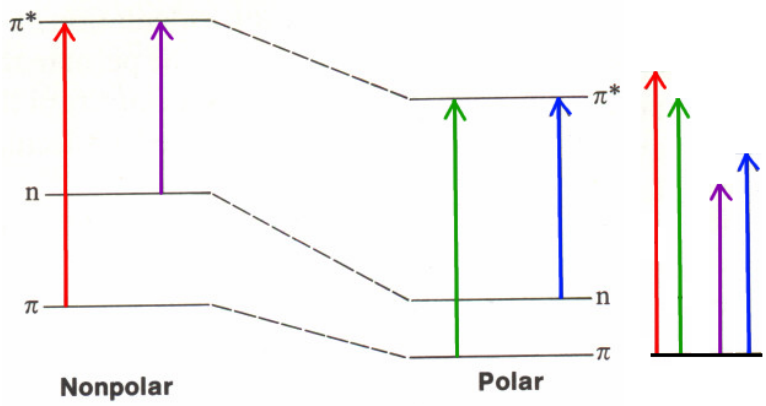
Homework Questions:

1. (20%) You are performing a light scattering experiment with a 488 nm laser. What is the smallest particle size (in nanometer units) that will allow you to detect an angular dependence (and therefore some shape information)?

Answer:
The particle will scatter as a Rayleigh scatterer (uniform scattering observed as a function of angle of observation) as long as it is 1/10 the size of the incident wavelength. Larger particles will display shape dependence (anisotropy) in their scattering intensity as a function of angle of observation. 1/10th of 488 nm is 48.8 nm. So anything larger 48.8 nm may provide shape dependence in the scattering profile.

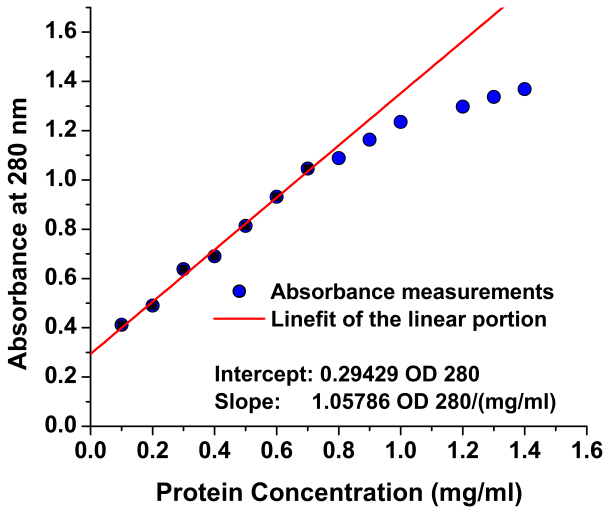
2. (30%) A macromolecule is moved from a non-polar solvent to a polar solvent, which causes a change in energy states as shown below. When moving to the polar solvent, do you expect a red or blue shift in the absorption profile, or none at all for (a) the n to π* transition, and (b) the π to π* transition? Explain why. Arrows on the right are drawn to scale for comparison.



Answer:
In this case, the energy for each transition changes as the molecules are moved from a non-polar to a polar environment. First, the energy of each electronic level is lower, but that is not what is observed in the absorbance spectrum. Here, only the sizes of the transitions matter. This is illustrated simply by comparing the height of the transitions. **a)** For the n to π^* transition, the energy increases as the molecules is moved to a polar environment, because the blue line is higher than the purple line. This means that the wavelength would have to be lower. A lower wavelength corresponds to a higher energy. So for the n to π^* transition, we see a blue shift. **b)** For the the π to π^* transition, the energy decreases, so there a red shift is observed.

3. (50%) A researcher carefully weighs out 10 mg of dry, lyophilized pure protein powder and dissolves it in a buffer, and prepares and measures the following dilution series on a spectrophotometer, which is blanked with distilled water:

mg/ml Protein Concentration	Absorbance Units (OD 280 nm)
0.1	0.412
0.2	0.490
0.3	0.638
0.4	0.690
0.5	0.814
0.6	0.932
0.7	1.046
0.8	1.089
0.9	1.164
1.0	1.236
1.2	1.298
1.3	1.337
1.4	1.369



Answers:

a. (5 %) At what OD level at 280 nm does this spectrophotometer become nonlinear? Explain with a graph.

b. (5 %) What advantage or disadvantage does the researcher have by blanking the spectrophotometer with distilled water rather than with the buffer?

c. (20 %) Is there any baseline absorbance present from the buffer? If so, how much?

d. (20 %) What is the molar extinction coefficient at 280 nm for this protein, given this was measured in a 1 cm cuvette, and the molar mass of the protein is 45,000 Dalton? Make sure not to include any non-linear data for this determination. Express your answer in $\frac{OD\,L}{mol\,cm}$ units.

Answers:

a. Plotting the data shows a leveling off of the absorbance starting at about 1.1 OD. So the spectrophotometer should be reliable up until 1.0 OD.

b. By blanking with water (which doesn't contribute any background absorbance) the researcher will immediately see if their buffer absorbs and "steals" away precious dynamic range to measure their protein.

c. Yes, extrapolating a fit of the linear region of these data to zero concentration shows a non-zero absorbance (the intercept of a linear fit), which is ~0.29 OD at 280 nm. This means the buffer contains some absorbing component(s).

d. The slope of the fit of the linear region is ~ 1.058 OD_{280}/(mg/ml*cm). Since the molar mass of the protein is 45,000 Dalton, a 1 mg/ml solution is 1/45,000 molar (=0.00002222, or 22.22 μM). Hence, the molar extinction coefficient is 1.058/0.00002222 = 47,604 OD_{280} L/(mol*cm)