Tutorial 4 is due on 11/2
Tutorial 5 will be posted on 11/9

Chem 151 Lecture 9

EXP 7

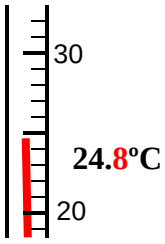
Iron in Biology: The Iron Storage Protein

1. **Introduction to a Quatitative Experiment**
2. **The Chemistry of Ferritin**
3. **Det'n of Concentration by Spectophotometry**
4. **Det'n of the Total Iron Content in a Ferritin Molecule and the Rate of Iron Released from Ferritin**
5. **Calculations in Exp 7**

All experimental results must be reported with correct number of significant figures (the certain digits + one guessing digit at the end.)

Examples:

Record the temperature read from a thermometer:



The last digit displayed on an equipment is the doubtful digit

The mass read from an analytical balance: 0.12344 g ± 0.00001 g

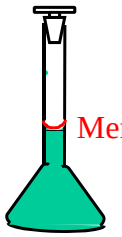
20.12344 g ± 0.00001 g

All computation results should be rounded to correct number of significant figures

Practice on DQ#7

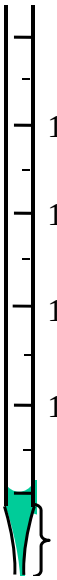
Transferring 0.50 mL of solution with a Graduated pipet

Volumetric Flask



25.00 mL ± 0.03 mL

How should one record the volume of soln in a 25-mL vol. flask ?



For most Mohr pipets in our lab, this part is not calibrated.

If you see droplets adhering on the inner wall of the pipet, your pipet is dirty.

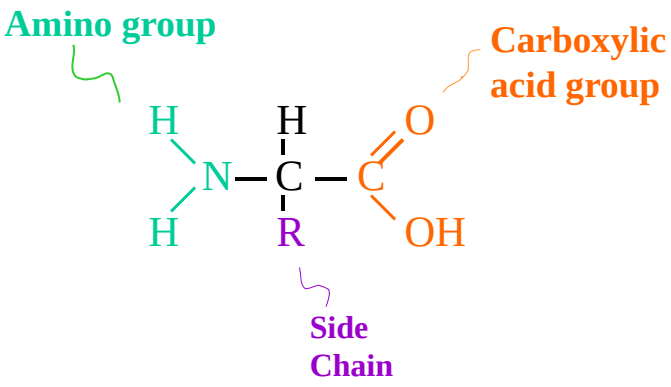
Always rinse your clean pipet with the solution to be transferred.

Be sure that you understand the markings on the pipet.

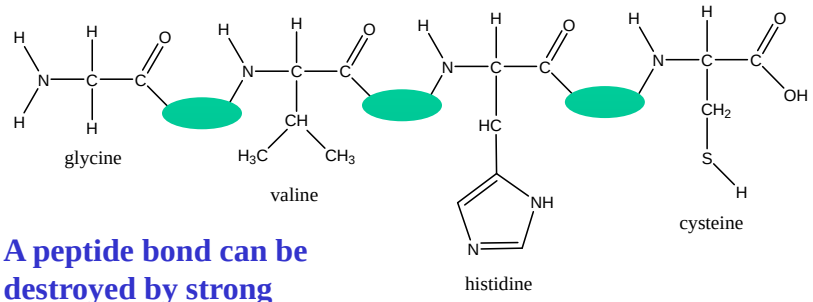
Never allow the meniscus to go below the last graduation mark.

The volume to be delivered is the difference between the initial and the final volume readings.

Amino Acid

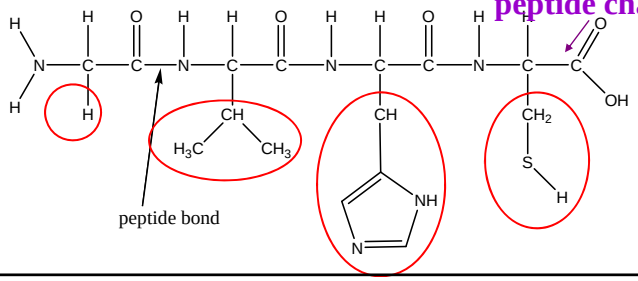


Amino acids link together to make a polypeptide

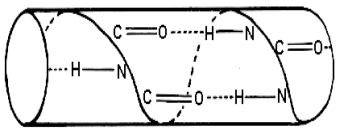


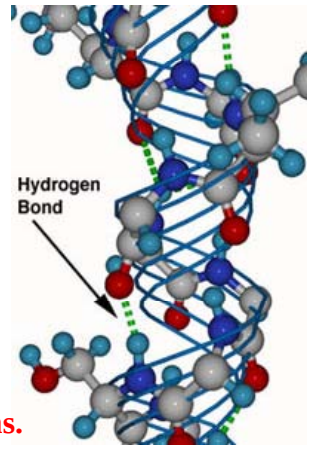
A peptide bond can be destroyed by strong acids or strong bases.

Backbone of the peptide chain.



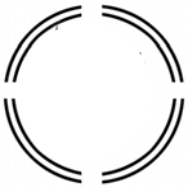
Hydrogen Bonding in α-Helix Peptide Chain





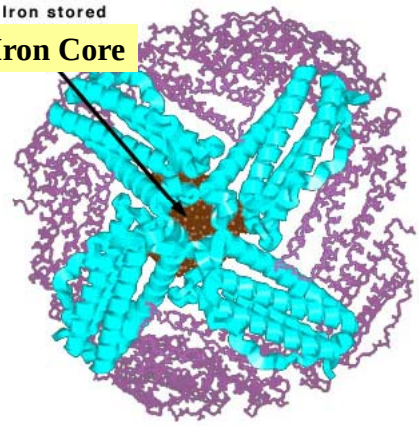
Protein consist of polypeptide chains.

Ferritin = Apoferritin + Iron Core



Apoferritin is an empty ferritin shell (without the iron core inside). Its molar mass is 474,000 g/mol.

A single ferritin can hold up to 4,500 iron atoms in the iron core.



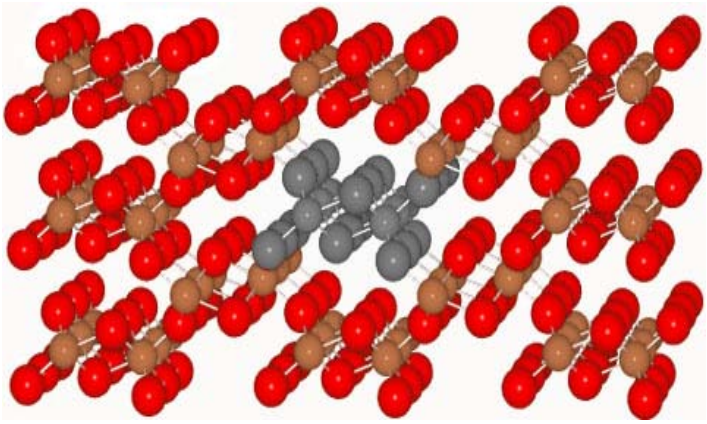
Human ferritin is a hollow protein sphere containing 24 polypeptide units.

Each polypeptide units contains 184 amino acid residues.

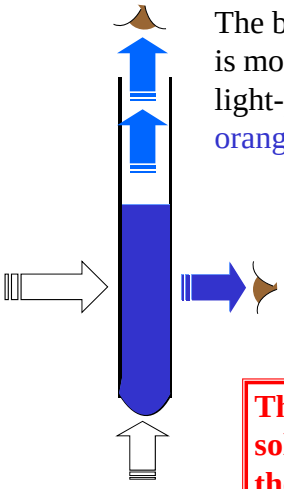
The Iron Core is Ferrihydrite

FeO(OH) or $[FeO(OH)]_8[FeO(H_2PO_4)]$

Molar mass = g/mol



Spectrophotometry



The blue color (as detected by the eyes) is more intense when the length of the light-path is longer, (because more orange photons have been absorbed.)

The blue color is more intense when the concentration of Cu^{2+} increases, (because more orange photons have been absorbed.)

The quantity of light absorbed by a solution is proportional to the length of the light-path (path length) and the concentration of the light absorbing species in the solution.

A Spectrophotometer can measure the quantity of light being absorbed/ transmitted.

Absorbance and Transmittance

%Transmittance, T = I / I_o **Absorbance A = log (1 /T) = log (I_o/ I)**

It is observed that $A_\lambda \alpha c$ **(conc. of light absorbing solute)**

and $A_\lambda \alpha b$ **(path length of sample cell)**

$\Rightarrow A_\lambda \alpha b \cdot c$

$$c = \frac{A_\lambda}{\varepsilon_\lambda b}$$

extinction coefficient (molar absorptivity)

$$A_\lambda = \varepsilon_\lambda b\, c$$

Beer-Lambert Law

The absorbance should be btw 0.15-0.85 to give a good result.

In Exp 7, you will

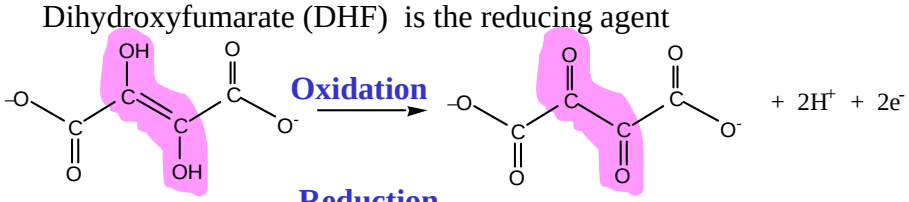
A. Determine the Total Iron Content in a Ferritin Molecule.

B. Determine the Rate of Iron Released from Ferritin in 25 minutes

C. Determine the Iron(II) Concentration in an Unknown Solution by spectrophotometry

First, the Fe(III) in the iron core is reduced to Fe(II).

Dihydroxyfumarate (DHF) is the reducing agent



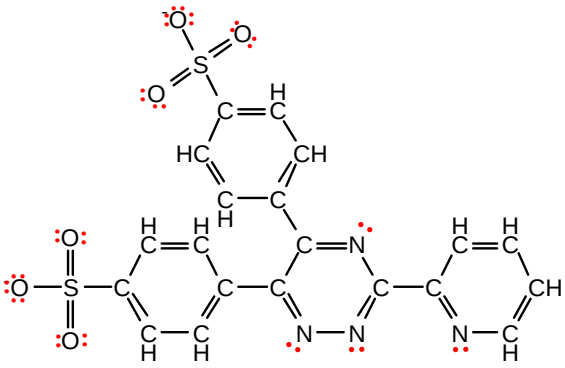
$Fe^{3+} + e^- \xrightarrow{\text{Reduction}} Fe^{2+}$

Then, Fe(II) is converted to a colored complex.

$Fe(H_2O)_6^{2+} + 3\ \text{ferrozine}^{2-} \rightarrow \mathbf{[Fe(ferrozine)_3]^{4-}} + 6\ H_2O$

ε_λ = $M^{-1}cm^{-1}$ at 561 nm

Ferrozine^{2-}



$Fe(ferrozine)_3^{4-}$

A. Determination of Total Iron in a Ferritin Molecule

In a 10-mL volumetric flask	In another 10-mL volumetric flask
I	**I_0**
1.00 mL of 0.100 mg/mL Ferritin	**Blank** ← **No ferritin**
1 mL 2 M H_2SO_4	**1 mL 2 M H_2SO_4**
1 mL DHF **Mix well**	**1 mL DHF**
Wait 30 minutes	~~**Wait 30 minutes**~~
2 mL 2.5 M CH_3COONa	**2 mL 2.5 M CH_3COONa**
2 mL ferrozine^{2-}	**2 mL ferrozine^{2-}**
Wait 30 minutes **Mix well**	~~**Wait 30 minutes**~~
Dilute to 10.00 mL	**Dilute to 10.00 mL**

Use the blank and measure the absorbance of the solution.

A. Determination of Total Iron in a Ferritin Molecule

$$\left[\begin{array}{l}\text{Iron content in one ferritin molecule}\end{array}\right] = \frac{\text{Number of Fe atoms}}{\text{Number of apoferritin}} = \frac{\left[\begin{array}{l}\text{Moles of Fe(II) in the 10-mL volumetric flask}\end{array}\right]\ \text{I}}{\left[\begin{array}{l}\text{Moles of apoferritin in the 10-mL volumetric flask}\end{array}\right]\ \text{II}}$$

I. Moles of Fe(II) in volumetric flask

Absorbance of purple solution and apply Beer's Law ⇨ Molarity of Fe(II) in the 10-mL volumetric flask ⇨ **Moles of Fe(II) in the 10-mL volumetric flask**

II. Moles of Apoferritin in volumetric flask

Moles of Fe(II) in the 10-mL vol. flask from I. ⇨ Moles of iron core in the 10-mL volumetric flask ⇨ Mass of iron core in the 10-mL volumetric flask

How many Fe in a ferrihydrite, $[FeO(OH)]_8FeO(H_2PO_4)$?

Mass of ferritin in the 10-mL vol. flask (0.100 mg) – [ ] = Mass of apoferritin in the 10-mL volumetric flask ⇨ **Moles of apoferritin in the 10-mL volumetric flask**

B. Det'd the Rate of Iron Release in the initial 25 min.

In a 10-mL volumetric flask

Add ferritin last and start timing

1 mL 2 M H_2SO_4
1 mL DHF
3 mL of H_2O
Wait 30 minutes

1 mL 2.5 M CH_3COONa
2 mL ferrozine^{2-}
Wait 30 minutes

Dilute to 10.00 mL

In another 10-mL volumetric flask

Blank — No ferritin

1 mL 2 M H_2SO_4
1 mL DHF

Wait 30 minutes

1 mL 2.5 M CH_3COONa
2 mL ferrozine^{2-}
Wait 30 minutes

Dilute to 10.00 mL

After 25 min., measure the absorbance of solution.

B. Det'd the % of Iron Released in 25 min.

Absorbance ⇨ molarity of Fe^{2+} in soln ⇨ moles of Fe^{2+} in soln

$$\%Fe\ released = \frac{moles\ of\ Fe\ released\ in\ 25\ min}{\text{Total iron in ferritin of Part A x 10}} \times 100\%$$

C. Determine the Iron Concentration in an Unknown

In a 25-mL volumetric flask

0.50 mL Fe(II) Unknown solution

1 mL DHF
2 mL ferrozine^{2-}
Wait 10 minutes

Dilute to mark with H_2O

Measure absorbance of solution in volumetric flask → **[Fe^{2+}] in flask**

Need to find [Fe^{2+}] in original unknown solution