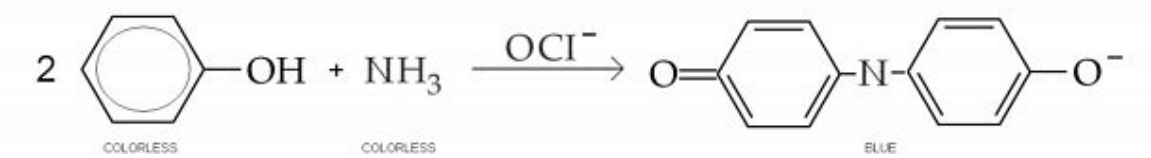
Instructor: Jorge Ramos (0.5) Name: _____

(**2.5**) Ammonia can be determined spectrophotometrically by reaction with phenol in the presence of hypochlorite (OCl^-) to yield a blue compound



A 0.12 g sample of certain nitrogen-containing compound was chemically digested to convert all of its nitrogen to ammonia, then treated with phenol and a sodium hypochlorite solution. The resulting blue solution was diluted to the mark in a 250.0 mL flask. For reference, a standard solution was prepared from 0.0075 g of NH_4Cl and diluting to the mark in a 100.0 mL flask. Here are the results of the spectrophotometric analysis done in a 1.00 cm cuvette:

Absorbance of standard from NH_4Cl (the 100.0 mL solution)	0.416
Absorbance of unknown (the 250.0 mL solution)	0.584

Compute (**a**) the extinction coefficient **ε** of the blue compound including units and (**b**) the percent nitrogen by mass in the unknown compound.

Mol of NH_4Cl = mol NH_3 = mol Blue Compound = [0.0075 g] / [53.45 g/mol] = 1.403 X 10^{-4} mol
Concentration of Blue Compound in standard solution:
C = mol / volume = [1.403 X 10^{-4} mol] / [100 X 10^{-3} L] = 0.001403 mol/L
Use any concentration units but keep using the same ones throughout the rest of the problem.
ε = [A] / [Cl] = [0.416] / [(0.001403 mol/L) X (1.00 cm)] = 296.5 $(mol/L)^{-1}$ cm^{-1}
Concentration of Blue Compound in solution made from unknown compound:
C = [A] / [**ε**l] = [0.584] / [(296.5 $(mol/L)^{-1}$ cm^{-1}) X (1.00 cm)] = 0.001970 mol/L
Mol Blue Compound = mol NH_3 = mol N = [0.001970 mol/L] X [250 X 10^{-3} L] = 4.925 X 10^{-4} mol
Mass of N = [4.925 X 10^{-4} mol] X [14.01 g/mol] = 0.006900 grams
% N = [(0.006900 grams)/(0.12)] X 100 = **5.75 %**

(**2.0**) A 22.00 miligram sample of haemoglobin was dissolved to make 100.0 mL of solution. The absorbance of the solution at 508 nm was found to be 0.150 when analyzed in a 1.00 cm cuvette. The absorbance is due to the iron content of haemoglobin [4 Fe atoms/molecule] and the solution has an extinction coefficient **ε** of 200 $(mg\ Fe/mL)^{-1}$ cm^{-1}.
Compute the molar mass of haemoglobin.

The absorbance comes from iron and the extinction coefficient **ε** is 200 $(mg/mL)^{-1}$ cm^{-1}.
A = **ε**Cl → C = A / [**ε**l]
C = 0.150 / [200 $(mg/mL)^{-1}$ cm^{-1} X 1.00 cm] = 7.50 X 10^{-4} mg Fe/mL
Mass of Fe = 7.50 X 10^{-4} mg Fe/mL X 100.0 mL = 7.50 X 10^{-2} mg Fe
For mmol of iron → 7.50 X 10^{-2} mg Fe / 55.85 mg/mmol = 1.34 X 10^{-3} mmol Fe
For mmol of hb = 1.34 X 10^{-3} mmol Fe / [4 mol Fe/mmol of hb] = 3.36 X 10^{-4} mmol of hb
Molar mass of haemoglobin = 22.00 mg / 3.36 X 10^{-4} mmol = 65.5 X 10^{3} mg/mmol
Because the 10^{-3} factor cancels, then mg/mmol = g/mol
Molar mass of haemoglobin is about **65,500 g/mol** or **65.5 kiloDaltons**