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A Release of Internal Original Research Documents

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Chemistry Vol III

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180 Sheets
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Chemistry

Volume III

July 09 2011

Duplication within Blood:

Can we question the same process occurring in the blood as within the culture?

Take blood sample, add NaOH + $CuSO_4$
ferric Citrate

1st Reaction:
$2Fe^{3+} + 6NaOH + 3CuSO_4 + H_2O$
$\rightarrow 2Fe(OH)_3 + 3Cu^{2+} + 3Na_2SO_4 + H_2O$

citrate $Fe(OH)_3 + C_6H_8O_7 \rightarrow FeC_6H_5O_7 + 3H_2O$
Could this happen in blood? pale brown

What is the reaction for Fe^{2+} instead?
ferrous Citrate:

$Fe^{2+} + NaOH + CuSO_4 + H_2O$
$\rightarrow Fe(OH)_2 + Cu^{2+} + Na_2SO_4 + H_2O$

balanced is:
$Fe^{+2} + 2NaOH + CuSO_4 + H_2O \rightarrow$
$Fe(OH)_2 + Cu^{2+} + Na_2SO_4 + H_2O$

citrate $Fe(OH)_2 + C_6H_8O_7 \rightarrow FeC_6H_6O_7 + 2H_2O$
green

The precipitate is a dark muddy green.
When citric acid added, the color seems to be in between the green and the brown.

This would make sense. Possibly does look more brown than green.

Fresh blood is giving a very different reaction
It has turned brown as Caroline.

With sufficient NaOH it is turning very green.

Turns muddy green. does indicate a combination of both Fe^{2} & Fe^{3+} in the blood

Sort of an olive green
(combination of green + brown)

Studying the pH of the culture w/ Copper

The pH of the culture is very basic (~ 8.5) due to the NaOH that was used to knock it down.

Now as you add your culture you see a precipitation of Ferric hydroxide.

Now you measure pH & you get 4.9
So it became very acidic.

Now why does the solution become more acidic & then somehow H^+ is being generated.
From what?

Ferric 1st Reaction: (R)

$2Fe^{3+} + 6NaOH + 3CuSO_4 + H_2O \rightarrow 2Fe(OH)_3 + 3Cu^{2+} + 3Na_2SO_4 + H_2O$

This wins?
↙ ?
$(+H^+ + OH^-)$

Now how would this turn from basic to acidic? ?
Somehow we have to increase the H^+ or H_3O^+
Where is the H^+ Coming from?
has to be a dissociation of water?
Water ionizes to some degree
pH Goes from 6.5 to 4.9 ???

Yes, water does ionize to a slight extent ($1E-5\%$)
$H_2O \rightleftharpoons H^+ + OH^-$
but H^+ Cannot actually exist in water!!!
They form a Covalent bond w/ water to form H_3O^+
Moore:
"The acidic properties of water are ascribed to H^+ or H_3O^+!!
So H^+ actually means H_3O^+ in water.

What type of reaction is this?

Four types of reactions:

1. Combination
2. Decomposition
3. ~~Single~~ Replacement
4. Double Replacement

Looks like a Combination or a replacement reaction to me
Fe combines with OH
Group
Sodium replaces Cu

Some things we need are

! ✓ OK Sulfuric Acid Test tube rack
Nitric Acid
Universal Indicator

There is something really important here that has not been mentioned!

In our first ferric reaction, it fails the ion test!!!
This means it is not an ~~atom~~ ion!
It must be bound.
Bound to what? We have a short list
eg Azide.

Repeat the test.

Take the original Culture & test for Fe^{2+} & Fe^{3+}

It fails both tests! This means it is not in ionic form.
But it does not mean that it does not exist in the Fe^{2+} or Fe^{3+} states but it means it could be bound as a Complex.

If it were bound, then the equation would be of the form

$2(Fe^{3+}\cdot R) + 6NaOH + 3CuSO_4 + H_2O \rightarrow 2Fe\cdot R?(OH)_3 + 3Cu^{2+} + \cdots SO_4 + H_2O$

Now we have tested proper for Cu^{2+}.
We also know the pH is low! eg 4.9
but we do not know why.

Could we have something like Copper Nitrate?
Would it ionize?

$Cu^{+2}(NO_3)_2^{-2}$ is highly soluble.

it would really help to test for Nitrate ion - a Nitrite?

$Cu(NO_2)_2$

"The addition of nitrite ions to a solution of cupric salts produces a deep green solution. due to formation of cupric nitrite complexes.

It is looking like the raw culture is passing the brown ring test for Nitrate ions.

But we were supposed to be using the $Fe(OH)_3$ solution to test it. Add copper first.

~~Proposed~~

$2\ Fe^{3+}N_3^-$

Unbalanced

$Fe^{3+}N_3^- + NaOH + CuSO_4 + H_2O$?

$\rightarrow Fe(OH)_3 + Cu^{2+} + Na_2SO_4 + NO_3^- + H_2O$?

~~The~~ brown ring test failed after the $CuSO_4$ was added but passed using the culture by itself.

I just got a very serious reaction.

1. Used the clear fluid after the $CuSO_4$ added.
2. Added the Fe^{2+} solution
3. Added the Fe^{3+} solution
   got one very serious lime green precipitate.

The test for Cu^{2+} came from your tube and my sheet of qualitative tests. When you add NaOH to Cu^{2+} it creates a nice blue precipitate

yes it clearly forms a significant lime green precipitate

1. Extract Clear result from $CuSO_4$ addition (we know that is acidic)
2. add 1,10 Fe^{2+} reagent
3. add sodium thiocyanate

a mega lime green precipitate forms.
What is it?

It is clearly not the reagents that are reacting.
What if it was only one of the reagents?

It positively requires both reagents & whatever is in the extraction of the $CuSO_4$.

Clearly a negative result for both Fe^{2+} and Fe^{3+} with the extraction fluid of the $CuSO_4$.

What are formula of 1,10 & Sodium Thiocyanide?
$C_{12}H_8N_2$ $NaSCN$ ← ↑ Sulfur? Nitrogen?

So extraction fluid + these cause what.

ND

It is now positive that the raw culture passes either the NO_3^- or NO_2^- test so now we have

Fe^{3+} ~~NO_3^-~~ + NaOH + $CuSO_4$ + H_2O → $Fe(OH)_3$ + Cu^{2+} + Na_2SO_4

no evidence of existence

or

~~Fe^{+3} NO_2~~

Ferric Nitrate is highly soluble & we have some!!!

The reaction looks amazingly similar to the Culture reaction.

When the 1,10 reagent & the Sod Thio reagent are added you get quite a precipitate also, but it is brown instead of lime green.

Fe^{3+} $(NO_3)_3$ + NaOH + $CuSO_4$ → $Fe(OH)_3$ + Na_2SO_4

We do have a reaction we observe & found.

Sulfuric acid reacts w/ most bases to give the corresponding sulfate.

Positively not. Fe^{+3} is bound — bound to what?
↙ but you cannot measure or find that!

$$6Fe^{+3} + 3(NO_3)_3 + 18NaOH + 9CuSO_4 + H_2O$$
$$\rightarrow 6Fe(OH)_3 + 9Na_2SO_4 + 9Cu(NO_3) + H_2O$$

does NO_3 sometime exist @ −2 state? ↑ This is possible

We are showing nitrate (or nitrite) ion in original culture but not afterwards

We are finding no Fe^{3+} ion in culture?

$Cu(NO_3)$ ← for this to be −2 Nitrogen would have to be in the +4 state. Is this possible?

O_3 3(−2) = −6

4 − 6 = −2

So $Cu^{+2}(N^{+4}O_3^{-6}) = -2 = 0$ ok

$Cu(NO_3)$ mean Nitrogen in the +4 oxidation state
Yes, Nitrogen can do this.
Can be +/− 3, 5, 4 & 2

$Cu(NO_3)$ is apparently Copper I nitrate?

We do seem to have Nitrate or Nitrite ion in culture along w/ Fe3 bound —

It cannot be in other form?

Explain
$Fe(NO_3)_3 + NaOH + H_2O \rightarrow$

This would be soluble and detectable because it is highly soluble and would dissociate into ions.

It does indeed match the spectrum of the culture very closely but we cannot detect the Fe^{3+} in an ionic form. This means that it is bound. Bound to what?

We also know that if Fe^{+3} was in ionic form that when it was mixed w/ NaOH it would form $Fe(OH)_3$! which is a precipitate! Which we cannot have in our culture solution so it could never be in ionic form.

So what form is it?

With the culture added:

1. Potassium nitrate tablets are making it cloudy
2. Cobalt Chloride - almost no reaction
3. $MgSO_4$ - nothing seen
4. Potassium nitrate is making a precipitate
5. Potassium Chloride is not
6. So why does KNO_3 & $CuSO_4$ make a reaction but not KCl?

Be careful, you had added $AgNO_3$ to it in addition to KNO_3 & that appears to be what has caused the precipitate.

Pure KNO_3 & the culture is doing nothing.

$AgNO_3$ & KNO_3 does nothing.
Add the culture & it will

$AgNO_3$ + KNO_3 + Culture (+ NaOH) forms a precipitate
expect to form $Fe(OH)_3$ and either Ag or K ions

The brown ring test w/ strong H_2SO_4 is quite interesting

1. The brown ring test might be unsuccessful if the band of clear separation of layers & I just shake it up and indiscriminately mixes the layers.

What is also interesting is you are getting a precipitate but it also disappears as soon as you shake it up.

It is not passing the nitrate test of the culture directly.

H_2SO_4 however, is precipitating the culture. $CuSO_4$ also precipitated
Any chance the could be $FeSO_4$ vs $Fe(OH)_3$?

$$Fe^{+3} + H_2SO_4 + NaOH \overset{?}{\rightarrow} Fe(OH)_3 + Na_2SO_4 + H_2O$$

$$H_2SO_4 + NaOH = Na_2SO_4 + H_2O$$

$$Fe^{+3} + NaOH = Fe(OH)_3$$

So

$$Fe^{+3} + NaOH + H_2SO_4 = Fe(OH)_3 + Na_2SO_4 + H_2O$$

I think I have it

Unbalanced $FeCl_3 + NaOH = Fe(OH)_3 + Na^+ + Cl^-$

Bal $FeCl_3 + 3NaOH \rightarrow Fe(OH)_3 + 3Na + 3Cl$

~~Next~~ and.

2 $Fe(OH)_3 + 3H_2SO_4 + H_2O \rightarrow 2Fe^{+3} + 3SO_4^{2-} + 7H_2O$

This one all works fine.

~~Fe^{+3}~~ $Fe^{+3}R^{-3} + NaOH = Fe^{+3}R^{-3} + Na^+ + OH^-$

$Fe^{+3} + Na^+ + OH^- + CuSO_4 \rightarrow Na_2SO_4 + Fe(OH)_3 + Cu^{+2} + R^{-3}$

??! Yes This is a valid reaction!

~~FeR +~~

Yes we can substitute but since N_3 is -1

$FeN_3 + 2Na + 3OH + CuSO_4 \rightarrow Na_2SO_4 + Fe(OH)_3 + Cu + 3N$

a viable reaction.

we change to:

$Fe(N_3)_3 + 2Na + 3OH + CuSO_4 \rightarrow Na_2SO_4 + Fe(OH)_3 + Cu + 9N$

I think you have it! this would have to react

Soluble! yes!!! yes!!! w/citrate yes!!! everything would be ions except $Fe(OH)_3$

Now I think you have an even more accurate reaction since N_2 would need to react to become N_2. This can happen under the following conditions.

Try w/ other Anions

$$2Fe(N_3)_3 + 2Na + 6OH^- + CuSO_4 + H_2O \Rightarrow$$

(but this is positive)

So this is positive

$$\rightarrow Na + SO_4 + 2Fe(OH)_3 + Cu^{+2} + 9N_2 + H_2O$$

(only unknown)

This is all feasible and agrees with all data given.

$Fe(OH)_3$ verified with citric acid solubility
Citric acid color
insoluble in H_2O

Cu^{2+} ions verified with ammonia

SO_4 ions verified w/ dilute HCl & $BaCl_2$

Sodium ions & Nitrogen not verified yet.

passes

Nitrogen has no color or smell
No effect on moist litmus paper
Thereby no specific test for N_2
Notable for its inertness.

"Azide iron Complex" a respiration inhibitor.

Binding Candidates

OH^- hydroxide
CN^- cyanide respiration inhibitor
N_3^- azide respiration inhibitor
NO_2^- nitrite

make up new .5M $FeSO_4 \cdot 7H_2O$ 1M $FeSO_4 \cdot 7H_2O$ = 278.02 gms/mole

$$\frac{278.02 \text{ gms}}{1000 \text{ ml}} = \frac{X}{60 \text{ ml}} \quad X = \frac{16.681 \text{ gms}}{60 \text{ ml}}$$

$$(1/2) = \frac{8.34 \text{ gms}}{60 \text{ ml}} = 0.5M \; FeSO_4 \cdot 7H_2O$$

We have a reaction that looks very solved @ this point.

(1) $2Fe(N_3)_3 + Na + 6OH + CuSO_4 + H_2O \rightarrow Na + SO_4 + 2Fe(OH)_3 + Cu^{2+} + 9N_2 + H_2O$

Is this acidic? why or why not?

Now look @ the citrate reaction.

(2) $Fe(OH)_3 + C_6H_8O_7 \rightarrow FeC_6H_5O_7 + 3H_2O$

ferric hydroxide — citric acid — ferric citrate pale brown

Now are ligands are

OH^- hydroxide
CN^- cyanide
N_3^- azide
NO_2^- nitrite

Let's look the other ligands to see what type of reaction take place

$Fe(CN)_3 + Na + OH + CuSO_4 + H_2O$
$\rightarrow Na + SO_4 + \cancel{Fe(SO_4)}\ Fe(OH)_3 + Cu^{2+} + H_2O$
$+ CN?$

So what is $Fe(CN)_3$ + what type of ligands

$Fe(CN)_3$ in water dissociates to
Fe^{+3} and CN^-
and then
$CN^- + H_2O \rightarrow HCN + OH^-$

ok, so now we have the equation

acidic? why or why not

$Fe(CN)_3 + Na + OH + CuSO_4 + 4H_2O \rightarrow$
$Na + SO_4 + Fe(OH)_3 + Cu^{+2} + 3\ HCN + H_2O + OH^-$

Ok you are doing well.
Remember that our solution is and seems to be very acidic.
What is HCN? Hydrogen Cyanide
It is hydrogen Cyanide.

Colorless, extremely poisonous liquid.
Is slight acidic.

Third example is OH^- ion.
It also leads to

$Fe(OH)_3 + \ldots$
$\rightarrow Fe(OH)_3$ which is not true.
You cannot end up with what you started with because we have a reaction that produces $Fe(OH)_3$.

And so our last one is NO_2^-

$Fe(NO_2)_3 + Na + OH + CuSO_4 + H_2O$

$\rightarrow Na + SO_4 + Fe(OH)_3 + Cu^{2+} + H_2O$

What is $Fe(NO_2)_3$? Ferric ~~Nitrate~~ Nitrite!!
~~And this is our chemical we brought~~! Not true
I cannot really find this?

???

Nitrites are $O=N-O$

Why didn't the brown ring form with ferric nitrate and $FeSO_4$ & H_2SO_4??

No browning!

Two problems:

1. Why is the NO_3^- test failing?
2. What is the nitrite reaction & its likelihood?
3. $Fe(N_3)_3$ is not the same as $[Fe(N_3)]^{+3\ -9}$ ~~+2~~ = -6?

We should actually have the latter.

Scan of Ardeth.

$$[Fe(N_4)]^{+2\ -12}_{-1} = -10?$$

Sulphuric acid detaches iron from the hemoglobin molecule.

Ammonium persulphate oxidizes the iron to the ferric state.

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yes, but what are the agents?

Methemoglobinemia usually results from exposure to an oxidizing agent.

Ascorbic acid may be used to reduce the level of methemoglobin

So it is

$[Fe^{+2}(N_4)]$ and $[Fe^{+3}(N_4)]$

but remember the article that says what it can bond to. N_3^-
OH^-
CN^-

I am partial to Lewis Dot structures since they show what is happening w.r.t. the octet rule.

K No 19 $1s^2 2s^2 2p^6 3s^2 3p^6 4s^1$
$= Ar\ 4s^1$ +1

As No 33 Ar: $4s^2 3d^{10} 4p^3$ −3 means +5
$= 3d^{10} 4s^2 4p^3$ means 5

Sr: No 38 +2 means +2 in outside shell

I: ⋮ −1 means 7in outside shell

So you can start to form bonds now.

Just what happens w/ NaCl

Na· ·Na

Cl = -1 = +1 ·Cl·

But doesn't Lewis structure only apply to covalent bonds?

You can tell by electronegativity

Na = 0.9

Cl = 3.2

Δ = 2.3 = ionic

Lewis structures are used to simply electron configuration notation

So what would FeN_4^{+2} look like?

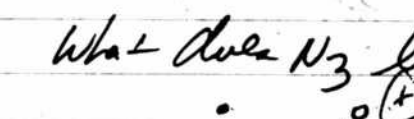
Fe·

It would be N N·Fe·N N

Coordinate Bond.

But we learned Fe^{+3} can bond to N_3^-

What does N_3 look like?

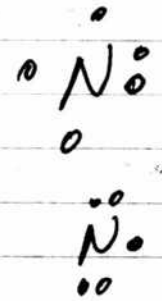


8 full
7 loose 8 − 7 = 1 − ??

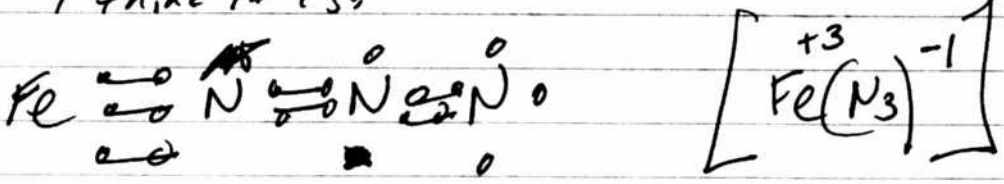
N is −3, means +5 in shell

N_3^- azide has a valence of −1. Why?

Valence electrons is the no. of electrons in the outer shell.

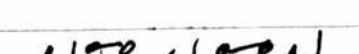


So I think it is:



$-3+2=(-1)$ Net Charge

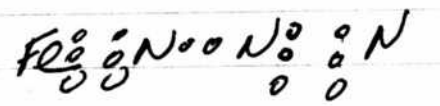
Then is easy to see.
We know N has $-3 = +5$ Valence



Try to fill up as close to 5 as possible
Seems to work.

And how would O_2 or N_2 bind to Fe+3

15+3=18
Total



Is there such a compound as ~~ferric~~ nitride?
I can not find it yet.

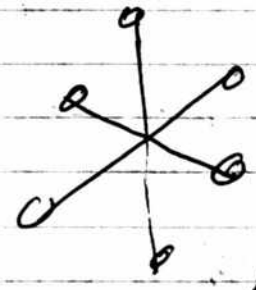
No it is azide, not nitride!

ferric azide

Ok, it is called "ferrihemoglobin azide"
or ferrihemoglobin is another name for methemoglobin.

$Fe^{+3}(N_3)^-$ contains octahedral covalent bonds to the iron atom.

So what are octahedral covalent bonds?
(8 faces to the structure)
It means 8 faces



How would this actually be

Ok, so one way another it bonds.

airbags use azide, they decompose to Nitrogen gas.

What if it was $2Fe^{+3}(N_3)^-$.

Some to my group members and I are:

Problems

1. Nitrate test fails - do not know why

2. Do not understand how Fe^{+3} would bind to ligands like N_3^-

3. Do not know the form of combination of Fe^{+3} in cuttlebone with ???
(Have a partial hypothesis)

This is truly a question

these are not lead

This is actually a strong hypothesis

$$\overset{+6\ \ -6}{2Fe(N_3)_3} + Na + 6OH + CuSO_4 + H_2O$$

known tested tested tested

$$\rightarrow Na + SO_4 + 2Fe(OH)_3 + Cu^{+2} + 9N_2 + H_2O$$

This is a proposed

We also know airbags use azide and they release a nitrogen gas.

This is a consistent reaction.

my uncertainty but fits airbag reaction

4. We also learn that we enjoy Lewis structures.

(1) Do how do you test for nitrates & why is our test flawed?

(2) Study Metal Complexes

(3) Our chemistry course

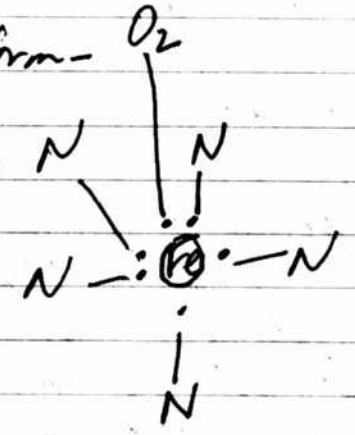
4. Lewis structure

5. Our research paper

We now have a list of 5 candidates that can bind to Fe^{+3}

OH^-
CN^-
N_3^-
NO_2^-
H_2O in ligand form -

Fe^{+2} means 6 electrons



see p 1035 Brown text

"A lone pair is a valence electron pair without bonding. Lone pairs are a subset of a molecules valence electrons."

We found another real possibility of flunking for the Fe^{+3} ion.

See Brown p 1020 bottom of page!

"Hydrated metal ions" are actually complex ions in which the ligand is water!

Thus $Fe^{+3}(aq)$ consists largely of

$\left[Fe(H_2O)_6\right]^{3+}$ $\quad +3+(+2-2)=\underline{\underline{+3}}$

This is one reason it may not be able to be detected! But it also says the ions like CN^- often replace the water molecule.

The water molecule is actually a ligand!

It is makes sense. We found a reference that ferrohemoglobin azide contains octahedral covalent bonds to the iron atom. The only way this can work is if you have 6 Nitrogens (a 6 Nitrogen ligand)

Well, look @ your equation, you have a multiple of N_3^- involved.

Either way you are starting to see how it can work!

Different molecules can bond to the Fe^{3+} ion.

You already have one reaction involving an azide that is consistent.
You could try other.

Next if the ligand were water, we could try with the idea:

$$\left[Fe(H_2O)_6\right]^{3+} + Na + OH + CuSO_4 + H_2O$$

$$\xrightarrow{?} Na + SO_4 + Fe(OH)_3 + Cu^{2+} + H_2O$$

Cannot get this to balance

But I do did get Cyanide ion CN^- to balance!

$$Fe(CN)_3 + Na + OH + CuSO_4 + 4H_2O$$

$$\rightarrow Na + SO_4 + Fe(OH)_3 + Cu + 3HCN + H_2O + OH$$

HCN = hydrogen cyanide a respiratory inhib.

Ok, I think you have made sufficient progress.

Our class has structure

Fe (EDTA) completely identified in Coordination Chemistry module 1.

"transferrin" helps us in our digestive tract to absorb Fe.

Chelating agents These are

Let's start looking @ blood #'s

R	G	B
250.5	91.6	42.4

[redacted]

251.2	123.5	84

The #'s are entirely different. The many different scanners will likely give different results. A Problem!

206.8	44.6	49.2

Leveled Images

	R	G	B
	218.7	38.4	12.9
	251.1	89.0	48.7
	241.9	79.7	38.5
	250.5	82.9	38.2
	249.8	93.5	45.3
	185.9	50.7	44.2
	207.9	46.4	50.6
$\bar{X}$	229.4	68.7	39.8
σ_{n-1}	25.7	22.7	12.7
E_{90}	42.3	37.3	20.9
90^+			
90^-			
σ^+	255.1	91.4	52.5
σ^-	203.7	46.0	27.1

$P_r = \frac{200}{\pi} \tan^{-1}(C \cdot \Delta x_i)$ $\quad C = \frac{1}{\Delta x_i} \tan\left(\frac{P_r}{\frac{200}{\pi}}\right)$

$\Delta x_i = \frac{1}{C} \tan\left(\frac{P_r}{\frac{200}{\pi}}\right)$

This is a very simple method of analysis.

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Prof Dean Harmon Ligands

gives other ligands that appear to be able to bind to Fe^{3+}

NH_3
H_2O
OH^-
CN
CH_3COO acetate
Cl

are these neutral or anions?
Figure out the charges!

We have another source as p272 Brockman demystified

In my ~~pt~~ previous work I found:

OH
CN
N_3
NO_2

So now the total combined list is

OH^-	NH_3
CN^-	H_2O
N_3^-	CH_3COO (acetate)
NO_2^-	Cl

~~ferrohemoglo~~ ferrichemoglobin may have preferences in the first ~~alum~~ column.

Drawing the chemical structure of an organic compound is a useful exercise. That is in Barrow's organic chemistry.

Lets try to make a molecule model

Now you understand the dotted lines

Heme B is $C_{34}H_{32}O_4N_4Fe$

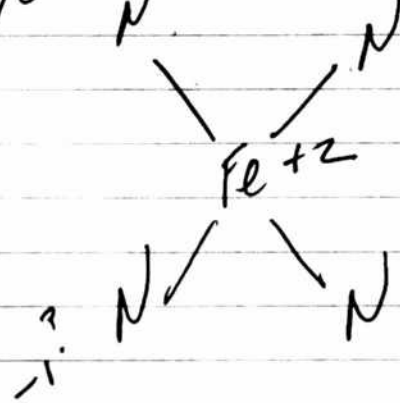
What is a Cytochrome?
a porphyrin

Cytochrome is a hemoprotein that contains heme groups
They are found in bacteria.

Porphyrin are organic compounds. Heme is a porphyrin.

FeN_4 Fe is +2

Oxygen is not soluble in aqueous blood.



A lone pair is a valence electron pair without bonding.

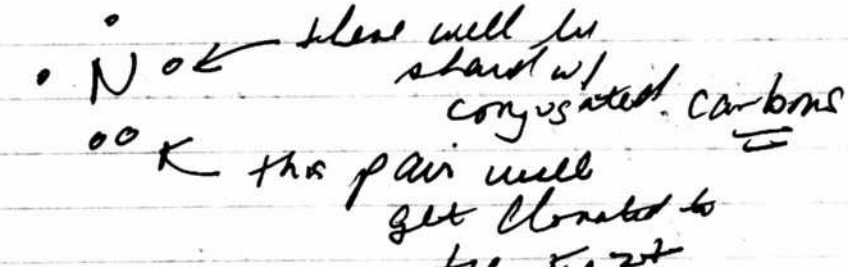
The iron in heme is actually forming 6 bonds.

It has a coordination no of 6!

Each nitrogen atom is donating 2 additional electrons.

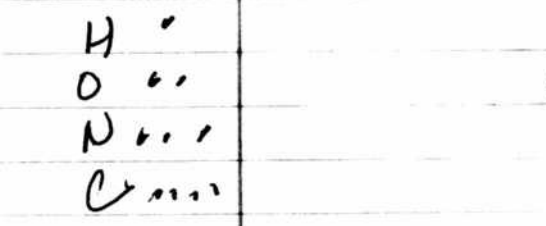
Nitrogen is $1s^2(2s^2 2p^3)$ means 5 electrons in outer shell.

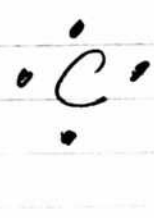
So Lewis diagram is



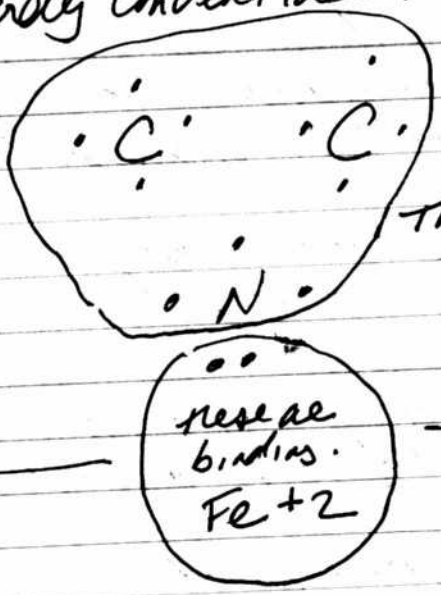
Carbon
Lewis Structure is:

$1s^2(2s^2 2p^2)$ 4 valence electrons





It is a very interesting molecule.
Hardly conventional.



So somehow

3 more times
4 Nitrogen, or iron

Now iron has Ne
Fe! ~~1s² 2s² 2p⁶ 3~~.

Ar $4p^2\ 3d^6$

:Fe: has a charge of +2

↑ This is apparently "held" in position in a +2 oxidation state.
No actual electron sharing takes place from the Fe^{+2} iron.

So, because the Fe^{+2} iron is "held" in place by coordinated covalent bonds and is further surrounded by resonant C–N ~~resonant~~ resonant structures.

Then a slow still "room" for O_2 & Histadine to bond.

The flow chart of Fe^{+2} & Fe^{+3}

Now, think about what happens when Fe^{+2} is oxidized to Fe^{+3}

1. least it take energy to remove an electron.

2. It also take energy to break the ? → Coordinated Covalent bond w/ Nitrogen

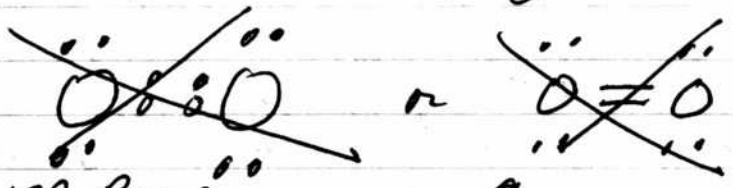
I wonder how much?

3. Then the Fe^{+3} can apparently bond w/ a whole host by candidates including N_3^- / & CN^-

4. But also the oxygen is released as a free radical / also very reactive!

5. And remember, bonds the Fe^{+2} to O_2 is a Class reaction, the more there is the harder, and vise versa, the less the harder it is to get started.

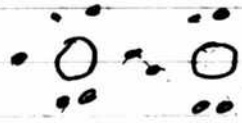
The Lewis Dot Structure for O_2 is:



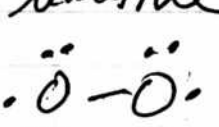
or

Actually p 129 Barrons

Organic



or



very reactive

Oxygen

$1s^2 2s^2 2p^4$ 6 electrons in outer shell

very reactive, want to accept 2 electron.

Strongest 3 antioxidants are:

1. Glutathion
2. Vit C
3. Vit E

How do you find the no. of valence electrons from the electronic orbital configuration

From CRC it gives bond strength of Fe-O on p 9-54 as 390 kJoules per mole.

Lets review energy consumption:

(1) We have

1st ionization potential	759.3 KJ/mol
2nd	1561.1
3rd	2957
⋮	

(2) Humans have 2.5E13 red blood cells.
(3) 4 iron atom per molecule.
(4) ~ 280E6 molecules of hemoglobin in each cell.

So no of iron atoms in the human body ≅

$(2.5E13)(280E6)\cdot 4 \cong 2.8E22$ iron atoms

(We should be able to check this).

Now if 1% have been oxidized to Fe^{+3} we have

$2.8E22(.01) = 1.96E21$ of Fe^{+3} atoms

and energy Δ is

$\frac{1.96E21}{6.02E23} = .00326$ moles of Fe^{+3} absorbed in body

$.00326 \text{ moles}(2957 - 1561.1) = 4.55 KJ$

in how long a time period.

Picking & Raising apple above head is ~ a joule.

Now we check on amt of Iron in the body.
3 to 4 gms.

So Atomic wgt of Iron is. 55.85 gms/mole

so $\frac{2.8E22 \text{ atoms}}{6.02E23} \approx .045$ moles. } Not bad. a reasonable estimate.

and $\frac{3.5 \text{ gms}}{55.85 \text{ gms}} = .062$ moles.

So our energy estimate is reasonable.

Now for bond dissassociation?

Fe-O is 390.4 KJ/mole.
now 2 mole atoms of Oxygen should be
~ 781 KJ per mole of $Fe-O_2$

a mole of FeO_2 is: 87.8 gms/mole.

Now we would have .00326 moles of Fe^{+2}

$.00326 \left(\frac{781 \text{ KJ}}{87.8 \text{ gms/mole}}\right) = 2.55$ KJ
to break FeO_2 bonds.

So we have combined estimate of $\begin{array}{r} 4.55 \\ +2.55 \\ \hline 7.10 \text{ KJ} \end{array}$

over 90 days = $\frac{7.10E3 \text{ J}}{90} \approx 80$ apples per day.

One apple ~ 150 gms or 5oz 80 × 5 = 25 lbs per day
16

Additional evidence of 397 nm line

We have lines visible for Iron(III) at

395.4 [redacted] from CRC p 10-34
396.9
397.9 (wi[redacted]2

and $\bar{X} = 396.7$ $\quad \frac{395.4 + 396.9 + 2(397.9)}{4} = 397.0$

Test it by using Fe^{2+}

II 624.8
III 654.6

See if you can test this???

Using Iodine:

A problem: Why does Iodine have peaks @ 397 & 448 also? Why???
It happens in both the cuvette & the test tubes. Why & how?

It does have a similar color to our culture. But it also means it is not unique.

The spectrum of Iodine is very different in a lower concentration.
Single peak is @ 377 nm
& nothing else.
Maybe you dry concentrate solution too much does it destroy the results?

The cuvette gives you a smoother result but the best table results are essentially the same. Higher concentrations may be distorting the results???

Maybe what you are seeing in the blood is a distortion also???

The curves do change & can shift as a function of concentration.

Why do we only see the peak @ 397?
With both Iodine & Iron —

Now blue food dye is COMPLETELY different. It does not give a peak @ 397.

So we learn from this that more than one substance gives a peak @ 397.
448
Ferric salts
Iodine
Formaldehyde
] Compare these 2 @ concentrations

Povidone Iodine has a formula
C_6H_9NO – how can it not
have Iodine

Now how can ~~it~~ be the same as
ferric nitrate w/ a spectrum

40 drops 30 ml

You had a little start. You were
concerned that spectrum of iodine
was essentially the same as ferric
nitrate. Well actually they are not that
close if you compare their behavior wrt
concentration.

Iodine spectrum disappears after a dilution
factor of 3. Ferric ~~is~~ nitrate is still
very strong after a dilution of 1000 times

~~As~~ on the surface the color is similar
on the surface but w.r.t. concentration
they are different. And actually if you
look @ the spectrum closely you
see that they are different

You do ask stellar holes.

So the lesson is:

If you happen to think that spectra are alike, then just look @ wvt Concentration. They will never be the same.

Lets think about where we are now.

The spectra revealed the alliteration.
We are in the process of finding out what the alliteration actually is.

We know we have a problem w/ oxidation.
We have a candidate list of binding agents & known free radicals.
We do not know positively what the binding subjects are. We are going to try to find out. We have a strong suggestion of N_3^- but not proven.

We now have tests for some organic compounds!
Hot dog! I found my mag magnets!

Look @ "the spectrochemical series"

Equation is consistent of N_3^-

Color is also consistent of N and C
Compounds like Co and Cyanide—

Magnetic properties are also important.
We expect the culture isolated to
be diamagnetic

We also had the organic qualitative ~~test~~.

The culture has no obvious magnetic
properties or reaction.

What is an "octahedral ligand set"

Paramagnetism occurs when there
are unpaired electron.

Green colored solutions are more likely
to be ~~para~~ paramagnetic. (lower
energy from absorption of red photons)
Orange solution are more likely to
be diamagnetic (higher energy
from absorption of blue photons)

CO, CN (strong field ligands) (orange colors) (higher energy) (diamagnetic)

Low spin means less unpaired electrons

High spin means more unpaired electrons (paramagnetic) (lower energy)

I, Br (weak field ligands) Cl, Fl (green colors)

square planar
tetrahedral
octahedral
} These are orbital geometries

We are making a new stock solution.
Our peak @ 397nm is ~~1.869~~ for A 1.797
We had previously calibrated a solution

@ 448 nm A = ~~1.906~~ ~~1.865~~ ~~1.847~~ 1.825 (after it settles)

We have a relationship:

Conc. in mg/ml = (A - .2943) * 2.49 @ 446 nm.

So our second solution is

$(\overset{1.825}{\cancel{1.906}} - .2943) * 2.49 = \overset{3.81}{\cancel{4.01}}$ mg/ml

a ≈ ~~4~~ mg/ml
3.8 mg/ml

Stock Solution

1. We know the concentration of the primary stock solution by direct mass measurement.

This is 14.67 mg/ml

Very concentrated.

2. Absorbance can not be used for high concentrations.
We can see that 5.86 mg/ml was the max detectable by spectrometer, otherwise clipping occurs.

3. Our relationship, as long as the final concentration is $\leq$ 5.86 mg/ml is

$$\text{Concentration in mg/ml} \cong \left(\text{Absorbance @ 447 nm} - .2943\right) * 2.49$$

So our secondary solution has a concentration of

$$\left(\underset{\text{@448nm}}{1.825} - .2943\right) * 2.49 = 3.81 \text{ mg/ml}$$

$$\cong 3.8 \text{ mg/ml}$$

Secondary Solution.

We also know that 1 drop = .06 ml

$\frac{1}{.06}$ = 16.7 drops / ml

so for our secondary solution:

$\frac{3.8\ mg}{ml} \times \frac{.06\ ml}{drop} = \frac{.23\ mg}{drop}$

So 1 drop = .23 mg

No of mg = # drops (.23)

The spectrometer has a limit of detection by 5.06 mg/ml.

This means our 2nd stock solution is ideal for testing @ 3.8 mg/ml.

Thus far good work.
You have plenty of stock solution to work with now.

You also have a Calcium + H_2O_2 test in progress.

Page 46

What is your main goal now?

To determine what the Fe^{3+} is most likely bonded to.

To solve why 397 nm is such a recurring theme between

1. The Culture
2. Ferric Salts
3. Formaldehyde

} What does 397 nm mean?

Remember we also do have ferric ions showing up @ 397 in the CFC. What does this mean?

The ideal range of detection in the spectrometer of the Cultures is in the range of 2-3 mg/mL. Too high introduces a lot of distortion.

Crystal Field Theory Gives Us this:

I^- Br^- Cl^- F^- OH^- H_2O Moderate or Neutral NH_3 CN^- CO

more electronegativity — less electronegativity

Weak field ligands (I, Br, Cl, Fl) — Strong field ligands (CO, CN)

Less electron Repulsion (all axis) — Greater electron repulsion (align w/ axes)

red purple

Green Colors — Orange Colors

Paramagnetic — Diamagnetic

Lower Energy — Higher Energy

Unpaired Electrons — Paired Electrons

High Spin — Low Spin

also metals Co can be sequenced!

Mn^{2+} Ni^{2+} Co^{2+} Fe^{2+} V^{2+} — Fe^{3+} Cr^{3+} V^{3+} Co^{3+}

Now in our culture, our strong peaks are @ 397 & 448 nm

397 means absorb Violet — Perceive Green

448 means absorb indigo — Perceive Yellow

497 of the solution is strong means

498 means absorb blue green, perceive red.

What happens if you mix green & yellow & red.

Actually the bigger picture is absorbance for 400 to 550

Then is spanning yellow-orange-purple is perceive

It is spanning Violet to green is absorb.

purple & orange give a muddy brown!!

which is just what we see

It matches!!!

The Muggle view of color

Ok, now we are getting the color correct.
You understand why you are seeing the color you are and what it means.

First of all, it is the transition metals that produce color. And we do have color even if it is an ugly brown.

Higher to Moderate energies. We are absorbing in the culture in the range of 400 - 550 nm

The corresponds to absorb violet - blue - green
Then means we see yellow - orange - purple

Guess what you get when you mix yellow orange & purple?

BROWN!!!

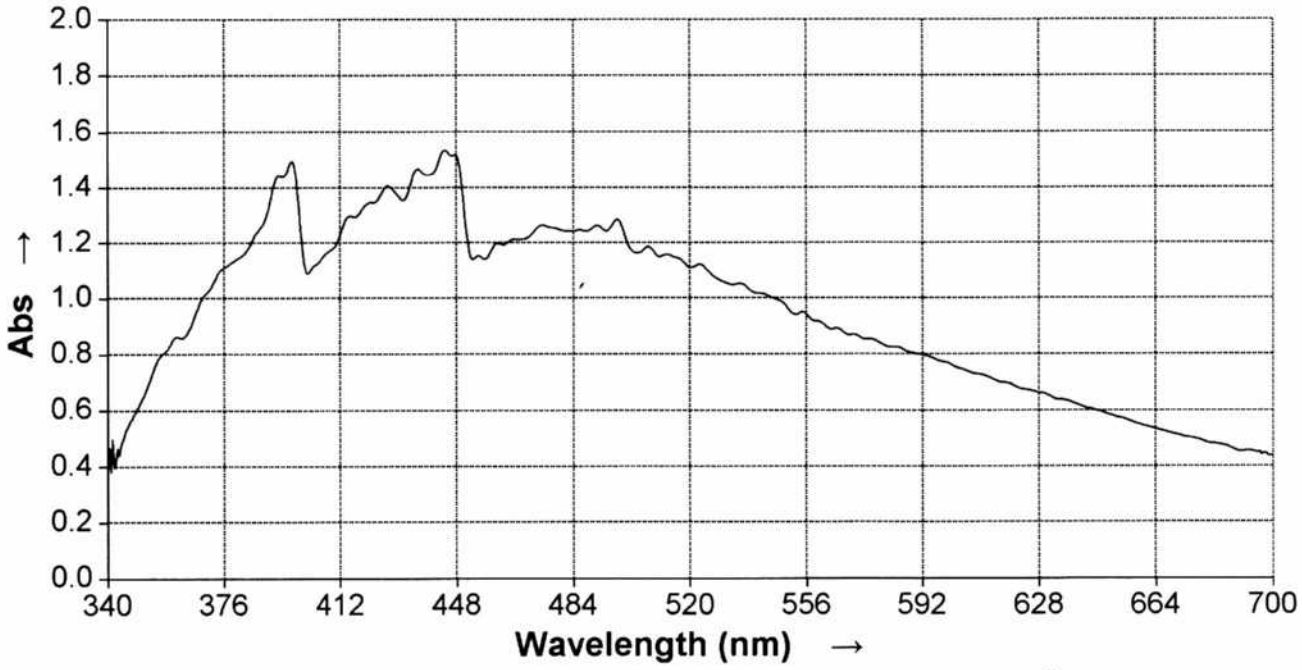
Exactly what you are seeing.

Now higher to moderate energy mean higher to moderate field ligands.
Who are these?

SPECTRONIC 200

Scan report

Spectrum of :	Scan2	Analysis date :	24 - Jul - 2011
Analyzed by :	Carnicom Institute	Analysis time :	12:10:35 PM
Channel # :	5	Print date :	24 - Jul - 2011
		Print time :	12:30:19 PM



This is the culture @ med Concentration ~ 3mg/ml
We are absorbing more strongly in the
400 – 550nm range.
This means we are absorbing violet – blue – green
This means we are seeing yellow – orange – purple

What do you get when you mix yellow orange purple?
Brown!!! exactly what we are seeing.

This should mean in general high to moderate
energy ligands.

Page 49A

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Yellow, purple & orange mixed.

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What color do you get when you mix yellow purple and orange?

In: Colors [Edit categories]

Sharpie Color Markers www.SharpieUncapped.com
See How People Express Themselves With Sharpie®. Visit Now!

Blick Art Materials ® DickBlick.com
Huge savings when you buy on-line. Outstanding service and selection.

Ads

Answer:

Improve

Brown. Purple is a mixture of red and blue. Orange is mixed red and yellow. Mixing red, blue and yellow together gives brown. You can alter the shade with different proportions, but it will be a shade of brown.

Color Checker Charts www.xritephoto.com/
Make quick color adjustments on site - color & white balance cards

Red Hair Colors 2011 GarnierUSA.com/Red+Hair+Colors
Even the Darkest Browns Can Become The Most Intense Reds - Learn How!

Get Perfect Lights Hair www.PerfectLights.com
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Ads

Brown!

First answer by Sikuan. Last edit by Sikuan. Contributor trust: 97 [recommend contributor]. Question popularity: 1 [recommend question].

Like Be the first of your friends to like this.

Can you answer these colors questions?

- Can a blue eyed mother and a brown eyed father have a brown eyed child?
- How motion blue different from radial blue?
- Who owns Orange Mobile?
- What color does dark purple and red make?

Related answers:

Which **color** do you get when you **mix yellow** and **purple**?
a dark green

What **colors** do you get when you **mix orange** and **yellow**?
Red

What **color** would you get when you **mix yellow** and **purple**?
purple yellow

What **colors** do you get when you **mix yellow** and **purple**?
brown

What **color** do you get when you **mix orange** and **yellow**?
yellow-orange, because you always say the primary color first when mixing them with secondary colors.

Research your answer

[redacted]

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Answer these

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In: Insects •

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In: Medical Fields •

How many songs has lady ga
In: Lady Gaga •

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Lets make a strong solution of $CoSO_4$ How about 1.0M.

Could we separate the ligands from the solution?

What are the strongest candidates?

The Plot Thickens.

We have separated the iron using Copper and now we have a yellow color left. (Makes sense) But we also have sulfuric acid!

Solution is mildly acidic 5.5
Could be a Complex such as Ferric

$$\left[Fe(H_2O)_5(OH)\right]^{2+}$$

We could also have a "ferricyanide ion"

Ferric solutions can be quite acidic from the reaction w/ water

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We could also have a "ferricyanide ion"

Ferric solutions can be quite acidic from the reaction w/ water

$CuSO_4 \cdot 5H_2O$
mw = 249.7 gms/mole

$\frac{249.7 \text{ gms}}{1000 \text{ ml}} = \frac{X}{60 \text{ ml}}$ $X = 14.98 \text{ gms}$

Too Strong. Make it 0.5 M = 8.50 gms OK Molar

Organic Cyanides are called nitriles.

The anion $N \equiv C^-$ is apparently called the Cyanide ion, reacts w/ the ferric form of Cytochrome and acts as an inhibitor of respiration.

nitrile
Carbonitrile

$N \equiv C^-$ is called a monovalent Cyano group

organic Compounds
$-C \equiv N$ is a nitrile.
$-N \equiv C$ is an ISOCyanide

Organic nitriles & ISOcyanide are less toxic

N_3^- is an azide ion

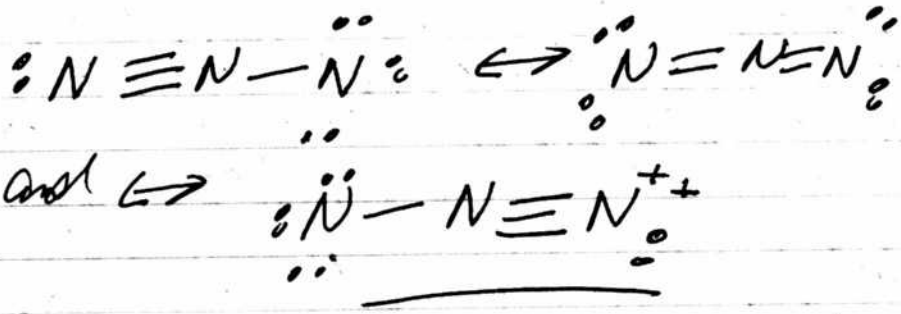
" As the oxidation of the metal increases, so also does the amount of splitting to of the d orbitals" (ie, shifts spectrum toward blue, perception toward yellow.

An example of something that bonds with azide N_3^- is hydrogen

makes sense

HN_3 is hydrogen azide.
Very ~~explosive~~ explosive.

N_3^- is actually a resonance hybrid of these Lewis structures.



and ↔

We do have a reaction with the extract!
It is with ammonia, turns blue green

This means the resulting solution absorbs in the orange & red region
(this means it has been lowered in energy)
likely reduced therefore.
What is it that has been reduced?

The extract has a yellow color.
It reacts w/ Ammonia to produce a blue (aq) color blue-green

The yellow fails the test for both Fe^{2+} & Fe^{3+} ~~to show~~ not that.

What is the test for Cu ions?
NH_3 & NaOH
both turn it blue.

Then tests both succeed. Even though the solution is yellow. It therefore appears to be a mix. We do have copper ions in the solution.

Somebody w/ $CuSO_4$ Cu^{2+} ions in it would OOO range but what is it that absorbs @ 182 - 183.

Cyanide test has
1. add Fe^{+2} (eg $CuSO_4$)
2. add HCl or H_2SO_4
it should turn blue in presence of CN^- ion. this test fails.

Looks like if the copper is fully oxidized that the final solution is essentially clear.

Another example is NaN_3 ($^{+1}\ ^{-1}$)
sodium azide

$[Fe^{+3} N_3^{-1}]^{+2}$???

seems to me we are most likely dealing w/ a ferric azide N_3^-
ferric cyanide CN^-
ferric Co_2 Co
complex.

We have a proposed reaction of the cultures:

$2Fe^{3+} + 6NaOH + 3CuSO_4 + H_2O \rightarrow$
$2Fe(OH)_3 + 3Cu^{2+} + 3Na_2SO_4 + H_2O$

However, NaOH is soluble & so is Na_2SO_4
your latest formulation is—

$2Fe(N_3)_3 + Na + 6OH + CuSO_4 + H_2O \rightarrow$

$Na + SO_4 + 2Fe(OH)_3 + Cu^{2+} + 9N_2 + H_2O$

by dissociation verified ↑ verified ↑ verified ↑

So your question is how could you involve a complex

$[Fe^{+3}(N_3)]^{+2} + Na + OH + CuSO_4 + H_2O \rightarrow$???

$Fe(OH)_3 + Cu^{2+} + Na_2SO_4 + H_2O + N_2$

You do not know how to have a reaction that has a complex in it.

Two scenarios:

$FeCN + Na + 3OH + CuSO_4 + 2H_2O \rightarrow$
$Na + SO_4 + Fe(OH)_3 + Cu + HCN + H_2O + OH$

but FeCN is actually in form $[Fe(CN)]^{+2}$

and alternately

$2\,FeN_3 + Na + 3OH + CuSO_4 + H_2O \rightarrow$
$Na + SO_4 + 2Fe(OH)_3 + Cu + 3N_2 + H_2O$

but FeN_3 is actually in form $[Fe(N_3)]^{+2}$

Indeed I did find a reference to a compound of form $Fe(N_3)_2$ which would be a ferrous metal organic network.

Guess what.
Our complex need not be
bound with anything other
than water and OH

The hydrated Fe^{3+} ion has the form

$$\left[Fe^{+3}(H_2O)_6^{0}\right]^{3+}$$ is +3

Fe^{3+}

OH & NH_3 both produce

$$\left[Fe^{+3}(H_2O)_3(OH_3)^{-3}\right]$$ is neutral

This should work!

It looks like we have it. The Fe^{+3}

does not have to be bound to
anything other than water & OH—

Google gave us idea under the
topic "Coordination Complex"
w/ a perfect color chart that
matches us exactly!

It is "Hydrated
~~appears~~ to be called Iron III hydroxide"
(ferric hydroxide)

So now we have a reasonable reaction

$Fe(H_2O)_3(OH)_3 + NaOH + CuSO_4 + H_2O \Rightarrow$

$Fe(OH)_3 + NaOH + SO_4 + Cu + 4H_2O$

So take a look @ this. The NaOH is neutral to the equation.

This means ← this came from mixing with lye.

$Fe(H_2O)_3(OH)_3 + CuSO_4 + H_2O \rightarrow$

$Fe(OH)_3 + SO_4^{2-} + Cu^{2+} + 4H_2O$

↑ tested ↑ tested ↑ tested

all verified!

So when you heat the organism with lye it forms a metal complex.

Who knows what complexes are formed in the body?
ferric oxides?
ferricyanides?
ferric hydroxides?

We may have something else going on here.

Our extract left us with a yellow solution. But we know it has Cu^{2+} ions in sol. When you added NH_3 it turned aqua, blue green

When you added NaOH it turned blue. Also when you added sodium thiocyanate it turned milky.

Carbonate ion w/ Copper turns blue green!

Also your reaction on the previous page is not complete.
The $CuSO_4$ forms the precipitate.

Fe^{3+} $Fe(H_2O)_6^{3+}$ is a yellow brown solution (you like we end up with on the extract.)

$Fe(H_2O)_3(OH)_3$ is a brown precipitate

Which is what we end up with not what we start with!!

Now we have some questions.

What is the difference between

the hydrated Fe^{3+} ion $[Fe(H_2O)_6]^{3+}$ yellow brown solution

and

$Fe(H_2O)_3(OH)_3$??? Brown precipitate

Notice also in our Copper reduced solution we have 3 distinct layers.

The Copper is replacing something to form a precipitate.

FeCO?

Something is wrong here.

We say we are starting out with hydrated iron oxide and then we are ending up pure iron hydroxide. This is not true. the $CuSO_4$ is replacing something.

The Fe^{+3} has to be combined w/ something + before you add it w/ $CuSO_4$ because a replacement reaction occurs at some point.

It is easy to show that Citric acid dissolves both Fe^{2+} & Fe^{3+} hydroxides. Make them by adding NaOH to salts of each dissolved in water.

Yellow & Green Colors. Colors. Now you understand why.

See green. (absorbs red) (lower state)
See yellow (absorbs both go) higher state.

You have proven it with the centrifuge

Citric acid dissolves the precipitate. It turns it back to a brown color. It does not completely separate like it did after you added the copper.

the copper replacement precipitate

What's the difference between iron hydroxide gel water and hydrated iron hydroxide in water?

CO, CN strong field ligands (orange colors) (higher energy) (diamagnetic)

Low spin means less unpaired electrons

High spin means more unpaired electrons (paramagnetic) (lower energy) (green colors)

I, Br (weak field ligands) Cl, Fl

square planar
tetrahedral
octahedral
} These are orbital geometries

We are making a new stock solution.
Our peak @ 397nm is ~~1.869~~ for A 1.797
We had previously calibrated a solution

after 1:10 dilution

@ 448 nm A = ~~1.906~~ ~~1.865~~ ~~1.771~~ 1.825

We have a relationship:

Conc. in mg/ml = (A - .2943) * 2.49 @ 446 nm!

So our second solution is

$(\overset{1.825}{\cancel{1.906}} - .2943) * 2.49 = \overset{3.81}{\cancel{4.01}}$ mg/ml

$\approx$

a = ~~4~~ mg/ml
3.8 mg/ml

Remember the reaction of ions in solution are independent of the substances?

$Fe + CuSO_4 \rightarrow FeSO_4 + Cu$

$Fe + NaOH + CuSO_4 \overset{?}{\rightarrow}$

We have a basic reaction of

$2Fe^{3+} + 6NaOH + 3CuSO_4 + H_2O \rightarrow$

$2Fe(OH)_3 + 3Cu^{2+} + 6Na^{+1} + 3SO_4^{-2} + H_2O$

Or we could have said:

$2Fe^{3+} + 6NaOH + 3CuSO_4 + H_2O \rightarrow$
$2Fe(OH)_3 + 3Cu^{2+} + 3Na_2SO_4 + H_2O$

but we also have
$Na_2SO_4 + H_2O \rightarrow 2Na + SO_4 + H_2O$
because Na_2SO_4 is soluble 4 gms in 1 Liter
NaCl is 35 gms/Liter

Test this reaction yourself!!!

(Mix yours is insoluble
$Fe^{+3} + NaOH$
FeCl) $+ CuSO_4 + H_2O$!

↑ this forms precipitate $Fe(OH)_3$

This reaction is not balanced.

Your reaction is not the same.

Fe^{+3} + NaOH alone produces a precipitate. But your Fe^{+3} is somehow in solution * then the copper precipitates out.

So something is different here.

Question: the hydrated Fe^{3+} soluble?

We have something to the effect of

$(Fe^{+3}$ in solution! not a precipitate$)$ + NaOH + $CuSO_4$ →

$Fe(OH)_3 + Cu^{2+} + SO_4^{2-}$

Is your final result acidic?

yes!!!! Why is it acidic??? has to have H^+

So somehow a piece of the Fe^{+3} in the ligand from precipitating out. It is bound somehow.

The Fe^{+3} is not in ionic form. This has been proven.

So it would have to be a strong ligand.

for instance
An ionic salt alone gives a precipitate

$FeCl + 3NaOH + H_2O \rightarrow Fe(OH)_3 + H_2O + 3Na + Cl$

if we were bound w/ CN^- ?

$FeCN^-$
FeN_3^-
$FeNO_2^-$
$FeOH^-$

Hydrated Fe^{3+} complex alone gives a brown solution

$[Fe(H_2O)_6]^{3+} + NaOH + CuSO_4 + H_2O \rightarrow$

$Fe(OH)_3 + Cu^{2+} + SO_4^{2-} + Na + OH$

Got it w/ Chemix!!!

$2[Fe(H_2O)_6]^{3+} + 6NaOH + 3CuSO_4 + H_2O \rightarrow$
brown solution!

$2Fe(OH)_3 + 3Cu^{2+} + 3\underline{\text{Soluble}}\ Na_2SO_4 + 13H_2O$

or could it be hydrated also?

Now the question is where does the acidity come from?
Another form would also:

Another form we'll also

Fe 3+ ion verified

$$\left[Fe(H_2O)_6\right]^{3+} + 6NaOH + 3CuSO_4 + H_2O$$

$$+ \cancel{Heat} \rightarrow 2\left[Fe(H_2O)_3(OH)_3\right] + 3Cu^{2+}$$

(Cu^{2+} verified in extract)

$$+ 3Na_2SO_4 + 7H_2O$$

(Na_2SO_4 verified in extract; H_2O dissassociates)

Now to get H^+ ions in there, as verified by a pH check we have

yellow or brown solution → $\left[Fe(H_2O)_6\right]^{3+}$ known + $2NaOH$ known + $CuSO_4$ known + H_2O →

$$\left[Fe(H_2O)_3(OH)_3\right] + Cu^{2+} + Na_2SO_4 + 3H_2O + H^+$$

brown jelly precipitate verified

(Cu^{2+} verified; Na_2SO_4 (soluble) verified clear/verified; H^+ verified)

This is a workable model

==

Cyanide Ion testing

~~$Fe(CN)_6$~~

$[Fe(CN)_6]^{3-}$ is hexacyanoferrate III

We found a paper on the oxidation of ascorbic acid by the $[Fe(CN)_6]^{3-}$ that it can be monitored by the disappearance of the yellow.

Today: when we add ascorbic acid to the culture, the solution lightens in color.

Acetic acid added to the culture causes exactly the same lightening of color.

$[Fe(CN)_6]^{3-}$ apparently is yellow in color

Yes it is. My solution does look close.

We know we have copper ions in the solution, also sulfate ions (positive test)
We know/bet does not have Fe^{2+} or Fe^{3+} in it.

Have both potassium ferrocyanide! $K_4[Fe(CN)_6]$
& ferricyanide! $K_3[Fe(CN)_6]$

Cyanide Ion testing.

There is a test for the Cyanide ion

1. add $FeSO_4$
2. add HCl or H_2SO_4
   should turn blue. (Prussian blue)

The yellow extract:

1. Conductivity is high 700 uS
2. pH is mildly acidic ~ 6.0
3. Peak is @ in 370 nm

I am getting a white precipitate w/ $AgNO_3$

This may mean a Complex of
(this is Fe^{2+})

Fe^{3+} $[Fe(CN)_6]^{4-}$

Silver nitrate forms a white precipitate w/ Chlorides

$AgNO_3 + NaCl \rightarrow AgCl$

Silve Nitrate forms a whit precipate with

Sodium Bromide	Silve Bromide
Sodium Chloride	Silve Chloride
Sodium Carbonate	Silver Carborate

We clearly have a white carbonate forms w/ addition of silver nitrate to the extract from the culture.

So now we know it has
1. Copper ions (added)
2. sulfate ions (added)
3. Carbonate ions ???

Does not seem as likely it is from Bromide or Chloride but not impossible.

It might be silver sulphate?

Yes, sulphate also form white precipitate w/ $AgNO_3$ & we know we have sulfate.

So what is the yellow color from???

We have some observations:
(1) The color wheel was yellow looks like it went to green after it had set long enough in the copper.
(2) Both yellow & green colors failed Fe^{2+} & Fe^{3+} ion tests
(3) We get a precipitate, green, light green color, we mix (1, 10), sodium thiocyanate & yellow solution. We have no idea what it means.

Lets think about the green color & what it mean

$C_{12}H_8N_2$ Phenanthroline or a Carbon Hydrogen Nitrogen metal chelator.

$NaSCN$ Sodium, Sulfur, Carbon, Nitrogen

$C_{12}H_8N_2 + NaSCN + H_2O$ (?) → light green precipitate

transition metal?

Green is 650 nm means it absorbs red (lower energy) $[M(phen)_2(NCS)_2]$

Spectrum appears to be flat across the board, how could that be?
to dense?
Very hard to say what this means.
We have no peaks of any kind.

But the yellow extract has a different spectrum
one peak around 340 and the other around 800 nm (Infra-red)

We do not know how to interpret this yet.

We know it has Copper ions
SO_4 ions
We may have Chloride ions also?
Remember our earlier paper indicated an increase in Fe^{2+} ions & Chloride ions

But apparently silver reacts w/ sulfate ions also to produce silver sulfate.

Ot, we may have more knowledge.

We have run a test for the Chloride ion and it definitely has passed it.
Method
1. Yellow solution
2. Add dilute nitric acid (we do have it)
3. Add $AgNO_3$
It is supposed to be a fairly unique test.

So in our yellow solution we have identified
Cu^{2+} ions
SO_4^{2-} ions
Cl^- ions
} these two have been added
} this one may be a new ion.

Do this mean it indicates somethings else as Cu dissociated w/ the Cl^-?
Mg, Ca, Sr, Ba??? Li, Na, K?

Guess what?

We have a green flame showing up!!

Remember we have a green precipitate that showed up.

Ba^{2+} is yellow green.
Cu^{2+} is blue w/ flashes of green.

Another source says Cu^{2+} does burn green.

Ba or Cu ??? (We know there is copper but something combined w/ Chloride)

Ba apparently burns apple green.
Cu burns green to blue green

You appear to be getting a green flame maybe observed to some degree

Green	Red
Copper	Calcium
Barium	Lithium
	Strontium

After all these years, we finally have a test for Barium Ions.

1. Have ions in solution of barium (used barium chloride solution)
2. add some ethanol
3. add some sulfuric acid

it form clear a white ppt precipitate.

Now we know our extract does not have barium ions in it

We do, however, pass a Chloride test by using dilute nitric acid followed by $AgNO_3$, form silver Chloride.

So what dissociates w/ the Chloride?

Could we have had ferric chloride as an original constituent?

Is there an iron Fe^{3+} w/ Chlorine?

We must now question if the original complex is

$FeCl_3 \cdot 6H_2O$

The topic is

ferric chloride complex

There are indeed ferric chloride complexes.

$[FeCl_4]^-$ is indeed a yellow ion. It is a complex.

~~$FeCl_3$~~

Ferric chloride / Iron III chloride

This is matching. It is yellow. It is a complex so Fe^{3+} for that will fail.

Why does the test for chloride ion succeed?

1. Dilute Nitric Acid
2. $AgNO_3$
3. $AgCl$ formed - white precipitate.

Maybe the nitric acid breaks the complex down??

A that we have found it tonight.
I believe it is a ferric chloride
complex that remains.

There is nothing to say that it is
bound to Chlorine in its original
ligand state upon the culture.

The chlorine determination is made
after the reduction of the iron (replacement?)
by the copper.

But hence Cl^- ions exist (we show
this, it may just form a complex
with it from the spectrochemical
series. Cl is weaker on
the series but at this point what
is left for the ferric ion to
bind to.

You would like to see the reaction
down.

How could you make this

Cl^- & Fe^{+3} ions?

Should be $[Fe(H_2O)_6Cl_4]^{-1}$

1. Test for Al^{+3}?

2. Heat urea after dye?

3 Composition of urine

4 Alignment w/ magnetic field?

5. Urine is $(NH_2)_2CO$ or CON_2H_4 Urea

(insoluble) $C_5H_4N_4O_3$ Uric Acid

Composition

Water 95%.
Urea 9.3 g/l
Chloride 1.87 g/l
sodium 1.17 g/l
potassium .750 g/l
Creatinine .67 g/l

ions form are Na^+, K^+, Cl^-, Mg^{2+} Ca^{2+}
ammonium NH_4^+, Sulfates SO_4^{2-}
and phosphates PO_4^{3-},

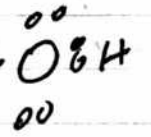
Notice!! Free radicals are electrically neutral
* (have an unpaired electron – they are not ions!)

Think about what is happening here.
Fenton reaction stands by itself.

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH + OH^-$$

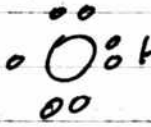
First, let's look @ oxidation states

OH^- This is an ion.
$-2 + 1 = -1$ ok



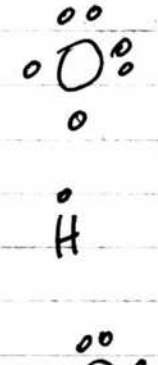
this is a free radical.

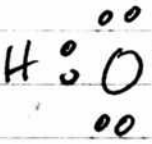
OH
$-1? + 1? = \emptyset$?
$-2 + 2 = \emptyset$?
potent oxidizing agent



~~H_2O_2~~ Flat O_2 has an oxidation state of $\emptyset$
H_2O -2
H_2O_2 -2

H_2 has oxidation state of $\emptyset$!
H has " ok +1





$-1 + 1 = \emptyset$

= This is the hydroxyl radical

-1

So sometimes O is in a -2 state & sometimes in a -1 state.

Now we understand the difference between an ion and a free radical. A free radical is electrically neutral but highly reactive.

Fenton's

$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH$

First of all with the presence of OH^- you would think that it would be alkaline.

But it is not - why? It measure at ~~5.2~~ 3.6 !!!
Why is this.

As indeed the growth environment for the culture is acidic but we do not know why.

So we have
$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + OH$ (acidic)
→ highly reactive
← why acidic?

Now

Culture + Fe^{3+} + OH^- + $OH \rightarrow$ rapid growth. + H_2O
(highly acidic)
why?

why?

?? $[Fe + Culture(H_2O)_6]^{3+}$

+ 2 NaOH + 3 $CuSO_4$ + $H_2O \rightarrow$ 2 $[Fe(H_2O)_3(OH)_3]$ +

$3Cu^{2+} + 3Na_2SO_4 + 5H_2O + H^+$

So can we get to form → joins w/ culture??

$Fe^{3+} + OH^- + OH + H_2O + NaOH + CuSO_4 + \text{Culture}$

??

$\rightarrow [Fe(H_2O)_3(OH)_3] + Cu^{2+} + Na_2SO_4 + H_2O + H^+$

$+ \text{Culture}$

Actually the only thing required here is for OH (free radical) to form with the culture!

Everything else fits

Page 78

Aug 07 2011 in Montana:

1. Situation: Very bad took situation. Weak still today but less pain. (by far!)

LOST the accounts on Tues AM

2. Market is in potential turmoil. US Debt rating gets lowered for first time in history.

3. A social obligation @ the same time. Manage all three? How is your social intelligence quotient?

Page 79

Coordinate Covalent Bond:

P 70 of Chemistry Workbook has an excellent description of why ya have Coordinate Covalent bonds take place.

It is a combination of empty valence shells available in the transition metals (Mostly group B on the periodic table) (the central portion of the table) and lone pairs (unpaired electrons) with the same orbital available from a ligand that will combine w/ the metal. (an electron donor)

Now that ya understand the basics of a coordination complex & how it forms. ya must also tackle the oxidation process.

The oxidizer steps away electrons
The reducer accepts the electrons.

Now the big question is in the case of Fe^{+2} & Fe^{+3} in the same molecule

What is it that steps away the electron?
i.e, what is the oxidizer?

So in the case of methemoglobinemia

what is it that commonly acts as an oxidizer?

Even if you do not know exactly what it is ~~that~~ oxidizers it, what are typical examples ~~that~~ that can cause it.

~~Guess~~ what:
We have already found an answer.

Biochemistry Demystified p268

Ex: A decrease in pH (ie more acidic

* decreases the affinity of hemoglobin for oxygen. (Therefore, if the oxygen is not bound to Fe, the iron is in the Fe^{+3} state

So an acidic condition will promote an Fe^{3+} state in the body.

We now have 12 Candidates as potential binders to Fe^{3+}:

Page 81

Ligands

Biochemistry Demystified — More binders to Fe^{+3} in methemo deoxy heme are listed on p272 Biochemistry Demystified

one source

- CO
- CN^-
- SO
- NO_2
- S_2^-
- good job. H_2S
- ~~the strongest oxidizing substance~~!

as our list is quite extensive know.

Total List Now Is:

should be not likely
- CO
- CN^-
- NH_3
- H_2O
- OH^-

Dept of Justice Paper — What is the source here?

We previously identified

identified or likely

- OH^- hydroxide
- CN^- Cyanide - respiration inhibitor
- N_3^- Azide - respiration inhibitor
- NO_2^- nitrate
- SO
- NO_2
- S_2^-
- N_3^-
- NO_2^-

the first paper found

Ferrihemoglobin azide is one such example of a complex. Ferrihemoglobin is another name for methemoglobinemia

$Fe^{+3}(N_3)^-$ is ferrohemoglobin azide.

Prof Dean Harmon also gave a list:

another source

- NH_3
- H_2O
- OH^-
- CN
- CH_3COO
- Cl

unknown: CH_3COO

not necessarily ~~less likely~~: Cl

this is actually a real Candidate. See Haemoglobin a molecular lung: 2 Peter E Child paper

Looks like the Dept of Justice Blood Document I sent to myself by email

Two questions:

1. What are examples of oxidizers?

2. Where is the paper that identified the first binders to Fe^{3+} (ligands) identified?

A sequence is
1. The oxidation of HbO_2

$$HbO_2 \rightarrow Hb + O_2^-$$

deoxy & superoxide anion are formed

Now we have another source that says ~~Fe+3~~ the O_2^- will get ~~replaced~~ displaced by Cl ion so the would be Cl^-

So this should be of some form to the effect

$$\left[Fe^{+3}(Cl)_4^{@}\right]^-$$

apparently it is in this form.

And then the hemoglobin is in the deoxy ~~state~~.

Now, do we not have evidence of chloride ions? ion our cultures?

Page 83 *

We have some fairly good evidence that a ferric chloride complex may be within the culture.

complex paper

We have a paper that says such a substitution can occur

the haem paper says Cl ions will displace

*

$HbO_2 \rightarrow Hb + O_2^-$ (free radical paper)

O_2^- can get displaced with chloride ion (Haemoglobin paper)

~~This~~ I anticipate this to lead to a ferric chloride complex & deoxy heme.

Should be of form $[Fe(Cl)_4]^-$

Now interestingly enough, we seem to move Fe^{+3} form w/ the copper reaction.

*

When this reaction is at least partially complete we are left w/ a yellow extract.

Guess what, this tests positive for the chloride ion & the yellow color indicates the ferric ion

This is one real possibility. This is from a culture result, not the body!

Try a real sample! Do you get the same result???

Does urine have chloride ions in it?

Now what exactly is an oxidizer and how does it work?

Ok, closing soon.

Free radicals are apparently very good oxidizers.

There are three ways you can look @ oxidation, not just in terms of electron loss.
You can, but you do not have to, view it in terms of

1. electron loss
2. ~~oxid~~ oxygen gain
3. hydrogen loss

A first simple example of oxidation / redox is $Na_{(solid)} + Cl_{(gas)} \rightarrow NaCl$ salt!

so Na (elemental, no charge) + Cl elemental, no charge → $Na^+ Cl^-$ salt, an ionic bond.

oxidation/reduction results from a transfer of electrons (not sharing)

We have learned that this means an ionic bond. (not covalent)

becomes positive ion loses an electron

So $Na \rightarrow Na^{+1} + e^-$ (loses electron)

~~$Cl \rightarrow Cl$~~

$Cl + e^- \rightarrow Cl^{-1}$ (gains an electron, becomes negative)

gains an electron becomes negative ion

If something is oxidized, it has lost an electron.
If something is an oxidizer, it steals the electron

If something is reduced, it gains an electron.
If something is a reducer, it gives an electron.

lost an electron

So

$Na \rightarrow Na^{+1} + e^-$ Sodium has been oxidized.

$Cl + e^- \rightarrow Cl^-$ Chlorine is the oxidizer.

gained an electron

$Na + Cl + e^- \rightarrow NaCl + e^-$

$Na + Cl \rightarrow NaCl$

a very interesting process. Redox reactions should be ionic in nature, not covalent.

When elemental iron is combined w/ O_2 it forms ferric oxide

Now iron is a great example

$Fe + O_2 \rightarrow Fe_2O_3$ (not balanced)

$\overset{+3}{Fe}_2\overset{-2}{O}_3$

$2Fe + 3O_2 \rightarrow 2Fe_2O_3$ (balanced)

So breaking this up:

$Fe \rightarrow Fe^{2+} + 2e^-$ oxidized.

$O_2 + 4e^- \rightarrow 2O_3$

notice this is a ferric form (oxide) not ferric hydroxide!

In the case of hydroxides

When ferric & ferrous ions are combined with hydroxide ion (not hydroxyl radical) they form ferrous & ferric hydroxide precipitate respectively

$Fe^{2+} + 2OH^- \rightarrow Fe(OH)_2$ green

and

$Fe^{3+} + 3OH^- \rightarrow Fe(OH)_3$ brown

So it depends upon what is there.

Redox is not always ionic.

Another example of a redox reaction is:

$C + O_2 = CO_2$ burning of coal

but CO_2 has an electronegativity Δ of 1.0
This would be a Covalent bond of
$\leq .5$ Covalent
0.5 - 1.7 polar covalent
> 1.7 ionic

This is hardly an ionic bond. So redox reactions do not have to be ionic, but they might be.

$\overset{+4}{C} + O_2 \rightarrow \overset{-4}{CO_2}$

What about iron oxide $2Fe_2O_3$ $1.8 - 3.5 = 1.7$
This is ionic

So we have a mix.
sometimes ionic, sometimes polar covalent.

Let's think about where we are:

1. You would still like to look @ what are good oxidizers.
2. How might these oxidizers interact w/ Fe^{2+} to produce Fe^{3+}
3. What are free radicals & how do they differ from oxidizers?
4. What are Lewis dot examples of both oxidation & free oxidized reactions?

An oxidizer steals electrons. So oxidizers would be on the R side of the periodic table.

So now we know why Cl, O, N, S & C would all be good oxidizer agents.

Now look @ our spectrochemical series:

$I^-, Br^-, Cl^-, F^-, OH^-$ H_2O NH_3 CN^- CO

And now we see that the list makes perfect sense.

The only one that does not "fit" is H but remember how H can be "moved" to the right side of the periodic table.

But there is very little surprise to the list. Notice the strong overlap!

I^-, Br^- & F^- would be obvious additions but most less available up in the body.

Notice the redox set: (types of redox)
1. ~~Loss~~ of oxygen Gain of oxygen!
2. Loss of hydrogen (lost hydrogen gets off in a gaseous form)
3. Loss of electrons

Now Sulfur is not really showing up on the spectrochemical series, but w/ this exception & some more study on H, my list is the same list!

Spectrochemical Candidates	My Candidates
Cl^-	Cl match
OH^-	O match
NH_3	N match
CN	C match
CO	(S)?
(H?)	

Now we have a good sense of what representative oxidizers are.

Actually is a gain of oxygen similar to a "loss of hydrogen"?

So these elements (and combinations of them) are good oxidizers.

This means that they could be candidates to turn Fe^{2+} to Fe^{3+}.

Remember the statements: (Mass College of Pharmacy Tara Stoppa, Pharm D Candidate)

"Methemoglobinemia usually results from 2000 exposure to an oxidizing agent."

So now we know what this means we now know the candidates. (Could be others)

Cl
O
N
C
} these elements could & will combine w/ one another

and this can easily lead to the spectrochemical series:

$I^-, Br^-, Cl^-, F^-, OH^-$ H_2O, NH_3 CN^-, CO^-

Now we learn about the role of free radicals.
~~Remember that free radicals are electrically neutral~~, but highly reactive.

Let's look @ an example.
The most reactive free radical that there is is the hydroxyl radical.

It is NOT TRUE that free radicals are electrically neutral. It may be but it does not have to be.

Radicals can have positive, negative or neutral charges. So they are hardly electrically neutral, that was a false statement.

Having free radicals is not unusual in itself, it is a matter how many.

Three major free radicals are

O_2^- superoxide → $\ddot{O}::\ddot{O}$ so O_2 stole an electron

O_2^{-2} peroxide → $\ddot{O}::\ddot{O}$ & O_2 stole two electrons!

OH hydroxyl → $\ddot{O}:H$ Single O combined w/ hydrogen & stole an electron herself.

~~Remember~~ $\ddot{O}:$ is highly reactive in itself and then goes on to steal an electron.

*

Page 92

An important summary of the problem

1. So the problem is that we have oxidation of Fe^{2+} in blood, ~~thus carbon~~ decreases the oxygen carrying capacity of blood.

2. Next, the oxidized iron in the Fe^{3+} state binds to other ligands, some of what themselves are respiratory inhibitors.

3. An added insult to injury, when Fe^{2+} in blood is oxidized it produces free radicals (also a chain reaction process)

A 3rd example: (deoxy) We know this happens

$HBO_2 \rightarrow HB + O_2^-$

a major free radical

A double whammy is what happens.

The organism must have oxidizing elements within it, & the ability to use oxidizing agents

Next, what exactly is the damage done from free radicals?

Free radicals react with proteins, lipids and nucleic acids which are constituents of membranes (cell walls) OR DNA & RNA & "wreck havoc in the living system".

Talk about a triple whammy!

1. Lowered oxygen (a chain reaction process)
2. Increase respiratory inhibitors
3. free radical creation to "wreak havoc in the living system". (also a chain reaction process)

ie, we are

1. pulling the oxygen out of the blood
2. replacing it w/ inhibitors
3. creating highly reactive free radicals

The big three

O_2^- superoxide
O_2^{-2} peroxide
OH hydroxyl

Free radicals can mutate DNA & genes
Biochemistry Demystified p 169

Deprivation of oxygen leads to
Cancer cell proliferation
There is huge. P 217 Biochemistry
Demystified.

Glycolysis is heavily linked to Cancer.

Chap 10 is glycolysis

Glycolysis is a process that ~~liberates~~
energy from a glucose molecule
under anerobic conditions! p196
BD

& guess what.? Biochemistry
Demystified

Most energy in
living cellular systems
comes from the oxidation of glucose
(ie normally in the presence of oxygen!)

~~Just it happens~~ Just it happens
in anerobic conditions it leads
to cancer!

Work directly w/ the oral sample now.

1. Cr replacement?

2. Cl ion detection?

3. Consider figuring out the main reaction.

"The most important reactants in free radical biochemistry in aerobic cells are oxygen and its radical derivatives (superoxide & hydroxyl radical), hydrogen peroxide and the transition metals."

British Medical Bulletin
Volume 49
Issue 3 p481-493
by K.H. Cheeseman

Lipid peroxidation is a measure of the damage.

Sound familiar? O_2^- O_2^{-2} OH + Transition Metals ie Fe!

Hydroxyl radicals react w/ all types of biologically important molecules
- nucleic acids
- proteins
- sugars
- lipids

producing radicals that undergo further reaction.

Lipid peroxidation refers to the oxidative degradation of lipids. Free radicals steal electrons from the lipids in cell membranes resulting in cell damage.

Lipid peroxidation means rancid oils!

Something called the

"Method of Continuous Variation"

Can be used to determine the formula of a complex ion. (in conjunction w/ a spectrophotometer).

You need to do this.

Now you must:

1. Work directly w/ the culture form
2. What is the "method of Continuous Variation" that can be used to determine the formula of a complex ion.
   This would be fantastic.
   also called Job's method

Significant Discovery:

The oral filaments dissolve in NaOH & heat directly Gives the very brown color I just like the culture.

Red Wine does have some similarity to the culture solution but not exactly the same. The oral sample does, however, absorb pigments from the wine.

We noticed how that wine is more flatter from 412 to 520. Red solution should show absorbance @ 490 which is correct. Also wine flattened out @ a higher no. ie 0.3

I believe you have the best isolation of the organism yet.

You have subtracted out were from the culture and it leaves a very distinctive peak @ 396 nm.

This corresponds to percenury yellow (same as Fe^{+3} ion) and absorbing violet.

This is another level of confirmation

Also we see that adding $FeSO_4$ (concentrated) to H_2O_2 and the culture is producing a darker color & there are dark specks developing.

We also learn that the oral filament dissolve easily in NaOH may be another reason for promoting an alkaline condition in the body.

We speak filaments sent by [redacted] are essentially impervious to NaOH vs the oral filaments. Oral filaments easily dissolve in NaOH.

Now on the culture we should test for Fe^{2+} in & the Cl ion!!! & for Cu displacement!!!

1. Test the Fe^{2+} and Fe^{3+} tests. This is expected. Hypothesis is that it is bound and not free as an ion.

2. Cu displacement test succeeds. Chemistry of oral sample is shown itself to be identical to the cultured sample.

3. Spectrometry of oral sample is also the same as the culture sample.

4. Now a test for Cl ion. The oral sample positively passes the test for the Chloride ion. Test in the original oral-lyse solution as well as in the extract after the Cu-Fe displacement reaction.

The struggle now that we have the culture and the oral sample that we have an ferric - chloride Coordination complex that exists.

There are indeed sodium salts in Wine so it is a possible reason why we are finding the Chloride ion.

So at this point we are not positive whether we have an Fe^{+3} Cl in Complex

$$\left[Fe^{+3}(Cl)_4\right]^{-1}$$ is the expected form.

The univ of Limerick in Ireland paper is incredibly important here

Haemoglobin - a molecular lung: 2
Peter E Childs

is the paper!

Lets try testing wire directly for Cl^-.

But remember our statement in an early paper that the Chloride ion appears to be increasing in Concentration???

It seems questionable that the filament would "absorb" the Chloride ion but I suppose it is possible.

Does red wire test positive for the Chloride ion?

Clearly we have a positive test for the Chloride ion in diluted wire. Now how about highly diluted wire. Yes, still easily detectable

Yes, even more diluted wire works. 1 drop of wire in about 5 ml of water, easily detects the Chloride ion. So this is actually a very sensitive test.

Preparation of oral culture:

1. Gather oral sample
2. Rinse repeatedly w/ distilled water.
3. Now add 4 drops lye to almost 50 ml of distilled water and the rinsed oral sample. Heat to boiling. The solution turn brown and the filaments completely dissolve.
4. Filter the solution. Then make the stock oral solution (actual concentration unknown).
5. We presume that the filament absorbs the dye in wire.

   Why??? What are the dyes?

   Is a ferric chloride coordinate complex toxic to the body?

Guess what causes the red color of red wine?

Anthocyanins.

Sound familiar - cyanide ion??

Spectrochemical series?

Fe^{3+} ion combines readily w/ the cyanide ion.

I believe we know now why red wine extracts the filaments.

Our gums have all kinds of capillaries in them. We can support that the iron w/ the blood of the gums has been in part turned to the Fe^{3+} state. Our gums are also highly acidic.

When we add red wine we are adding anthocyanin. Cyanide ion combines w/ Fe^{+3} ion in an acidic environment produces a red filament???

Pigments in wine react w/ saliva?

Pigments in wine react w/ baking soda?

Wine + NaOH gives a green solution

Now we understand why anthocyanins —

The cyanide ion has the structure

$$\left[:\ddot{C} \equiv N: \right]^-$$

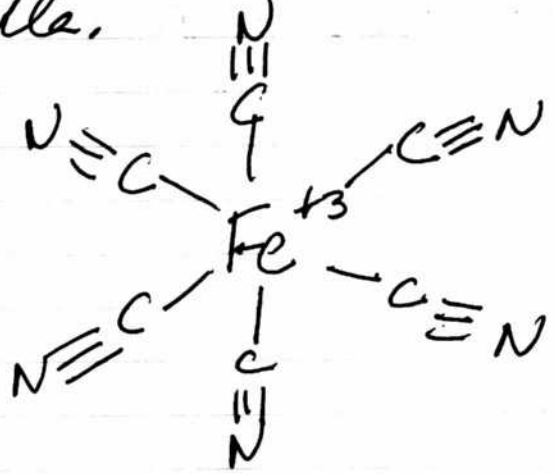
Now what do anthocyanins have

Guess what

ferricyanide looks key to our situation. $[Fe(CN)_6]^{3-}$

is the formula.

"iron(3+) hexacyanide"



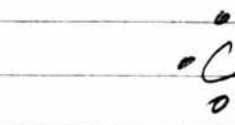
Now how does the structure of anthocyanin fall into place here??

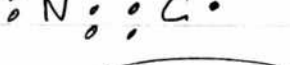
What is the flavylium ion?

flavylium

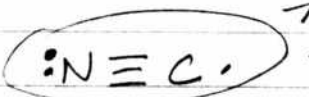
If the carbons in the anthocyanins bond to nitrogens you would have cyanide ions available! Cyanide ions form w/ Fe^{3+} to form coordination complexes.

H +1
O -2
N -3
C -4

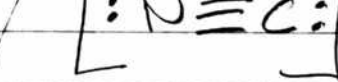




This would be a covalent bond now if we add another electron it has a negative charge and it becomes a cyanide ion



This would be the neutral form



→ if it combines w/ hydrogen

it forms hydrogen cyanide so lots of this can happen.

Now in the case of red wine we know we have anthocyanins available.

This means free carbons available.

These seem like they could easily combine w/ nitrogen. It is in the air!

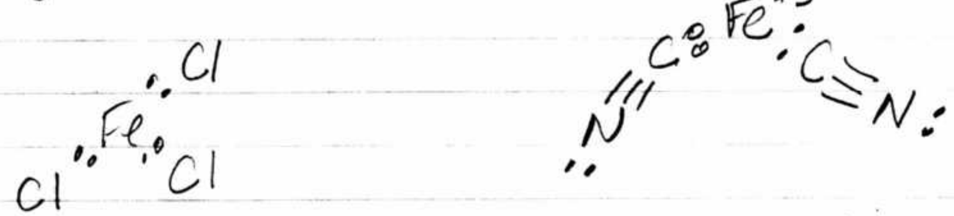
This would lead to <u>neutral</u> covalent bonds

$\cdot C \equiv N:$

but if there were somehow to steal ~~an~~ or share an electron from something, eg Fe^{3+}

Fe^{+3} ⇒ ~~Fe:~~ $\cdot\dot{Fe}\cdot$

+3 $\cdot\dot{Fe}\cdot$ $Cl\cdot$



$[Fe(CN)_3]$

So this suggests that the wine is forming a ferric cyanide complex with the red wine.

It is the most reactive ligand

We need to learn about these Lewis dot structures.

We need a test for the ferric cyanide complexes (should be present in oral samples)

Ascorbic acid has a formula of $C_6H_8O_6$
Molecular wgt is 176.13

In solution, it rapidly oxidizes.

There is no question that addition of ascorbic acid turns the oral stock solution a lighter color

It is actually producing a precipitate. It is not doing this in urine.

So now we have a copper reaction and an ascorbic acid reaction they both produce a precipitate

We clearly have a gas being produced. And a precipitate

We have a very successful test.

Oxydation of the ferricyanide ion by ascorbic acid produces hydrogen ~~oxygen~~ gas and iron in the Fe^{+2} state to ferrocyanide complex.

Oral sample extract does not test positive for Fe^{3+} ion. It appears indeed to something.

You add ascorbic acid. It causes an oxidation reactor that produces Hydrogen gas and a clear solution.
When you test it for Fe^{2+} ions it is positive!

THIS IS IT - The oral sample reaction

We have the answer to the Chemistry of the oral sample.

It is a ferric cyanide complex.

When you add ascorbic acid the $Fe(CN_6)^{3-}$ oxidizes the ascorbic acid to/release hydrogen gas
2. produce a colorless solution
3. form a ferrous cyanide complex
4. which tests positive for the ferrous ion.

Anthocyanin has nothing to do with the cyanide ion.
But ph & heat may make it a factor.

!!! Ferric chloride reacts w/ wine to produce exactly the color we are getting.

Tonight we will work on more Chemistry but in the meantime, a section on valence electrons. Valence electrons are any thing beyond the noble gas previous.

eg Na: $1s^2 2s^2 2p^6 | 3s^1$ valence electrons

or $Ne\ 3s^1$ so Na.

Cl: Ne $3s^2 3p^5$ so 7 valence electrons

Iron: ~~Ar~~ Ar $4s^2 3d^6$ = 8 valence electrons

Fe^{+2} mean two electron have been stolen

means :Fe: Fe^{+3} mean 5 :Fe:

FeCl Chlorine gives 3 $FeCl_3$ = $^{+3}_{\ }$ $^{-3}_{\ }$ = 0

O_2 has 6 :O:

Everybody wants 8. Oxygen gains two leads to charge of -2

Cu:

Ar $4s^2 3d^{10} 4s^1$??? Listen to Khan!

Conflict w/ Khan

d has a max of 10. from Moore p31.

Khan on valence electron:

Fe Ar $4s^2 3d^6$

→ Khan says this is the outer shell & shells backfill

it is the outer shell that reacts.

We have a conflict between Khan & Thinkwell.

Khan would say Cr is [Ar] $4s^2 3d^9$

Khan would say outer shell has 2 valence electron

There is a way a conflict between Khan & everyone else, including Thinkwell.

Thinkwell seems to have a great deg. not.

Khan seems to have a usable practical instructive system.

Who is right here?

Now let see the nature of the problem.

The nature of the problem:

Thinkwell defines the valence electrons as any that lie beyond the previous noble gas configuration.

Khan seems to define the valence electrons as those in the outer shell.

These two definitions are not the same.

Example

Fe configuration is $[Ar]\,4s^2\,3d^6$

by Thinkwell, the valence electrons are 8.

by Khan, the outer shell is 4 (less energy than outer shell) so Khan is saying the outer electrons are 2 in number

These are hardly the same.

What is the true definition of the valence electrons?

EZ Chemistry p 37

defining valence

With the big thick book by Brown in the glossary the definition says both Thinkwell and Khan!

range 1. outermost shells
2. beyond the noble gas configuration
3. the electrons actually used in bonding.

Fascinating, then points out the problem! These are not necessarily the same.

No wonder you have been confused! p248 & 249 Brown - Chemistry at least discloses the confusion but w/ no real answers.

so I see alternative interpretations

CW: Khan: [Ar] $4s^2 3d^9$

Brown [Ar] $3d^{10} 4s^1$ p249

Fe: [Ar] $4s^2 3d^6$ ↓

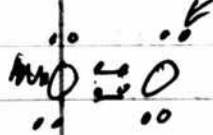
Fe [Ar] $3d^6 4s^2$ ↑ Thinkwell, Brown

Now what are the number of valence electrons?
Khan would say 2
Brown would say 1
Thinkwell would say 11 (those beyond noble gas configuration)
but completely ignored d or f so not included.

Practice determining Valence electrons

O: $[He]2s^2 2p^4$ both level two, so it is 6

is 2 deficient, oxidation no = −2



O_2 double bond

Mg: $[Ne]3s^2$

Important

Exceptions to He & H – they only seek 2 electrons not 8

do the Lewis (valence electron) tell you what it can do.

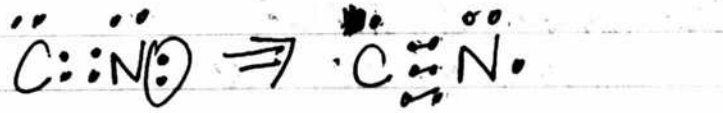
the CN^- ion

C: $[He]2s^2 2p^2$ = 4 valence

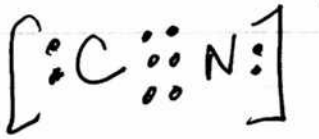
N: $[He]2s^2 2p^3$ = 5 valence

minus charge = 1

10 valence electron



Close, answer is given as definitely not following octet rule



$[Fe(CN)_6]^{-3}$

so now being in Fe^{+3}

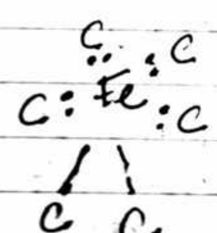
Fe: $4s^2 3d^6$ = 2 valence electrons
but we have a +3 charge
so this means we had to steal 3 electrons.
so you don't have enough so you need to
include the d shell.
so valence electrons in this case should be 8

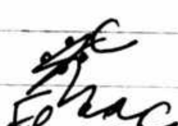
:Fe: but we steal 3, this leaves 5

Fe $^{+3}$ needs to combine with $[:C:::N:]^{-1}$

How would this happen?
This is a very interesting problem.
I am not sure how we know it has 6
Another way to think about this
It just steals one from the d shell

·Fe· How to join? $[:C:::N:]^{-1}$





You are on the right track.

you donated by ligand?

Looks like 3 bond types.

You figured out the $[CN]^-$ cyanide ion OE

but apparently the transition materials do not follow the octet rule.

Sometimes apparently it can be an 18 electron rule, not 8.

Notice in the ferricyanide we have 6 covalent bonds w/ Carbon.

A chelate occurs when the ligand binds to the metal in two or more places. In ferricyanide it is binding in 6 places!!

Yes, it positively is a chelate!

Here is an example given of the 18 electron rule: $[Fe(CN)_6]^{4-}$

Fe 8 valence (remember full depletion of valence electrons) this is Fe^{2+}

Charge of 4⁻ 4

6 x CN 6

18 electrons

this is called coordinately saturated.

Ok, the ferricyanide $[Fe(CN)_6]^{3-}$ is one heck of a chelate. It should be difficult to break down.

Now let's start working w/ the wine chemistry.

Start by testing wine. Does it have Fe^{+2} & Fe^{3+} ions in it?

Fe^{+2} in wine? No. Fails test. Phenanthrolen solution positively works w/ $FeSO_4$ test.

Fe^{+3} in wine? No. Fails test. This means if wine has iron in it, it is not in the ionic form.

Now, looking @ our wine composition, we see that wine does have iron in it but @ level of 0.2 to 0.4 mg. So what form does iron exist in wine?

Red Wine generally has $\leq$ 2 mg per liter. If iron is present in wine, it appears that it will be in a Fe^{2+} ion ~~state~~.

Now lets deliberately add Fe^{2+} & Fe^{3+} to wine and test again.

Red Wine + Fe^{2+}

test: yes positive

Red Wine + Fe^{3+}

test: yes, however it does disappear after mixing so much harder to detect.

Now look @ the Cu displacement issue:
Red wine + $CuSO_4$? Do we get a displacement? or a Precipitate?

No, nothing apparent. This indicates that there is no detectable component of iron within the red wine.

Now add ferric chloride to wine.

Positively turns the wine brown. This indicates to me that we now have the Fe^{+3} hydrated ion coordination complex

Postulate: $[Fe(H_2O)_6]^{3+}$

I think the is correct. The Fe^{3+} test turns weakly red just as the drop is placed on, and then dissipates.

So it appears to me we have a hydrated ferric ~~ion~~ complex that forms in wine and turns it the same color.

Ascorbic acid test:

Red wine + $FeCl_3$ turns it brown.
Suspect $[Fe^{3+}(H_2O)_6]^{3+}$ complex

Now if you add ascorbic acid, the wine immediately turns back to its original color. So the influence of the complex is removed.

~~Citrate~~
Citric Acid or
Ascorbic Acid. ???

watch this!!!
You may have an error!
Ascorbic is for the cyanide ion
Citric acid is for Ferric hydroxide!

Our conclusion @ the time.

The chemistry of the red wine & of the oral sample stock solution be entirely different from one another.

Oral sample solution
1. Produces precipitate w/ use of Cu.
2. Produces clear solution & gas when mixed w/ ascorbic acid.

Both reactions are consistent w/ a $[Fe(CN)_6]^{3-}$ complex that has formed in the oral sample.

The red wine does neither of these.

Slowly, fungi & bacteria do produce their own cyanide ion so it should be available for Monday.

The $[Fe(CN)_6]^{3-}$ is an extremely strong chelate that should lock up the iron in the Fe^{3+} state.

The $[Fe(CN)_6]^{3-}$ is highly consistent w/ the spectrochemical series and our reference that shows CN- binding is extremely favorable to Fe^{3+}.

Now in the case of red wine we know we have anthocyanins available.

The means free carbons available.

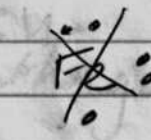
These also like they could easily combine w/ nitrogen. It is in the air!

This would lead to neutral covalent bonds

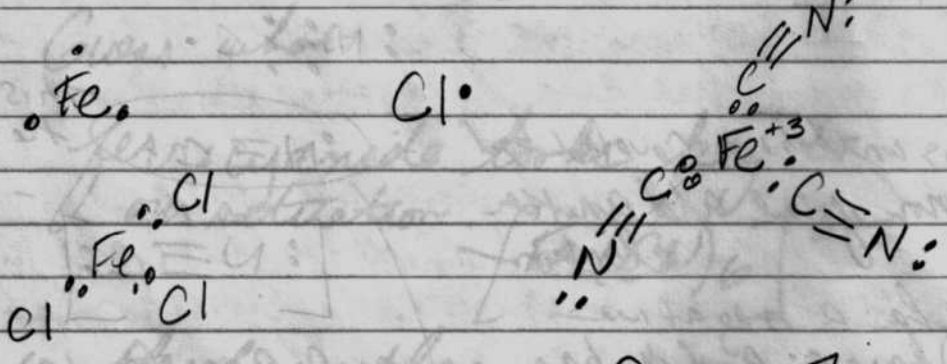
$$\cdot C \equiv N :$$

1 Share

bc if this were somehow to steal an electron for something, eg Fe^{3+}

$Fe^{+3} \Rightarrow$  $\cdot Fe \cdot$

+3



So this suggests that the wine is primary a ferric cyanide complex with the red wine.

$[Fe(CN)_3]$

It is the most reactive ligand.

We may also have binding to the Cl^- to give Fe^{+3} complex.

$HbO_2 \rightleftharpoons Hb + O_2^-$

O_2^- can get displaced w/ chloride ion

Question:
Why does the oral stock solution, when $FeSO_4$ solution is added, produce a clear solution and a dark precipitate?

The same as when you add $CuSO_4$?

Why is this???

What does this mean?
No difference in reactions.
Iron ions not replace iron.

Something else is happening that depends upon Fe^{2+} or Cu^{2+}, not just Cu. What is it??

Cobalt Chloride produces the same precipitate and Silver Nitrate turns a rich deep red brown color.
What is going on here?

With the culture we have a precipitate also using the $FeSO_4$ & the $CuSO_4$

but not the same reaction with the Cobalt Chloride & the silver nitrate

So we have a problem.

We have a precipitate, but we do not really know what it is.

What precipitate form with Cu & Fe??
They are both sulfates.

What was your original hypothesis?

$X^{+2} + NaOH + FeSO_4 + H_2O \rightarrow ?$ Fe^{+2} positive!

$X^{+2} + NaOH + CuSO_4 + H_2O \rightarrow$ Cu^{2+} yes!

Same reaction occurs w/ culture as with oral sample.

What do we have here??

Samples tonight

1. Wine + Lye
2. Oral samples (Compared to Wine + Lye)
3. Culture Stock (Compared to Wine + Lye)
4. Wine + Lye (Compared to wine)

5. Potential Problems:
   Wine + Substantial Lye spectrum look almost identical to

   Culture Stock compared to Wine + Lye

I am not sure if we have a conflict or not here.

You must now be careful that you are not primarily getting a spectrum of Wine + Lye

The culture specimen and the oral sample spectrum are surprisingly very similar. The oral sample appears to have more influence from culture contamination however. This is most likely where the deviations from 412 to 500 occur w/ the oral sample spectrum.

So I think we are ok w/ this.

Lets try to burn the filament sample.

Lets regroup or where we are in the paper.

You are now describing the heme molecule in detail, Oxy VS Deoxy states

Next you were going into qualitative Chemical Analysis of the culture and you have run into a big surprise.

You discovered initially that Fe2+ & Fe3+ ions do not show up in the culture.

We clearly show that when test fails However, we leave open strongly the possibility that iron still exists in a bound condition, not ionic the is only hypothetical.

One case would be a hydrated ferric ion. You tried by accident that $CuSO_4$ produces a precipitate. You think it is

We believe that the reactor
w/ copper produces ferric hydroxide
But it is only a belief @ this time.

Make ferrous hydroxide
Make ferric hydroxide

ferrous hydroxide is green
($FeSO_4 + NaOH$) an insoluble precipitate

ferric hydroxide is yellow-brown
($Fe^{+3}Cl_3 + NaOH$) an insoluble precipitate

ferric hydroxide is soluble in citric acid.

$Fe(OH)_3 + C_6H_8O_7 \rightarrow FeC_6H_5O_7 + 3H_2O$

pale brown
ferric citrate

Principles of Pharmacy, Henry Vinecome Arny
Google books p1127

American Druggist Vol 22 p191
is a new better.
$Fe(OH)_3$ does dissolve in citric acid.

They both immediately dissolve in citric acid & go back to their original colors
ferrous hydroxide → pale green solution
ferric → goes back to brown.

W/ oral samples we indeed have a strong precipitate form.

Copper + wire do not produce the precipitate. ~~the te~~

Copper, Wire & Lye does produce a thick green precipitate.
This indicates ferrous ions in solution in wire and ~~the~~ replacement reaction with Copper. The bottle that the oral culture solution is unique.

Wine by itself does not pass the Fe2+ test (1,10)

The oral sample does immediately dissolve what appears to be ferric hydroxide

Show tests w/ standards & then with oral sample.

Method:

Prepare standards

$FeSO_4$ in solution
add NaOH
forms green precipitate
this is ferrous hydroxide
add citric acid
dissolves immediately
returns to green

$FeCl_3$ in solution
add NaOH
forms brown precipitate
this is ferric
add citric acid
dissolves immediately
returns to brown.

Now perform the test w/ the oral or culture sample

Oral
add $CuSO_4$
a displacement occurs
brown precipitate forms
presume ferrous hydroxide
ferric
add citric acid
dissolves immediately
& returns to original brown color.

Culture
add $CuSO_4$
a displacement occurs
brown precipitate forms
presume ferric
hydroxide
add citric acid

Wine does not do this. (a totally different reaction)
Wine + Lye + Copper produces
a dark blue precipitate
(looks like copper sulfate)

Actually it is making a lot of sense that our original Compound is the Hydrated Fe^{+3} ion.

Then when we add the OH^- it may be that we are getting the $[Fe_2(H_2O)_3(OH)_3]$ brown precipitate.

And it may be that that is dissolving in citric acid. It is almost the same as $Fe(OH)_3$

Copper seems to be the critical element

* But somhow adding $FeSO_4$ to the culture also produces a dark
* precipitate which also dissolves
* in citric acid and also goes back to the brown color, not green!!

What does this mean???
There is something called an adduct

$$12FeSO_4 + 3O_2 \rightarrow 4Fe_2(SO_4)_3 + 2Fe_2O_3$$

(+2 on $FeSO_4$, −2 on SO_4)

iron sulfate — ferric sulfate (ferrous) — ferric oxide

So it says to me it can produce ferric compounds

Citric Acid appears to dissolve what I believe to be a ferric hydroxide compound.

We might check the oxalate Fe^{3+} ions.

But the test for Fe^{3+} ion fails

So it still may not be an ionic form.

What are other tests for ferric hydroxide

The compound directly dissolves in HCl

Yes, testing directly makes ferric hydroxide (ferric Chloride + NaOH) HCl can dissolve it.

So now we know HCl & Citric Acid both dissolve

We can now recall that citric acid and ascorbic acid are involved in completely different qualitative Chemical Analyses.

Citric acid is used to demonstrate solubility of both ferrous & ferric hydroxides.

Ascorbic acid [illegible] with a cyanide ion reaction. Let's recall that.

Another huge question down the road is why does the filament reaction of the ant to cyanide of wine?

But for now, ascorbic acid & CN^- reaction.

What is molecular formula for ascorbic acid?
$C_6H_8O_6$
Can apparently also be $C_3H_4O_3$
Dehydroascorbic acid is $C_6H_6O_6$ (DHA)
Molar mass is [illegible] gm/mol
DHA is an oxidized form of ascorbic acid.

Vit C donates electrons. So it is a reducer. A reducer is an antioxidant.

$$\frac{176.12 \text{ grams}}{\text{mole}} \qquad \frac{176.12 \text{ grams}}{1000 \text{ ml}} = \frac{X}{32 \text{ ml}} \qquad X = 5.64 \text{ grams}$$

solubility is 33 grams / 100 ml - OK

In the ascorbic acid $Fe(CN_6)^{3-}$ reacts the ascorbic acid is oxidized. This means it loses electrons. This means it donates electrons. This mean that ascorbic acid is a reducer. This mean it is an "antioxidant". An antioxidant donates electrons A reducer is a provider of electrons An oxidizer is a stealer of electrons

Fe^{+3} state goes to Fe^{2+} state means it has gained an electron. It gained it from the reducer.

Vit C does not turn urine clear so it is not oxidizing reducing urine.

It positively turns the oral sample more clear.

$$\left[Fe(CN)_6\right]^{3-} \rightarrow \left[Fe(CN_6)\right]^{4-}$$

means it gained an electron.

However, the test for $[Fe^{2+}(CN)_6]^{4-}$ seems to be failing. I am having trouble repeating this.

~~What if it was FeCl~~

!! Indeed it has turned red after !!
Centrifusing w/ (L10)

!! The is monumental. It shows Fe^{3+} has been reduced to Fe^{2+} !!!!

A very light tinge of red but it is very visible after centrifusing to a clear solution.

The mean ox should be able to produce the blue color also.

Fe^{3+} test does not produce red.

So we have it in the Fe^{2+} reduced state.
The confirms the color reaction w/ ascorbic acid.

Good work!!!

We have reduced Iron from Fe^{3+} state to Fe^{2+} with the use of ~~citric~~ ascorbic acid applied to the real sample stock solution.

We are applying ascorbic ~~citric~~ acid to wine by itself. No clear solution is created so it is definitely not the same as the real sample.

Now add ~~water~~ lye to the wine sample. When we add the ascorbic acid to the stock it does not produce a clear solution. ~~Reacts~~ The wine is entirely different from the real sample.

We have proven the reducta process.

Page 135

A fantastic demonstration of how Vit C acts as an antioxidant (reducer)

1. Put Fe^{3+} ions in solution (eg ferric chloride) - yellow solution

2. add Ascorbic Acid. (Vit C) Immediately turns colorless (Fe^{3+} is being reduced to Fe^{2+})

3. Add Fe^{2+} indicator, bright red!

A GREAT DEMO for Workshop

Fe^{3+} indicator fails (little red turns) immediately colorless.

You have a problem.
You cannot repeat the test
of ascorbic acid + oral sample
+ Centrifuge + Fe^{2+} test.

Why?

You have some unusual things happening.

Oral sample + $CuSO_4$ gives precipitate,
after centrifuge.

I put citric acid in it
& it did not dissolve ???

And not only that it turns green?

We do have a problem.

Page 137

Here is what we do know.

Oral Sample + $CuSO_4$ produce a dark precipitate + colorless solution upon centrifugation.

Perfectly clear. You believe it to be free by decision but how do you know.

If it was ferric ~~hydroxide~~ it would dissolve in acid??

We also know it turns it slightly acidic

Not true - the original oral sample solution is not even basic OH- Why & how?

The solution is indeed basic @ 9.0 with the meter.

When you add Copper it clearly becomes acidic

drops from 9.0 to 5.9

The means the precipitation is removing hydroxyl ions - the makes it more acidic.

Brown soled matches.

Everything seems to say we are producing $Fe(OH)_3$ when we add copper.

1. Clear solution
2. Brown precipitate
3. Has acided from 9.0 to 5.9
4. We know that OH^- ion are available from the lye applied to the oral sample.

Now if it is ferric hydroxide why can't we probe it.

It looks like it is hard to dissolve.
Nitric acid may be worky

I may be getting a red tint from Fe^{2+} test after adding weak HNO_3 to proposed ferric hydroxide precipitate

It is starting to look like there may be a combination of precipitate. When adding acid something is dissolving right away. Something else seems to stay.

It does appear that there is a slight red tinge to the Fe^{2+} test and not the Fe^{3+} test. There is after adding acid. (H_2SO_4)

Yes the Fe^{2+} test is actually absolutely shows a slight tinge of red & the Fe^{3+} test is not.

Then was after using H_2SO_4
The says it does not have to be ascorbic add acid,
& may be okay it with nitric acid, sulfuric acid & maybe ascorbic acid.

We do have a Color Change it is very weak but we do have it.

after adding acid, it looks like the (1,10) is reacting w/ the precipitate left. The (1,10) tube is more red than the Fe^{3} tubes

Proving Fe^{3+} in the oral sample

I have found the answer!

What a hunch it was!

The oral sample must be fresh! When it has been sitting for a while something changes.

So the steps are

1. Prepare fresh oral sample & rinse until clear water
2. Add lye, heat & strain
3. Add ascorbic acid (vit C)
4. Centrifuge
5. Separate liquid from precipitate @ bottom
6. Add (1, 10) & the Fe^{2+} test Come out very positive.

The proves reduction of Fe^{3+} to Fe^{2+} (based on electrons) by Vit C.

7. Positive red color, Positive a Fe^{2+} test.

Then is why it worked last night.

The sample was fresh.

Something happens in the jar where it is no longer potent in the same way.

Now, would it work w/ any acid?

HCl does not produce the same result!

We also learn that waiting even 10 minutes reduces dramatically Fe3+ in the reactor. Somehow the ions are being taken up and bound w/ something in the stock solution. They are no longer in ionic form.

You have proven that Fe^{3+} exists within the real sample.

Here is a question however. How do we know that the original Fe was not in a +2 state and was oxidized by the lye & heat

Try that w/ $FeSO_4$.

Well if it was ferrous, it would produce ferrous hydroxide which is green!

You can finally start write your paper.

Further verification it is unique:

Fe^{2+} heated w/ lye (used $FeSO_4$) passes only the Fe^{2+} test
It does not pass the Fe^{3+} test.
The mean light & heat does not oxidize Fe^{2+} to a Fe^{3+} test.

So what is it that can do this?
An oxidizer, like peroxide.

Our next question to ask is why the filament reacts with wine.
We now know the filament has Fe^{3+} in it.
What is anthocyanin?

ferric ion is soluble @ pH < 3.5
" " insoluble forms an orange/yellow precipitate @ pH > 3.5

ferrous ferrous ion always completely soluble.

metal ions (Fe^{3+}) react w/ anthocyanins.
I have found it.

They are called metallo complexes.

ferricyanins is another term

Ok, ferricyanide is not the same as ferric anthocyanin complexes but they are both related & important to our discoveries.

We have

$[Fe(CN)_6]^{3-}$ this is ferricyanide

$[Fe(CN)_6]^{4-}$ this is ferrocyanide

ferricyanide ~~has gained~~ has lost an electron relative to ferrocyanide. (Less negative means more positive, means it lost a electron).

This means the complex has been oxidized.

$[Fe(CN)_6]^{4-} \rightarrow [Fe(CN)_6]^{3-}$ this is our model.

this has been oxidized — to produce this.

$[Fe(CN)_6]^{3-} \rightarrow [Fe(CN)_6]^{4-}$

this has been reduced — has gained an electron. to produce this.

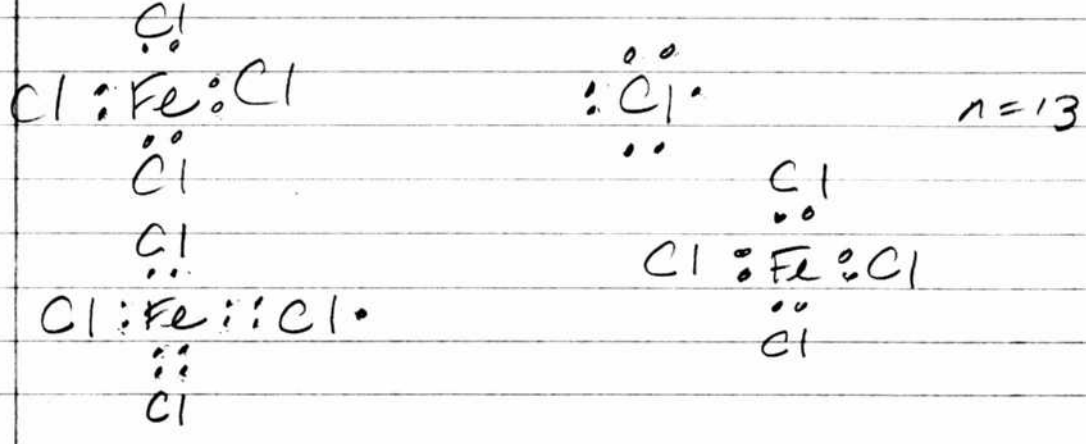
We have two separate things:

ferric metal complexes
explain the above reaction

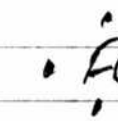
ferricyanides are most likely in
low fields (from spectrochemical
series).

Fe: Ar $4s^2 3d^6$ OK outer shell is 4 energy

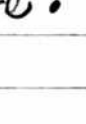
Cl: Ne $3s^2 3p^5$



Interesting: The electron configuration of Fe^{3+} is
Ar $4s^2 3d^6 \rightarrow$ Ar $3d^5$ (lost 3 electrons)
So it is



This means Cl^{-1} should be



C≡N bond is extremely strong
on par w/ explosives like
Nitroglycerin

This is under topic bond enthalpy
p 330 Brown

Note
* * Iron is required for bacterial
* growth in the blood.

Brown p1036

09/03/11

Current Questions

1. How do I know that $[FeCl_4]^-$ forms?

   Why not just $FeCl_3$?

2. Why would the Fe^{2+} state exist so shortly?

3. So why do some complexes form? Remember the spectrochemical series?
   Because of valence shell electrons
   Coulombs of lone pairs of ligands
   see page CCB these notes

4. Source list: See page "Ligands" these notes

   CO
   CN^-
   NH_3
   H_2O
   OH^-
   SO
   NO_2
   S_2^-
   N_3^-
   NO_2^-

   Spectrochemical series:

   $I^- Br^- Cl^- F^- OH^- H_2O NH_3 CN^- CO$

5. What is a free radical? P926 Brown
   any substance with one or more unpaired electrons

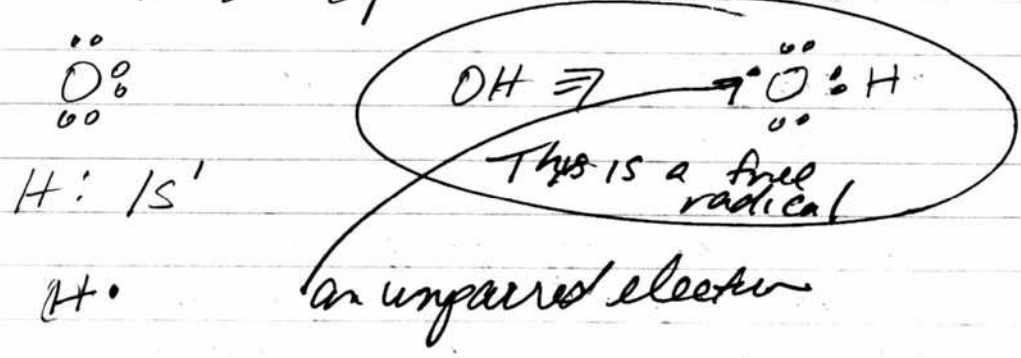
6. What is the method of continuous variation?

Free radical examples:

OH

O: $He\,2s^2 2p^4$ = 6 valence electrons

OH hydroxyl



H: $1s^1$

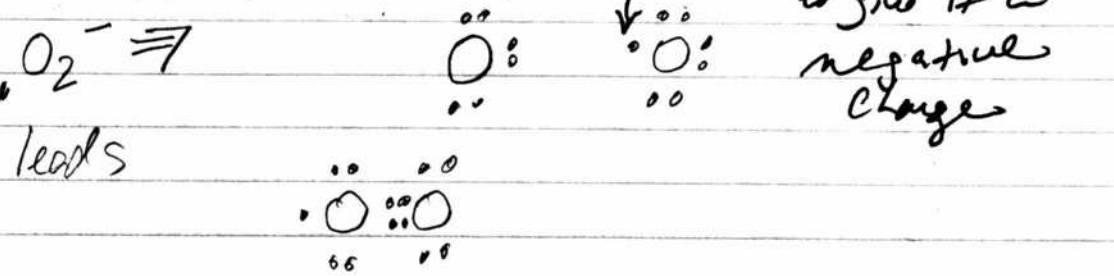
H•

Free radicals may have positive, negative or neutral charge

The three most important free radicals are OH, H_2O_2 and O_2

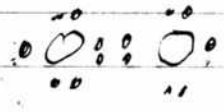
peroxide O_2^{-2} superoxide

O_2^{-1} superoxide



leads

O_2^{-2} peroxide



Back to Ligands

The azide ion is $N=N=N$ (with charges $-$, $+$, $-$)

The azede functional group is $R-N=\overset{+}{N}=\overset{-}{N}$

Azide is N_3^{-1}

Inhibits respiration

It is difficult to isolate

Cytochrome c is a protein associated w/ mitochondria and is involved in electron transport.

Cytochrome c is also a heme protein (ie, contains iron) when mitochondria that can also create a bloody problem.

So it is not just the blood. It is also the mitochondria that would be affected.

What are the common oxidizers in the body?

Oxygen
hydrogen peroxide
MMS ??

What are common oxidizers?

Fluorine
Ozone
Hydrogen Peroxide
Chlorine, Dioxide
Chlorine

A more complete list of oxidants is:

1. Oxygen
2. Ozone
3. Hydrogen peroxide
4. Chlorite, hypochlorite, bleach

Ok, see this:
Normal blood look like

$Fe^{2+} - O - O$

When blood gets oxidized we have
Fe^{+3} and a free radical O_2^-

Well since oxygen itself is an oxidizer
all that it requires is a breaking of
the original bond
of $Fe^{2+} - O - O$

Then you have an oxidizer available! O_2!

So it looks like the only real requirement is that "something" (ie, the organism) breaks down the original bond of Fe^{2+} to O_2

How would such a thing happen?

By the way, free radicals are not the same thing. Oxidizers can create free radicals.

Oxidizers steal electrons.
Free radicals have an unpaired electron.

So back to oxidizing iron in blood
ie, what causes methemoglobinemia

(1) Nitrites oxidize iron from Fe^{2+} to Fe^{3+}
Nitrites are NO_2^-

(2) When it moves out of the plane, it hits the nitrogen atom & gets knocked off.
(what causes it to move out of the plane?)
(Biological Physics, Nield Czarek Google Books p186)

3. The bond between oxygen and heme is a loose one.

4. Oxidizers cause the bond to break!
Like ~~peroxide~~ hydrogen peroxide.

So you protect the iron with chelators (substances that bind with iron in the Fe^{2+} state)

Lactoferrin
Transferrin

The liver produces transferrin

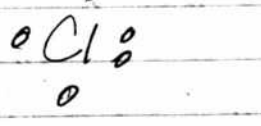
Iron is most available to the body when chelated with to amino acids

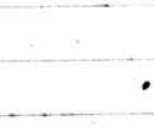
Glycine is the most commonly used amino acid.

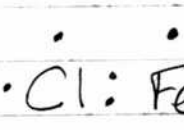
$Fe \Rightarrow Ar\ 4s^2\ 3d^6$ Fe^{+7} valence = 5

Fe° no, this is elemental

$Cl \Rightarrow Ne\ 3s^2\ 3p^5$ Cl^- = 8 valence valence =







Iron Behaving Badly is a major paper.
2400 References. Jan 2009

Douglas B Kell
Univ. of Manchester

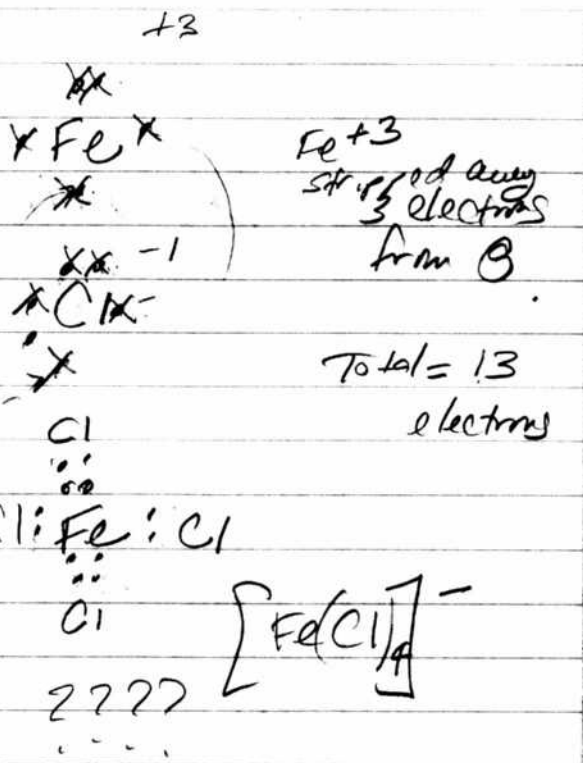
Criteria:

1. Blood - Visual
2. Blood - Spectroscopic
   Scan RGB
3. Urine pH
4. Temperature
5. Oral samples

Add to set of Six
1. Ellestathmin
2. Transferrin - Liver?

What is the method of continuous variation?

The stoic no. lone pairs only refers to the central atom.



This makes sense to me
Electronic Configuration

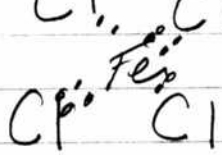
Fe = Ar $4s^2$ $3d^6$
Fe^{+2} = Ar $3d^6$
Fe^{+3} Ar $3d^5$ 5 valence
This makes sense

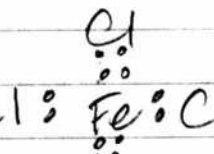
so Chlorine:

Cl Ne $3s^2$ $3p^5$
Cl^- Ne $3s^2$ $3p^6$
but we have 4 Cl ions!

~~0 valence~~ = 32!
13 Total

If you take 0 valence

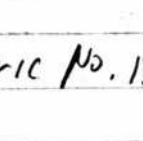




Seems to me it should be like this

Notice 77 Double Bonds

This seems right to me but my book is showing bonds.



Steric No. is 4

$[Fe^{+3}Cl_4^{-4}]^{-1}$

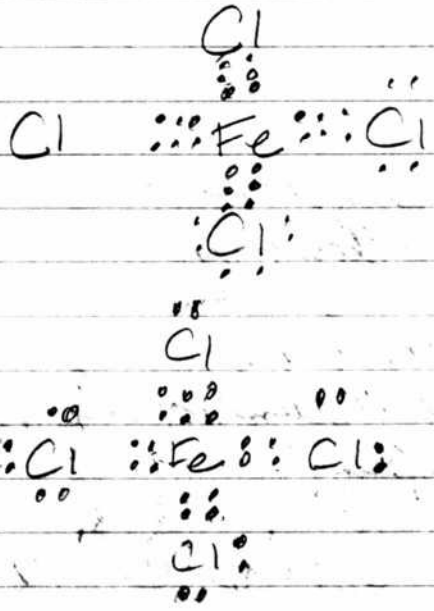
$[Fe(Cl)_4]^{-1}$

Fe^{+3} = Ar $3s^2 3p^6$ Valence = 5

Cl^{-1} = Ne $3s^2 3p^6$ Valence = 8

but we have 4 Cl ions so we have 4(8) = 32

$\frac{+\ 5}{37\ \text{Total}}$



plus you have an other electron. Where do you put it?

32 now 5 more?

I do not think that transition metals follow the "octet rule"

True. Transition metals do not follow the octet rule. If anything it is the 18 rule.

It looks like no one is sure exactly what the molecular structure of $[Fe(Cl)_4]^{-1}$ is. Well here is to let it slide now.

Ferrichrome, another incredible finding.

Fe^{+3} Can bow as many as 4 wants up to 18

Fe^{+3} ion is acidic, by itself!

See p 709 Brown

* Amazing on p 1036

Bacteria, beside needing iron to grow, work in the following way

Bacteria produce siderophores. A siderophore is an iron binding ligand. It binds w/ Fe^{3+} to form

"ferrichrome" $C_{27}H_{42}FeN_9O_{12}$

Ferrichrome is produced by both fungi & bacteria.

Ferrichrome is absorbed by the bacteria or fungus.

Within the bacteria or fungi the ferrichrome is bound to oxygen (6!) and used for its own metabolism.

Quantum Review:

1. Does the culture vs oral form produce the same Fe^{2+} titration?

2. Can the method of continuous variation be used to your advantage?

Answer:

The culture does not produce the same Fe^{2+} reaction as the oral sample does. It does not mean that Fe^{2+} is not there, it just means it is not in ionic form. Remember the bone sensitive tissue and the culture used was hardly "live", it was ~~dried~~ dried form.

So the truth is we do not know if the culture can react in the same way or not.

3. How do you test a solution for its magnetic properties?

4. What is an Fe^{3+} complex w/ CO vs CN^-

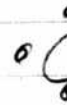
Metal Carbonyls (The CO problem)

We are now looking @ the CO ligand.

A known compound is $[Fe(CO)_5]$

It is electrically neutral.

C ⇒ $1s^2\ 2s^2\ 2p^2$ 4 valence electrons

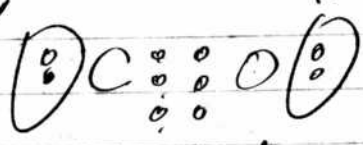


O ⇒ $1s^2\ 2s^2\ 2p^4$ valence = 6

Total Electrons = 10

You should have been able to do this!

lone pair

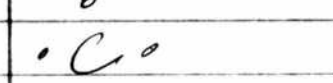


6 + 4 = 10

Triple Bond.

Metal Carbonyls, eg Iron Pentacarbonyl is another iron coordination complex. It is neutral. It is toxic. Forms Carboxyhemoglobin, which will not bind O_2.

This is the smoker problem.



4 valence electrons

Valence - 6 electrons

Combine to form 8 electrons around each atom

wants 8

Triple bonds.

We notice having three colors in our oral sample w/ ascorbic acid added. Ascorbic acid is a reducer and turns Fe^{3+} to Fe^{2+}.

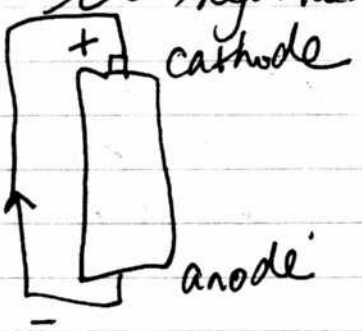
Now we notice when we wait after centrifuging the solution is less red (be, orange). Orange means it absorbs blue, which is higher energy than perceived red (which means absorbs blue green). So the longer we wait, ~~the more~~ after centrifuging, the higher the energy the solution has. The more ~~the~~, more electrons are being removed

It is time for Electrolysis!
of the oral sample.

Conductivity of the oral sample solution ~ 230 uS.
pH of the oral sample solution ~ 8.5

We already see that a gas is being produced @ the negative terminal and a dark deposit is being produced on the positive terminal.

In a battery, electrons emit from the negative terminal.



Activity Level of Metals:
Iron replaces copper
e.g. place iron in a Copper Ion solution,
~~like iron~~
e.g. assume you put iron ions in solution
$FeSO_4 + Cu + H_2O \rightarrow CuSO_4 + Fe + H_2O$??

?? Not sure

Anode is where OXIDATION IS occurring
Cathode is where reduction is occurring

From Chemistry Comes Alive
In metal nitrate solution

Oxygen Oxygen is generated @ the anode

In some cases hydrogen is produced @ the cathode
WHILE IN OTHERS METAL DEPOSITS FORM

this seems to be us allright.

The reaction @ the cathode correlate pretty well w/ the reduction potential of the metal ions.

$Fe^{3+} + e^- \rightarrow Fe^{2+}$ $\quad$ .77V

$Cu^{2+} + 2e^- \rightarrow Cu$ $\quad$ .34V

Pure electrolytic, apparently iron compound is not particularly soluble in either HCl or H_2SO_4.

The rust indicates very powerful bonding.

* It is dissolving, but very very slowly.
Appears to be a form of hematite
Fe_2O_3 ferric oxide or iron(III) oxide

The Electrolytic Proof of Fe_2O_3

You have a motor w/ iron oxide

Fe_2O_3 by electrolysis

1. Color (brown - red)
2. Dissolves very slowly in H_2SO_4
3. Insoluble in water
4. Reaction for sulfuric acid is

$$Fe_2O_3 + H_2SO_4 \rightarrow Fe_2(SO_4)_3 + H_2O$$

(-6) (-6)

Oxides react w/ acids to form a salt & water.

But what is this salt? It should be ferric sulfate

5. Centrifuge quickly (heavy) the most stable form of iron oxide. "black"
6. Very magnetic (will not stick to steel) (weak ferromagnetism)
7. Iron Oxide can react violently w/ hydrogen peroxide which it does.

Conductivity of Oral Solution after electrolysis is ~ 470 mS

pH of oral solution after electrolysis is 10.4

So it becomes more alkaline and more conductive

B. We have a positive vs zero reaction with H_2O_2.

The iron oxide is a catalyst. The iron oxide itself does not change.

It is a catalytic reaction!

(9) Electrolysis happens to begin with.

Amount of iron collected:

$\sim \frac{0.2 \text{ gms}}{5 \text{ samples}} \quad \frac{4 \text{ gms}}{X} \quad X = 100 \text{ samples}$

So 100 samples $\cong$ 4 gms
1 sample $\cong$ .04 gms $\cong 1\%$ of body iron.

However the molar wt of Fe_2O_3 is

159.7 gms

70% is the iron

So

$\frac{(0.2)(.7)\text{ gms}}{5 \text{ samples}} = \frac{4 \text{ gms}}{X}$ $X = 143$

So approx $\frac{150 \text{ samples}}{100\% \text{ of iron in block}}$ $\frac{1 \text{ sample}}{X}$

$X = 2/3\%$ of 1%

$X \doteq .67\%$ of iron is contained in 1 sample.

Call it 0.5% approx.

So in 200 days you would produce enough to account for all the iron in your block.

Hydrated Ferric Ion $[Fe(H_2O)_6]^{3+}$

We measure 0.1 mA in our electrolysis.

We have found an analytical method on p 133 of Moore. Fe_2O_3

We estimate .034 gms produced in 8 hrs of electrolysis w/ 0.1 mA

What is the equivalent mass?

Page 165

$FeX + NaOH + CuSO_4 \rightarrow Fe(OH)_3 + Cu^{2+} + SO_4^-$

??

$FeCl_3 + NaOH + CuSO_4 + H_2O \rightarrow$

$Fe(OH)_3 + Cu^{2+}$

A B C

$Fe^{3+} + 2Na + OH^- + CuSO_4 + H_2O \rightarrow$

$Fe(OH)_3 + Cu^{2+} + Na_2SO_4 + H_2O$

My plausible reaction. $(= 2Na + SO_4)^2$

This works

$Fe^{3+} + Na^+ + 3OH^- + CuSO_4 + H_2O \rightarrow$

$Fe(OH)_3 + Cu^{2+} + SO_4^{2-} + Na^+ + H_2O$

Another reported reaction that is consistent is:

$$\left[Fe(H_2O)_6\right]^{3+} + Na^+ + 3OH^- + CuSO_4 \rightarrow$$

$$Fe(OH)_3 + Cu^{2+} + Na^+ + SO_4^{2-} + 6H_2O$$

We now know to some degree what is happening.

Now we need to know how it is happening.

We should start by looking @ our data but it is looking very good.

Important Biology 2

Bio

! Many enzymes do not work w/out vitamins.

Enzymes are proteins

Catalyst do not have not to be a protein
ie ferric oxide + hydrogen peroxide.

Oxidation is a form of release of energy!
(Think about it if it loses an electron)
An electron is energy !!!

Glucose is oxidized. (reservation !!!)
Glucose + Oxygen → $CO_2 + H_2O$ + Energy

The Energy produces ATP (storage tank)
(ATP store energy in high powered bonds)

Cells use ATP to supply their energy needs.
(ATP also gets oxidized, so again it releases energy)

We are oxidizing our iron instead of glucose!
Misplaced energy.

Instead of producing ATP
It feeds the garden.

Wolfe has a great experiment
Sugar + $KClO_3$ + H_2SO_4 ⇒ energy!

Sugar + Bleach + H_2SO_4 → same thing!

+ Enzymes make ATP happen

Something very strange has happened.

Organic Sugar + Bleach + H_2SO_4 → Energy + Fibes ??!!

Sugar + Bleach + HCl → also works but it releases Chlorine gas and is more violent.

Sulfuric Bleach

$H_2SO_4 + NaOCl \Rightarrow HCl + SO_2 + Na_2SO_4$

Acid

Fumes are dangerous. toxic ↑ toxic ↑

Bleach + Ammonia → Mustard Gas

What is mustard gas

A very dangerous & toxic reaction

(Bleach & Urine same problem!)

This is what respiration is:
(pyruvate in between)

Glucose + Oxygen → CO_2 + H_2O + Energy Stored in ATP

* you need enzymes to make the happen!

(Pyruvate in between) — w/ oxygen debt is a problem

$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O$ + energy 36 ATP

Enzymes are required!

An oxygen deficit does not allow the happen favors anaerobic processes (fermentation).

Remember on liver or Cancer cause?
Biochemistry Asymptopia.

Glycolysis is the first half of respiration!
(no oxygen required)

The Krebs cycle is the other half.
(It uses oxygen & produces a lot more energy)

How to explain oxidation:

Proposition:

Charge of +2 minus energy creates an electron

Same +3

+2 + +2 − (−1) = +3

I have a +2

Math approach

I have something that is a (−1)

How do I get a +2 to go to +3?

+2 − (−1) = +3

+2 minus (an electron) = +3

+2 minus energy = +3

Perform Chemistry Experiments:

Chemistry approach

Fe^{+2} + H_2O_2 (an oxidizer) in your body = Fe^{+3} + loss of energy

Fe^{+3} + Ascorbic Acid (a reducer) an antioxidant in your body = Fe^{+2} + gain of energy

$FeSO_4$ or liquid iron can be used.

every electron changes through a shell releases energy.

Heme protein is in mitochondria but it does not bind oxygen!

A mitochondria is 5-10 μm.
So it is visible.
Mitochondria metabolize sugars

Mitochondria consume oxygen and produce ATP

The mitochondria produce ATP

Krebs: Glycolysis breaks up the carbon backbone of glucose into smaller carbon fragments.

Urine composition:

95% water
urea 9.3 g/L
chloride 1.010 g/L
sodium 1.17 g/L
potassium .75 g/L
~~Creatine~~
creatinine .67 g/L

Sterile to urethra.
Not toxic

But breakdown of urea produces
asphyxiating ammonia.

Strong odor due to bacterial action

Urea is $(NH_2)_2CO$
highly soluble & non-toxic

Highly concentrated urine can smell like ammonia.
~~Ammonia is~~
Ammonia is NH_3 It is a gas.

Reacts with water to form ammonium hydroxide
Necrosis of tissues, cellular ~~damage~~ destruction

New York State Dept of Health

Bacteria breakdown proteins in the intestine and create ammonia.

The liver converts ammonia into urea, which is eliminated in urine, to be

ammonia levels in the blood rise when the liver is not able to convert ammonia to urea. Cirrhosis and hepatitis can cause this.

Wolff - important to maintain a stable pH in the body

You have now successfully tested for protein!

NaOH & $CuSO_4$

(more)

3 drops 1 drop

Heating w/ HCl did help & make a difference

The oral sample positively fails the test for proteins

Bacteria do have proteins but
our test of the dal culture
lack the proteins.

---

all! Enzymes are proteins!

Bond Dissociation Energy

From table

Fe - O bond requires 409 ~~4.184~~ KJ/mol

We learned there are estimate .0023 moles of iron ions in the Fe 3+ state.

$$\frac{.0023 \text{ moles}}{}(409 \text{ KJ/mol}) = .941 \text{ K Joules}$$

$$= 941 \text{ Joules}$$

Walking 3 mph 280 cal/hr

1 joule = .24 Calories

Pathology: Cotran

Hypoxia is @ the forefront.

Loss (↓ decrease?) in O_2 carrying capacity of blood.

Four systems are especially vulnerable.
1. cell membrane
2. respiration and production of ATP
3. enzymes
4. genetic apparatus RNA, DNA

First point of attack is aerobic respiration by mitochondria & reduction of ATP production.

Decrease in ATP causes an increase in anaerobic glycolysis (remember cancer?)

pH is reduced.

Reduction of ATP - fats do not break down properly.

Free radicals ~~are~~ are initiated by oxidative reactions.

O_2^-
H_2O_2
OH (not OH^-)

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I am not sure you learn anything different from this other than there is some electrical potential here

Battery Test
Zn - Cu
electrodes give +.96V

Al - Cu give 0.6V

Pb - Cu = 0.35V

Ni - Cu 0.1V

Fe - Cu 0.55

Mg - Cu 1.42V

Guess what.

The Iron oxide collected from the coal sample is by electrolysis indeed magnetic!

Mg - Cu 1.46 from weak coal solution
Conductivity 465 μS (slightly conductive)
pH = 9.3

Not ~~really strong~~ but it is magnetic

NaOH alone w/ Mg - Cu gives 1.25V
pH ≅ 9.7
Conductivity is 533

Fe_2O_3 is ferromagnetic

So the lye alone could account for everything going on.

Iron itself is ferromagnetic

Hard & Soft Metal

refers to the magnetic properties not whether the material is "hard" & "soft"

hard metals retain the domain charge

Iron is the metal of the stronger metal alloys."

Iron III is by definition a hard metal since it has a high ionic charge

---

Full reference on the iron source has been found

"The development of iron chelators for Clinical Use"
by Raymond J Bergeron & Gary M Brittenham
Google Books.

In this reference

Projects:

1. Electrolysis of Fe^{2+} and Fe^{3+}

2. Chromatography

Electrolysis of a ferric salt (ferric nitrate) is entirely different than electrolysis of ferrous sulfate.

ferric nitrate gives an clear deposit, deep and dark.

ferrous sulfate bowl, & gives two results, namely a green precipitate (appears to be ferrous hydroxide) and a foamy dust colored surrounds the ferrous.

In ferrous sulfate, the green precipitate is the dominant form, the foam is less.

In ferric nitrate, solid deposit, very little material.

Observations for [redacted]
moderately

1. Water soluble Crystals
2. Can be transparent in Crystal form.
3. absorbs moisture from air
4. Melts reading to a gel like material, Recrystalizes
5. Not exactly soluble in Strong HCl either
6. Not soluble in Ethanol (least impact)
7. Slightly soluble in NaOH

all failing to some degree

8. The means I can not get it into solution for the spectrometer. Also it has no color so can not use for visible spectrometry

Electronegativity of Ligands:

We have a relation.

Covalent Bonding % $\cong 4.72\Delta x^3 - 21.9\Delta x^2 - 7.31\Delta x + 101.9$
(Electronegative)

CO : $\Delta x = 2.5 - 3.5 = 1.0$ } less electronegative — 11.4 %

CN $\Delta x = 2.5 - 3.0 = .5$ } less electronegative — 93.4 %

NH_3 $\Delta x = 3.0 - 2.1 = 0.9$ — 86.6 %

Cl = $\Delta x = 0$ } more electronegative — 101.9 %

Fl $\Delta x = 0$ } more electronegative — 101.9 %

Notice they are all covalent, it is a matter of degree

More electronegative ligands hold their electrons (electrons) more tightly, therefore they will not interact as much w/ d orbitals. These are weak field ligands.

Less electronegative ligands do not hold on to electrons as tightly, therefore they do interact w/ the d orbitals more so. This causes higher energy splits.

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Ligands tend to come in along the xyz axis.

This is the repulsive force.

CN^- & CO cause the greatest splitting of the d orbitals.

1. Absorption tells us something about expected Color

2. Electronegativity tells us something about Color

3. Geometry of the molecule tells us something about the color

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Does a specific frequency correspond to a particular Energy?

$E = h\nu$ $\quad h = 6.626E-34$ joule-seconds

ν = frequency
so the could have a wobble

$E_{hf} = (6.626E-34\ j\text{-}sec) \cdot \frac{C}{\lambda}$

$C = \lambda \cdot f$
$f = C/\lambda$
let $\lambda_1 = 420nm$
$\lambda_2 = 650nm$

420nm $E_1 = (6.626E-34\ j\text{-}sec) \frac{3E8\ m/sec}{420E-9\ m} = 4.73E-19$ Joules
$= 2.85E5$ joules/mol $= 285$ KJ/mole

650nm $E_2 = (6626E-34\ j\text{-}sec) \frac{(3E8)}{650E-9} = 3.06E-19$ Joules
$= 1.84$ Joules/mol $= 184$ KJ/mole
E5

$E = h\nu$ simple form

$E_1 = \left[\frac{(6.626E-34)(3E8)}{1E-9} (6.02E23) \right] \cdot \frac{1}{420nm}$

$\frac{1.2E5}{\lambda nm}$ $\quad E$ in KJ

$= \frac{1.20E8}{\lambda nm \cdot 1E3} = \frac{1200}{\lambda nm} = \frac{1200}{420} = 2.86$

It looks like we have a reaction of Fe 3+ w/ NaOH

$$\left[Fe(H_2O)_6\right]^{3+} + NaOH \rightarrow Fe(H_2O)_3(OH)_3$$

orange → dark orange

~~under Chemguide. co. uk~~ after

www.Chemguide.co.uk/inorganic/transition/iron.html

This fits this to a tee.

It is called iron(III) hydroxide.
I buy it. Question is, does the culture produce it? This came from a rain sample + Liquid iron + H_2O_2

& Soda + NaOH

This tells us in the rain culture we had $\left[Fe(H_2O)_6\right]^{3+}$ now a question is is the rain culture producing this?
It seems like it because the rain culture is different from the control.

This is enlightful.

Energy in kJ from wavelength only
(This is the "enthalpy of reactions")

Chap 10 in Thinkwell has p. bonds

a chemical bond results from the overlap of two orbitals, each of which is half filled.

Ya have come up w/ a method for quickly determining the energy in kJ ~~simply from the~~ wavelength in nm

$$\text{Energy in kJ} \cong \frac{1.2E5}{\lambda \text{nm}}$$

Example: 420nm

$$\text{Energy}_{\text{kJ}} \cong \frac{1.2E5}{420\text{nm}} \cong 286\text{kJ}$$

Lewis dot diagram do not tell the whole story. Molecular orbital theory is more comprehensive.

We have spoken this lecture CH 10 on molecular orbital theory and the transition metals.

Pi acceptor donors cause a greater splitting of d orbitals.

CO must be a pi acceptor donor
CN^- " " " " " "

Pi donors are weaker field ligands.

CO, CN^- are both pi acceptor molecules

Remember our spectrochemical series & molecular orbital theory pretty much tell us what they are.

Metabolism

Enterobactin
is a very powerful siderophore
that binds to Fe (3+).

Most common in gram negatives.

Guess what - we are gram negative.

* So we are dealing w dealing w/ a
bacterial form (or modified) that
likely contains enterobactin, a
siderophore that loves Fe (3+)

Discovery

How did you just discover this?
What was the process?

I looked up pi acceptors
& found a reference to cyanide as
being a pi acceptor.
This confirmed suspicions along w/
the lecture of determination
requirements from Chemistry 102.

Within a pdf paper, you found reference
to enterobactin w/ likely strong
bond to Fe3+.

And voila at last point.

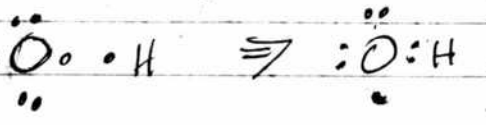
Oxidation takes place in 3 forms:

1. The gain of oxygen
2. The loss of electrons
3. The loss of hydrogen

Fenton's reaction:

Also in general, metals serve as catalysts for oxidation.

$Fe^{+2} + H_2O_2 \rightarrow Fe^{3+} + OH + OH^-$



You are working w/ heat.

Oxides react w/ acids to form a salt & water

+3 -2

In this case

$Fe_2O_3 + H_2SO_4 \rightarrow Fe_2(SO_4)_3 + 3H_2O$

(ferric sulfate)

$2Al + 6HCl \rightarrow 2AlCl_3 + 3H_2$

↑ very dangerous. Explodes if contained. Will form a bomb

Think about reaction before proceeding. Open water glass, small scale operator would be fine.

$n_{Al}(C_p(Al))\,\Delta T_{Al} = -n_{H_2O}(C_p(H_2O))\,\Delta T_{H_2O}$

↑ solve for this.

You still have to assume the identity of the substance.

It could be anything, it does not have to be Aluminum.

but you need to know the molecular formula to get the no. of moles.

Iron Heat Capacity Experiment

14.27
- 12.72
1.55 gms

Water 100gm
$T_1 = 21.9°C$
T_2 23.6

Iron
$T_1 = 179°C$

$Cp\ H_2O = 75.3\ J/mol$

$n_{Fe} C_p(Fe) \Delta T_{Fe} = - n_{H_2O} C_p(H_2O) \Delta T_{H_2O}$

$C_p(Fe) = - n_{H_2O} \frac{(75.3\ J/mol)\ 1.7°}{n_{Fe}\ 155.4}$

$n_{Fe} = \frac{1.55\ gms}{55.85} = .0278\ moles\ Fe$

$= - \frac{5.56}{.0278} \frac{(75.3)(1.7)}{(155.4)}$

$n_{H_2O} = \frac{100\ gms}{18} \quad 5.56$

$= 164.75$

Iron shows up @ 25.1 J/mol

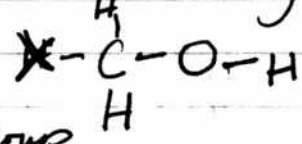
Why would you have the much error?

Carbon $1s^2 2s^2 2p^2$

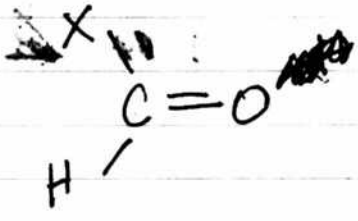
The two's mean it has 4 electrons in the outer shell

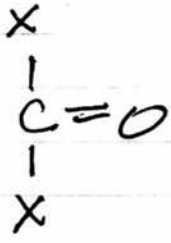
Functional Side Groups

1. Hydroxyl : OH (give polarity)



2. Carbonyl Group





3. Carboxyl (COOH) Group

4. Amino

5. Sulfhydryl

6. Phosphate

Oct 29 2011

The Conference has been ~~Completed~~ Completed.

You deserve a rest.

New problems:

1. New fibrous sample. Best use?

✓ 2. Culture - separation of form.

3. Cultures - spectrometric analysis of what cultures vs non ~~cultures~~

4. Dictostylium species?

[redacted]

NO 5. DNA production from culture solution?

Single bonds are σ bonds
Double bonds are one σ & one π
Triple are one σ and two π bonds

Common ferrous salts are

ferrous sulfate
ferrous gluconate
ferrous fumarate

Fenton is actually w/ $FeSO_4$ is

$FeSO_4 + 2H_2O_2 \rightarrow Fe^{3+} + OH + 3(OH)^- + SO_4$

or

$FeSO_4 + H_2O_2 \rightarrow Fe^{3+} + OH + (OH)^- + SO_4^{2-}$

The is probably better.

Someone else says the SO_4^{2-} is a spectator ion, so leave it out. But why is it so acidic otherwise?

$2H + H_2O_2 + 2Fe^{2+} \rightarrow 2H_2O + 2Fe^{3+}$

Carbon

$1s^2 2s^2 2p^2$

or

He $2s^2 2p^2$

+ electrons in outer shell.

2 + (4C) +

A good Chromatograph column is made by

1. Paper towel vs coffee filter sealing the bottom
2. Fill ½ w/ alcohol
3. Fill w/ sugar
4. pack gently between sugar & alcohol
5. top w/ white sand
6. let dye just sink into sand
7. Top w/ alcohol

In order for it to work, the dye cannot interact w/ the sugar.

Lye & sugar probably interact.
Yes, sugar is dissolving in lye so it can not work.

You must use a solvent for the culture a oral sample that does not dissolve sugar!

Acetone must have dissolved the paper.

It broke down & damaged the column.

Could have been the lye in the culture also though. Yes ~~it~~ actually very likely.

I don't think you can use any lye.

You are after something which dissolves ~~the~~ culture but does not react w/ sugar. Hard to find & ~~not~~ sure.

What if you flooded a column w/ base?

Acetone damaged the plastic cap!

DO NOT USE ACETONE!

IT WILL DESTROY THE APPARATUS!

We had some real problems w/ Chromatography.

Ya learned some things but ya were not successful as a whole.

1. Ya could not replicate your results.
2. Ya could not [illegible] a stationary phase. Everything had [illegible] problems:
   1. [illegible]
   2. Sugar
   3. Lime
   4. Silica gel - Coarse

   } and all kinds of combinations
3. Ya also made a complete mess [illegible]
4. One time ya succeeded w/ food [illegible] but ya could not repeat it.

5. TLC is a much cleaner & simpler method of Chromatography. But it can not be used for extraction. Also my culture is not dissolving in any solvent?

6. ACETONE DESTROYS PLASTIC & THE EQUIPMENT!

What you are trying to do is:

1. Determine the molecular composition of the organism or at least knowing how many components there are.

2. Determine if the [redacted] has DNA.

3. What is the [redacted] spectroscopic analysis of the Fe-[redacted] culture?

You now have the spectroscopic analysis of the [redacted] culture. It was [redacted] that anything dissolved or [redacted] down. You end up getting the same results as before. You graph is in comparison to water. We are not certain how much the Fe-H_2O background reacted as contaminate of the results.

Lesson you do not have a good way of breaking down the [redacted] But look at the color deepened!

It is a deep red rust color!

Result: Under the scope Lyse, Heat & HCl do affect the underlying sub micron organism.

We know now that the culture growth form has magnetic properties.

Expected to occur because of the ion properties of the organism.

Does that mean that the electromagnetic properties?

As a consequence of Einstein's theory of special relativity electricity and magnetism are fundamentally interlinked.

Magellan: Magnetism & electricity

Our mix - H_2O_2 culture is highly acidic
pH ≅ 3.

Fe^{2+} + ~~H_2SO_4~~ H_2O_2 alone produces a very acidic solution.
It is not the culture that make it acidic.

$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH + OH^-$

you would think OH^- it would become pH. Why does it become so acidic?

$FeSO_4 + H_2O_2 \rightarrow Fe(OH)_3 + H_2SO_4$

The other does not necessarily mean you get the OH

$Fe^{2+} + SO_4^{2-} + H_2O_2 \rightarrow Fe^{3+} + OH + OH^- + SO_4^{2-}$

So what if you used $FeCl_3$ instead?
The is ferric!

Problems:

1. You have no way of breaking down the filament in the culture from entirely. You do have a way of breaking down the oral filament samples.

2. DNA extraction of the sample would be helpful!

The means can be Categorized.

You must keep repeating the DNA test until you find out how repeatable or not. If your test that succeeded your solution was brown. If your test that failed the color was went colored.

What if you dissolve the oral sample in

Did we use a mature culture?

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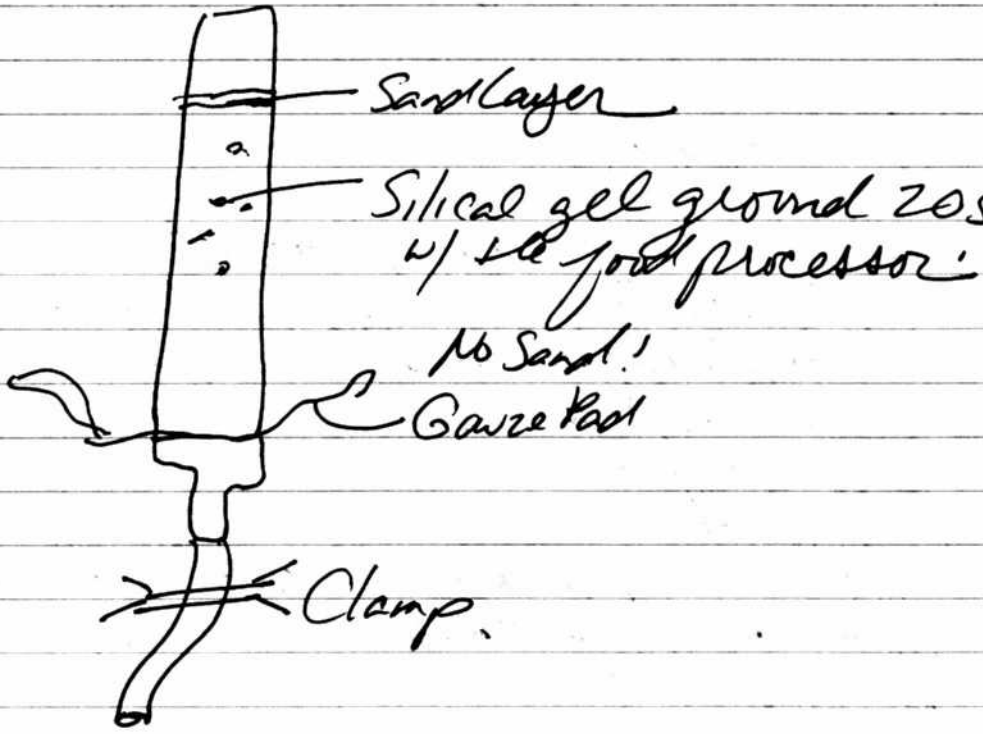
We finally have Chromatography working in a reliable & repeatable fashion!

It has taken a lot of time.



Let it drain through the sand before adding more water on the top

I have achieved separation w/ ethanol

Why do ya want to know molecule shape?

It helps to predict reactivity.

How does it do this?

Chromatography:

Blood is too strongly attracted to the stationary phase, therefore they stuck permanently w/ in the column.

Shape affects:
Melting Point
Function of a biological molecule
Polarity of the molecule (affects polarity)
Boiling Point

eg water is more polar than alcohol.
Alcohol caused blood to move through the column, water caused blood to get stuck.

In general, non polar molecules do not react w/ polar molecules

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Why is shape of a molecule important?

Shape determines reactivity of the molecule

Shape determines the physical & Chemical properties of the molecule.

Well guess what, you can't get any more important than that.

Now we need to learn how and why this is the case. It will radically increase your application skill in Chemistry.

Separation of ~~some~~ forms is
taking place!

1-4 use either a water as the solvent
Tends toward clarity from brown
(indicate ~~polar~~ polar components)

Start w/ 5 we use ethanol as a
solvent

5 starts becoming more cloudy
(indicate a non polar reaction)

6 is now very cloudy.

We have positive separation.

This is the same type of thing that
happened w/ hemoglobin
Hemoglobin got stuck, alcohol
freed the column.

Our hypothesis that the cloudiness may
be due to a protein like what
happened w/ hemoglobin
Test the cloudiness for a ~~pH~~ protein

The larger the difference in electronegativity, the more polarized the bond.

This should also be in reverse, the more polarized the bond, the larger the difference in electronegativity.

Be careful though, a polarized bond does not necessarily mean a polarized molecule.

The solution is very basic (pH higher) because it was dissolved in lye!

By No. 10 it is clearing up considerably.

Making your column 6" instead of 8" using 20 sec ground silica gel (kitty litter) is producing very good results.

We learn that $CuSO_4$ reacts with the whitish solution to form a precipitate & clears up the solution but it does not seem to pass a test for protein.

It is clear. You have a successful separation using both water (polar) and ethanol (non polar)

Now you just need to know what is in the cloudy solution.

You know it reacts w/ $CuSO_4$.

$$CuSO_4 + 2NaOH \rightarrow Cu(OH)_2(s) + Na_2SO_4(aq)$$

blue precipitate
Then a match.

But we still have to wonder, why does it clear up the cloudiness of the solution?

There must be something else happening.

Centrifuge does not, in any way clear out the cloudy solution.

The separation test produces a cloudy precipitate.

The fails the test for protein.

It may be passing a test for fat?

Fails a test for starch.

Fails the test for sugars w/ Benedicts Solution
Benedicts test requires heating of the solution

Benedicts solution contains:

1. Copper Sulphate
2. Sodium Citrate
3. Sodium Carbonate (Washing Soda)

I may be on to something.
We may be dealing w/ a lipid component.
Add ethanol to the culture and it does become cloudy.

Spectra of lipids falls primarily in the UV spectrum

Fat
Starch
Glucose
] Carbohydrates
Protein

We now have the idea of using salt to get eluent to flow within a column.
Ammonium sulfate?

$(NH_4)_2SO_4$ Molecular weight is 132.14 at

$\frac{132.14}{1000\,ml} : \frac{X}{60\,ml}$ $X = 7.93$ gms for a 1M Solution

200 ml = 26.4 gms for a 1M Solution

We got a green complex somehow?

1. Culture + $(NH_4)_2SO_4$
2. Then Alcohol
3. Then water - Came out green

This interesting and new. It has never been seen before.

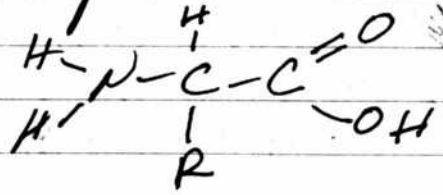
"all soluble proteins on addition of a copper salt in alkaline solution ($NaOH$) give a purple colored complex which is known as "biuret".

The reaction occurs in the peptide bonds of tripeptides & larger.

austincc.edu

We are starting to learn the elemental composition of the culture w/ our AA spectrometer.

Proteins



So we know now the growth has Fe^{3+}

C, N, H, O, + R (and it should be a non polar R?)

We are getting a definite precipitate reaction.)

We suspect $MgSO_4$

Potential reaction ions here!

$MgSO_4 + BaCl_2 \rightarrow BaSO_4 + MgCl_2$ ← white precipitate

Alternative

$(NH_4)_2SO_4 + BaCl_2 \rightarrow BaSO_4 + 2NH_4Cl$

So you would need to
distinguish between
ammonia & magnesium ion

It appears you'd clear wd have
a $BaSO_4$ Compound

plus a salt. (ions in solution)
either ammonia Chloride
or magnesium Chloride

How can you tell which one?

No white precipitate formed
w/ addition of $NaOH$ to original
X. SO_4 solution
The water against magnesium

11/19/11

We have made a new column.
We have learned that the blue crystals in the kitty litter is Cobalt Chloride.

You can remove the color by adding bleach.

Your Column w/ bleach would not allow the oral sample to flow. When you added ethanol it flowed very well. This has produced a cloudy yellow solution. When you add water to the tot well stuff not separate in a centrifuge. This does raise the question of whether there is a lipid or not. It appears it may be a lipid.

Now we deal w/ our blue solution. Is it possible the blue came from a reaction w/ Cobalt Chloride? Now when you test the blue solution it appears to pass the test for a protein. (NaOH + $CuSO_4$ / Biuret)

So do we have oral + cobalt Chloride + alcohol → protein???

Ammonium Sulfate + Ethanol

produce a stringy white precipitate

I do believe we have consistently produced a lipid. By definition a lipid is insoluble in water.

Now I believe we have also produced a protein but it needs to be verified under bleached conditions.

You produced a precipitate

$(NH_4)_2SO_4$ Molecular wt is 132.14

We have 250 ml of H_2O we need 80% saturation

Saturation is 3.9 M

$$(.80)\ \frac{3.9M\ (132.14\text{gms})}{1000\text{ml}} = \frac{X}{250\text{ml}} \qquad X = 103.06\text{gms}$$

Solvents you have used are:

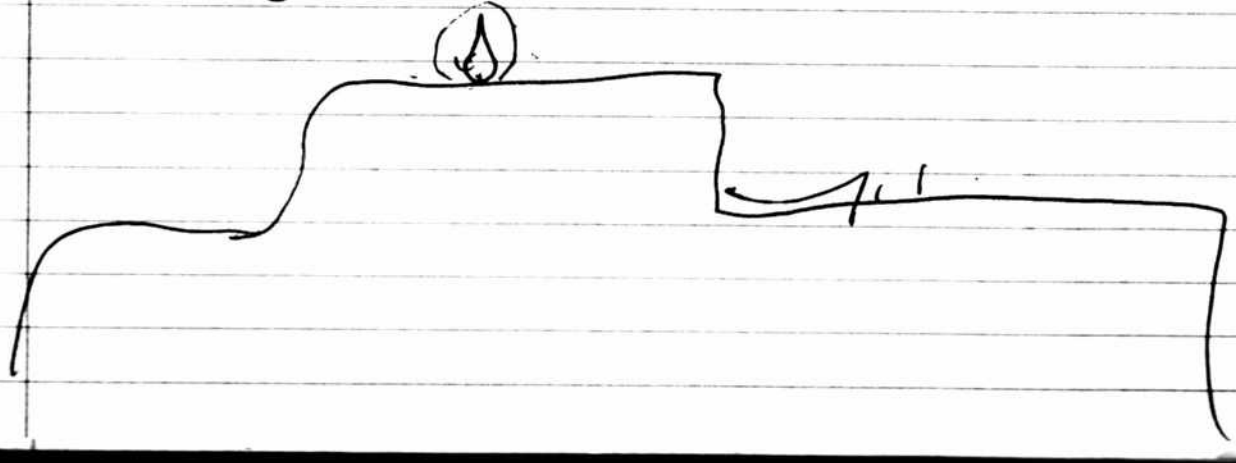
Ethanol
Water
Isopropyl alcohol
Acetic Acid & (Vinegar)
Acetone (problem w/ plastic)
Gasoline (problem w/ plastic)
Ammonium sulfate

Current Gas —

II. Separation

1. Iron
2. Lipid
3. Protein

Are you writing up anything on your thyroid work?



11/19/11

Created a new column tonight.

Prepped the column w/ $(NH_4)_2SO_4$ instead of just water.

Added Isopropyl alcohol as 1st solvent.
Had minor separation of a brown band.

Have added (replaced) with ethanol.

A very significant green band developing.

You had seen the light
The bleach used destroyed the pucer.

Make up 1M NaOH solution

MW of NaOH = 40.0 gm/mol

$\frac{40 \text{ gm}}{100 \text{ ml}} = \frac{x}{60 \text{ ml}}$ $x = 24 \text{ gms}$

Column Observations:

We learn that when you add the red extract to a column that has been prepped w/ H_2O, it turns greenish.

Then is a band that forms but it seems to shell out.

Brown is Carrying at blue-green
how & why?

We now have positive proof of a protein.

Sequence appears to be

1. Column w/ water
2. Add Culture
3. Add Alcohol
4. Add 20% saturated Amm Sulfate
5. Add Alcohol again (comes out blue)
6. Now we are flushing w/ water and Vinegar

— w/ the blue solution

Now in primarily water, the solution comes out blue.
In alcohol we get a precipitate and a solid purple color

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Today we learn that the blue green color is coming out in the ethanol but it is actually being forced out by water.
And that the Ammon. sulfate was required to get the whole process started.

We also learn that the darker material went to the top layer & that they seem hard to free up.

The columns becoming more & more clear but it still produces the blue green color so fun is a continual flush.

Cu^{2+} ion w/ ammonia creates a rich blue solution (cloudy) but our blue green extract does not do that so the blue to blue green color is not from copper ion but it still would be from a copper complex.

Ammonia is NH_3
Ammonium sulfate is $(NH_4)_2SO_4$

Cobalt Chloride w/ Ammonia produces a precipitate - blue green. This is not what we have.

Ammonium Sulfate + $CoCl_2$
does not produce any reaction.

$(NH_4)_2SO_4$ + $CoCl_2$ also does not
produce any visible reaction.

Nothing we do is matching our
suspected protein.

We found a reference that shows
clearly a blue solution that results
from Biuret test, and it is
a PEPTONE.

albumen gives a violet color.

Our candidate is a peptide.

a peptone is a type of peptide

specifically we suspect a dipeptide.
Peptones are used in the growth
of bacteria and fungi.

The argument for a peptide is strong (ie dipeptide)

1. Blue color - passes biuret test.

2. Paper that says blue color is from 2 amino acids

3. Does not coagulate upon heating.

4. Used to support culture growth of bacteria & fungi

5. Water soluble

6. Spectral analysis?

Peptones are not precipitated from solution by saturation with ammonium sulfate.

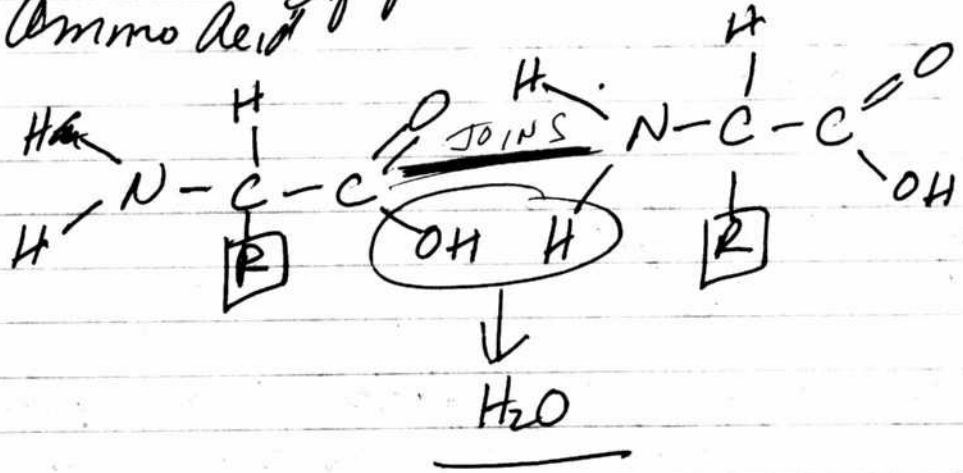
Peptones are the result of digestion in the stomach. The stomach breaks large proteins into smaller peptides and peptones.

Ethanol Ammonia Mix may have caused the dark portion to drop further down the column but it still did not separate.

Ammonia turns the culture stock area more duo green. Bleach turns it yellow.

Bleach destroys proteins! Watch out!

Amino Acid Dipeptide



We believe that we know we are likely to have a

non polar dipeptide

seems to be extremely non polar

CH_3?? CH_1, SH are candidates

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Could we not also have a

"blue copper po protein?"

highly non polar

Copper based?

dypeptide

Ammonia bleaches the column very nicely. It does not seem to destroy the solution like bleach did (bleach destroys proteins).

Ammonia is polar.

adding NH_3 to lactoferrin in water did not change the Biuret reaction one iota. Both tubes came out perfectly purple.

We know that "our molecule" is highly polar because of its

does a dypeptide bond to iron?

Chromatography Results -

We have learned a lot over the last few days from the Chromatography work.

First of all NH_3 with $CuSO_4$ produces a pale blue precipitate, NOT A clear purple solution!

$$Cu^{2+} + 4NH_3 \rightarrow Cu(NH_3)_4^{2-}$$

It is a Complex called ~~Tetraamine~~ Tetraamine cupate(II) Complex

The color of the solution is blue, not purple!!!

Violet is a bluish purple.

We have primary peak region from 590 to 605.5

This avg is @ 597.75

The protein has been proven w/ the purple color.

Method

1. Eliminate Contamination issue + question by flushing the column w/ ammonia. This turns the silica gel clear.
2. Now start flushing it with water.
3. Now add the culture
4. Now add ~ 20% saturated ammonium sulfate
5. Now add ethanol.
6. The well gave a separation of a mildly greenish elute which will test positive for Lowry.
7. Ammonia will flush through straight.

You are getting both purple and blue colors.

also you extract them w/ polar solvents eg ethanol

This suggests a polar dipeptide as one candidate (ya blue vs purple)

Carnosine is a polar dipeptide

Other polar "residues" that can be incorporated into dipeptides include

1. serine
2. asparagine
3. threonine
4. tyrosine
5. tryptophan

When you add the culture + ammonia it bleaches bleaches the column.

It does not appear to be bleached at upper the culture
this means an additional reaction w/the Columns of some sort is taking place.

Our whey is 78.6% protein.
This is very high.

You should be able to calculate a ppb protein solution.

From this you should be able to determine the Concentration of your eluate. But what do we do about the blue vs purple question?

You would need a dipeptide to compare to.
Where do I get me?
Aspartame???

We have essentially proven that we do have a dipeptide now.

1. The paper that says R=2

2. The blue color with R=2

3. No coagulation upon heating.

4. Direct Control Comparison w/ Aspartame.
#26
w/ aspartame we have a max absorbance of 607.5 nm.
Amax = .172
We have essentially the same peak @ 607.5 w/ our protein elute.
Amax = .534
Ya now have a means of determining Concentration.

5. lets put 3 packages in 20 ml of water, Aspartame.

Control will be 4 pks Aspartame

20 ml H_2O

4 ~~8~~ 12 drop $CuSO_4$

~~4~~ ~~6~~ 8 NaOH

Final volume 25 ml

2 ml Straight
$2 + 2H_2O = .5$
$1ml + 2 H_2O = .33$
$1ml + 3 H_2O = .25$

Ok, we have a solution.

Concentration = 4 packets
20 ml water
4 drops $CuSO_4$
8 drops NaOH

1 drop = .06 ml
12 drops (.06) = .72 ml

1 packet = 1gm ≅ 1ml

20 ml
.72
= 20.72

~ 25 - 20.72 = 4.28 ml

$\frac{4.28 ml}{4 packs} = \frac{1.07 ml}{1 pk}$

so each pack is approx 1 ml
Now we measure absorbance @ 607.5 nm

Absorbance = .275 * Conc.

Concentration = $\frac{Absorbance}{.275}$

Now we are in position to determine concentration of diode

Abs @ 607.5 nm = .618

Therefore

Concentration = $\frac{.618}{.275} = 2.25$

It is an extrapolation, but this is not unreasonable.

Equivalent to 2.25(4) = 9 pks of heptane in 20 ml of H_2O.

This seems fairly substantial.

$\frac{9 \text{ pks}}{29 \text{ ml}} = \frac{1 \text{ pk}}{3.2 \text{ ml}}$

so our sample is equivalent to approx 1 pk heptane in 3 ml of H_2O

Not unreasonable

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We now have an estimate for the mass of aspartame.

100 time as sweet as sugar

1 pt = 2 tsp of sugar

Density in bags $\approx \frac{100\ kg}{m^3} = \frac{.0007\ kg}{cm^3} = \frac{.7\ gms}{cm^3}$

1 tsp = 4.93 cm3

So $1\ cm^3 = \frac{4.93}{cm^3}(.7\ gms) = 3.45\ gms$

Now 2 tsps (1 packet) = 6.90 gms

and $1/100(6.90\ gms) = .038\ gm = 38.3\ mg$

So now we know 1 packet $\approx$ 38.3 mg
So in our work

Not a bad estimate

The actual amount from the company we contacted is 33 mg

Now our Concentration in facts is 2.25 for our dilute

$2.25 \approx \frac{(4\ packets)(38.3\ mg/packet)}{29\ ml\ of\ H_2O} \approx \frac{11.9\ mg}{ml\ H_2O}$

The peptide in our culture solution

and 1 packet should be 38.3 mg
So we are equivalent to about 1/3 package dissolved in 1 ml of H_2O

So our generalized result is:

$$\text{Concentration} = \frac{\text{Absorbance}}{.275}$$

but our unit of concentration is $\frac{4 \text{ packets}}{25 \text{ ml of water}}$.

$= \frac{1 \text{ packet}}{6.5 \text{ ml of } H_2O}$

and we are calling this "1"

$\cong \frac{38.3 \text{ mg}}{6.5 \text{ ml } H_2O} \cong \frac{5.9 \text{ mg}}{1 \text{ ml } H_2O} = \text{"1"}$

So our modified formula is

$$\text{Concentration in } \frac{\text{mg}}{\text{ml}} \cong \frac{\text{Absorbance}}{.275} \times \frac{5.9 \text{ mg}}{\text{ml } H_2O}$$

!!! $\text{Concentration in } \frac{\text{mg}}{\text{ml}} \cong 21.4 \times \text{Absorbance}$

~~Straight~~ $A = .618$

$.618 (21.4) = 13.2 \frac{\text{mg}}{\text{ml}}$

looks good

You should be able to determine a calibration graph for powdered milk also. (protein in general)

Dissolving Ammonium Sulfate in water:

$(NH_4)_2SO_4 + H_2O \rightarrow NH_4OH + H_2SO_4$

Question, is the acidic or basic?
Direct measurement 7.6
So, slightly basic, but not by much.

Review the chemical structure of a dipeptide:

After flushing w/ NH_3 to bleach the Coleman, addition culture stock, & alcohol it flushed straight through.

So this tells us it must need $(NH_4)_2SO_4$

Dear Harmon

90% of all molecular reaction involve the polarity of the molecules.

Acid-base reactions are due to polarity w/ the hydrogen atom.

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Hydrogen

How do you make H^+ ?

1. If you take away the electron.

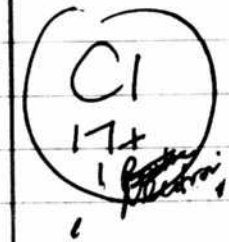
An acid is a proton donor

A base is a proton acceptor.

So what does it mean to donate a proton.

HCl $H^+ Cl^-$

17 electron



If you get rid of 1 proton
You have 16 protons
& 17 electrons
This makes Cl^-
Cl should be an acid?

If you take away the electron you are left with ⊕ ie, a proton

This is an H^+

Arrhenaus ios defins acid in terms of H^+ & OH^-

Brownsted lowry defines acid & base in terms of what they do.

So an H^+ (electron zone) is a proton

Brosted Lowry is defined only in terms of what happens to the H^+

an H^+ IS a proton, ie a "naked proton"

Thus is really interesting.

1/4 NH_3 + 3/4 Ethanol right on top of culture on silica is giving good flow

No ammonium sulfate added.
Green color is showing up right away.

Suggested path for column:

1. Water in column
2. add culture, sink below
3. an inch or so of $(NH_4)_2SO_4$, sink below
4. 10% Ammonia, 90% Ethanol
5. see what happens

This worked.

Concentration in latex used
21.4(1.007) = 21.5 mg/ml

But if you ~~subtract~~ the reference
which is what you should be
doing (ie, the blue green elution)
you get
21.4(.642) = 13.7 mg/ml

Which is matching perfectly:

13.2
13.8
& we had another 13~
so avg ≅ 13.5 mg/ml

Another round subtracting the reference

$21.4(.40) = 8.6$ mg/mL

Avg of all samples to date is:

$21.4(.691) = 14.8$ mg/mL

Our chemical tests for Copper fail.
This is interesting

Cu^{2+} + HCl gives a yellow solution (failed)

Cu^{2+} + NH_3 gives a dark blue solution (failed)

So both tests failed!
So what is the blue green color from?

It also fails the flame test for being green.

It fails all tests. It is not copper ions.

It might be a copper complex but it
is not Cu^{2+} ions.

We need red cabbage & lantern
cabbage for paper electrophoresis.

We know some things ...

1. We have a dipeptide

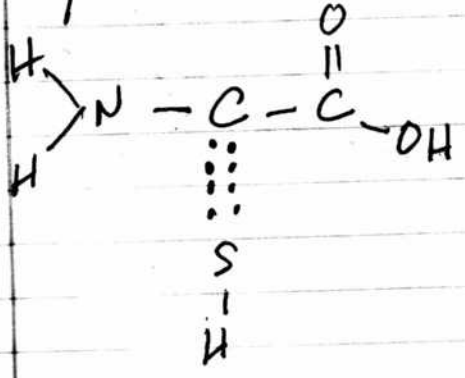
2. The dipeptide is polar

3. Polar amino acids have
S, O, N in them

4. We believe we have disulfide bonds
from the resistance of the material
to chemical damage.

5. It almost certainly has to have
an Fe 3+ state.

Cystein has the structure



Another one
will lead to
the sulfur

We may have something that is highly
positively charged ??

a protein w a preponderance of basic amino acids will have an overall positive charge (thus in neutral aqueous solution.

a protein w/ many acidic amino acids will have a overall negative charge in neutral solution

The SH bond is only slightly polar

We have a problem w/ our biuret protein - dipeptide hypotheses.

Ammonium Sulfate + $CuSO_4$ + NaOH

→ gives a perfectly blue color

just like the biuret test can with a dipeptide.

You may have a false positive!

This weakness is stated in my new lab manual & it looks like you must use an alternate method to establish positively the existence of the protein a dipeptide.

This is a problem.

However:

Amm. Sulfate we get the max peak @ ~ 630 nm - quite blue.

With our "protein or digestive" hypothesis we get our peak @ 604.

Thus is noticeably different.

We learn some more things. Adding too much Copper shifts the absorbance peak to the right (more red).

So you must have a standard to compare any thing.

Suggest 3 ml of solution
1 drop 1% $CuSO_4$
4 drops NaOH

	Peak:	Mag
$(NH_4)_2SO_4$	602.5	0.70
Aspartame	639	.72
Elvie Clear	604.5	.65
Elvie Cu Colored	643	.67

Same?

Same?

So now the evidence indicates indeed that you still do have a dipeptide but that it only is within

the Copper Colored Blue

and
NOT THE Clear Blue.

This indicates it is somehow tied in w/ a reaction w/ the kitty litter!

And that when it is bleached out you no longer have a reaction!

So what is in the kitty litter a question??

What appears to be happening is that the protein a dipeptide is reacting with a copper compound in the kitty litter.

It appears to be forming a blue Copper protein complex.

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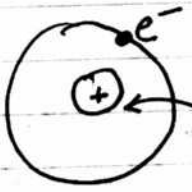
P. H^+ Concentration is the same as proton Concentration.

Why is H^+ equivalent to a proton?

Because



When the electron is taken away, all that is left is no proton. Then is what is remaining of the hydrogen atom.
So the hydrogen atom is when core becomes a proton.

The proton is also called the hydronium ion.

List.

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need Kasco

Pipets (gw made it!)
Universal ind. color 100ml 12.20
methyl red 5gms 10.05 OK
nitric acid 500ml 18.75 OK
pH meter

NaOH

Micro Pipette

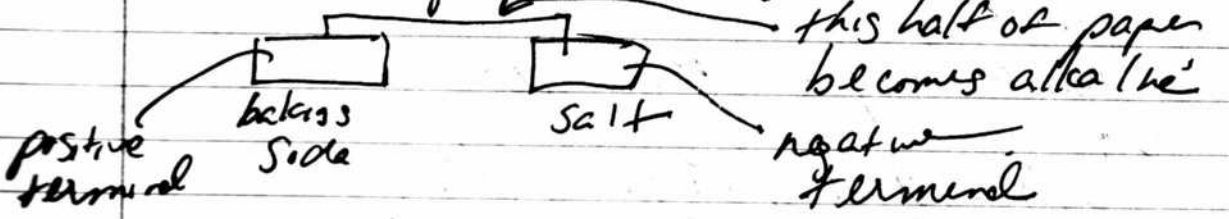
commissie blue, bromthol blue

30 drops / 1 drop / X = .0667ml
20ml / X
= 67 ul

Pasteur Pipettes might work.
p 169 16.90 thkgs
16.40 bulbs

Carolina ka
a student Pipette 10ul off 26.75
Tab standard P.p.s.

The electrophoresis bridge



Need to

1. Make culture stock
2. Repeat aspartame ~~test~~.
3. Learn how to make a starch gel.

12% gel

36 gms in 300 ml

6mm thick

Buffer.

Want a ~~heated~~ buffer w/ hydrolyzed potato starch

Citric Acid - Sodium Citrate Buffer:

pH = 5

millimoles per liter = 10

Use:

Citric Acid, 77 gms / liter

Sodium Citrate 1.06 gms / liter

Starch hydrolysis.

We are going to use a 13% solution.

Boiled 15 min in dilute HCl

(Maybe 5 drops 6.7M in 13.5 cm x 8.5 cm x 0.6 cm

= 68.9 cm^3

/ lets use 100 cm^3 = 100 ml = 100 gms)

13% = 13 gms (by weight

pH is about 2 w/ 5 drops in ~100 ml H_2O

It looks to me like we are successful!

We obtained a yellow elute. Lets see if we can recall how. We added $CuSO_4$ also added $(NH_4)_2SO_4$ salts. Also added alcohol, ammonia. Nothing seems to pull out the blue color (blue copper protein complex?). But then we add methyl ethyl ketone (MEK) we get a yellow elute w/ the blue (entone blue) still left behind. The yellow elute is alkaline @ pH 9.5.

Now we are trying HCl.

And then we will try vinegar.

We have a ~~lead~~ lead that our blue copper protein may be a

"Type I Copper Centre (T1Cu)"

also known as a "cupredoxin"

It consists of a Cu atom coordinated by two histidine residues & a cysteine residue.

Guess what cysteine is? The SH side group!

The suggests that the amino acids in our "dipeptide may involve histidine & cysteine...??? & that it is likely binding to Iron

Cupredoxins have strong absorbance near 600nm

591 peak in the blue copper proteins

I think we are on to something

We are likely dealing w/ a

"metallodipeptide"

You made this term up

vs a

metallopeptide protein.

There is indeed such a term.

It is called

metallo-dipeptide

(we expect iron, histidine, cysteine).

Another possibility is two cysteine groups

There is a Cysteine cysteine test!

add NaOH & weak lead nitrate

Where can we get lead nitrate? $PbNO_2$

12/13

It appears that we have successfully hydrolyzed the blue copper protein w/ HCl.

We are gradually removing the blue color from the tube & the eluate come out clear.

Subjected to burner test, however we get a strong blue color w/ max peak @ ~~650~~ 645 nm

I believe that eluate is going to have the amino acids [Methionine / Cysteine] none or both

Fascinating that it comes out clear even though it is coming from the blue color of the tube.

We need to test - is it Cystine or Cysteine.

Cysteine has the single SH bond

Cystine has the disulfide bond.

Cystine should be a dipeptide?

yes - Cystine is a dipeptide of cysteine.

Now, how would it bind with iron?

And also histidine possibilities.

We have our gel box:

Gel Tray dimension:

7cm x 7cm x .5 cm = 24.5 cm^3 = 25 ml

Now a 10% gel is

2.45 gm starch.

We used 5 drops for 100 ml H_2O
we only need 2 drops now.

Use 40 ml a
(I measured) 4% starch = 4 gms
2 drops HCl
This worked perfectly
13% = 5.2 gms

Now let's see how much buffer we need to cover it.

4cm x 4.1cm x 7.6cm = 142.9 cm^3
per segment of gel device

* 2 trays = 285.8 cm^3 +

7.6 x 18.5 * 1cm to cover = 140.6 cm^3

So we need 286 cm^3
+141 cm^3
427 ml to play on a session of citrate buffer.

Essentially then 500 ml.

We can make the buffer in the container itself.

$$\frac{2.1\text{gm Citric acid}}{100\text{ml}} = \frac{1.05\text{gms}}{500\text{ml}}$$

Then use lye to bring the pH to 6.

NaOH preparation

$$\frac{40\text{gm}}{\text{liter (100)}} = \frac{X}{60\text{ml}} \qquad X = 2.4\text{gms}$$

We know that the eosin stained protein appears to be negatively charged because it migrated to the positive terminal.

Now
eosin is an acid analine dye which stains the more basic proteins.

Next:
a protein with a preponderance of basic amino acids will have an overall positive charge in neutral aqueous solution.

Then is not matching.

1 eosin

1 eosin

1 eosin + clear protein

1 eosin + clear protein

1 meth. blue + clear protein

1 meth blue + clear protein

1 color protein + eosin

1 color protein + meth blue

Acidic dyes w/ a negatively charged chromophore bind to the positively charged molecules.

Eosin is such an example.

So now we know that we expect eosin ly likely to migrate to the positive terminal, which it is.

Methylene blue is positively charged.

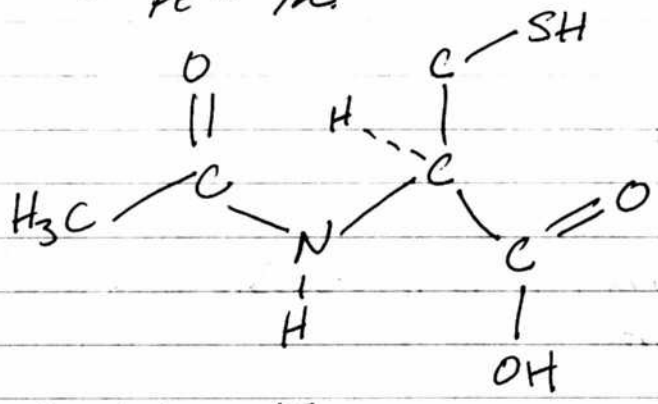
Ther mean the dye ly likely will move toward the negative terminal which it is.

Now, how do ya tell if a molecule is positive a negatively charged?

basic = positive charge
acidic = negative charge
} why?

Next, how do ya tell if a molecule is acidic or basic?

Oxalic Acid reduces the Color to a green color but it does not release the Fe^{2+} ion.



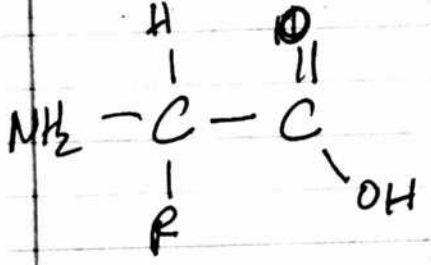
Acetyl cysteine

604 nm is the ammonia or ~~ammonia~~ ammonia salts
650 is the dipeptide

The iron test is succeeding in both cases when NAC is added

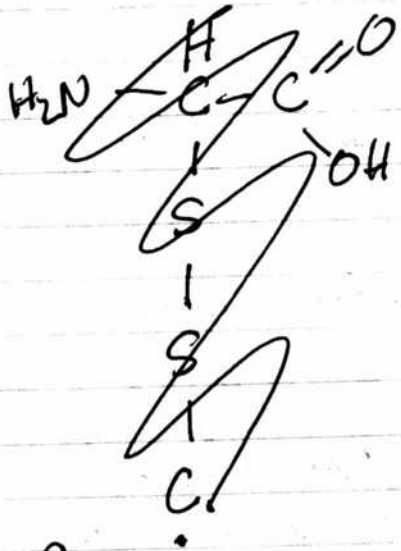
It would appear that NAC breaks disulfide bonds and releases the iron into Fe^{2+} ionic form.

Remember the amino acid structure?

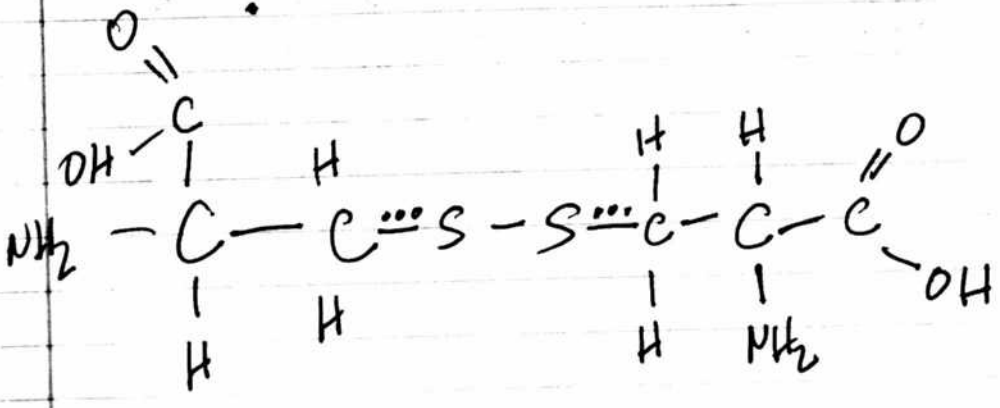


In the case of cysteine, R is a covalent bond of SH

Two cysteins together form a cystine



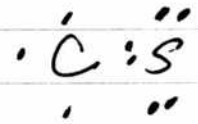
Also metal atoms bind. See p 7 McGilvery -



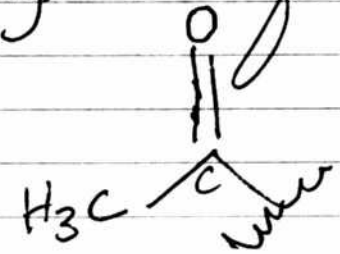
Iron Fe^{3+} is likely attached to this. But exactly how is

Carbon 4 valence electrons
Sulfur has 6

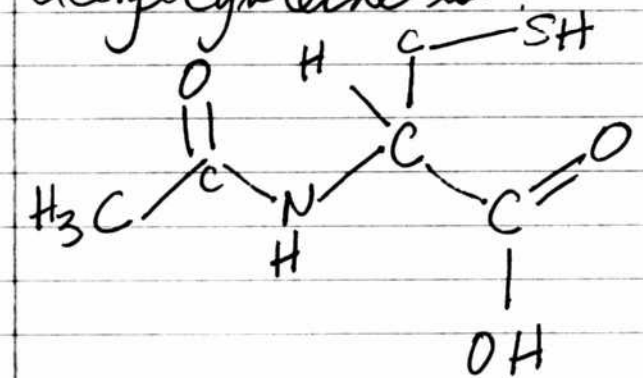
What we have



Acetyl is a functional group.



Acetylcysteine is:



We have a situation where the nal sample is filtering almost clear.

The may create some difficulties

The gravity / filter elute definitely does not react to Fe(2+ test.

Protein Separation Dec 21 2012

We now know the sequence:

Culture
$CuSO_4$
Ammonia salts (~25%)
turns it stray blue.
Flushing w/ water
We start to get stray blue elute.
This elute is ~~640~~ 604 nm

This is ammonia salt - Not PROTEIN!

Protein require hydrolysis w/ acid.

This will be 650 nm (Aspartame)

We have some confusion.
Top Aspartame is coming out @ 604 nm.
1 drop $CuSO_4$, 4 drops NaOH in 10ml H_2O
We are working on concentration standard
1 pck. in 10 ml H_2O (33mg)

It is the Copper Concentration that is affecting it!
Now 2 drops $CuSO_4$, 8 drops NaOH!
in 10ml H_2O

Now it moves to 616 nm

Now 3 drops $CuSO_4$, 12 drops NaOH in 10ml

Now it moves to 625 nm.

Now 4 drops $CuSO_4$, 16 drops NaOH

634 nm

So it is shifty to the right with more $CuSO_4$ & NaOH

Now what does ammonia salts do?

Ammonia Salts

2 drops $CuSO_4$ + 8 drops NaOH in ~2ml

= 604 nm

We have a finer distinction to make.

Amm. salts turn things blue w/ Cu.

So does the hydrolyzed protein complex.

But the protein complex (aspartame) also turns darker blue & spectrum shifts to right as more Cu & NaOH are added.

You must start by thoroughly studying the Amm. salts.

Start w/ 2.5 ml of Amm. salts

		Max
1 drop $CuSO_4$, 4 drops NaOH	597 nm	.796
2 drops $CuSO_4$, 8 drops NaOH	597 nm	1.18

a beautiful clear blue color.

What we see here, and we have seen it before, is that max absorbance of Amm. salts is the same even though we vary the concentration of buret.

Notice the peak absorbance also declines around 340 nm.

Now lets look @ aspartame.

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Proof of distinction between Amm. salts & a dipeptide upon exposure to Biuret.

Aspartame

1 pt in 10 ml H_2O (11 gm/ml)

623 620	1 drop Cu,	4 drops NaOH	.28
632	2	8	.49
639	3	12	.72
644	4	16	.92
641	5	20	1.06

2 pts in 10 ml H_2O (12 ml)

624 626	1 drop Cu,	4 drops NaOH	.24
4 mins 614	2	8	.54
626	3	12	.81
635	4	16	1.04
644	5	20	1.28

So there is clearly a shift to the right w/ increased biuret reagent.

This is what the dipeptides do as opposed to Amm. salts which maintain a constant max @ 591 nm.

What is the reaction of Amm. Salt & $CuSO_4$ + NaOH?

One more test on strong Amm salt w/ high concentration of biuret.

591 Full strength Amm. salts + 5 drops $CuSO_4$, .88
+ 20 drops NaOH - this is full strength.

This proves the distinction between amm salts & the dipeptide complex.

Notice the peak are also near 340
(estimate around 340)

We now have a way of positively distinguishing between the amm salts & our protein.

It is 597 nm vs 620-653 nm
amm. salts dipeptides

We have proven our dipeptide again

λmax @ 653.5 A= .83

increasing peak @ 340 also.
a perfect match.

We truly seem to have formed a match.
Now reduce the blue to clear again.
Passes Fe2+ test.

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B.

Protein (Clear)

+ $CuSO_4$ + NaOH → blue

+ NAC (turns it clear)

+ (1,10) turns it red. (Fe^{2+} available)
binds broken.

Protein (Clear)
+ NAC + (1,10) does not pass Fe^{2+}

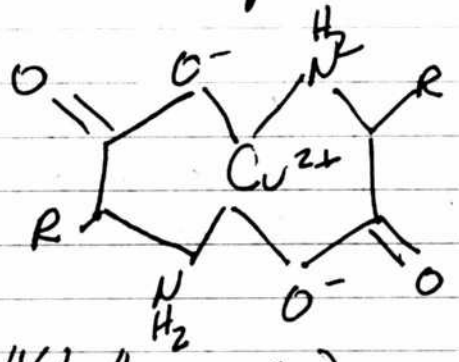
add NaOH still does not pass Fe^{2+}

add $CuSO_4$ now it passes the Fe^{2+} test.

So we have a question.
What is actually breaking the bonds
& freeing the iron?

$CuSO_4$
NaOH
NAC
} all three required?

Amino Acids and Cu^{2+} form a blue complex



(a "blue" complex)

Now in our case there has to be an iron (Fe^{3+}) in here also somehow as it is released by the NAC.

So we learn in a copper alkaline environment NAC breaks bonds & releases free iron (Fe^{2+})

Interesting. The Cu was very important.

1. Take protein (clear) solution
2. Add Cu^{2+}
3. Turn blue green
4. Add NAC
   turns blue green (or purple) & then turn clear!
5. Then Fe^{2+} test is positive

We can make our own Bradford reagent.

We need

need
- Coomassie Blue
- Ethanol
- Phosphoric Acid

] Not too hard Carolina has Phosphoric acid

Bradford Reagent:
100 mg Coomassie Blue
50 ml 95% ethanol
100 ml 85% W/V Phosphoric acid
dilute to 1 liter

What is TBE?

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Orders:

Carolina:	Enasco	Home Science
✓ Phosphoric Acid		pH meter
✓ Sodium Citrate		petri dishes
✓ Nitric Acid 1M		600 ml beaker
✓ Ninhydrin 5g		1000 ml beaker
✓ Agarose 5g		10 ml pipette
✓ Universal Indicator		
Coomassie Blue		
✓ NaOH		
✓ Desiain		
✓ still need Coomassie blue		

73.15 ✓

~~65.20~~

~~51.45~~

+Ship

53.45

* Protein Separation: Dec 22, 2011

In the first time ever, we have a reliable method of reproducing the proteinaceous Complex.

The method in the Chromatography column is:

1. Fe culture in the column
2. Add $CuSO_4$
3. Add amm. salts, estimated ~20% saturation
4. Flush w/ water. The weak flush out amm. salts which have λ_{max} of 597 nm
5. Add hydrochloric acid. A very gradual process w/ several titrations. You also get some solids in the process. Separate only the liquid which may have a slight blue tint. Biuret tests proves @ 640-650 nm. The is the proteinaceous Complex

maybe ammonia added?

Make $CuSO_4$ solution

$$\frac{246.68 \text{ gms}}{\text{mole}} \rightarrow \frac{246.68 \text{ gm}}{1000 \text{ ml}} = \frac{X}{60}$$

$X = 14.8$ gms / 60 ml for 1 M solution

but we want 0.5 M so $(.5)(14.8 \text{ gm}) = 7.4 \text{ gms}$

$$\frac{7.4 \text{ gms}}{60 \text{ ml } H_2O} = 0.5 \text{ M Solution}$$

We discover from our Indian speaker that BME (beta mercaptoethanol) breaks disulfide bonds. Creasing of the protein.

BME can be toxic but in small quantities

BME appears to be toxic except may be very small quantities.

BME & urea section in biochemistry lecture on fractionating.

Cystine is the S-S dipeptide
Cysteine is the SH bond.

Problems:

1. You have not completely replicated your ~~reproducto~~ production of the protein.

You have a sequence but it is not complete.

Solvents I've used are

Water
Alcohol (Isopropyl = alcohol)
Acetone
MEK Methyl Ethyl ketone
Ammonia
Ammonia Salts, estimate 20%
Acetic Acid (Vinegar)

We have a tentative sequence of

1. Iron Culture
2. $CuSO_4$ added
3. Amm Salts
4. Flood w/ water? & add ammonia? Anything else? MEK? Alcohol?, Acetic Acid
5. HCl is the only way of removing the complexes at today 2-3 days

do you need to recover the process.

Next, problems in the gel:

1. Be very gentle in you love everything
2. Caution w/ Comb extraction
3. Stacky the protein w/ glycerol?
4. You do not have the dye you need!
   Commassie Blue
5. Trial work in between
   Met Blue as an a pH indicator
   Eosin
   Iodine

Proteins (in Glycrol?)
Milk
Asparagus
Own solution (blue & clear)
pH Blue
Albumen?

Dyes
Cabbage

Important:

The blue form of the protein
w/ NAC does pass the Fe^{2+} test!

Alone, w/out any additional

What is the pH of this solution?

It is actually fairly neutral!

What about the clear form?

NAC by itself does not matter.

NAC w/ Cu^{2+} turns it in w/ a dark orange color.

What is the pH of this form?

It is highly alkaline pH 9.

Now the question is
what is the role of pH?

What if we neutralize the clear solution? Would we still get the same reaction?

Yes, but it is very mild.

1. Clear form is highly alkaline pH 9
2. add NaC
3. add Fe^{2+} test
4. No major reaction, possibly very mild
5. add Cu, a reaction to Fe^{2+} test. (it is orange more than red)

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Copper recommended 3 mg per day.

Assume we have 1500 ml
@ 100 ml per day.

$\frac{3 \text{ gms}}{1500 \text{ ml}} = \frac{X}{1 \text{ ml}}$ $X = .002 \text{ gms} = \frac{2 \text{ mg}}{\text{ml}}$

x 100 ml = 200 mg per day, way way to high.

Now choose 0.3 gms per 1500 ml

$\frac{.3 \text{ gms}}{1500 \text{ ml}} = \frac{X}{1 \text{ ml}}$ $X = .0002 \text{ gms}$ $X = .2 \text{ mg/ml}$

.2(100 ml) = 20 mg/day

But $CuSO_4 \cdot 5H_2O$ is not pure copper!

It is only 25% of the mass.

So 20(.25) = 5 mg/day perfect.

So .6 gms for 2 bottles

You now have 5 times too much.
Cut it in half.

We have not produced the protein reliably.

We now have
Culture
$CuSO_4$
@ this point we get a dark blue complex and a light blue complex.

Now what to do @ this point?

Do we add NH_4, alcohol, HCl?
We are trying HCl this time.

Notice we got the blue complex w/ out adding anything???
We know Cu & Fe precipitate in some way.

Try Culture + NH_4 + Cu

When you add $CuSO_4$ to the culture & centrifuge you have clear separation.

Interesting though the pH is close to neutral you broke your water glass.

We have a decent sel to use
What do you want to use?

1. Met Blue w/ Glycerol

2. M.B.G. w/ Milk

3. MBG w/ clear

4. MBG w/ copper color

5. MBG w/ egg yolk

1 MBG	1 MBG
2. MBG + Alb	2 MBG + Milk
3. " "	3 " "
4. MBG + Milk	4. ———
5. MBG	5. + Copper
6 MBG + clear	6 +
7 MBG + Copper.	
8. MBG + Clear	8. Clear

Problems 12/27/11

Problems

1. Can not reliably produce the protein with a known chromatography sequence
2. The stained gels are very delicate & must be treated very carefully.
3. You cannot yet effectively dye the gels & obtain any reproducible effects the dye process is a huge problem. What exactly to do?
4. You do not know the molecular structure of the metallodipeptide.
5. You do not know the chemistry of the metallodipeptide cuprous alkaline NAC reaction
   You only know it seems to work.

How do we start making progress on these problems?

4. Write #4, you should be able to learn something about the general chemical nature of a metallopeptide.

You found a strong reference in the "Chap 5 pdf" that SH groups bind to Copper and metal (iron).

You have several properties to reduce the disulfide bond

also Horton
1. NAC
2. BME (toxic?) (urea?) from online, in India
3. Oxalic acid.
4. Peracetic acid (Horton textbook)
5. Pyruvic Acid

In the idea is
1. Iron is bound
2. Copper replaces the iron and binds are broken w/ NAC in an alkaline environment.

We see now that
Cultine + Copper create a bound
complex composed of two parts.
Turquoise + blue section

It stays bound even with acid. HCl

Now when you add Amm. Salts (~20-30%)
the blue section starts to separate
down the column. The turquoise
stays bound.

Culture Info.

We have learned that raw sulfur
is not soluble. It is the
sulfate ion that is absorbed
not raw sulfur.

Cysteine positively binds to iron
causes oxidative stress when
accumulated in the brain
the iron rich globus pallidus

A Iron Discovery

We have just made what appears to be an important discovery.

The culture, when boiled in HCl & the liquid filtered off tests positive for Fe^{2+} !!!!

The beef-milk culture medium boiled w/ HCl does not pass the test!

This means the iron is coming from the culture

This is a big deal.

It is the simplest most direct method of proving the existence of iron in the culture.

Now, does HCl ever act as a reducer???

Now, does the real ☉ sample do the same thing?

Wine Wine, by itself also fails the ~~test~~ boiled w/ HCl

The oral sample, however, boiled w/ acid

FAILS

This suggests a difference between the culture and the oral samples.

The wine solution is however, red, so it ~~contains~~ may contaminate the results.

Well, ya had an important discovery the culture has iron directly ~~added~~ HCl & boiling.

HCl is not an oxidizing agent. Apparently it is actually a reducer.

Wait a ~~minute~~ minute!

Our $FeSO_4$ has both Fe^{2+} & Fe^{3+} in it now. It has been partially oxidized.

Even Liquid Iron is passing both $Fe2+$ and $Fe3+$ test. This is Confusing.

Another big discovery.

Your $FeSO_4$ as well as your liquid iron has been partially oxidized and they both have Fe^{2+} & Fe^{3+} ions in them.

This is not good for testing purposes.

Next we learn (remember) that citric acid is a reducing agent.

So if you add Citric Acid to $FeSO_4$ it reduces it so that only Fe^{2+} is back in solution.

So the citric acid is acting as a "preservative".

Consider adding some citric acid to your $FeSO_4$ solution to keep it in the Fe^{2+} state.

I did it. This change really complicated matters.

It would now be possible to
Calculate the amt of Fe^{++}
in the hydrolyzed (HCl)
Culture.

The is not critical,

It is more important to recover
the protein.

Test the metallo dipeptide
cuprous NAC alkaline reaction.

pH of clear protein appears to be about 9.

but when you add NAC pH is now about 6.

There is a very slight positive Fe^{++} test at this stage

Conclusion:

If in the presence of the protein
Complex we create a ~~cuprous~~ cupric
alkaline environment
and then we add NAC

the protein complex is reduced and
free iron in Fe^{++} state exists.

There is a mild reaction in a ~~cuprous~~ cupric neutral
environment but alkaline is a
stronger reaction (more iron released)

Yes positively proven by taking the neutral environment and adding more NaOH
It becomes brighter orange.

What if no copper is added?
A mild reaction does take place.

Copper seems to intensify
Alkali seems to enhance

12/28

On the column, we have pulled away from the ammonia + HCl which did not seem to do much to a progression of MEK and now xylene.

All that can be said @ this point is that it is draging very fast now and that the dark blue may be very slowly moving down the column.

The turquoise is hung up. What is dragging is the light brown to yellow solute.

Draging very very fast after adding the xylene.

Yes we can see that the dark blue has positively moved down the column now. So it does indeed appear that xylene has broken it up some.

Polarity

2.5 XYLENE is indeed highly non polar. MEK is
5.2 Ethanol is mildly ~~polar~~ non polar 4.7
9.0 Water is highly polar

Ammonia next. The dark blue is moving slowly. Turquoise is now gone.

Removed Ammonia because it seems to be stuck.

Back now to adding MEK to try and get things many again.

Want:

Watch glasses
distillation equipment
lab coat

SDS Page materials

Here is the question can we have

dipeptide: Cys – S – Fe – S – Cys ?

or

dipeptide: Cys – S – Fe – Histidine

vs 4 cysteines

or

2 cysteines & 2 histidines?

a Proposal

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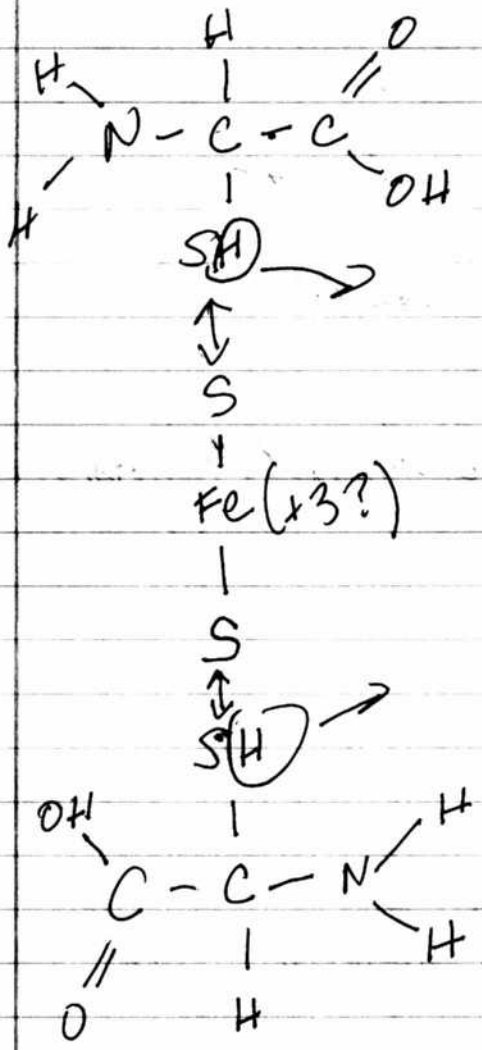


Dec 31, 2011 –

ok I have 640 nm again.

Colors w/ non polar solvents

HCl produces the final result

Ok, I have it again. The dipeptide form (clear) (clear)

The general method:

1. Culture
2. $CuSO_4$
3. Amm salts (~20%) believed to be important
3.5 Maybe ammonia.
4. Non polar solvents appear to be important.
   MEK
   xylene – (highly polar but still reacts static blue state)

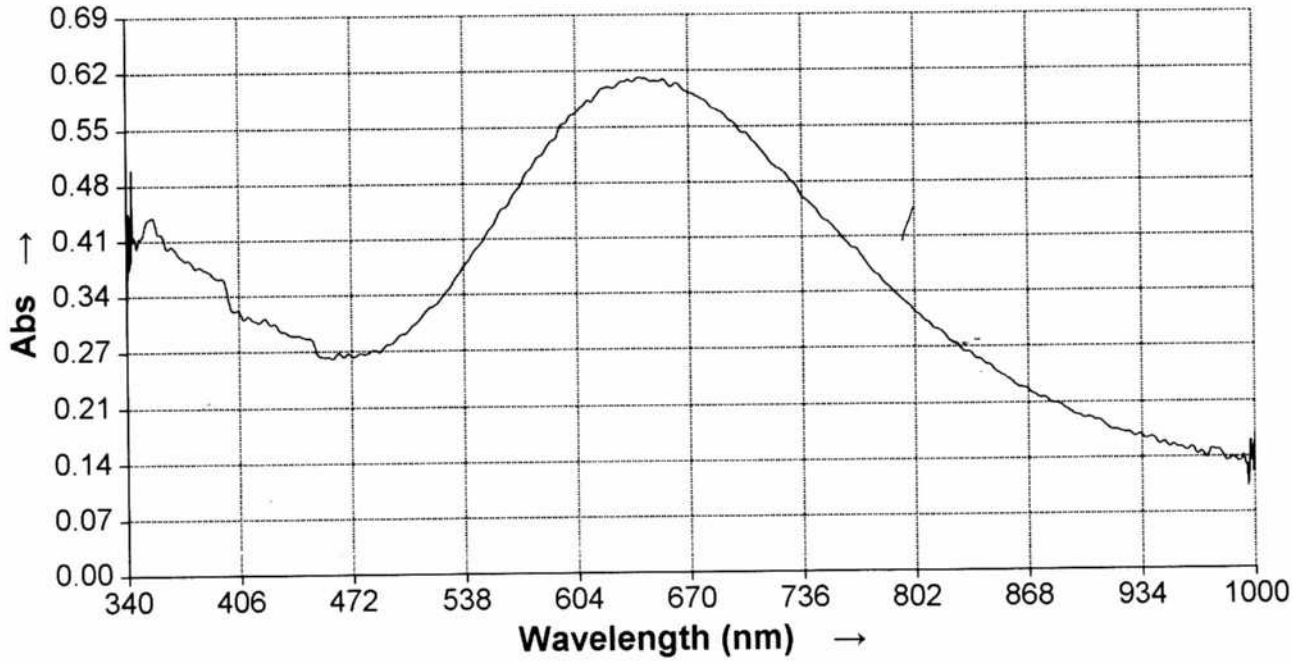
Whatever it takes to turn it ~~a~~ confirming blue, not turquoise

5. HCl (blue comes out clear but passes the 640nm test)

SPECTRONIC 200

Scan report

Spectrum of :	Scan1	Analysis date :	31 - Dec - 201
Analyzed by :	User	Analysis time :	7:56:56 PM
Channel # :	8	Print date :	31 - Dec - 201
		Print time :	7:57:45 PM



Dec 31, 2011 New Years Eve

I have succeeded. 642 nm is the dipeptide

This is now the third time for successful separation.

Now the next question is

Why did I buy

Phosphoric Acid
Sodium Citrate
Ninhydrin

Because

Bradford Reagent is used for protein testing
and Bradford reagent contains

100 mg Coomassie Blue
50 ml 95% ethanol
100 ml 85% w/v phosphoric acid
dilute to 1 liter

There is why!!!

But what was purchased is already
the Bradford reagent. It has alcohol
and acetic acid instead of alcohol
& phosphoric acid.

From Thermo Scientific we learn that
the Bradford test does not work
for peptides & smaller proteins
(certainly not dipeptides)

We proved themselves by testing aspartame. There is no change in the spectrum from the dye itself.

This may not be exactly true!

Aspartame did indeed register a shift

		Max Absorbance	May
6	Dye itself:	585	.795
6-7	Clear Extract (most recent)	617	.912
9	Aspartame	602.5	.61
10	Dye	587	.82

A very clear spectrum w/ the dye itself.

11	Milk	600	1.62
12	Co Colored Extract	599.5	.76
13	Clear Extract (Earlier Version)	586.5	.62

This one fails the test.
All other cases pass the test.

The Bradford test appears to be less reliable than the Biuret tests when testing dipeptides.

fails also.

14	Clear Extract	588.5	.78

From Pierce Net:

Development of color in Bradford assay has been associated w certain

BASIC AMINO ACIDS

primarily arginine, lysine & histidine.

No of dye ligands is proportional to the no. of positive charges on the protein.

The Bradford test does not appear to be useful for a dipeptide as stated.

We sometimes have the protein being eluted. ~640 λ nm over and over. Sometimes we are getting a precipitate so the clear solution needs to be separated. It is the copper & NaOH that is producing the precipitate.

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TLC again

looks to me like we can make TLC work.

We know now have ninhydrin.!

We make a spray reagent!
Make TLC plates
go to town!

Ninhydrin solution can be made by

.15 to .20 gms
25ml acetone

1oz = 29.6 ml ≈ 30
so 2oz = 60ml

So

$$\frac{.175 \text{ gms}}{25 \text{ ml}} = \frac{X}{60 \text{ ml}}$$

X = .42 gms perfect

You get 12 bottles worth for 5 gms

Another suggestion is

$$\frac{20 \text{ gms}}{600 \text{ ml ethanol}} = \frac{2 \text{ gms}}{60 \text{ ml}}$$

Sounds to me like I should split the difference

Use $\frac{1\text{ gm}}{60\text{ ml}}$ ethanol

It is also said the solvent does not matter.

Another one

$\frac{25\text{ gms}}{4000\text{ ml}} = \frac{X}{60\text{ ml}}$ $X = .375\text{ gms}$

So .375
2
.42
$\Sigma = .93\text{ gms}$

I suspect $\frac{0.5\text{ gms}}{60\text{ ml}}$ is fine.

Need
1. smaller glass plates. Your coating was too thick
2. Butane Cylender

We previously have a protein. Look @ milk vis ours. Buret comes out clear by itself, it forms a precipitate!

pH of Eluate:

So here is a fascinating finding.

Even though you are adding HCl to the blue copper complex

and it comes out clear at the bottom

the eluate clear has a pH of 9.1 ?!!?

Why!

How can you be adding acid after acid runs and the pH is 9.1 ???

This seems to mean that the hydrolyzate process of is forming a

"basic proteinaceous complex".

Going back, recall the statement:

A protein w/ a preponderance of "basic amino acids" will have an overall positive charge (within a neutral aqueous solution).

This would indicate that our proteinaceous complex may contain basic amino acids.

Also we see the dot moves in alcohol, not xylene.

The indicator we have a polar basic amino acid involved??

Who fits the category??

Arginine, Histidine, Lysine } Basic and polar

Cysteine is neutral & slightly polar. (This would fit)

If the side chain contains an amine functional group, the amino acid produces a basic solution because the extra amine group is not neutralized by the acid (carboxyl) group. Amino acids which have basic side chains include lysine, arginine & histidine.

Question: How did you come to the conclusion that cysteine (and cystine) is involved?

Because blue copper proteins have the Cupredoxin: Cu atom coordinated by two histidine residues & a cysteine residue..

Could we not titrate the basic solution? What would it tell us?

We should also be able to get concentration??

Each aspartame package has 33 mg of aspartame.

We can now determine concentrations of the clear protein complexes.

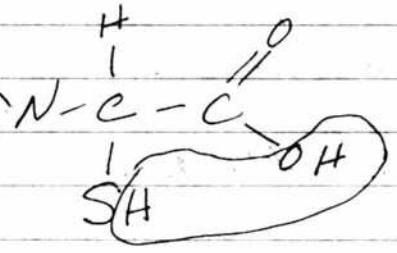
Sulfur Ne $3s^2 3p^4$ 6 valence electrons

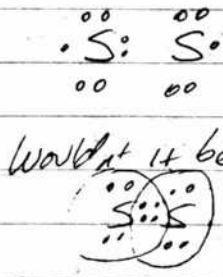
Fe Ar $3d^6 4s^2$

(normally 2 valence electrons
but since d & s are so
close in energy it may be
considered as 8

Fe^{+2} is Ar $3d^6$ or 6 valence electrons
Fe^{+3} is Ar $3d^5$ or 5 valence electrons

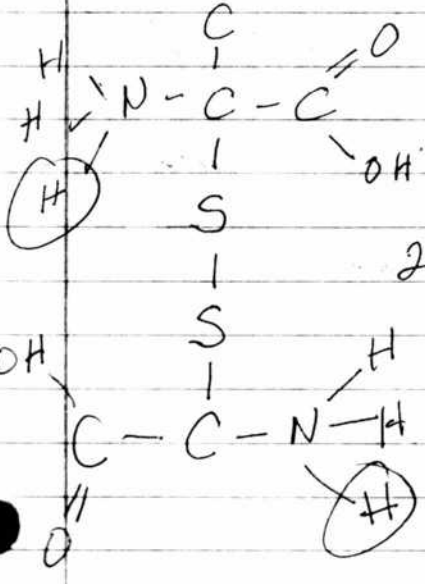
So Complexes can have a charge!



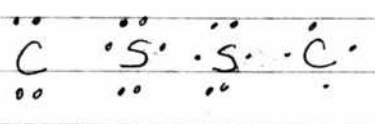


would it be?

yes, without Carbon



$2H^+ + 2e^-$



but w/ Carbon it would look like this

Revisited an earlier proof.

Cu, Amm Salts & NH_3 Ammonia produce a nice blue color solution.

But it is not the same!
It has a peak ~ 597 nm

It also does not have the peak in the 340 ng/m.

The blue is not the same as the protein blue.

Professor Lee says sulfur can have up to 10 valence electrons.

(3d row and above can have more than 8 electrons in the outer shell)

sulfur = Ne $3s^2 3p^4$ = 6 normally.

Makes you wonder how it can have 10?

Later he has sulfur even going to 12 electrons!

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We now have 6 amino acids + aspartame
You are truly getting the lab set up now.

1. Arginine — Polar, Basic
2. Cysteine — Slightly Polar, Neutral
3. Glutamine — Polar, Acidic
4. Glycine — Non Polar, Neutral
5. Histidine — Polar, Basic
6. Lysine — Polar, Basic

Amino Acids are:

Polar: or Non Polar
Basic (Arg, His, Lys) & Neutral (Gly)
Neutral (Cys) Glutamine
or Acidic (Glutamic Not Glutamine!)
(We do not have one)

We therefore have a very good assortment.

Call this Elute III
Protein Elute - Even Simpler

It looks like you have given a simpler method of now eluting the proteinaceous complex.

Sequence:

1. Culture (added guide a bit, 2-3 iterations below below gravel.
2. Add $CuSO_4$ (2 iterations) and beneath gravel
3. Add Ammn sulfate salts (approx 30% saturation?)
4. Nx add Ammonia.

If you add ammonia before the ammon salts go below the gravel, it turns the solution blue. But this blue is not the protein, it is @ 597 nm.

Your 1st elute after this (No HCl needed) is coming out of the correct buret spectrum. (640 nm & second peak near ~ 360 nm).

We can also see that in the Coleman it is turning blue and the blue represents a shift from 597 to 640 nm.

Question: Is the elute alkaline?

Buret took left 7 NaOH because it took to acidic, it took about 8 drops for 1-4 ml.

Notice it is slightly Copper colored like our original elute.

No! It is acidic! pH ≈ 3

Yes, both the paper and the meter have the pH @ ~ 3.0

How can this be?

Polar, acidic amino acids are:

1. Aspartic acid
2. Glutamic acid

} We do not have either one available right now.

We do not have one of these. What about aspartame, is it acidic a basic?

Aspartame in water ~~is completely~~ neutral.

TLC

if not moving fast enough, make mobile phase more polar (solvent)

if moving too fast, make the solvent less polar.

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$Rf_3 = \frac{4.3 - 6.5}{4.3} = .85$

Unknown

$Rf_3 \quad \frac{12.3}{50} = .25$ Glutamine

Glutamine:

~~We know now that our unknown is more polar than glutamine.~~

Glutamine is Polar & neutral.

"The most polar compound will have the lowest Rf in normal phase Chromatography.

Example:
Alanine .70
Glycine .50
Arginine .72
Leucine .91

Also from wikipedia Thin Layer Chromatography
"the less polar compound moves higher up the plate (higher Rf value)

So Rf_2 = Glutamine is more polar than our unknown.

$Rf_{X_4} = \frac{44}{54.5} = .81$

$Rf_4 = \frac{6.5}{49} = .17$ Glycine

Glycine is also more polar than our unknown.

$Rf_{X_2} = \frac{31.2}{44.8} = .83$ ~~Cysteine~~ Unknown

$Rf_2 = \frac{14.5}{42.5} = .34$ Cysteine

Cysteine is more polar than our unknown

Unknown $Rf_{X_1} = \frac{31.8}{44.2} = .86$

$Rf_1 = \frac{11.3}{50} = .23$ = Arginine

Arginine is more polar than our unknown

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Our average Rf is .05
.01
.03
.06

$\bar{X} = .038 = .04$

Our amino acid
ranking is:

29.4	.17	Glycine Non Polar, Neutral	More Polar ↑
25.6	.23	Arginine	
34.4?	.25	Glutamine	
6.9	.34	Cysteine	↓ Less Polar

Notice the data on
Cysteine slightly polar, neutral
and Glycine: non polar, neutral
hardly moved

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By order of the dots moving:

Arginine polar, basic (Notice color change most movement)

Glutamine polar, neutral

Cysteine slightly polar, neutral

Glycine non polar, neutral (least movement)

These results seem to match.

We learn our unknown is much less polar than these amino acids.

Maybe a fat soluble protein??

Our compound is highly non-polar

We actually have a very clean extraction of the protein complex going on. Strong smooth peak @ ~ 640 nm. Also the small peak @ ~ 355 nm.

This is a very good match. The elute is generally very clear w/ the slight copper color to it. It is not at all the original blue @ 597 that is going in by combining Cu, Ammonium salts & Ammonia.

* One thing to remember here is that you used a stronger concentration of Ammonium salts, probably on the order of 30-40% vs 20%.

The column is becoming quite blue and is eluting quite nicely w/ ammonia alone @ this stage.

The pH is now about 5. It was ~ 3. So it is very gradually shifting now but it is still acidic.

Notice that no hydrolysis w/ acid (HCl) is being used here!

The combined pH. of Extract III @ this point is about 3.5 so it is still very acidic.

I need longer capillary tubes. It takes a lot of work to keep between each run with my needle. But it does work.

Wikipedia is telling us how to make our own plates.

silica gel mixed w/ small amount Calcium sulfate & water.

Notice our plaster of paris already has some crystalline silica in it.

If we grind up our silica very fine and add plaster of paris it should be similar.

By changing the solvent, the separation of components can be adjusted.

Potassium permanganate & iodine can also be used to visualize! We have $KMnO_4$!!!

Now our problem is that we would like our unknown to move up the plate less & the amino acids to move up more.

Less polar Compounds move higher up the plate

So our unknown is very non polar.

Our amino acids are relatively polar.

In terms of dissolution the amino acids should be

~~most polar~~ less polar

Polar, Basic	1	Arginine
Polar, Neutral	3	Glutamine
Non Polar, Neutral	4	Glycine
Slightly Pola, Neutral	2	Cystine

~~least polar~~ more polar

What is actually the case?

Some correspondence, but may not be exact?

"If not moving fast enough, make the solvent more polar.

If moving too fast, make the solvent less polar"

So for us, the amino acids should be placed into a more polar solvent.

The unknown should be placed in a less polar solvent.

Ethanol has a polarity index of 5.2
Thus is very polar.

The unknown should be placed in a less polar solvent,
such as MEK 4.7
or Xylene 2.5

The amino acids should be placed in a more polar solvent such as

Acetic acid 6.2
Water 9.0

Let a wad w/ the unknown. Spray
MEK 4.7 Beaker No 1
+ Xylene 2.5 Beaker No 2

Also spray proteins

So one of the things we are seeing here is that our unknown seems to be of a highly non polar nature.

However NH_3 (ammonia is polar) and this is what is reacting fairly well in the column.
But the question is is it going to get stuck?

So far HCl is the only thing which positively cleaned out the column.

So there is some real uncertainty here.

A hydrophobicity scale helps us out.
We see from the type of chart that Cysteine is clearly non polar

and Arginine is most polar.

Therefore in our HC TLC plate we see that Arginine moves the most
and that cysteine moves the least.
So in our TLC plates with ethanol
the most polar compounds
are moving the most and the non polar compounds are moving the least.

This suggests that our unknown is highly polar.

Now we also change the solvent to MEK & xylene, both very non polar solvents.

We see that the unknown does not move at all. This means that we went way too far the wrong direction.

This says to me we want to reduce the polarity of the solvent only a little but. not too much.

Run a series w/ acetone & vinegar

HCl is definitely polar.

Electronegativity difference $\hat{=} \Delta = .9$
This is straightforward.
Everything says our compound is highly polar.

Set up w/ unknown

6 = acetone - dots clearly did not move.
7 = acetic acid - vinegar - did not stay up the plate

The Lewis structures are really valuable because you get the steare number from it

Multiple bonds count as one
regular bonds count as one
lone pairs count as one

and the steare number gives you the balloon. which gives you the geometry of the molecule.

We see that acetic acid (& most likely water as well) is not moving up the TLC plate hardly at all. so there is a problem w/ that.

This says a minor difference in the polarity of the solvent may have a huge difference in the results.

This strongly suggests the use of acetone.

Our results of TLC appear to be in the opposite direction of silica based plates, ie polarity of ours seems to be inverted.

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Green series

1 Arginine
2 Cysteine
3 Histidine
4 Lysine
5 Aspartame

We are beginning to wonder if indeed the plates require "activation". Acetone looks like a complete bust. Why? Because of the solvent or because of the plates not being activated?

We have an interesting result. Nothing moved on acetone, including the unknown. Why?

We also see #1, 3, & 4 produced a very dark spot. Why?

1 = Arginine
3 = Histidine
4 = Lysine

] These are the basic & polar set.

So there is something unique about these.

Should we consider isopropyl alcohol now?

I did just run a control test.

Ninhydrin does seem to react w/ NH3 very weakly but no dot moved.

It also seemed to react more strongly w/ the ammonium salts but the dot also did not move in the same way. We have a very strong migration taking place on the PLC plate.

It looks like we have made progress.

We have a separation of the HIS amino acid that is extremely similar to our compound. We can also see that the HIS is actually a mixture. We have something that turns green w/ ninhydrin but it is not an amino acid.

The green color is not an amino acid reaction let's look @ system again.

#1 Histidine in ethanol
2 Cysteine in ethanol

Interesting work. You are getting now the same results w/ Histidine & Cysteine that you are w/ the unknown.

This is not what you got before! Why?

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Maybe actually you did get the
left lane and you did not realize
it. Its getting tricky.

You can not tell any differences. ??

Everyone is the same. Why?

No 3: Cysteine in MEK

The results are not at all clear.

Some purple, some not?

Right now we have no idea what is up.
Right or left, up or down.

The TLC plates one time did nothing w/ MEK,
next time it did. ? Activation of plates?
Different colors than purple - impurities?
Up to the solvent line - Rf ≅ 1.0 -
what is this about?
Yellow color?

so how do we learn what we really have?

Polarity of Solvents.			
Non Polar	Xylene	2.5	4.7
both pola & non MEK	Acetone	5.1	
both pola & non	Ethanol	5.2	
Pola	Acetic Acid	6.2	
Polar	Water	10	

Try Cystine in

Xylene	No dot movement
Acetone	large movement Rf ≅ .6
Ethanol	modest movement, good definite Rf ≅ .6
Water	poor movement.

What if we used paper in the electrophoresis
Container?

Conclusion: Ethanol actually works very well,
& gave very good separation. No real band
on the first three times

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The dot spreads out, it does not just move. $\frac{20}{39} \cong 0.51$

$\frac{16}{31} = .52$ $\frac{17}{32} = .53$

So we actually have surprisingly consistent results w/ cysteine.
3 different trials in ethanol w/ the same results and $\bar{x} = .51$

The means our protein may not actually be reaching the end point yet.

HIS:

$\frac{9.5}{17.5} = .56$ $\frac{10}{18} = .56$ $\frac{21}{40} = .53$

Avg = .55

surprisingly close to cysteine

Arginine also looked definite but may only be $\frac{12}{46} = .25$

The work maybe more successful than you think.

Argenine, Cysteine, Histidine

Lets do argenine

Next, never breathe ninhydrin again!

If you can determine the steareo number (which is not too hard, if you can Construct a Lewis dot diagram of the molecule) you can then determine the geometry of the molecule.

We are now testing argenine 1, 2, 3, 4
We do have generally a more greenish color which we saw earlier. Mostly we have a strong separation

1	10/31 = .32		
2	15/26 = .58	14/26 = .54	
3	11/27 .41		
4	14/26 .54	12/27 = .44	

$\bar{X} = .47$ $\sigma = .099$ or ≈ 0.10

So 68% of between .37 & .57

It could easily be the same as the others

So basically we have 3 amino acids giving essentially similar results

1	.53	$n=3$	$\sigma_s = .01$
2	.55	$n=3$	$\sigma_s = .02$
3	.41	$n=6$	$\sigma_s = .099$

So w/in experimental error they are giving essentially the same result.

So we have to wonder, is there a contaminant that is common to all or is it truly the amino acids?

I do not know.

Tonight we learn ~~two~~ two things

Aspartame ~~bt~~ behaves similar to our Glycine-Glutamate. There is no intermediate band. It goes up to the end of the solute & stains purple

This suggests our amino acids have a fairly common component to them which is to be dismissed.

We also learn that blood does not show anything w/ ninhydrin even though it moved in the electrophoresis suggests it does not have amino acids detectable.

I need: ~~Gel~~ Electrophoresis

1. A micropipette!!!

2. Glycerine in solution?

Sure enough, we are supposed to mix the sample w/ glycerol to help it settle down.

Thin Stem Plastic Pipettes & Capillary tubes may work just fine!

We have a series in electrophoresis

0 Dye MB
1. Elvis
2. Aspartame
3. Histidine

Centrifuging is also important

Start working w/ milk, egg yolk, blood

Start working w/ dialysis solution, bags

SDS (a detergent) needs to be added to the proteins prior to the PAGE electrophoresis. It is important.

A Great DNA Extraction Sequence:

1. Cut up onion (do not blend yet)
2. 30ml H_2O, 1 tsp salt, stir
3. 2 tsp Soap, stir, avoid bubbles
4. put onion into blender, add salt & soap mixture
5. Blend 1-2 minutes, get it smooth
6. Heat this mixture in a beaker 1-2 min stirring occasionally lightly
7. Strain it
8. add gently to ethanol in a petri dish

from patsscienceclub on youtube

The method made a lot of DNA in the petri dish.

We have separation of blood into both negative & positive directions @ pH ~ 5.0.

Isoelectric point of red blood cells is ~ 4.6

On the alkaline side (which is 5.8 eg) the charge carried is negative (this means it would migrate to positive)

Which one side does.

But then what is the big dark point to the negative terminal?

	Isoelectric point	Net Charge @ pH 6
Myoglobin	~~6.2~~ 7.2	Negative
Hemoglobin	6.8	Negative
Serum Albumin	4.8	Very negative

Cytochrome C is range & positive but it is not supposed to be there.

[redacted]

1. Blood serum?
2. What is the range spot that has a positive charge?

01/14/12

We are distilling our protein complex. It has a slight blue-copper color. It appears to be successful.

Our boiling temperature appears to be ~ 86°C.

The distillate (elute) does appear to have an odor to it, but not really like alcohol.

You are taking the distillate very slowly because the body of it is fairly close to water. It does seem pure.

Some sources say you can not distill w/ $\Delta < 30°C$ but I do not believe that. If you are willing to take things more slowly & stress you can operate @ a temp $\leq 10°C$ but is, however, a very slow process.

The ester odor definitely says it has a contaminant of some type in it but it does not smell like alcohol. It smells like a very weak form of the xylene. Your temp range has been from 82 to 88°C.

Boiling temp of xylene is ~ 140°C.

So it is not xylene!

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Results are not great.
Peak is @ ~ 611
1 drop $CuSO_4$, 4 drops NaOH

pH ≈ 5.5

1 & drops more $CuSO_4$, & 2 drops NaOH

= 2 drops $CuSO_4$, 6 drops NaOH

We clearly have a separation that has taken place. But the results are not entirely clear.

The remaining distillate is very blue w/ Cu & NaOH however the peak is near 597 (~604) so the corresponds more closely w/ the amino salts
The elute from distillation (BP ~86°C) comes out turbid more like a completely negative biuret reaction
The pH of the is ~ 10.0 very alkaline.

What is the pH of Amino salts? 6.4
almost neutral.

The ammonium salts come out surprisingly similar. This means you do not have a positive test at this point.

A question is, does heat destroy peptide bonds?

Heating completely changed the aspartame. It does not even slightly give a positive biuret test.

It is orange!

This means heat destroys your proteins.

So now you have destroyed your protein complex of the last few weeks with distillation.

So you must learn to do it all over again.

Need
1. capillary tubes (longer)

Next test w/ Unknown

1 Ethanol
2 IsoPropl
3 Acetone

Running a test only w/ the solvent.
Isopropolyl moved very slowly —

Future Candidate
aspartame
milk
blood
egg yolk
amino acids

We learn that the unknown moves very well in ethanol.

It does not move in acetone! Why???

What does this mean? Why would this be?

"Activation" just means heat it up to drive off water so results are not contaminated.

Potential problem. It is stated that Ninhydrin reacts w/ ammonia.