle, however, now also has a bunch of waters bound in addition, so its molecular weight would go up, but that's not what was asked.

3) (15%) In going from high salt to low salt, would the sedimentation coefficient increase or decrease or stay the same? Explain your answer.

Now we are talking about the sedimenting particle that includes the bound waters. Since the $\bar{v}$ is increased, the term (1- $\bar{v}$ ρ) is smaller, which makes the sedimentation coefficient smaller.

4) (15%) In going from high salt to low salt, would its diffusion coefficient increase or decrease or stay the same? Explain your answer. Hint: $D=RT/(Nf)$, where D is the diffusion coefficient, R is the universal gas constant, T is the temperature, N is Avogadro's number, and f is the frictional coefficient.

When the small Na+ ions are replaced by a bunch of bound water molecules, which are about twice as large as a Na+ ion, the sedimenting particle becomes larger. This will increase its friction, which decreases D.

5) (15%) Compared to a sedimentation speed in a velocity experiment performed at 40,000 rpm, the same sample measured at 20,000 rpm will sediment... (write down the equation for the relevant force term)

a) twice as fast
b) four times as fast
c) half as fast
d) a quarter as fast ← Correct answer
e) the same
f) it will float

Equation: $s = v/(\omega^2 r)$, and $v = s\ \omega^2 r$ (slide 46), so cutting the rotor speed in half will result in 1/4th the sedimentation speed, with ($\omega = rpm * \pi/30$, slide 45)

6) (25%) For the reaction A + B <=> AB calculate the molar concentration of [B] that must be ***added*** to the reaction to achieve 30% binding of A at equilibrium if the Kd is 500 nM and you start with 300 nM [A] (total) in the mixture. Also calculate the amount of [B] that remains unbound at equilibrium.

At equilibrium we have: $Kd = 500\,nM = \frac{[A][B]}{[AB]}$. We have 300 nM [A] in the mixture, part of this will be free [A] and 30% of that will be in complexed form. Since 30% is complexed, 70% is free. Hence: [A] = 0.7 * 300 nM = 210 nM, [AB] = 0.3*300 nM = 90 nM, and [B] = 500 nM * 90 nM/(210 nM) = 214.3 nM. The total concentration of B needed for this equilibrium therefore must be [B] + [AB], so it is 214.3 + 90 nM = 304.3 nM. The percentage of [B] that is not bound is 100*214.3nM/304.3nm = 70.42 %.