CARNICOM INSTITUTE LEGACY PROJECT

A Release of Internal Original Research Documents

Authored
by
Clifford E Carnicom
President, Carnicom Institute

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Chemistry Vol IV

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5 SUBJECT

180 Sheets

COLLEGE RULED

Chemistry Vol IV

January 19, 2012

Clyffard's Birthday

We have had a little break here.

I have been sick for most of these weeks w/ various relapses. I seem to be pulling out ok w/ only a residual cough now.

What are your biggest problems right now?

1. Identify to any degree possible, the proteinaceous complex'

2. Reliably produce the proteinaceous complex P.C.

3. Learn the molecular structure of the P.C.

4. Explain the chemistry of the cupric alkaline NAC P.C. reaction.

Our last lesson is that distillation destroys the proteinaceous Complex.

Use aspartame (how to dye?) in gel electrophoresis.

Page 2

We have actually apparently acquired some of the PC w/ the use of HCl even though it has a slightly yellowish tint. The column is very slow but the fact is that we have some.

A very good looking graph with peak @ 642 and use near 340.

Also, it is very alkaline (at pH 9) even though you are using HCl

Reviewing our distillation result we in fact have a peak @ 622 nm. We also do have a rise @ 340 nm. The indicates we may actually have some PC in the solution.
What is the pH of it?

pH is mildly acidic, ~ 6.
It is mostly odorless.
It has a slight blue tint to it.

The distillation process clearly separated the solutions. I am tempted to call the distillation successful.
The elute is clear but highly alkaline from the turgovon I used bromcresol test.

A replat test w/ amm. sulfate.

It is indeed not the same.
Peak @ 604 nm. Also no
peak whatsoever @ 340.
I think we actually must have the
protein opto distillate.

You now have 3 jars to work with!

One Cu colored
One very weakly Cu colored
One clear

We once again also prove our
case. The distillate,
subject to a
Cuprex
alkaline
sufficiently NAC environment

breaks the peptide bonds of
the particular P.C.
and when tested for Fe2+, tests positive!

This means it unlocks w/ the
peptide bonds & releases iron in
the Fe2+ state

This is stupendous

You have done quite well here Clifford.
Now you must succeed w/ electrophoresis

Somehow we must dye

Aspartame & our P.C.

---

4 are Aspartame
3 are P.C.
PH is 5.0

Conjugate base

Conjugate acid

Protated - Lowry Acid - Base definition
donated a proton — gains a proton — gained a proton

$HNO_3(aq) + H_2O(l) \rightarrow NO_3^-(aq) + H_3O^+(aq)$

Acid — Water acts as base — lost a hydrogen (a new base) — gained a hydrogen.. (a new acid) ~~base~~

Nitric acid in water, (how would we know this there is no OH^-)?

There appears to be plus.

Strong acids Completely ~~dissociate~~ disassociate

pH ≈ 5.0

The dye appears to be migrating very smoothly through the starch gel.

At this point it appears the aspartame is moving more quickly towards the negative terminal than the PC is.

If true, this would say that aspartame is more positively charged than our P.C.

!!!

What is the isoelectric pt of aspartame?

The isoelectric point of aspartame is stated to be 5.2 (this is near our pH)

At pH below the I.P. aspartame carries a net positive charge.

@ pH above the I.P. aspartame carries a net negative charge.

Another source states the IP of ASP is 5.7
Another source has it as 5.9
Another source has 5.4

pH is now 5.2
The means it should start slowing down.

We are now proving in more than one way that we have amino acids on a P.C.

1. First method is spectrometry.

2. Second method is the distillate a elute from the column put directly on a piece of paper & dried & heated w/ ninhydrin. (Purple & brown) Also ya just dip the paper in the distillate and it also turns purple.

3. Third method is the starch gel.
Right spot is the P.C.
The left spot is aspartame.

We are definitely getting the protein from the column again w/ HCl.
It is looking very good & clear solution pH remains very alkaline even though you are using HCl.

Lab test aspartame w/ Ninhydrin.
Very clear success.

We now have a new starch run
4 dots aspartame w/ glycerol dye
4 dots P.C. w/ glycerol dye

The pH of our run has been adjusted to 3.0

We keep the water level slightly below the starch gel

The starch gel has 2 1/2 tsps tsps in about 40 ml H_2O. w/ HCl added.

We see now from example in web that TLC migrations occur in the darkest part of the streak, no matter how long the streak is!

They say to use a weaker solvent if the spot moves too quickly.

Stronger if the spots do not move fast enough.

Weaker means less polar
Stronger means more polar.

TLC Runs w/ Aspartame

	Polarity	
1. Ethanol	5.2	Centrifuged the
2. Acetone	5.1	No movement Aspartame
3. MEK	4.7	No movement
4. Xylene	2.5	No movement

Why does it not move w/ any of the other solvents?

Centrifuging the aspartame has purified it.

~~Methylene Chloride paper now comes out straight purple - looks just like Aspartame.~~

We have a real problem of separation here. We have not succeeded yet through TLC or starch electrophoresis or distillation for that matter.

How do we succeed? TLC is working only in ethanol but is not separating.

Starch electrophoresis is not showing anything definite?? Theory is wrong -

We presume there may be a disulfide bond.

We found several things that break disulfide bonds.

NAC, etc

Let us find that list again.

Agents that break disulfide bonds by reduction include:

1. 2-Mercaptoethanol
2. Dithiothreitol
3. TCEP (2-Carboxyethyl) phosphine)

We have found an incredibly strong reaction between our protein complex & harpern, in our case ammonium thioglycolate

We seem to have found another powerful method of determining the presence of iron in the Fe^{3+} state using the extracted P.C.

It uses ammonium thioglycolate (hair perm) which absolutely breaks down disulfide bonds.

It gives a strong purple color.
It is also reduced with the vitamin C.

The test is incredibly sensitive, a micro drop of iron(III) nitrate and a microdrop of ammonium thioglycolate produces the violet purple. You will practically be able to determine the concentration of iron.

Also the test fails w/ aspartame which does not have iron! So it should fail!

The test is also highly suggestive of the disulfide bonds of cystine. You now have two known methods to indicate disulfide bonds being broken, NAC + Cu^{+2} + alkaline (blues) w/ the Fe^{+3} test!
and direct on the protein w/ amm. thioglycolate.

You are planning to prove the case of cystine.

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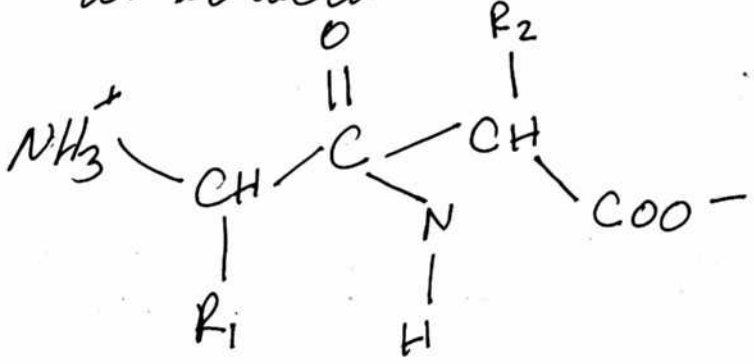
Our structure should be



Where R1 Candidates are
Cysteine (two would form cystine)
along w/ Iron in the Fe3+ state.

A strand of hair has 4 chemical bonds:
1. peptide] notice the similarity?
2. disulfide]
3. salt
4. hydrogen

Next discovery.

Iodine reacts with certain amino acid side groups.

You do indeed have a reaction of the protein w/ iodine.

You must use the micro pipette (Smart) w/ 2 small iodine drops to demonstrate this otherwise you overwhelm the reaction w/ iodine

Reference

Water +

+ 2 micro drops 10^{-2} iodine (almost perfectly clear)

Spectrum

Protein extract

+ 2 drops microdrops 10^{-2} iodine (yellowish color)

We have a very distinctive spectrum here. Peak @ 391, another @ 420, & another about 774 nm.

Compare this to histidine.

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After careful preparation of both cysteine and ~~histidine~~ histidine

1. ~~Dissolve~~
2. Centrifuge
3. Separate & ~~Extract~~ 1 ml
4. Dilute to 2 ml

and then add iodine, ~~there is no~~ there is no reaction to either, so certainly it is not the same as the ~~prot~~ protein complex

We have both a ninhydrin and an iodine reaction to our protein.

We set a white precipitate when we add Curley lotion to cysteine w/ the iodine added, the histine is already cloudy so it's hard to tell anything.

Actually there is a huge reaction taking place between iodine & Cysteine.

You can add all the iodine you want, it still does not change the Color of the solution!

It is stated that "the sulfur groups in Cysteine are Oxidized by iodine"!

The same thing is happening for histidine. It takes longer and not as dramatic but the same thing happens. The iodine Color is reversed, & negated by the histidine.

Not sure what it has means yet but obviously a reaction between iodine, cysteine & histidine

---

Clearly there is a reaction of iodine w/ the protein elute, what does it mean?

We set a very strong reaction of iodine w/ aspartame - a very purple color. Why?

You keep seeing references to butanol acetic acid as a solvent for TLC. Why?

12/22/12

Very clear protein fraction in column elute.

Still highly alkaline even w/ use of HCl
Peak @ 643 nm

Strong rise to 340 nm.
Reliably produces the protein complex

Where are we at?

1. We are reliably collecting the protein complex. We are close to having the method identified.

2. We have found another method of identifying iron deficiency in the protein complex using ammonium thioglycolate. A strong purple complex that can be reduced w/ Vitamin C.

3. We have learned that both Cysteine & histidine can completely undo the addition of iodine to the amino acid. So we know these two amino acids are reactive w/ iodine.

4. We also know we have a reaction (the opposite direction) it turns it color when we add iodine to our protein complex.

5. Iodine reacts w/ aspartame to create a purple color.

The reference says that iodine is an oxidizer. Is it oxidizing amino acids, esp Cysteine & histidine?

Another source says that iodine denatures the proteins that contain tyrosine & histidine.

Statement:
Iodine oxidizes cysteine in an acidic environment.

At pH 3.2 I have found one molecule of cysteine consumes three molecules of "iodine"

Yes this is exactly what happens, the iodine disappears!

We do not know what our reaction of iodine & the protein means. It turned it yellow and gives a distinctive spectrum.

Guess what:
In our elute II (most recent from Column, not distilled, very good spectrum)

We do have a clearing of the iodine color within the elute! This does suggest reduction of histidine & cysteine.

~~Not true!~~ Yes, it is true.
No data dilution of elute II allowed.

Good Work

You now have two ways of suggesting Cysteine & histidine may be involved.

1. Blue copper proteins (Type I) are commonly formed with histidine and cysteine.

2. Reduction of iodine is indeed taking place (iodine is then consumed) This happens w/ both histidine & Cysteine by direct observation,

The is a very sensitive test. The protein elute II is weak. You are not allowed to dilute it. You then can only add 2 microdrops of 10% iodine to detect the reduction. On our photograph, the clear solution to the left is the protein elute w/ 2 microdrops iodine added (turns clear). The solution on the right is yellowish in color when 2a the iodine (2 microdrops) in water.

3. We also have the reaction of thioglycolate which detects iron (III) but only when thioglycolate is added. This breaks disulfide bonds, which is why Cysteine has in it. Therefore Cysteine is the stronger candidate

Another big discovery.

The distilled elute is what is testing strong purple w/ ammn. thioglycolate.

Not the straight elute II

There is therefore an important difference between the distilled elute and the straight elute w/ respect to the presence of ammn. thioglycolate and the ability to detect iron.

The distilled elute fails the test for Fe^{3+} (ferric ionic form w/ sodium thiocyanate).

But also the straight elute when ammn. thioglycolate is added does not turn purple. This indicates that distillation was somehow important in freeing up the iron.

But it also says the straight elute is the one that contains Cysteine/histidine and that is the only form that is passing the iodine reduction (clearing solution) test.

The distilled elute acts in reverse, it turns more yellow w/ the addition of iodine. I do not know what this means

So clearly there are important differences between the straight elute and the distilled elute.

* The distilled shows the non-presence.

* The straight shows the cysteine / histidine amino acid presence.

At the simplest level, amino acids can be divided into polar and non polar.

Then you take polar, and divide it into basic acid neutral

and the non polars are always neutral

There is a nice way to think about things.

Now here is a great question:

When you hydrolyze Type I Ca Proteins what do you get???

Still highly alkaline elute liquid produced.

Histidine is basic polar
Cysteine is basic neutral

} We have alkaline elute liquid produced. Thus it contains.

Still a very smooth spectrum liquid produced.

Consideration.

Also, we may now have a test for cysteine!

Requires lead nitrate (we have it!)
and NaOH (we have it)

1. 10 drops protein
2. ~~15~~ drops NaOH (1M) use 10 drops
3. 2 drops 0.1 M Lead Nitrate

& Heat for ~~5~~ (10) minutes in a boiling water bath!

It looks like the test has/! succeeded w/ the control!!

The cysteine test has succeeded for the Control!

Fantastic!

A black precipitate ~~from~~ forms

We now have a test for cysteine!

It took close to 10 minutes.

Buy some lead nitrate!

I have altered our concentration w/ our elute to

1. 15 drops protein elute
2. 15 drops NaOH
3. 3 drops 0.1 M lead nitrate

Copper sulfate measurement

Ø.39 gms 10 nodules

≅ .039 gms each

= 39 mg each ≅ 40 mg each

* 25% for pure copper

≅ 10 mg each nodule.

Recommended is 1 mg per day.

We should probably take about 5 mg/day.

We were set up for 20 mg day.

So 10 should be ok.

1 nodule per day.

300 mg in 150 ml = .3 gms

but remember only 25% is pure copper.

So measured would have been 1.2 gms.

12/26

We have done an excellent job of collecty the protein

It is highly alkaline.
Very repeatable collection.

We have actually made some progress.
We found a test for Cysteine using lead nitrate and it worked remarkably well.

This is very good but now it suggests histidine
But we do not have a simple test for histidine.
We need one.

I use [redacted] Chemstrip tonight on the protein extract.

I get a slight positive test for protein and a definite positive test for nitrite.

This gives another level of confirmation on protein

1. spectral analysis of dipeptide
2. Ninhydrin test
3. Chemstrip test

I do not know what the nitrite positive test means or results from yet.

We found a paper that says Benedicts test description are wrong.
Only pure glucose gives red color.

Histidine and creatinine turn it more yellowish.

We are getting a very yellowish color w/ our glucose tablet

But mixing w/ histidine (protein) a. glucose together.

So this says whatever is in the pot protein is neutralizing the impact of the glucose in the Benedicts reaction.

The glucose is now getting a bit darker.

The Protein completely negated the glucose test.
Sugar cement yellow light glucose
Come out yelow brown brown,
protein came out clear.

Possible breakthrough

We may have made a major breakthrough today.

The ammonia involved may be plasma. It is the only one to give a yellowish color w/ reacting w/ ninhydrin in solution.

We have a yellow solution w ninhydrin.

Can we get to the spectral analysis?

When we add acid it turns perfectly clear.

440 nm is the max.

Here we go—

You are also running the column.

1. Kitty Little partial

2. Culture

3. Amm. Sulf. Salts

4. Ammonia → We seemed to have accomplished the equivalent of the Lucas test at this point! We have a dark blue elute coming out of the column. It is alkaline from Ammonia It is cupric from Kitty Little We have a perfect peak @ 640 nm and a nice rise towards 340.

5 ~~Isopropyl~~

Back to Ammonia

~~We can also perform~~ Identical to previous work.

We can also positively prove the reduction of the peptide bonds w/ NAC & the folding, ie, release of iron in the Fe(II) state.

Without NAC added, the Fe(II) test fails

Spectral work:

Rey e protein by itself:

We are getty a peak around 360-370nm.
This is matchy a paper we found on cysteine.

The paper also says 390 for ty tryptophan

1-7 Amino Acids
8 Elute
9 Distillate → Ruhemann's Purple D @ 571. Not bad @ all.

$\bar{X}$=577

			Color
Arginine	1	397, 557	Light Brown
Cysteine	2	397, strong peak	Yellow
Glutamine	3	397, 592	Purple
Glycine	4	397, 500	Purple-
Histidine	5	397, ~~506~~ (it is 568)	Purple
Lysine	6	397, 414	Purple-Brown
Asparlane	7	~~360~~ 397, 540	Purple Yellow
Elute	8	360, also a drop @ 397	Light yellow
Distillate	9	355, 776	Clear

We have learned something.

It is not just proline that makes a yellow complex.

Cysteine also makes a yellow complex.

What we do know is that it is not histidine.
Another reference gives 390 nm for tryptophan

So the now gives us candidates of

Cysteine
~~proline~~ proline
tryptophan

Now lets write our peak.
One source said that proline is @ 440 nm.
We are definitely not at that point.
Another source gives tryptophan @ 390.
We are definitely not there.

Our closest match is cysteine
We get 360 & 397.
We should be able to prove cysteine.
w/ lead & NaOH.

Next we go after cysteine
w/ the NaOH Lead Nitrate test

What you have done here is come up
w/ an independent method of testing.
Besides the Biuret test

It is the ninhydrin test.
Our numbers are also matchy.

The proline result is to be
yellow orange.

This indeeds shift the wavelength
away from yellow and toward orange.

This corresponds to the 440 nm
statement or another source

Yellow orange is now eliminated
proline

This drops us to
Cysteine and tryptophan

With our most recent spectral analyses

We have a definite drop off @ 397.

We also have the second small peak @ 416.

Cysteine Control ~~has~~ a very sharp peak @ 397
and @ ~~409~~ 419 (secondary small peak).

I believe we have it. It is the only one
that is close. well.

I believe we are dealing w/ very low
concentration and some
impurities. ~~or~~ less

You are very close

Cysteine is now proven by

1. Copper protein complex formed (Type I & II)
2. Nitroprusside paper test
3. Sulfur bond indicated by absorbance material
4. Color of solution in ninhydrin test
5. Ability to bind w/ iron - metallopeptides
6. Spectrometry VS Control.

Histidine has been disproven as well as all
purple complexes. Proline & Tryptophan also proven

You should now be able to titrate the amino acid

"When the amino group is secondary the condensation product is yellow.

What is a secondary amino group?

We now have 3 elutes:

Clear 1. Clear
2. Turquoise - Aqua
3. Dark Blue

all of them have the protein on it and all release the iron (II) w/ NaSCN added.

You seem to know the chemistry of the column now.

1. The culture is added

2. Cu is added to the column either through the existence of kitty litter or by adding $CuSO_4$.

As $CuSO_4$ is added it stays local @ the top of the column. If you use kitty litter the $CuSO_4$ is distributed throughout the column.

3. Now you add Ammon. sulfate salts, this time in pure saturated form.

4. Now you add Ammonia, which is alkaline & w/ kitty litter, everything turned dark blue. & passes the spectronic test.

You have pretty much grown the cysteine iron dipeptide complex and the reversal strategy w/ [illegible].

You made tremendous progress w/ the spectronic and ninhydrin. Your amino acid controls were very helpful.

Directly on a beaker we added

1. Culture
2. $CuSO_4$
3. Ammonia salts
4. Ammonia
5. NAC → (turns it yellow brown)
6. Iron II test succeeds!!!

It looks like we have major progress.

When we have the biuret complex from the column, i.e.
1. Culture
2. $CuSO_4$
3. Amm. salts, stirring
4. Ammonia
produces strong blue electo (biuret solution)

that
w/ NAC added frees the iron (II)
and frees the cysteine.

Proven by adding NAC, then ninhydrin,
then heating, then spectrometry.
Matches control cysteine superbly.

Now acetylization means combination usually
w/ acetic acid (vinegar).
Unfortunately vinegar does not clear the iron.
Testing vinegar w/ ninhydrin now.

Now vinegar combined w/ cysteine
amino acid added to the biuret
electo produces a black precipitate.
No idea what it is.

We have some new things happening.

We are getting a definite red color when

$II + Cu + NaOH + NAC + Heat \rightarrow Red$

(Cu: biuret)

the biuret is also giving a precipitate form more than eluate IV!

What we are learning is that eluate II has a contaminant in it, some kind.

It is red. Eluate II spectrum is indeed matchy cystatine.

Method is:
1. Eluate (can be diluted by 1/2 to save material)
+ 2 drops $CuSO_4$ + 4 drops NaOH
+ NAC + Ninhydrin + Heat

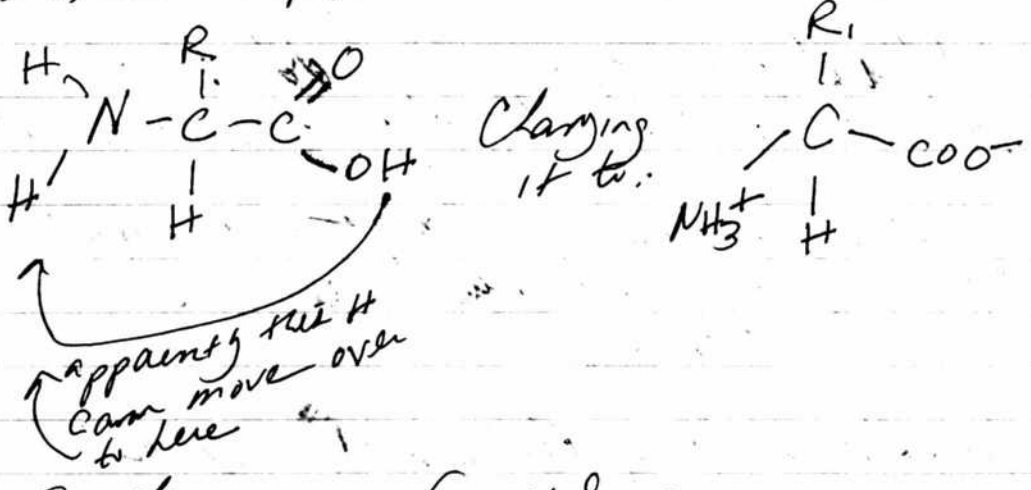
The red color in eluate II means there is something there you don't know about yet.

Lysine is more reddish color

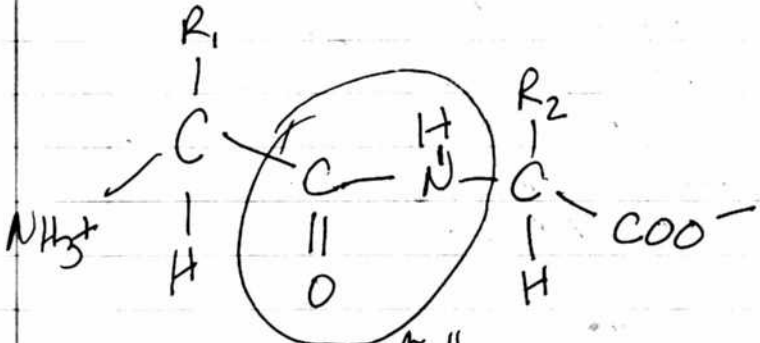
The heated test does not really produce much of a difference between Eluate (E) EII & EIV.

And yet when you treat them w/ NAC, Ninhydrin & heat there is some kind of difference.

General Dipeptide form



General Dipeptide



~~E.A~~
Cysteine has a defenite verified spectrum.

E4 + Nac + Ninhydrin did nothing.
add 1drop $CuSO_4$ – mega white precipitate formed.
still clear

add 4 drop NaOH we get a reddish color.
and luckily it disentegrates the precipitate.

Spectrum has some similarities to Cysteine.
Not exact, but somewhat similar.
A much broader peak

The solution already alkaline. Why did NaOH
make a difference.

E4 + NAC + Fe2 test did nothing.
add NaOH – nothing
add $CuSO_4$ – turns black & then orange.
Looks like Cu is somehow releasing Nitrogen
in the presence of NAC

Yes

E4 + NAC + $CuSO_4$ + Fe^{2} test goes positive
w/Nt adding NaOH
remember it was already alkaline

Seems to be Confirmed.

Fe^{2+} is released in the presence of

E4 + NAC + $CuSO_4$ + alkaline

The says ~~nothing~~ about the amino acid arrangement

Important ~~results~~
E4 + Ninhydrin = yellow
spectrum similar to Cysteine - but not Hexane -

E4 + NAC + Ninhydrin = red!!!

It looks like we have lysine involved!
A rather strong match w/ the reference spectra
Now what was interesting here is
that NAC was needed to bring it out.

Amine acc

Lysine is basic This could explain our alkaline

Next major discovery of color

E4 + NAC + Ninhydrin really does nothing.

But when you add NaOH and I mean lots of NaOH it turns very red.

pH of E4 is 8.7. So strong pH caused a reaction.

When you added to one w/ NAC & one w/NT along w/ ninhydrin and one turned purple and one turned red.

The purple is very intensely. peak @ 542 & 570 (sharp)

It is actually purple!

E4 + NaOH + Ninhydrin ?

It is looking like the pH is a huge factor in what color evolves.

We know ~~that~~ ~~NH~~ is turning it red - making amines?

So now we know the pH is important.

pH of EII is 8.9 amazingly similar. Let dilute. It has been diluted by a factor of 2 but pH is still 8.9 good.

Bogen indicator gives about 8. (~2 ml)
1 drop NaOH turn it to ~ 9

Start setting controls
E2 + 2 drops NaOH + 4 drops ~~NaOH~~ Ninhydrin = yellow
It seems to match cysteine reasonably well.

adding ~~more~~ NaOH is not changing color.
~~but~~ plat ever looks a little closer to cysteine control. Copper added just turns it blue yellow & forms precipitate

E2 + 4 drops NaOH + 4 drops Ninydrin = yellow.

We are running a control w/ Cysteine w/ 4 drops NaOH & Nin
It is turning very red

~~Cysteine is red @ pH~~

Cysteine is yellow @ neutral pH

Cysteine is red @ strongly alkaline

Cysteine + Neutral + Nin = Yellow
Distinctive spectrum ~~created~~

E2 (which is already alkaline) & then adding more NaOH is what gives us yellow. Not exactly the same.

You are putting two more amino acids in the control tests. Do not use more.

1 drop NaOH in 2 ml of H_2O is strongly alkaline.

I think you need to run your amino acid control tests in an alkaline environment.

Not very much amino acids back.

March 11 2017

I must write down what I have discovered.

E4 + Biuret + NAC + Ninhydrin + Heat
Gives a very clear yellow solution.

Spectrum of the matcha neutral system exactly

The solution clouds very quickly & the spectrum becomes useless, it must be taken right after it comes out of the hot water bath.

Proposal: Biuret is $CuSO_4$ + NaOH.
It measures & shows the existence of dipeptide bonds.

NAC reduces the color to clear so something important happens here.

Ninhydrin forms the yellow complex.
Short term spectra

When it cools it becomes cloudy — copper reacts with something to form precipitate ? ?

Lets learn what part of the sequence is needed to produce cysteine & iron.

[redacted]

Currently:

1. Extract
2. Biuret
3. NAC — iron test?
4. Ninhydrin
5. Heat
6. Spectrum

First we learn that the solution is clear when heated & cloudy when cooling.

Iron is showing up - —

Reduction w/ NAC alone will make the iron —

Blue complex does not show the iron w/out NAC

Is NAC required to have the cystein spectrum? Are there other things that do the same?

Vit C also reduces.

Vit C is also causing a reduction reaction & reaction w/ ninhydrin!!!

Also Vit C in the blue solution (eluate) turned it brown like the unk color

We have a major purple color occurring w/ Vitamin C!!!

It was a boiling water bath remember?

E3 + Vit C + Nin → purple complex
with heat

When you continue heating it it turns yellow & matches Cysteine.

But what is the purple & what does it mean?

E4 has a shift in the purple peak to about 569 nm.
E3 and other may have the purple peak around 577.
E4 gives a very clean spectrum.

Histidine has a double peak.
looks like a very good match. w/ E4

Histidine is indeed basic.
it is also polar.

Guess what - Nin in hemoglobin is coordinated to a histidine.

other authors divide in other ways.
The way seems to make a lot
of sense to me.

Aug 23 2012
A typo has occurred.
Need to verify cysteine & histidine
George Wolfe - Think well Biology - the Greatest
Amino Acids R Groups
He divides into 3 groups

Electrically Charged Amino Acids (Charges react)
1. Aspartic Acid (neg charge)
2. Glutamic Acid (neg charge)
3. Lysine (+ charge)
4. Arginine
* 5. Histidine (+ charge)

Polar Amino Acids: (OH on the end is polar) hydrophilic (water loving)
1. Serine
2. Threonine
* 3. Cysteine (SH on the end is polar) → CH_2 → SH
4. Tyrosine
5. Asparagine
6. Glutamine

Non Polar Amino Acids (Hydrophobic) (water fearing) (CH on end)
1. Glycine
2. Alanine
3. Valine
4. Leucine.
5. Isoleucine.
6. Phenylalanine
7. Tryptophan
8. Proline
9. Methionine

New Business Idea

Page 47

Cost	Soil	Water	
147	Chemical Composition Kit	Test Strips	60
		Water Quality Kit	224
	% Water		
	Organic Content	Amaw (or) ~~TDS meter Amazon #4~~	
	pH	Chlorine Test Strips	#3
	Calcium		
	Carbonates		
	Magnesium		
	Phosphates		
	Potassium	Test Strips	
	Sulfates	Phosphate	
	Nitrate	Iron	
	Ammonium	Hardness	
	Iron	Fluoride	
59	Auger	Nitrate	
		Nitrite	
		~~TDS w/meter~~	
14	Soil Screen Simplified (Amazon)	pH w/meter	

Enasco:

Chemical Composition of Soil
Auger
Soil Science Simplified
Water Test Strips
Water Quality kit or
Pollution kit?

Amazon

TDS Meter
Chlorine Test Kit
separate

Actual Tests we perform
~20
Water
Phosphate (g)
Iron (g)
Hardness (g)
Fluoride (g)
Nitrate (g)
Nitrate (g)
Chloride (g)

Ammonia gw
Chlorine gw
Chromium gw
Copper gw
Cyanide gw
Iron gw
Nitrate gw
Phosphate gw
Silica gw
Sulfide gw

TDS, uS, pH (g)

Soil
pH g w/meter
Nitrogen g
Phosphorus g
Potash g

% water
% organic
calcium
carbonates
magnesium
phosphates
potassium
sulfates
nitrate
ammonium
iron.

(Ion Count) extra

E3 already has a peak color.
Method:

1. E3 diluted by 1/2
2. Add some Vit C - the turns the solution clear indicating some form of reduction is taking place
3. Add 3 drops Ninhydrin
4. Heated @ 72°C It is turning a nice purple w/in 5 minutes
5. Very clear double peak spectrum
   Peaks @ 391 °
   508 (what does this mean.

pH is fairly neutral @ 6
About 3 drops 1M NaOH
But now, when you add NaOH and it become very basic, it changes to a very dark purple with peaks @ 397
565

So it clearly affects the color but I don't know how you can say that by looking at it seems to match a whole host of amino acids.
The pH is now 7-8

adding more NaOH did not shift the peak. They remain @
397
566
essentially identical
pH is now ~ 10

We see that the color theyre is very pH dependent.

Now when you continue to heat the solution (now very basic pH) it turns to a reddish color. (approx 90°C)

It turns orange red, much broader peak, peaks @ 397
447

And we have learned that cysteine is very red @ high pH & yellow @ neutral.

Next we put Control histidine & water in a test tube and heat to about 70. pH bee should be close to neutral

We all some purple right away but most of a red brown color.

Now we have added more NaOH (4 drops) estimate pH ~ 8.

Histydine by itself in water is already turning a nice purple. Needed to add very ~~histidine~~ little histidine to get the nice purple color. We believe we are @ neutral pH.

Case peaks 391
541

~~Now add NaOH~~ It's just not pure.
2nd peak a very broad.
May need to centrifuge
Did it. Not much of a difference.

We do learn that the spectrum is very much pH related & that the process is not entirely reversible from base to acid to base, etc. Histidine turns red to rose to clear in acid. You cannot go

straight back to purple.

Ty you need some tight controls on pH to be measuring the spectral controls.

You would need to set the pH of each solution and then measure the spectrum at the same temperature and duration.

We actually have an extremely good match between 2 control lactodene and E3 + VitC + Niah + Leat

1. E3 Straight (~ 3 ml)
2. Little Vit C added
3. 4 drops NaOH
4. 3 drops Ninhydrin
5. 70 – 140° C

Ok I had extremely good results w/ elute II. Histidine spectrum flicked in right away w/ strong deep purple

1. Elute II
2. A little more Vit C
3. 4 Drops NaOH
4. 4 Drops Ninhydrin
5. 90°C Bath for 5 minutes

a perfect match of spectrum

Now let's see if you can get Cysteine out of it.

Vit C is apparently very important to break it down to a ~~dipeptide~~ form Amino acid form

Ok, after enough, it is turney yellow after about 15 minutes in a mildly boiley bath.
ie, Elute II

Very good results, we are getting a transition to yellow after a secondary water bath in block case of Elute III and Elute II.

Elute II is not acidic by itself. You only get the purple color by adding NaOH, Vit C & Nin to the elute.

Also, you are not getting the purple from the stock solution. The say Chromatography & all that the entailed WAS necessary.

Whoa
With the stock solution.
1. Stock solution (4 drops in 3 ml)
2. Vit C added
3. NaOH added (make it alkaline!)
4. Ninhydrin

* We get a deep red color.

So obviously, what is this reaction???

When put strongly alkaline it turns dark red.

A deep purple red

The spectrum of this is complicated which indicates that it probably has many components & it. Peak seems to be near 446 but complicated & broad

Iron
Histidine
Cysteine etc

Interesting. When I take

Fe 3+
Cysteine
Histidine

] Combine, gives a sharp blue color which then disappears.

Now add NaOH, Ninh & heat.

Then dves ended appear to be some similar to our stock solution result. Need to increase the concentration of the amino acids.

We are positively getting a strong red color reaction w/ the

1. Stock (6 drops in 5 mL)
2. Vit C added
3. 4 drops NaOH
4. 4 drops Ninhydrin
5. Heat @ ~70°C

Gives a dark red color.

Now, what about w/out Vit C
Yes it still turns red.

Heating in a controlled test. We know it initially turns yellow, but we don't know how long it takes. Initially turns red/purple C

Let's make a reference solution of
4 mL H_2O
4 drops culture
Vit C
4 Drops NaOH but no ninhydrin!

I also have a dual set w/ everything but Vit C to see if reduction has a role here.

1st Water Test – Residence Drinker
08/31/12

pH 7.5 Chemical
Ammonia 2ppm
phosplate 0.5 ppm

Nitrite 0 ppm
Nitrate 5ppm

pH meter 8.1
TDS 350 ppm
uS 685

pH Chemstrip 7.5

Alkalinity – Test Strip 120ppm
Total Hardness 25 ppm

Note:
High Alkalinity
High pH
~~Ammonia~~ Ammonia Presence
TDS high
Some phosphates

Water Works Strips ?

Nitrate 4 ppm
Nitrite 0 ppm
Iron 0 ppm
Phosphates 10 ppm (Test Strip seems way too high)
Hardness 20 ppm

Pecos River Water 09/03/12

pH meter ~~B.10~~ 7.9
ppm TDS 270
uS 610

Test Strip
pH ~~7.2~~ 7.6
alkalinity 120
free Chlorine 0
total Chlorine .5
total hardness 200 ppm

Nitrate 0.2ppm. (Water Works Test Strips)
Nitrite 0 ppm
IRON 0 ppm
? Phosphates 25 ppm (Test Strip seems way too high)
Hardness 150 ppm

pH Chemical 7.6

Ammonia 0.25 ppm

API Phosphates 0.5 ppm

Waldorf 09/03

meter
TDS 250
uS 540
pH ~~8.0~~ ~~7.2~~ 7.1

Pool Check
pH ~~8.0~~ ~~8.0~~ 7.4
Alk 240ppm
Free Chlorine 0
Total Chloride 0
Hardness 400ppm

Nasco
Iron 1ppm
Phosphates 10ppm. too high again?
Nitrate 20ppm
Nitrite 0ppm.
Hardness 125ppm

API
pH Chemical 7.5
Ammonia 0.25ppm
Phosphates 0.25ppm.

Vit C caused a reductate to produce the red form.

No VitC in stock solution NaOH was all produces yellow direct.

You have absolutely proven histidine w/ EG3.

Now extended heating (area of stock solution w/out Vit C) produce a yellow color. Cysteine is the only reference that produced a yellow color.

You have a set of reference proteins
yellow - neutral pH
orange - moderate alkaline
red/red - highly alkaline.

What is happening here is that histidine has been proven by elute III.

Cysteine is proven by further heating of elute III w/ Vit C

But cysteine is also proven by direct protein heating of the stock solution w/ NaHydrox. No Vit C is required. Just heat the stock solution w/ NaHydrox.

You can not get histidine from the stock solution, only cysteine. Histidine had to come from the elute.

[redacted]

Pricing Structure

Single Water Test $60
Each Additional Water Test eg (pond fish keep) $40
Soil Test $60

You want 3 things

1. Sulfate testing
2. SH_2 & ICH test - on its way
3. Collyon testing

SO_4 test can be done w/ HCl & any barium salt (you have $BaCl_2$) but you do not get concentration.

$BaCl_2 \cdot 2H_2O$ Molecular Weight is
I have already made some!

You have a good test for SO_4.

~ 4 ml water
add Sulfate suspected solution
Lets create 1M HCl Solution (?)
Add 1 drop 0.1 M $BaCl_2$
(it does not take much)
White precipitate forms

What is the concentration of 1 drop .5M $CuSO_4$ in 4 ml H_2O?

$$\frac{60 \text{ ml}}{8.7\text{M HCl}} = \frac{x}{1\text{M HCl}} \qquad x = 6.9 \text{ milliml}$$

Today we put
1.0 ml of 6.7 M HCl into a
60 ml bottle we will have 1M HCl

---

3 ml H_2O
1 drop Ø.5 $CuSO_4$
1 drop 1M. HCl
1 drop Ø.1 M $BaCl_2$

turns light white

Lets get Concentration

159.6 gms/mol
$CuSO_4$ Set
this does not
account for Pentahydrate

$\frac{22 \text{ drops}}{2 \text{ ml}}$ $\frac{1 \text{ drop}}{x}$ $x = .09 \text{ ml} \cong Ø.1 \text{ ml}$

1.0 M $CuSO_4 \cdot 5H_2O$ = 249.7 gms/mol
So Ø.5 M $CuSO_4 \cdot 5H_2O$ = 124.85 $\frac{\text{gms}}{\text{mol}}$

~~249.7~~ gms

$\frac{124.85 \text{ gms}}{1000 \text{ ml}} = \frac{x}{Ø.1 \text{ ml (drop)}}$ $x = .0125$ gms per drop

So in 3 ml H_2O we have

$\frac{.0125 \text{ gms}}{3 \text{ ml } H_2O} = \frac{X}{1000 \text{ ml } H_2O}$ $X = 4.167 \frac{\text{gms}}{\text{liter}}$

Now for the sulfate ion concentration. Concentration. This is high.

$SO_4 = 96.1 \text{ gms/mol}$

So $\frac{96.1 \text{ gms mol}}{249.7 \text{ gms mol}} = .38$

and $.38 (4.167) = 1.604 \frac{\text{gms}}{\text{liter}}$

This would be our concentration level, which is way way too high.

Lets try to dilute,
Your test is not sensitive enough!

Dilution test
(01 ml) 1 drop $CuSO_4$ (0.5%) in 10 ml water

~~.0125 gms~~/drop

$\frac{\text{.01 ml}}{10 \text{ ml}} = \frac{1}{X}$ $X = 100$ dilution factor.

So this means our dilution solution is $.0125/100$

$\frac{.0125 \text{ gms}}{10 \text{ ml}} = \frac{1}{X}$ $\quad X = 800$

$= 1$ in 800 by weight.

Either way, it's not sensitive enough.

Let's check our detection level.
1/2 0.5M $CuSO_4$ in approx 1 ml is barely detectable after a couple of minutes.

MW of $CuSO_4 \cdot 5H_2O$ is 250 gms / mole
So a 1M solution is $\frac{250 \text{ gms}}{1000 \text{ ml}}$. We have a 0.5M solution so we have $\frac{125 \text{ gms}}{1000 \text{ ml}}$

Now we used 1/2 drop $CuSO_4$ so this is approx .05 ml
So we have approx $\frac{125 \text{ gms}}{1000 \text{ ml}} (.05 \text{ ml}) \doteq .0063$ gms

a 6.3 mcg.

Now we placed this w/in approx 1 ml of water & it is detectable.

$\frac{6.3 \text{ mcg}}{1 \text{ ml}} = \frac{X}{1000 \text{ ml}}$ $\quad X = \frac{900 \text{ mcg}}{1000 \text{ ml}}$

$= 900$ ppm.

Thus means we can detect sulfates on the order of 900 ppm?

But wait, the sulfate portion only has a MW of 96 gms/mol.

So we really have a detection limit of approx

$\frac{96}{250}(900) = 345$ ppm And this is useful.

US Public Health recommends sulfates not exceed 250 ppm.

Thus means we now have a useful detection test!!

Also we have Calcium Carbonate.

And we have Cu^{2+}.

So 3 new tests to add to the set!! Great.

And fix the Iron Test!
Strips or Chemical?

Sure enough, Sodium Thiocyanate only has a (Fe^{3+}) shelf life of 4 months!
Phenanthroline (Fe^{2+}) solution is still working fine

Here is a big question.
Can you use life cell analysis?

Make a molecular model
of iron sulfa protein

What is the reason about organic
molecules turning Cola (yellow
or brown) in NaOH?
does the acid not work?
It did not in one case.

Functional Groups
Hydroxyl OH

Carbonyl

Amino

Sulfhydrl

Phosphate

Carboxyl

Page 68A

Biochemical reactions involve specific chemical bonds or parts of a molecule. These sites of reactivity, or *functional groups*, can be classified into a few common types, as shown in Figure 1·7. The mechanisms of the reactions between functional groups in biomolecules have been studied by a variety of techniques, including comparison to well studied chemical reactions.

(a) *Functional groups*

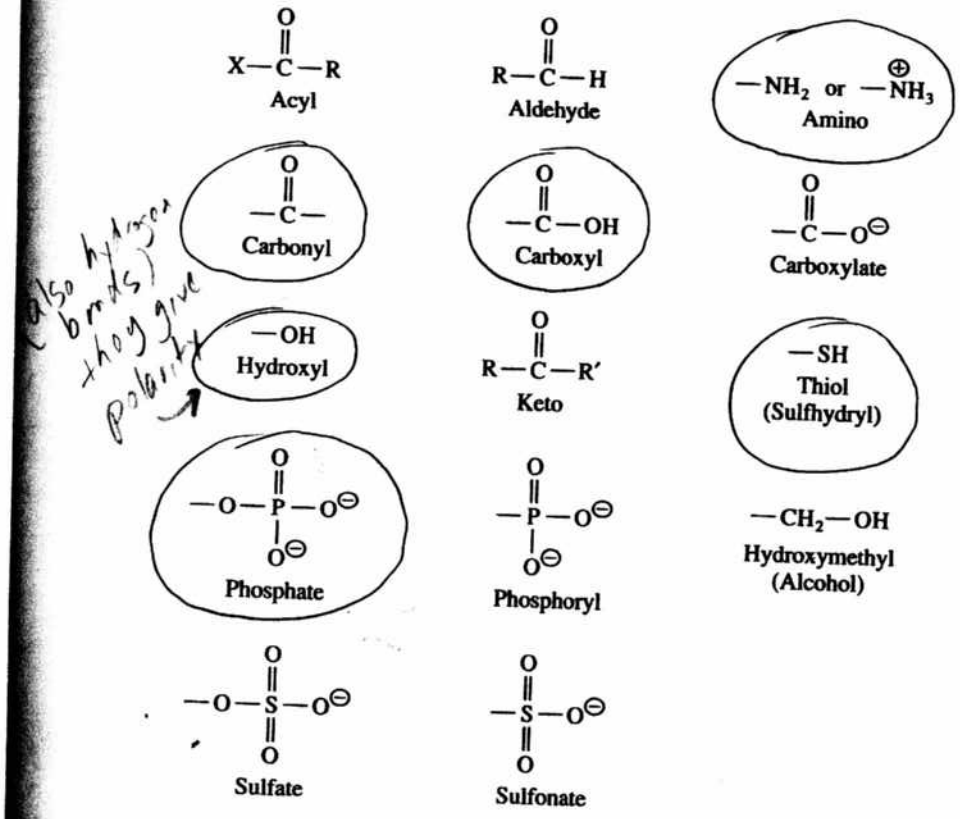


Figure 1·7
Important functional groups (a) and bonds and linkages (b) in biochemistry. R represents an alkyl group, a group that contains carbon atoms plus hydrogen. X represents any group other than an alkyl group or hydrogen.

Circled set
IS George Wolfe's
Emphasis

Functional Side Groups

Page 68

Page 68 A

Biochemical reactions involve specific chemical bonds or parts of a molecule. These sites of reactivity, or *functional groups*, can be classified into a few common types, as shown in Figure 1·7. The mechanisms of the reactions between functional groups in biomolecules have been studied by a variety of techniques, including comparison to well studied chemical reactions.

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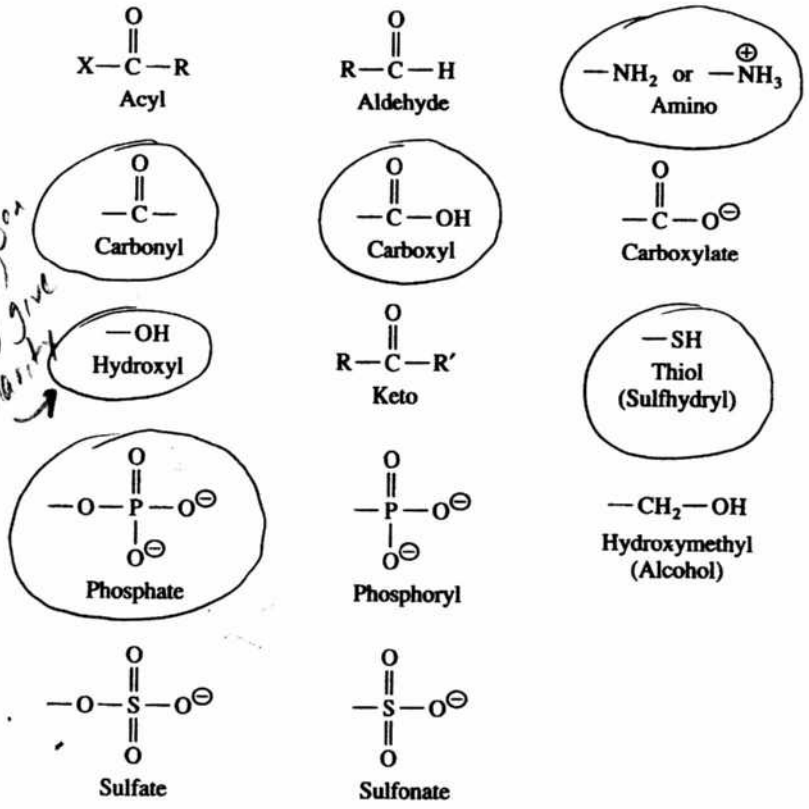
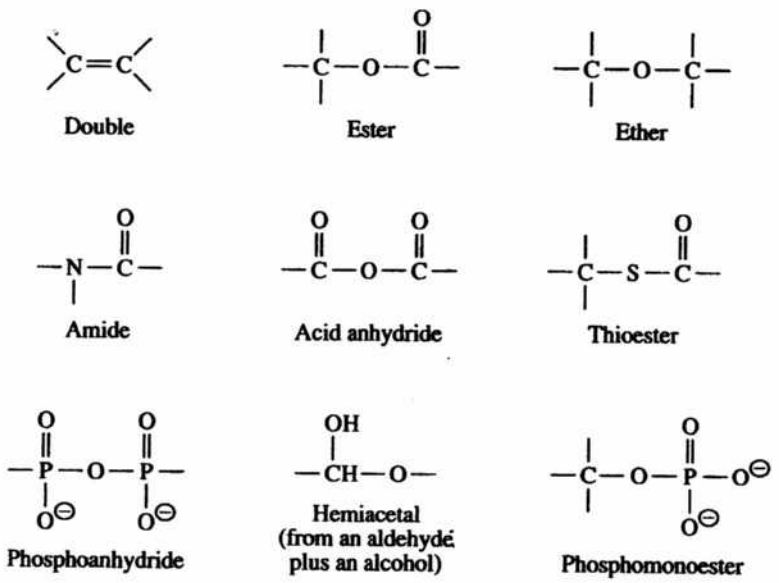


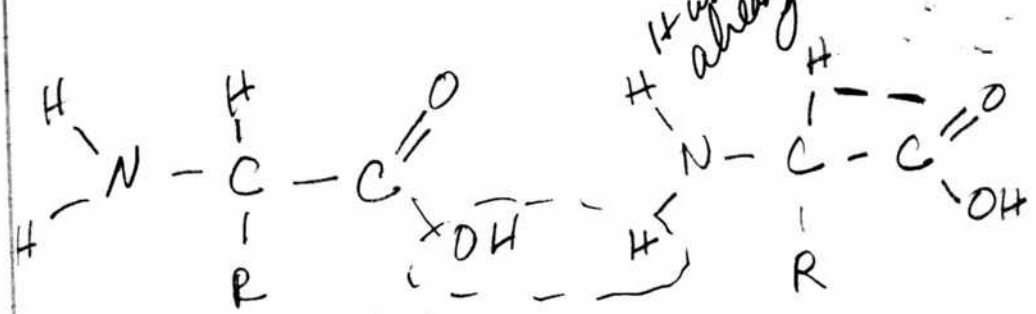
Figure 1·7
Important functional groups (a) and bonds and linkages (b) in biochemistry. R represents an alkyl group, a group that contains carbon atoms plus hydrogen. X represents any group other than an alkyl group or hydrogen.

Circled set is George Wolfe's emphasis

(b) *Bonds and linkages*

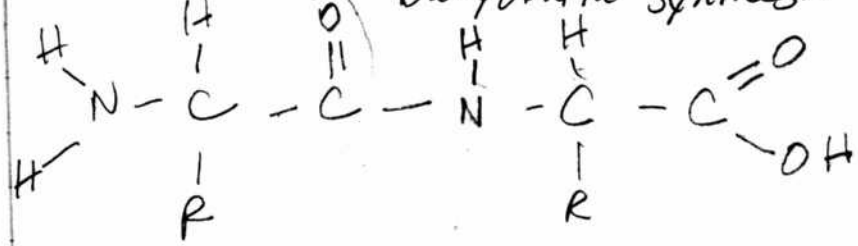


Amino Acid Construct.—



Carbonyl The H_2O goes away. "Dehydration Synthesis"

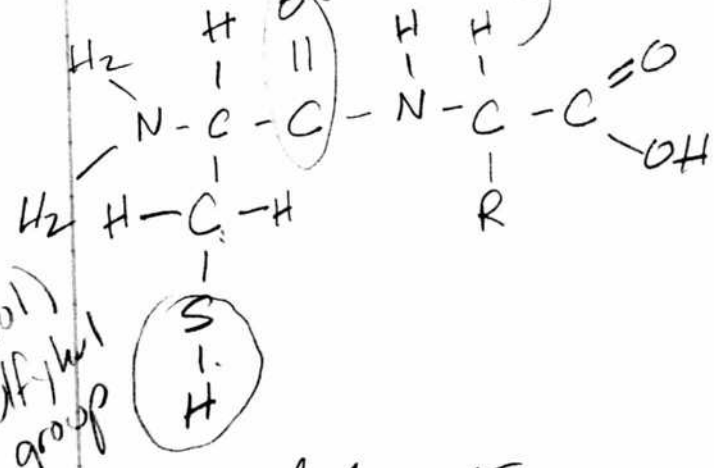
Peptide Bond



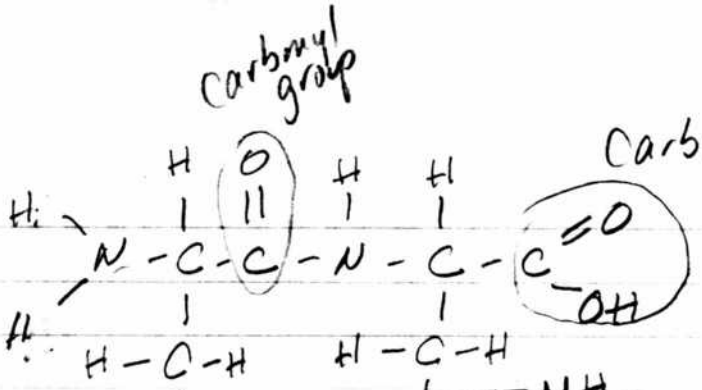
OK! Where does the H come from?

It was already there!!

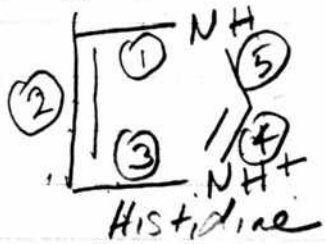
Now we have an R of cysteine CH_2SH or histidine



Now it includes cysteine.



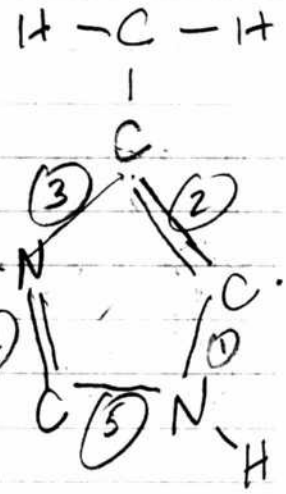
Sulfhydryl group (polar end)



Carbn to a NH
Carbn to a Carbn =
Carbn to a NH+
Carbn to NH+ =
Carbn to a NH

Electrically Charged.

So what exactly is a histidine ring?
It has an "imidazole" functional group.
Must be



also called an imidazole functional group

(1) Carbn to a NH
(2) Carbn to a Carbn =
(3) Carbn to a NH+
(4) Carbn to a NH+ =
(5) Carbn to a NH

All the bonds are here. This is a histidine

Now we know what the polypeptide looks like.
Now we look at the disulfur bond (proteins)

Notice the bond here is between (2) & (3) while in George Wolf diagram it is between (1) & (2) Apparently it does not matter as long as the CH_2 bridge to another C in the ring somewhere. There must be multiple bonding locations available

NON POLAR AMINO ACIDS (WATER FEARING - HIDE from Water)

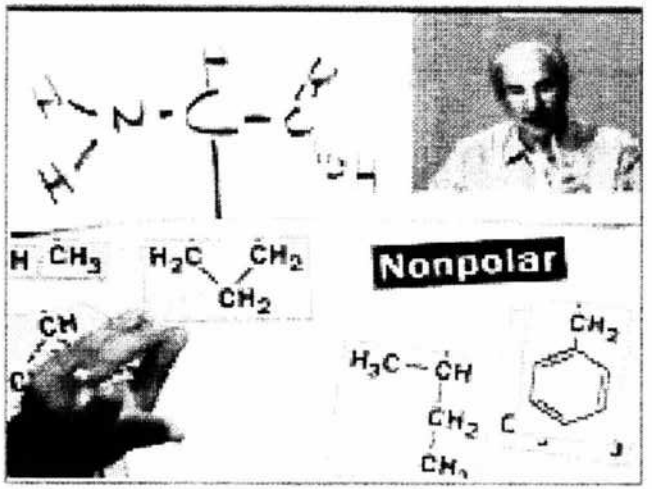
Thinkwell Lecture Player

Biology

with George Wolfe

Amino Acids: The R Groups

> amino acid structure in review • electrically charged amino acids • polar amino acids • nonpolar amino acids



Key Ideas

- *Proteins are polymers of amino acids.*
- *There are 20 different amino acids, each with a different R group.*
- *The properties of different R groups lead to interactions between R groups within a protein, which in turn determines the conformation of the protein.*
- *Amino acids can be placed into one of three general categories based on their R groups:*
  - *CHARGED*
  - *POLAR*

amino acids found in proteins

Page 71 A

- ***Nonpolar amino acids tend to have R groups consisting of many carbons surrounded by hydrogens. This makes them HYDROPHOBIC, or unable to interact with water molecules.***

nonpolar amino acids

glycine, alanine, valine, leucine, isoleucine, methionine, phenylalanine, proline, tryptophan

= carbon ring of **phenylalanine**

◄◄ ► ►► 10:27 / 10:51 Table of Contents

ELECTRICALLY CHARGED AMINO ACIDS (Charges React)

Thinkwell Lecture Player

Biology

with George Wolfe

Amino Acids: The R Groups

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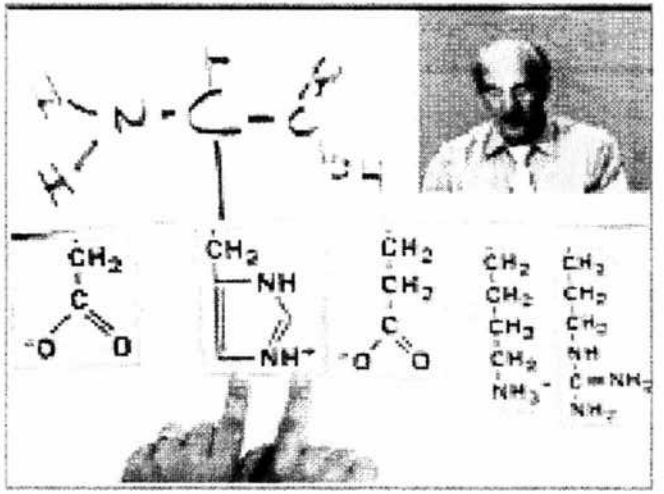

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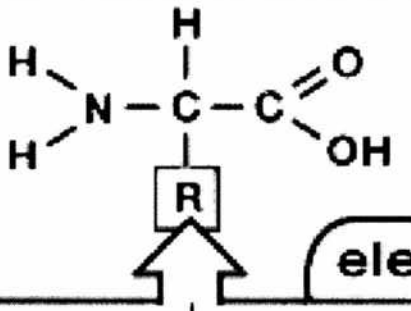
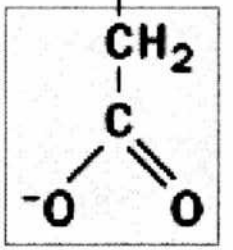
Page 71 C

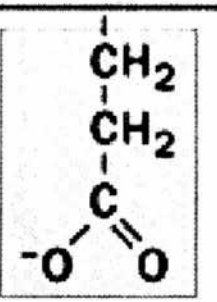


electrically charged amino acids

basic

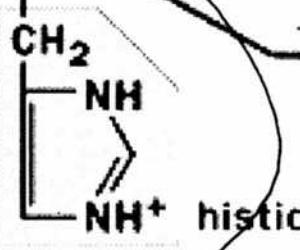


aspartic acid

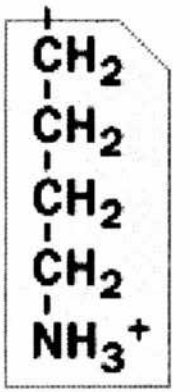


glutamic acid

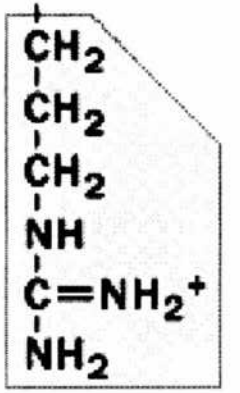
acidic



histidine



lysine



arganine

POLAR AMINO ACIDS (Water Loving (Water Reacting))

Thinkwell Lecture Player

Biology

with George Wolfe

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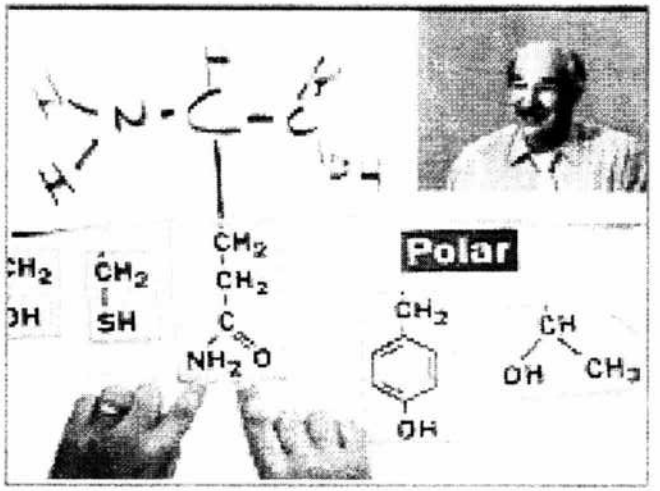

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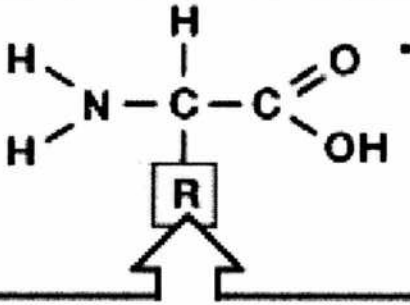
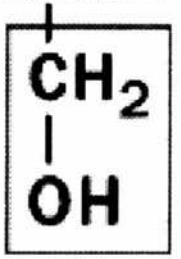
amino acids found in proteins

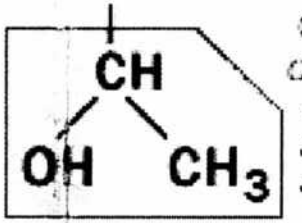
Page 71E



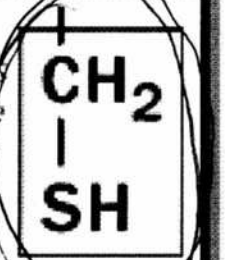
- *The polarity (partial charge) of "polar" amino acids allows them to interact with each other. They can also interact with water molecules.* ***(HYDROPHILIC)***

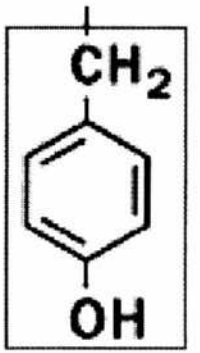
polar amino acids

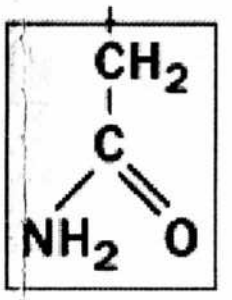

serine

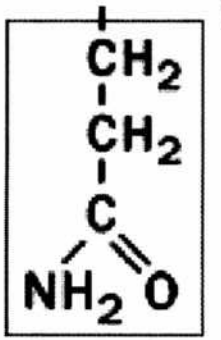

threonine

Cysteine can form disulfide bridges with other cysteine amino acids within a polypeptide.


cysteine


tyrosine


asparagine


glutamine

disulfide bridges: ***covalent bonds between the sulfur atoms of two sulfhydryl groups.***

The Hypothesis

Here is the development of our hypothesis.

Two cysteines can bond to iron
Two histidines can bond to iron
We appear to have both cysteine & histidine
There is a dipeptide iron identified.

How can we combine all of the above?

1. ~~Join two cysteines~~ together
1. Join a cysteine and a histidine together (a dipeptide.

2. Make another one of them (a copy)

3. Now join the two cysteines together w/iron

4. & join the two histidines together w/iron

5. & join both with iron

And you still only have a dipeptide.
All conditions above are satisfied.

The problem:
Break the iron bonds in a non toxic fashion.

We have a successful result w/ NAC & the culture.

Both VitC & NAC have the effect of reducing the iron to Fe +2. and severing the bonds.

NAC appears to be slightly more effective and precipitates out the color in

---

Now we are testing
Culture + VitC + ~~NAC~~ } No Phenanthroline
Culture + ~~NAC~~ + NaOH }

+ Heat
add 2 drops NaOH because of pH influence upon color.

NaOH had a absolutely positive effect upon Color.

VitC is giving a weak color
NAC is giving the darker color

You get the same results

What we have is a definite yellow Cysteine color on the left (Culture alone

Both w/ VitC & NAC change the color indicates a reduction of the chypeptide

$FeSO_4 + NaOH \Rightarrow Fe(OH)_2 + NaSO_4$

(+2 over Fe in $FeSO_4$; +2 over Fe in $Fe(OH)_2$)

The means the Fe^{+2} ion is not oxidized by NaOH (lye) it combines with the OH ion to form a hydroxide

? $FeX + NaOH \Rightarrow$ Metallic Protein $+ Na + OH$

---

yes NAC + high heat alone is producing a precipitate.

This is important.
Without NAC no precipitate.

Iron oxide (FeO) is black

Iron Oxide Fe_2O_3 is dark red

We are producing Iron oxide FeO

NAC appears to be more successful @ precipitating at the iron @ high heat than VIT C.

However Vit C also eventually works.

Page 75 Wolfe Notes

" " " "

SPONCH vs SPHNACH

The ☆ 99% of our biology.

1. Orbitals hold 2e⁻
2. Orbitals combine to form shells (Shells are ~~rooms~~ energy levels: S, P, D, F)
3. Shell 1 has 1 orbital

So what exactly is 4n+2

	4n+2	
	n	~~0~~
s	0	2
p	1	6
d	2	10
f	3	14

it is the no of electrons in a shell

4. The second shell has 4 orbitals (4 orbitals * 2e⁻ in each orbital) = 8e⁻

5. One electron goes into each orbital before any orbital gets a second electron.

6. The outer shell is reactive if it isn't full (This is the valence shell)

7. Further electrons (those in the outer shells) have more potential energy.

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From Chemistry Made Simple

Energy Level 1 : Has only S

Energy Level 2 : Has S & P (these are called subshells)

Energy Level 3 Has S, P & d

Energy Level 4 Has S, P, d & f

Now look @ 4n+2

	n	4n+2
s	0	2
p	1	6
d	2	10
f	3	14

Notice the exact match with the periodic table here.

What Wolfe is calling a "shell"
EZ Chem is calling an energy level.

So when Wolfe says shell #2 has "4 orbitals"
what he means is that it is
Energy level 2
which has S & P
S has 2 max electrons
P has 6
= 8

and Wolfe is saying this equals
4 × 2 = 8

Not so sure I like Wolfe's simplification so far. He's not distinguishing between S & p & d & f

I can't say I entirely am accepting of Wolfe's explanation of atomic structure. He never speaks of spdf (2, 6, 10, 14) which seems closer to reality to me.

What we are saying is that the periods of the periodic table represent ~~the~~ energy levels

Remember the d energy level is dropped by one number.

eg Cr should be $[Ar]\ 4s^2\ 3p\ 3d^4$

My book gives $[Ar]\ 4s^1\ 3d^5$
So it shifts one.
Chromium is an anomaly it appears.
You are on the right track.

Iron is $[Ar]\ 4s^2\ 3d^6$
No! So how does Fe have a valence of Fe^{+2}, Fe^{+3}
No! Fe^{+2} would be $[Ar]\ 4s^2\ 3d^4$
Fe^{+3} would be $[Ar]\ 4s^2\ 3d^3$

Yes this has an error. Shell 4 loses first.
Should be
Fe(II) is $[Ar]\ 3d^6$
Fe(III) is $[Ar]\ 3d^5$

There are wrong answers on the net!

In Argus, we have found out how to select a group and rotate it. Select something, then Edit, a Attach to Manipulator. This is great!

Cysteine — Histidine

Iron Sulfur Junction — Iron Sulfur Junction

Cysteine — Histidine

Ok, we have a tentative model in place.

Now get this.

Ascorbic acid is used to remove dissolved metal stains, such as iron, from fiberglass swimming pool surfaces (wikipedia)

It can be shown that Ascorbic acid reduces Fe^{3+} to the Fe^{2+} state.

So this expected to be a part of the solution

From Scripps, we learn that the oxidation states of iron in the Reuke protein are either two Fe^{+3} iron ions (in the oxidized state) or one Fe^{3+} and one Fe^{2+} in the "reduced protein."

~~The matches~~ matches.

Cl: Electronic Configuration

$1s^2\ 2s^2\ 2p^6\ 3s^2\ 3p^5$

or $[Ne]\ 3s^2\ 3p^5$ this means 7 e^- in 3rd energy level.

Now take Bromine.

$[Ar]\ 4s^2\ 3d^{10}\ 4s^5$ so 7 e^- in outer energy levels.

that's why VIIA all have 7 e^- in outer energy levels.

So the Lewis diagram shows only the electrons in the highest energy level

"shell" means "energy level"

We now understand the coordination of the Rieske protein.

$\left[(Fe^{+3}, Fe^{+3}), S_2^{-2}\right]^{2+}$ oxidized form

$= +6 \quad = -4$

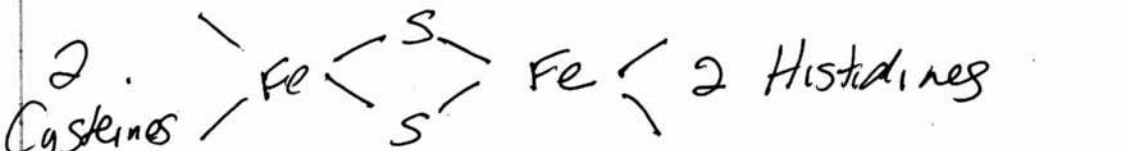
$6 - 4 = +2$

and

$\left[(Fe^{+3}, Fe^{+2}), S_2^{-2}\right]^{+}$ reduced form

$= +5 \quad -4$

$5 - 4 = +1$



Now, can we understand the bonding structure?

Fe: $1s^2 2s^2 2p^6 3s^2 3p^6 4s^2 3d^6$

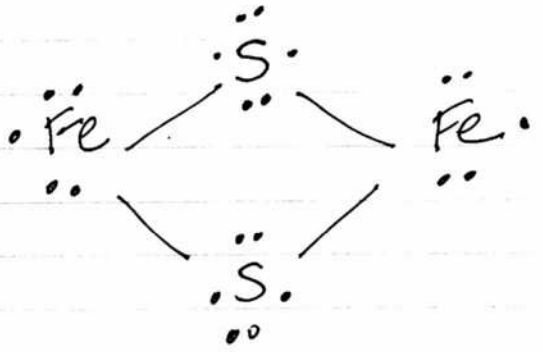
$= [Ar] 4s^2 3d^6$

So $Fe^{3+} = [Ar] 3d^5$ so there are 5 electrons in outer shell.

and $Fe^{2+} = [Ar] 3d^6$

Sulfur = $[Ne]\ 3s^2 3p^4$

@ level three, there are therefore 6 electrons in outer shell.



Now we know a way you can get 8 electrons?

Ascorbic Acid is $C_6H_8O_6$

$C_6H_8O_6 + Fe^{3+} \rightarrow Fe^{2+} + ?$

$C_6H_8O_6 + 2Fe^{3+} \rightarrow C_6H_6O_6 + 2Fe^{2+} + 2H^+$

It is "Oxidized" Vit C.

We have a source that says cysteine & histidine will reduce Fe^{3+} to Fe^{2+}. I tried it. It was true, but cysteine is a superoxide. So then I tried the culture w/ cysteine vs NAC. It definitely does not work. So the culture has additional complications that allow Vit C & NAC to work but not cysteine & histidine by itself.

File:Rieske Fe2S2.svg

From Wikipedia, the free encyclopedia

$[Fe^{+3}, Fe^{+2}, S_2]$ $[Fe_2S_2]^{+}$ (+5, −2)

Fe^{+3}_2 $[Fe_2S_2]^{2+}$ (+6, −2)

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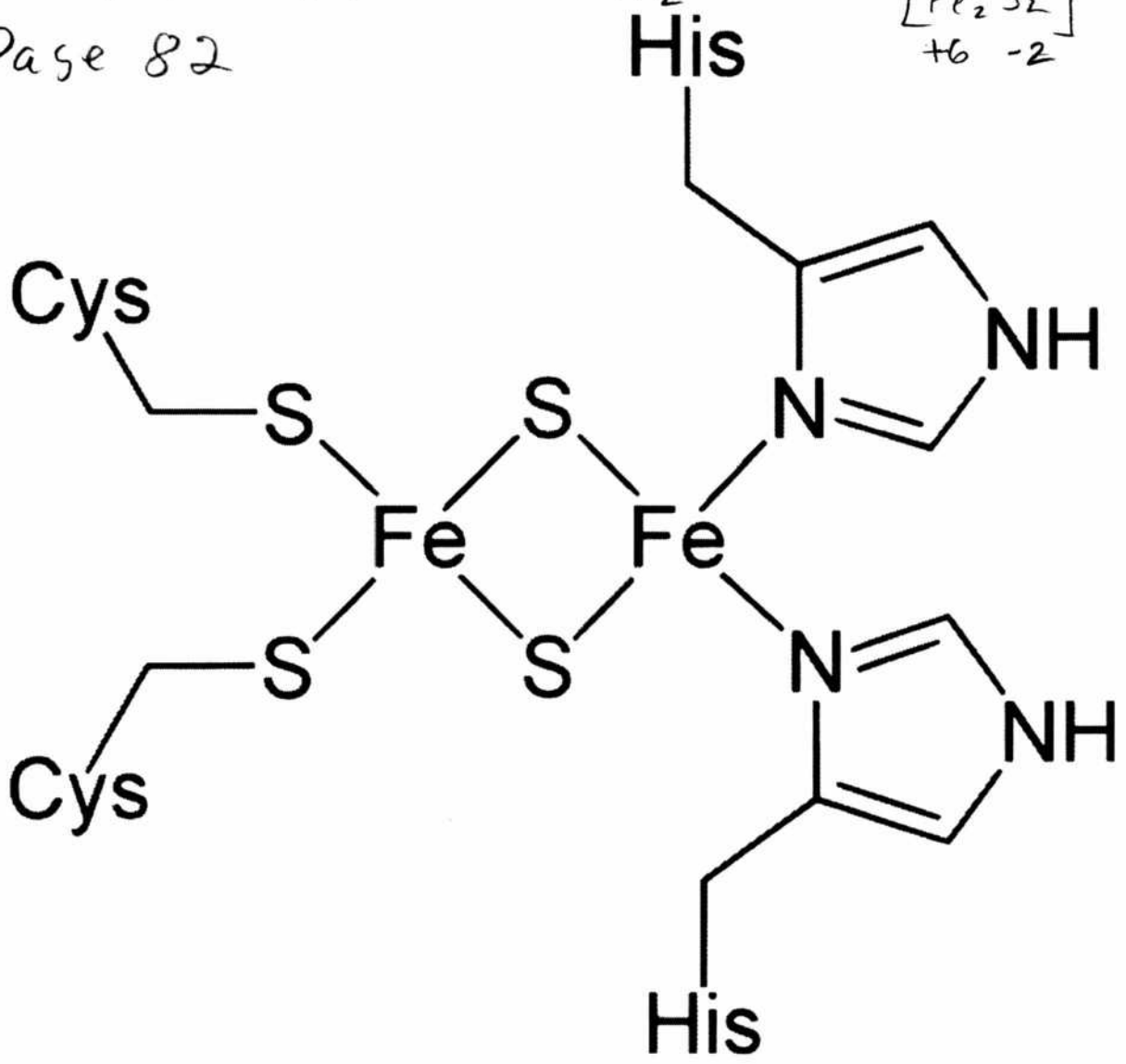

No higher resolution available.
Rieske_Fe2S2.svg (SVG file, nominally 620 × 582 pixels, file size: 12 KB)

This image rendered as PNG in other sizes: 200px, 500px, 1000px, 2000px.

This is a file from the Wikimedia Commons. Information from its **description page there** is shown below.
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Description **English:** Rieske iron-sulfur cluster. Created by Metalloid using MarvinSketch 4.0.3 by ChemAxon.

Date 2006-01-13 (first version); 2006-01-13 (last version)

Source Transfered from en.wikipedia (//en.wikipedia.org/)

Bronsted - Lowry Acid Definition

Acid is a ~~proton~~ proton (singular) donor
Base is a proton ~~(plural)~~ ~~pair~~ acceptor

Lewis

Acid - an electron pair acceptor
Base - an electron pair donor

No wonder you have been confused!
Notice they are in opposite directions.

In our case on Disk Organic Chemistry - Dean Hara

~~H~~ + ~~A~~ ~~H~~

(an acid) H+ wants to accept electrons to neutralize

A⁻ (wants to give electrons to neutralize) (C, O, N) (a base) eg

A is more electronegative than H. It pulls (attracts) the electron away. This makes A a Lewis electron pair acceptor. This make it act as an acid in the Lewis definition. So C O N act as acids in this case.

(I think Sulfur will count also)

Notice it is electrons here, not protons.

Is this true? Step it through

I bet this is wrong. :-)

So there are 3 different definitions or interpretations of acids & bases.

Bronsted:
acids donate protons (H+) ← this means H has a proton to donate.
bases accept protons (H+)

Notice it is all in terms of H+ (PROTONS! not electrons)

H^+ (proton donor) Cl^- (proton needer)

look, it is protons that are moving, not electrons

Three Methods for Acids!

1. H^+ vs OH^-
2. Proton donor vs acceptor
3. Electron pair acceptor (acid) vs electron pair donor (base)

This is all very amazing. Lewis acids and bases are used to explain Coordination Complexes.

If something is positively charged, it wants electrons, so it can be a Lewis acid (electron acceptor)

If something is highly negatively charged, it wants to get rid of electrons, so it is an electron donor, so it can be a base.

Notice it must be a pair of electrons. Which is what Coordination Compounds are about,

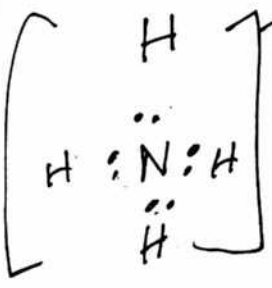
~~You need~~ OK. Found it.

~~1. Eosin Stain~~

1. How would you ~~construct~~ a Lewis diagram for a polyatomic ion, like nitrate NO_3^-?

It can indeed be done eg NH_4^+

N: $1s^2 2s^2 2p^3$ ⇒ 5 valence electrons
H $1s^1$ (but it is positively charged, so it is missing one)



This is very cool.
So it wants to accept an electron.
∴ we could say it is in position to donate a proton.
This makes it acidic.

Then it confirmed on wikipedia, FANTASTIC!

So logically, if something has a charge of any kind, it makes it acidic or basic respectively!

Left side of Periodic Table.

They are positively charged.
Therefore they can either donate protons (acidic) or they can accept electron (also acidic)

The right side of the periodic table is generally negatively charged as ions.
The means they want ~~to accept protons (which means basic)~~ or are deficient in protons so they want to accept protons. This means basic.

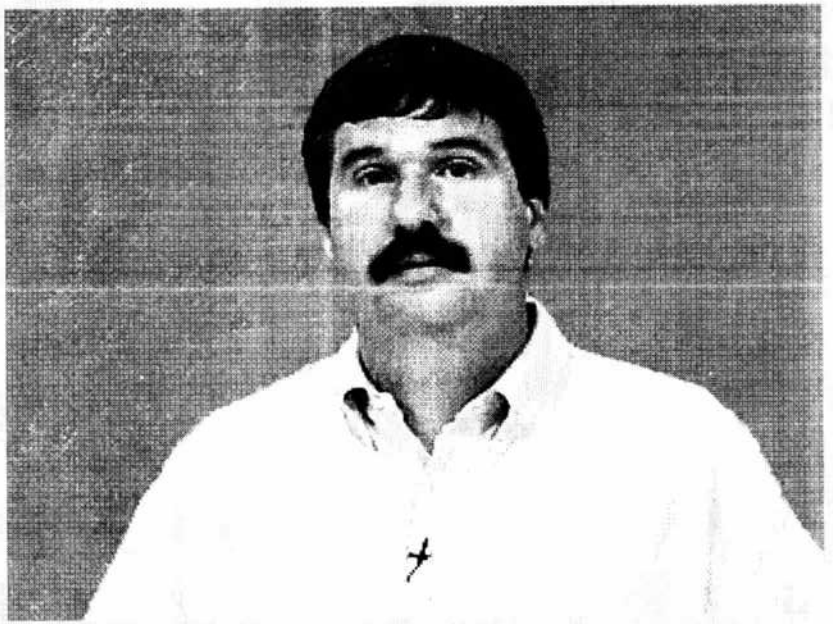
Now in term of election pairs, they would like to give away election pairs or donate pro ~~elec~~ election pairs and this mean a base!!

So generally

left side of Periodic is acidic Right side is basic

90% of Organic Chemistry Reactions and Prob

Thinkwell Lecture Player



professor
Dean Harman

Professor of Chemistry
University of Virginia
Charlottesville, VA

Degree:
PhD Chemistry from Stanford University

Specialization:
Organometallic Chemistry

key ideas

- **Predicting chemical reactions**
  - Organic chemistry focuses on how molecules react with each other.
- **Chemical bonds and reactivity**
  - Much of the reactivity of molecules relates to the polarity of bonds.
  - Bonds can be cleaved heterolytically or homolytically.
- **Heterolytic cleavage reactions**
  - The more polarized a bond, the easier it is to break that bond.
  - There are two general types of heterolytic cleavage reactions in which the more electronegative atom accepts both electrons from the polarized bond.

Thinkwell's Chemistry

Unit: Introduction to Organic Reactions

Introduction to Reactivity

Heterolytic cleavage reactions

Reactions of polar bonds:

polar bond between A and H

A = C,N,O... This process is a general acid-base reaction.

bond between A and a very electronegative atom X

A = C,N,O...

In both cases, the more electronegative of the two atoms accepts both electrons from the polarized bonds.

These two general types of reactions illustrate how most bonds are broken.

The structure of a molecule can be used to predict the degree of acidity or basicity of that molecule.

chemical reaction tips

A bond is made of two electrons.

↷ = *movement of electron(s) to form or break a bond*

nucleophile – *a "nucleus-loving" atom or molecule*

thinkwell

Replay

Table of Contents

acidity ~~Issues~~. Discussion

The discussion relates solely to a hydrogen bond!

Page 88

The oxidation of two cysteines forms a disulfide bridge.

So what is it that would oxidize amino acids? Sure enough, Oxidation of amino acids is bad news.

Bond ~~Dissos~~ Dissociation Energy:

~~C-O~~ ~~C-F~~

~~C-S~~ ~~C-C~~

HO	111	HF	135
HS	81	HCl	103
		HBr	87.5
		HI	71

A small change this way make a big difference

↓ Bond Dissociation Energy dominates this direction of periodic table

→ Electron affinity dominates this direction on the periodic table

The more polarized the bond the easier it is to break. (Electronegativity)

- single
a bond is by definition a sharing of 2 electrons.
So if you break a bond two electrons by definition are left behind.

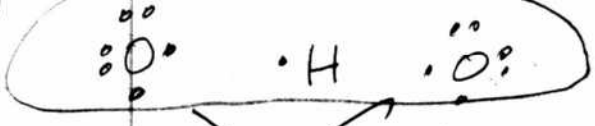
Lewis Dot: CO_2 vs H_2O

C 4 valence electrons $1s^2 2s^{(2)} 2p^{(2)}$
H 1 valence e⁻ $1s^1$
O 6 valence e⁻ $1s^2 2s^{(2)} 2p^{(4)}$

16 total valence electrons

⇒ :O::C::O: only Carbon has 4!

Steric Number
number of bonds = 2 (one atom only!) not 6
no of lone pairs on the central atom = 0
Steric # = 2 ? balloons = linear
(we have a triple bond)



looks polar to me.

:O:H:O: Hydrogen can only have one electron!

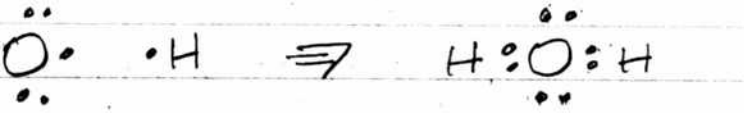
Oh!!! I see it It is H_2O not HO_2!!

:Ö:H O: Hydrogen fills the duet rule not the octet

NO!!
Hydrogen somehow has two valence electrons - why?

H_2O again! Notice it is H_2O Twoth's!!!

H $1s^1$ 1 valence e⁻
H $1s^1$ "
O $1s^2 2s^2 2p^4$ 6 e⁻



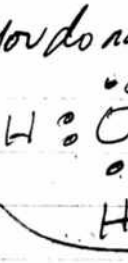
Steric No = 2 bnds + 1 lone pair? = 3.?

We have a total of 8 Valence electrons

We must

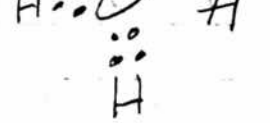
H_2O is

you do not see lone pairs!



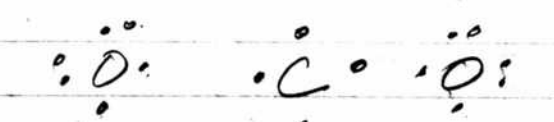
What is the steric no of water. Seems it should be 4

Why could we not do:

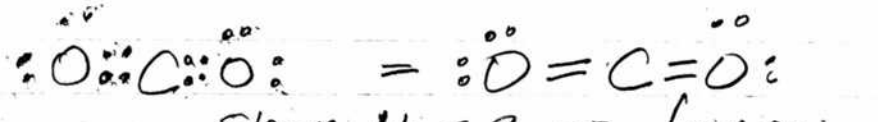


H can not have 4?

CO_2 is



6 + 4 + 6 = 16



Steric No = 2 → linear

Yes, the steric no of H_2O is 4
but it is not tetrahedral - why?
Because it is "distorted" by the two lone pairs.

Steric No = 2 is indeed linear.

But
Steric no 3 can be trigonal planar
OR BENT!

Steric No 4 Can be
tetrahedral
trigonal pyramid
or BENT!!

(water is bent!)

* 1. You do not see lone pairs!

* 2. Lone pairs occupy a little more space than bonds do.

These two rules show why water is "bent"

Pull out one of the sticks for each lone pair you have. For water, you pull out two sticks. Now understand the lone pairs distort the bond angle & occupy a little more space.

You are starting to understand why Molecules look the way they do.

Shape of the molecule affects reactivity.

We now understand why water is polar!

Now we are on Valence Bond Theory
a bond is the overlap of two orbitals, each of which is half filled

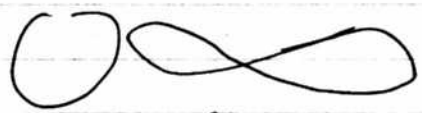
HF
orbitals
H is $1s^1$
F is $1s^2 2p^5$.

S ~~shells~~ (energy levels) can have 2
p energy levels (orbitals) can have 6

so H is missing 1 } they can overlap
F is missing 1 } and form HF

$4n+2$

n	$4n+2$
0	2
1	6
2	10
3	14

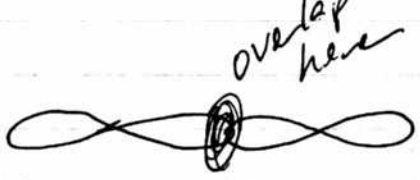


1s 2p

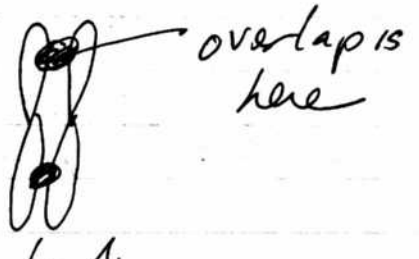
along axis is sigma bond.

O is $2s^2 2p^4$

O_2



p can have 6.
so two p orbitals



Borm: B: $1s^2 2s^2 2p^1$ needs 5
F_3 F: $1s^2 2s^2 2p^5$ needs 1

but we have 3 of them. So it needs 3
Seems like 6 bonds are desired.

NO. What Yee is saying is
that
B $1s^2 2s^2 2p^1$ mean 1 unfilled p orbital.

So even though p orbitals can have a max of 6 electrons (3 orbitals)
they do not have this, ie
Boron only has 1 p orbital half filled.
So it is only missing 1 electron.

Now Fluoride is also missing 1 but probably in a different orientation
and there are 3 of them.
So I bet we have 4 bonds that form.
Let's see.

Back to C a H.

C $1s^2 2s^2 2p^2$
H is $1s^1$

He says "Carbon has 2 unpaired electrons in 2 p orbitals".
How does he say this?

Seems to me it has no unpaired electrons for a single p orbital. What gives here?

OK, we have the answer from wikibooks.org. They fill up each orbital first before they double up.

So Now on Carbon: $1s^2 2s^2 2p^2$
It is putting 1 electron each in 2 p orbitals before proceeding. So there are two unfilled p orbitals. (2 bonds sought)

It 1s' means an electron - means it is seeking 1 bond.
so the prop had was CH_2 but he will show that this does not work in nature.
Nature seeks CH_4

Hybridization Theory is very messy and does not really reflect reality

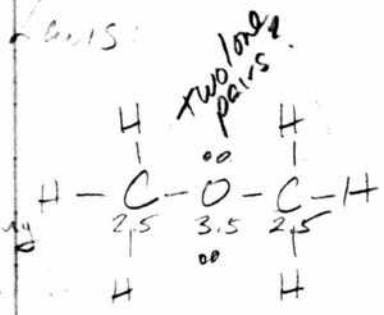
Molecular orbital theory is vly cool and does reflect reality

Like
1. Lewis dot
2. Steric nos. & VSPR theory for geometry
3. Do not like hybridization theory
4. Like Molecular orbital Theory

Page 95

Here is a great problem from the organic chemistry book.
Predict which molecules are polar:
CH_3OCH_3 CH_3OH CH_2Cl_2 CF_4

Ans:

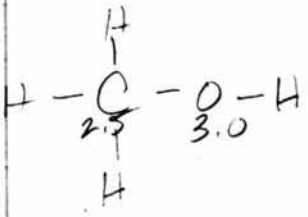


Dimethyl Ether
It is bent. Why? lone pairs?

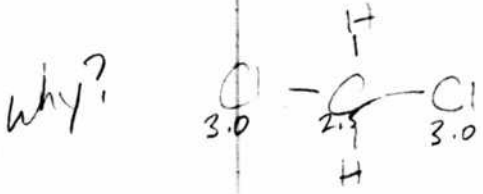
Why? look electronegativity

looks symmetric
But it is polar!
Why, it looks symmetric

Why is it bent?

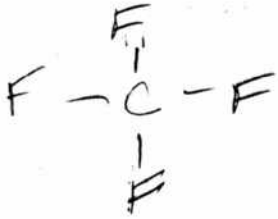


asymmetric and polar



Why?

looks symmetric
but it is polar

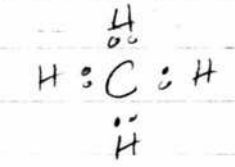


This is symmetric
and non polar.

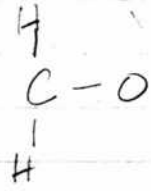
So the answer is when the Δ Electronegativity is > 0.4 it is a polar bond.

Lewis Dot Practice

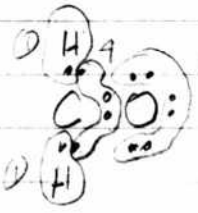
CH_4

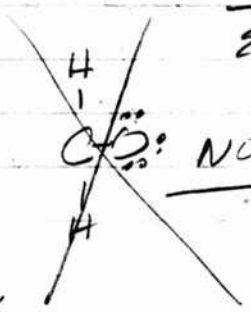


H_2CO

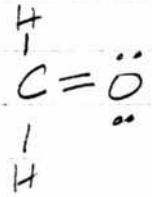


2	H:	$1s^1$	= 1
4	C:	$1s^2 2s^2 2p^2$	= 4
6	O:	$1s^2 2s^2 2p^4$	= 6
		$\Sigma = 12$	





NO! It is



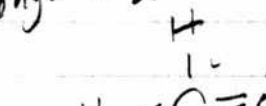
Formaldehyde

H has its duet satisfied
C now has octet satisfied

Great example CH_2O (most bonds)

(4)	C:	$1s^2 2s^2 2p^2$	Octet
2·(1)=2	H:	$1s^1$	Duet
(6)	O:	$1s^2 2s^2 2p^4$	Octet
12			

formaldehyde 2.1



2.1 H – C = O: 2.5 (8 taken) OK

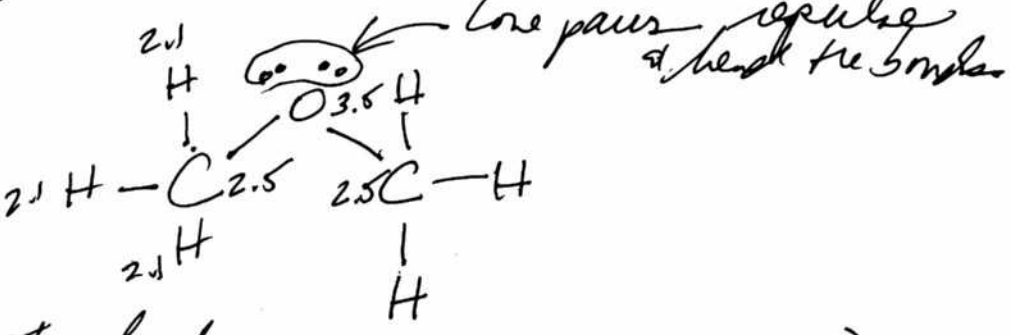
2.5 4 left

Now what is the shape?
Steric Number = 3 Trigonal Planar? GOOD JOB!
No lone pairs on central carbon atom
O has two lone pairs
Is it polar or non polar? Should be polar GOOD JOB!

Very good job!
Right on geometry & polarity!

Lone pairs have a greater repulsive effect than bonding pairs.

CH_3OCH_3



This bending makes the molecule (dimethyl ether) polar.

What is the steric number? should be 4 bent w/ bent geometry.

You are getting better

You are starting to get an idea of the geometry & polarity of the molecule.

Lewis Structure

P70 Chemistry Workbook (for Dummies)

P64 P214 Cliffs Study Solver - Chemistry

has excellent solutions.

We need to know the shape of molecules to know how they react.

Also P23 of Organic Chemistry Demystified has a description of the method

$[ClF_2]^+$: Cl $[Ne]3s^2 3p^5$ (7) 7

F $[He]2s^2 2p^5$ (7)·2 = 14

21

formation − 1

= 20

16 left

4.0 3.0 4.0

:F–Cl–F:

Symmetric in electronegativity but 2 lone pairs. Should be bent

polar

Steric no is 4 Excellent!

Could this be tetrahedral bent?

4.0 3.0 4.0

Geometries are on p 78 of Cliffs Study Solver.

Most empty valence shell is usually the central atom.

Page 99

all from Thinkwell,
Predicting Molecular Characteristics Using VSPR

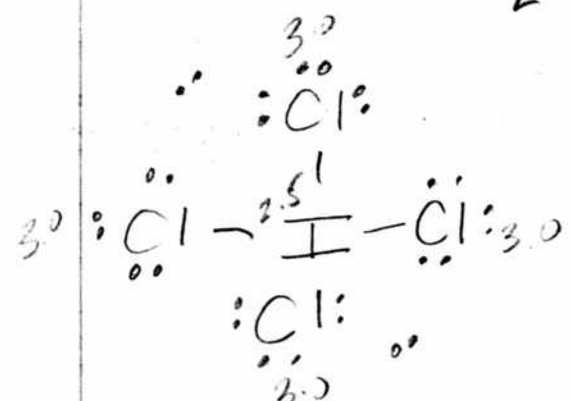
$[ICl_4]^-$ .. I: [Kr] $5s^2 4p^{10} 5p^5$ = 7 → 7

Cl: [Ne] $3s^2 3p^5$ = 7 (4) = 28

35

(ion) + 1

= 36



8

Should be non polar

Steric No is 6, 6 is octahedral GOOD!
Looks like it would be in a plane.
because of 2 lone pairs.
(This is called square planar. You were right here also! GOOD!
Very good work here.

Now for a more complex molecule Oxalic Acid

H – O – C – C – O – H ← bent! actually it is ~~seems to most likely~~ (NON) ~~to~~ polar

Lewis Structure is given → bent

What are bond angles in ...

∠HOC? Steric No is 4 which is tetrahedral bent — Yes, Good.

∠OCO? Steric = 3 Trigonal planar GOOD!

∠OCC Steric = 3 GOOD! "

It is non-polar because the bonds are symmetric.

It is said to be slightly soluble in water which makes sense

What you are often in all of these is the shape & its polarity.

You see how to get shape, or with more complex molecules, the internal bond angles.

Polarity is governed by electronegativity & by the dipole moments.

Shape is important in

1. Melting & Boiling Points
2. Reactivity w/other molecules
3. Biological molecules fitting

Here is a question.

How do I prove the existence of sulfur?

Actually, you have already proven it!

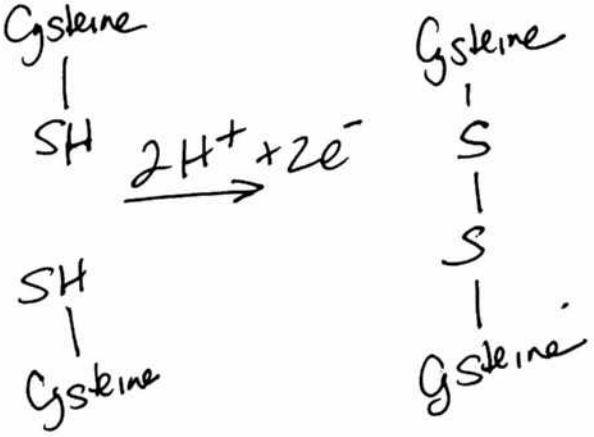
If you have proven Cysteine, you have proven sulfur!

We know sulfur is there, you know there is a backbone in there and that it is a dipeptide.

These strongly suggest how sulfur protects you actually have it.

What we really need to know is how glutathione works.

Aaron's Biochem Videos Ch3 Part 1 YouTube Explains Formation of Cystine Im 4BS



So it is a process of Oxidation.

Now it seems to recall oxidation
in Chemistry Essentials by Denniston p 112

Oxidation is

1. The loss of electrons aka "OIL RIG"
2. The GAIN of Oxygen
3. The LOSS of Hydrogen

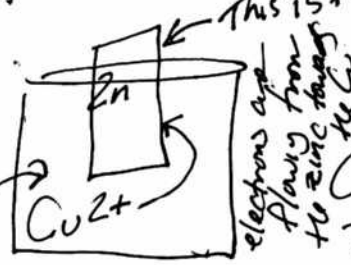
Guess what we have here? The loss of hydrogen!!!
What a great real life example.

To reduce the bonds
the "redox potential" of glutathione is
sufficiently negative (i.e. a reducing agent)
to reduce oxidized cysteines back to
their SH form.

Redox potential of glutathione is -240 mV.

Two sources now
So what does redox potential mean?

Redox Reactions



Zinc metal in Cu^{2+} solution.

Cu forms on top of the zinc metal.

This is amazing, isn't it?

This solution is the cathode

$Cu^{2+} + 2e^- \quad .34\,V \rightarrow Cu(s)$

$Zn^{2+} + 2e^- \rightarrow -.76\,V \rightarrow Zn(s)$

So we are presuming Zn^{2+} is a reducing agent and Cu^{2+} is an oxidizing agent.

Is this true?

Zinc is losing electrons & Cu^{2+} is gaining electrons to form copper metal.

So Cu^{2+} is being reduced, and zinc is indeed the reducer.

Zn is being oxidized, & Cu^{2+} is the reducing agent.

So the potential tells you if it is a reducing agent or a oxidizing agent.

(anode) Zinc is being oxidized

$Zn_{(s)} \rightarrow Zn^{2+} + 2e^- \quad +.76V$ (a reducing agent provides electrons)

Cu^{2+} is being reduced (cathode)

$Cu^{2+} + 2e^- \rightarrow Cu(s) \quad +.34V$

$1.10\,V$

As we must learn that oxidation takes place at the anode (– terminal) and reduction takes place at the cathode (+ terminal). Electrons flow from the anode to the cathode.

Try this reaction (s)!

Did it - entirely successful

Now how could we find the "minimum" metal that will react w/ Cu^{2+} solution?

$-X + .34 \geq 0$

$-X \geq .34$

$X \leq -.34$

Looks like $Fe^{2+} + 2e^- \rightarrow Fe(s)$ $-.44V$

would be the most practical metal available.

Look @ Aluminum! it is $-1.66V$

Try these @ home.

$Al_{(s)} \rightarrow Al^{3+} + 3e^-$ $+1.66V$

$Cu^{2+} + 2e^- \rightarrow Cu(s)$

n

$2Al(s) \rightarrow 2Al^{3+} + 6e^-$

$3Cu^{2+} + 6e^- \rightarrow 3Cu(s)$

Σ:

$2Al(s) + 3Cu^{2+} + \cancel{6e^-} \rightarrow 2Al^{3+} + 3Cu(s) + \cancel{6e^-}$

$2Al(s) + 3Cu^{2+} \rightarrow 2Al^{3+} + 3Cu(s)$

So what happens when you apply electricity?

How do you know how it all cats & reacts?

You could do the reaction also!

~~P351~~ P351 Outline of Chemistry

We start to see what happens.
We put a current through $CuSO_4$
w/ inert platinum electrodes.

We have Cu^{2+}, SO_4^{2-} & H_2O ions in solution.

(1) Oxidation always occurs at the anode.
(ie, stealing of electrons always happens @ the anode)
(This is electrolysis!)

$2H_2O(l) \rightarrow O_2(g) + 4H(aq) + 4e^-$ (anode)

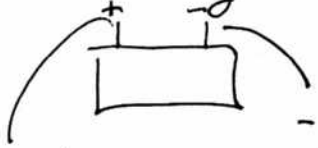
(2) Reduction occurs @ the Cathode

$2Cu^{2+}(aq) + 4e^- \rightarrow$ ~~$2Cu(s)$~~ $2Cu(s)$

So total reaction is:

$2H_2O(l) + 2Cu^{2+}(aq) \rightarrow O_2(g) + 4H^+(aq) + 2Cu(s)$

In a battery



+ is the anode

- is the Cathode

In a Galvanic cell

+ terminal is the Cathode

- terminal is the anode

So indeed they are reversed!

Because you know that in a battery, electrons actually flow from the negative terminal to the positive.

Another reaction to test.

Page 106

So it is a great question, how do end up w/ ferric oxide (black) by electrolysis? How would this happen?

$Fe^{+2}O_2 \rightarrow 3Fe(O_2)_3 \rightarrow 3Fe_2O_2$

$3Fe^{+2}(O_2^{-})_3$ should be black colored.

$Fe^{+3}O_2 \rightarrow 2Fe^{+3}(O_2^{-2})_3$ (should be rust colored)

+6 -6

+6 -6

So what if we had reduction taking place.

$Fe^{+3}X + e^- \rightarrow 3Fe^{+2}(O_2)_3$

This would indeed be a reduction so this make sense.

This would indeed be a reduction, (This should be taking place at the negative terminal of a battery (cathode)

What happens @ the anode?
Do we get oxygen released? We could check this
This would be electrolysis.

So the expectation is that we have electrolysis of water taking place plus the reduction of a ferric ion compound to produce a ferrous ion compound.

Now let's try to predict the voltage needed for these reactions to occur

First, assume no voltage w/ $CuSO_4$ & H_2O

$2H_2O(l) \rightarrow O_2(s) + 4H^+(aq) + 4e^-$ $-1.23V$

This is oxidation.

$2Cu^{2+}(aq) + 4e^- \rightarrow 2Cu_{(s)}$ $+.34$

$= -0.89$

means the reaction will not take place spontaneously until you add about 1 volt!

Now do ours:

oxidation $2H_2O(l) \rightleftharpoons O_2(s) + 4H^+(aq) + 4e^-$ $-1.23V$

reduction $Fe^{3+} + e^- \rightarrow Fe^{2+}$ $.77$

-0.46

So if we add about 1/2 volt the reaction should start.

There are also ways to determine concentration by current flow.

A Table of Standard Reduction Potentials is always very valuable.

Let's look @ glutathione + iron.

Glutathione has a reduction potential of $-.23V$.

If Fe^{+2} is oxidized, then

$Fe^{+2} \rightarrow Fe^{+3} + e^-$ (oxidized) $= -.77$

Glutathione reduced is

Iron is reduced, Glutathione is oxidized

$Fe^{+3} + e^- \rightarrow Fe^{+2}$ $+.77V$

Glutathione oxidized is $+.23V$

$1.00V$

This should mean the reaction proceeds spontaneously.

Glutathione is a spontaneous reaction (has the advantage of recycling itself)

Redox is a famously broad of chemistry it can be fused to.

1. Identify many many reaction that take place
2. Predict whether a reaction is spontaneous or not or if it requires external energy.
3. Predict what the reactants are likely to be.

Lets look @ Glutathione more closely.

Oxidant / Reductant

Glutathion → Glutathione 2e⁻ −.23
(oxidized) (reduced)

(oxidized)

so

Glutathion → Glutathione + 2e⁻ +.23V
(Reduced) (Oxidized)

by observation

Fe^{+3} + e⁻ → Fe^{+2} +.77

reduced

1.00V

Now to balance

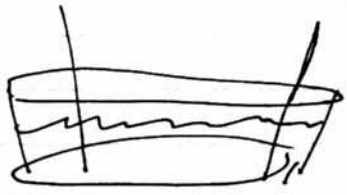
2 Glutathion + $2Fe^{3+}$ → Glutathione + $2Fe^{+2}$
(Reduced) (GSSG) (Oxidized)
(GSH)

These have names

GSH is $C_{10}H_{17}N_3O_6S$

Worked perfectly.

We have proven an electrolytic cell



Filled w/ solution of Ferric (III) Chloride
Subject to 24V

White Terminal
—

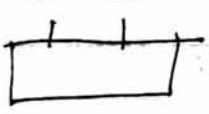
Green Terminal
+

Fe^{+2} Oxide is forming @ the cathode (reduction)

We know reduction is taking place here.

Oxidation is taking place here.

anode + cathode −



With a battery

(reversed from a galvanic cell)

yes, oxidation is taking place at the anode
reduction is taking place at the cathode

You have also done a test of reducing $Fe^{+2}Cl_3$ w/ glutathione. Worked perfectly. Fe^{+2} test is highly positive.

Without glutathione, the Fe^{+2} test in ferric chloride is negative. You predicted this would happen.

Ascorbic acid has a redox potential of +.06V = 60mV

$GSH \rightarrow GSSG + 2e^-$ (.23V)
reduced Oxidized

Cysteine $+ 2e^- \rightarrow$ Cystine (.34)
$\mathcal{E} = .57V$

The left to the reaction should occur spontaneously.

Now, lets go over

What is an oxidizer
What is an oxidant
What is a reducer
What is a reductant.
Do oxidizers get reduced, & what? & vice versa
What is a reducing agent?
What is an oxidizing agent?

yes.
The substance that gains electrons is the oxidizing agent. It is also the substance that is reduced.

Lets look at our Cu Fe example

$Cu^{2+} + 2e^- \rightarrow Cu$ $+.34V$

$Fe \rightarrow Fe^{2+} + 2e^-$ $+.44V$

$.78V$

Spontaneous Reaction

Now here is what is happening:
Cu is being reduced.
Fe is being oxidized.
Cu is the oxidizing agent.
Fe is the reducing agent.

We also know that oxidation is:
1. the loss of electron
2. the loss of hydrogen
3. the gain of oxygen

Oxidation is actually a flow of electricity, a flow of current.

So ~~alternately~~ redox will always be a gain & loss of electrons

Here is an example of oxygen & Copper.

$\overset{0}{2Cu} + \overset{0}{O_2} \rightarrow \overset{+2}{2Cu}\overset{-2}{O}$

But what is really happening is:

$Cu \rightarrow Cu^{2+} + 2e^-$ Cu is oxidized

$1/2O_2 + 2e^- \rightarrow O^{2-}$ O_2 is reduced.

How would ya know this?
Well, look @ oxidation state of 2CuO

$2Cu \rightarrow 2Cu^{2+} + 4e^-$

$O_2 + 4e^- \rightarrow 2O^{2-}$

or

$2Cu + O_2 + \cancel{4e^-} \rightarrow 2Cu^{2+} + 2O^{2-} + \cancel{4e^-}$

$2Cu + O_2 \rightarrow 2\overset{+2\ -2}{CuO}$

Well Cu has gone to Cu^{2+} means it has lost ~~oxygens~~ electrons & this shows the Cu has been oxidized.

Are more reactions redox or ionic?

* Redox reactions are the most common chemical reactions in biology.

2 reactions are either

redox

or

~~Ionic~~ (electrostatic)
Ionic
Covalent

Now let's think about oxidizers & reducers
vs the right & left sides of the periodic table.
Reducers give electrons
Oxidizers steal electrons.

If something is reduced, it gains electrons.
If something is oxidized, it loses electrons.
(steals)
Oxygen wants to gain electron.
The would mean that oxygen should
is reduced, but that it acts as
an oxidizing agent.

Fe wants to give electrons. The means that
iron also is oxidized. Then it means
that iron acts as a reducing agent.

A great summary

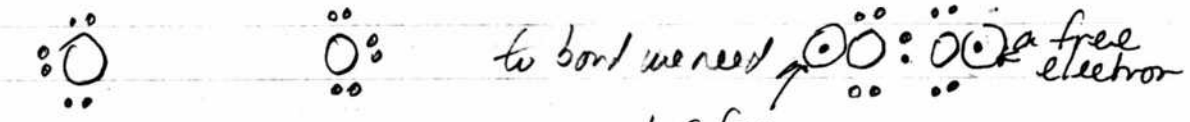
* Free radicals are a reductive process applied to free oxygen, ie, the gain of successive electron.

Free Radicals - A study. THIS PDF PAPER IS SUPERB!
We have some good pdf's in the reader now.

Page 116

O_2 itself is a "diradical"
A radical is anything that has a free electron (not a pair)
A pair of electron is called a lone pair.

O is $1s^2 2s^2 2p^4$ so 6e⁻ in the outer shell
so O_2 is



so O_2 itself is "pretty radical" / a free electron

This came from our pdf file on our reader.

"Called Free Radicals, Oxidative Stress and Diseases" by Enrique Cadenas

Now the key to understanding is that we follow a sequence:
The sequence is

superoxide → hydrogen peroxide → hydroxyl radical → water

$O_2 \rightarrow O_2^- \rightarrow H_2O_2 \rightarrow OH \rightarrow H_2O$

- O_2: it is a diradical
- O_2^-: one unpaired electron, it is a radical (actually not all that reactive)
  - We add one electron (this is reduction) this gives O_2 a negative charge (becomes an anion)
- H_2O_2: no unpaired electrons, it is NOT a radical
  - Now we add another electron (Notice this now satisfies the octet rule) even though it is not a radical it is an oxidant
- OH: the most reactive oxygen species
  - Now we add another electron and it creates OH and water combined
- These come out together as the result of adding another electron

Glutathion BOTH:

1. Breaks Disulfide Bonds!
2. Breaks down Hydrogen Peroxide!

No wonder it is incredibly powerful.

So if something steals electron and make them available to O_2 (a duradical itself) every thing goes downhill from there.

$O_2 \rightarrow O_2^- \rightarrow H_2O_2 \rightarrow$ (OH → H_2O)

~~also the Fenton~~ Fe^{+2} and Fe^{+3} in combination with O_2 & O_2^- & H_2O_2 is really bad news as it make the of hydroxyl radical,

The pdf by Enrique Cadenas is amazing.

What are your most pressing questions @ the time?

We have made a major breakthrough in discovery
the effect of glutathione
1. breaks down disulfide bonds
2. reduces iron from +3 to +2
3. destroys H_2O_2 and
the major oxidants.

The biggest question is how to increase
glutathione levels in the body.

Microscope Imaging Oct 22 2012

You have great results with

1. Light full bright
2. stage midway (small adjustments can make a significant difference)
3. Exposure max
4. Gain very low, no more than 1/3 up
5. Bright near 2/3 up, small changes make a big difference
6. Contrast 1/3 up
7. Color up 1/3
8. White balance up 1/3

Auto flicker off

Stating all the difference between

Ionic bonds
Covalent bonds

and Redox Reactions.

Ionic bonds involve a transfer of electrons (between metals & non metals)
Covalent bonds involve a share of electron (between non metal atoms)
A Redox reaction involves the exchange of electrons.
Redox is a type of reaction, not a type of bond!

It seems that ionic bonds and covalent bonds can both very much lead to redox reactions.

Al^{+3} Ion Test!

I now have a means to test for Al^{+3} ion in solution.

I made $AlCl_3$ (Aluminum Chloride) by reacting Aluminum foil w/ Hydrochloric Acid.

The test for Al^{3} is adding aqueous ammonia.

It form a white precipitate.

The reaction is:

$$Al^{+3}(aq) + 3NH_3(aq) + 3H_2O \Rightarrow Al(OH)_3(s) + 3NH_4^{+}(aq)$$

so it forms of white gelatinous precipitate of $Al(OH)_3$ ie aluminum hydroxide.

Now a question is what dissolves $Al(OH_3)$?

Rainwater Volume Summary

800 ml
+ 500 ml
+ 190
1490 ml total → 175 ml Factor 8.51

If we have 1000 ml left
our concentration ratio is $\frac{1490}{1000} = 1.49$

We are now measuring
TDS of 30

so $\frac{30}{1.49} = \underline{\underline{20}}$ TDS in original sample

A little higher than I would suspect.

We anticipate reduction to ~ 200 ml
This will lead to a TDS of 149.

$\text{Wavenumber} = \frac{1}{\lambda_{\text{microns}}} \cdot 1E4$

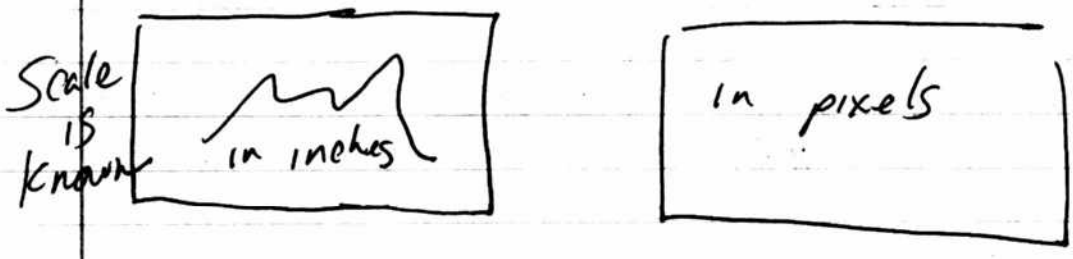
or $\lambda_{\text{microns}} = \frac{1}{\text{Wavenumber}} \cdot 1E4$

Water resonance ~ 3 μm.

$C = \lambda \cdot f \qquad f = \frac{C}{\lambda} = \frac{1E8}{3E-6} = 3E13$

Digitizing a spectrum:

I have an idea how we can digitize a spectrum.



transform the two into common ground. Then can be done. This will give you a digital spectrum that you can key into specview in 32.

Idea, Can't we have a molecular resonant frequency?

We have learned about your first important organic reaction with a functional group.

Acetylization

Amino groups (they are in amino acids for example) can be acetylated.

Acetyl is CH_3CO
When it attaches to something it helps it to cross the blood brain barrier. It is how aspirin is created.

Acetylization is forming the acetyl group w/ a molecule.

Also, "Carboxyl groups can be esterified" This is the type of rxn. you are seeking.

We are starting to see how it works.

Nothing in the holder it should read @ 100.
Nothing but Chart & time need to be set
Gain is set for a 10% reduction (i.e 90%)
when the gain light is on.

The manual slot does not need to be on.

The wheel sets it to 100 even.

Your hand blocks it, goes to Ø

Make sure the sample is aligned.

Always turn on WITH NO SAMPLE!
& No Sample!
Gain 158
Transmittance @ 100 w/ wheel w/ Gain ~~off~~
Hand blocks drives it to zero. Gain 150-240
I have got it!
3 min Chart
No manual slot needed! Ø5

It was an oil in gear problem!

A Reset may be needed if you turn it on INSIDE w/ a SAMPLE

10 100
100 to 90

1. Let instrument to boot up
2. Set Chart & Time to 0.5, 3 mm
3. Push gain up so that it hits at about 90%
4. Turn gain off & it should be about 10
5. 10% difference 100 → 90 between (Gain Off) (Gain On)
6. Zero transmittance to 10 if needed.

Petroleum Jelly on Polyethylene

4000	96
3538	96
3462	100
3116	92
2968	43
2916	12
2843	
2609	85
2345	94
1999	96
1789	100
1543	97
1455	

4000	96
3900	96
3800	96
3500	96
3400	100
3300	100
3200	100
3100	100
3000	90
2900	75
2800	75
2700	75
2600	93
2500	84
2400	87
2300	87
2200	87
2100	—
20	—
19	—
18	—
17	95
16	95
15	96
14	92
13	87
12	

40	95
39	98
38	100
37	100
36	100
35	—
34	—
33	—
32	—
31	95
30	90
29	5
28	10
27	85
26	95
25	95
24	98
23	100
22	91
21	100
20	100
19	95
18	100
17	91
1650	90
1600	95
15	100
1450	80
1425	40
14	80

1350	60
1300	85

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Vaseline

Lows @

2950	10
2850	0
1440	30
1370	30
720	60

Nujol (liquid paraffin) has peaks @ 2900 & 1450 (mulling agent) (p 30)

Compare to polyethylene

Look CnHn(2n+2) eg C_4H_4 C_2H_6

2950	~20
2850	~20
1435	80
1350	
850	70
700	70

Alkanes Carboxylic Acids

Alkanes, Carboxylic Acids

S=O (Sulfate), Aromatics C-C in ring

Sulfates

Blood is interesting.

Culture

4000 ~~9BB2~~

3200	~~75~~	3100	~~75~~
1600	70	1600	70
1100	63	1100	63

3200 is Caboxylic Acid

1600 is Carboxylic Acid
Amides
Amines
Alkenes
N=O Nitroso
C-C in Rig

1100 is Alkyl halides
Alcohols
Ethers
~~✓~~ Amines m
~~✓~~ Amines m
thio carbonyl
Carboxylic Acids

IR Spectra.

We have some very good information
Convey to.

Cleeha Polyethylene

Peaks @ 3270, 3210, 1830, 800

From Control Chart:
Peaks @ 2900, 2850, 1500 & 700

$\Delta =$ 370 360 330 100 $\quad \bar{X} = 290 \neq 300$

Why is this? Why $\Delta = 300$

I visually read peaks @
2900 2850 1450 700
~~3200 2896 1519 which appear better.~~

So what is true here?
Which met the control chart perfectly.

The indicate our chart is too high by 350. Why?

350 too high adjust to

2920, 2860, 1480, 450 (out of range?)

Cathie:
Our plot has peaks @

3575, 2000, 1700 & 1175

We read visually peaks @

3288, 2096 & 1579. This is quite different.

We need some control spectra.

I think our plots are up too high by 350.
Why?

Look at this
In the instruction book we have
FOR 1.0 Chart

4000 - 2000 2000 - 600

100 cm^{-1} / cm 50 cm^{-1} / cm

are recorded.
So Scales are not constant

But we are using 0.5 Chart

4000 - 2000 cm 2000 - 600

200 cm^{-1} / cm 100 cm^{-1} / cm

Culture Plot:

3450 Alcohols
~~~3350~~ ~~Carboxylic Acids~~ ~~R$\overset{O}{\overset{\|}{C}}$-OH~~
~~C=C-C$\overset{O}{\overset{\|}{C}}$-OH~~

Amines R2NH
Phenols Ar O - H

~2800-2850 Carboxylic Acids 3400-2800 broad

This is New?

2250 Isocyanide R-N-C
Subject to polymerization

1560-1580 Carboxylic Acids
Amines

1400-1450 ~~Carboxylic~~ Acids
(Sulfates)

500?. Disulfide
(~~550~~ 540-500)

Carboxylic Acids are supposed to have a sharp band between 1680 & 1725 cm^{-1}

You are not seeing this!
~~~

In both the culture and the blood we have a wide strong peak @ ~3425

We also have a strong peak (sharper) @ 2850 to 2950

This matches a phenol coupled with a alkane.

Phenol Ar O–H bonded } ?

alkane R–CH_2CH_3 }

The carboxylic acid couplings not matching up.

Blood & Culture

3400 appears to be an alcohol or phenol OH Stretch
broad band It can be alcohol or phenol

~~3000~~ -
2850 - 3000 is alkane CH Stretch

We could have an amine double dip up within the alcohol. This would lead to a peak @ ~1600 for an amine.
We have that.

We do seem to have an alkane from 3000 - 2850. This means it could be a phenol instead of an alcohol.

This 2110-2165 looks like a prospect of isocyanide.

But this region can also be an alkyne from 2100 to 2140 The a close.

But if it was an alkyne I should have other regions @
3310-3210 I do not.
700 - 600 I do not.

This casts weight toward isocyanide.

Now we have another peak @ 1400

This could be either a Carboxylic acid which would be a very sharp peak (which it is not) Carboxylic acid
or an sulfate which is dead center. and looks very reasonable.

But we also could have a Carboxylic acid doubled up @ 1400 which expects a Carboxylic acid from 1550 to 1610 which we did not have.
It could also overlap w/ an amine.

restore

[redacted]

Xerox your spectra to be able to write notes upon them.

Anything w/ water in it (eg, culture stock solution, actual samples etc) appear to have false peaks (maybe ether blend?)
@ ~ 3329 OH group
~ 1657 (alkenes dominate)

We have a problem.
1. Interference from water
2. Interference from polyethylene

Polyethylene is hardly ideal.

Polyethylene w/ H_2O ($NaOH$ is not really much

How can I really break down the filament?

I think that water soluble samples are giving you very distorted spectra.

Grim 11B

Manual Slit On
w/ Slit adjust to 173

Is giving some interesting results.

It is actually very similar to previous plot but at the slightly lower resolution.

To go the default on the slit width. A right mouse click widens the slit, allows more light, decreases the resolution.

A low mouse click increases the resolution but allows less light in.

I have a problem. I am not sure that I am able to reliably collect a spectrum of a water based sample such as:

1. oral filament ?
2. Culture ?
3. blood ?

} why do they all come out the same?

4. Vitamin C
5. Citric Acid

Polar solvents such as water & alcohol are seldom used because they absorb strongly in the mid IR range and they react with alkali metal halides such as NaCl.

Need BaF_2
$AgCl$

High absorbance may be leady to the narrow band of the spectrum. I am not sure how that is to be exaggerated.
Manual slit width narrowed?
Gain increased?

What do we learn?

1. Water in itself has a fairly significant spectrum

Does it match, or do we have significant contamination from polyethylene?

So clearly you have contamination from poly e!

So clearly your spectrum of water is a combination of polyethylene + water.

We would therefore expect this for most anything else.

But if we add water to poly e we should be removing most of the contamination issue

Non polar substances, such as alcohol seem to give good results. on poly e.

Notice on the blood culture oral sample } specimens we do not seem to be getting any polyethylene contamination.

We also have good plots of polyethylene, & plexiglass.

We have problems getting a plot of

1. the EPA filament
2. Hair
3. Ascorbic Acid } why?
4. Citric Acid
5. Acetic Acid -

Also we have a problem. I think we need salt crystals

Question:

Why can't not get a broader range of transmission with

Citric acid ?
ascorbic acid ?
acetic acid ?

When I compare the polyol + water?

Acetic acid looks like it starts to work when water is subtracted but we cannot get enough range - why?

I have improved certainty dramatically

Gain = 265

adjust transmission & Gain to 10%
w/ sample in

~~Hit Manual Set Calibration!~~

Ok it looks like I have found it.

Gain is set to 265

Wheel is set to transmittance @ start of graph

We should be able to duplicate this now

Turn wheel up - maybe only 5% deflection instead of 10%

The gain is varying now between 265 & 445

Looks like weak solution need high gain, strong solution low gain

I believe that you are making progress.

Gain varies between 250 & 450.

Transmittance to be set @ highest point of spectrum.

Samples must be very timely because they dry out.

We believe our spectrums of
1. oral sample
2. culture
3. blood are

are viable.

We do not understand why we can not interpret any.

1. citric acid
2. ascorbic acid
3. EPA sample
4. hair

Why do you have the problem?

I think the sample size have to be between 2 - 15 u.

I have good plots of real sample w/ scan ranging between 265 - 450.
Transmittance set to ~ 95% after calibration.

It looks to me like we get good graphs

1. sample size between 2 - 15 u
2. Gain set 250 - 450 350 avg looks fine
3. Transmittance @ max point set to approx 95%
4. Scan deflection of 10% does seem OK.

You have a successful culture plot by concentrating the solution by partial vaporation

Gain is 350
T is 95°

I am zeroing in on the influence of H_2O

Water	Polyethylene
3404	2920 – 2850
2127	1464
1643	719

Now we see that this combination produces much of what we see in the influence of both water and polyethylene.

So what happens if we try to remove it?

So here are some questions:

If I know what influence water & polye have, and I do w/ very good accuracy, why can't I remove it?

Why doesn't Lair dissolve in strong sulfuric acid?

Question: If something is transmitting too much light, why not just move the card up to knock down some light?

Remember to always clean the trays before boating!

SBS SDBS Search

C_6H_5BrFN 2-bromo-4-fluoroaniline
C_5H_4BrNO 2-bromopyridine-N-oxide-hydrochloride

Using all peaks below 2400

Matches with:
3350
2350
2080
1820
1550
1300
1000
700

3825 3350 2350 gives C_5H_4BrNO HCl
Close
2-bromopyridine N-oxide hydrochloride
3825
3350 +/- 40
2350
2080
1820
1300
1000
700

add 1550.!

3350
≀
1550 added

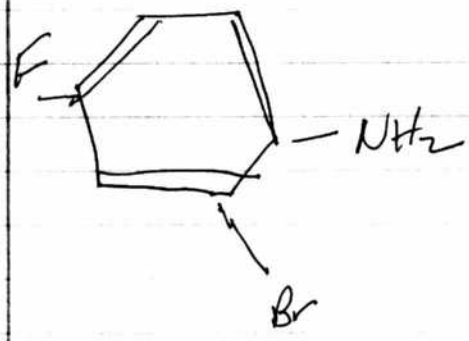
Br F OK +/- 50
also HCl version

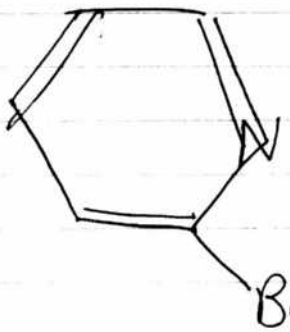
HCl only +/- 40

2350
≀
1550 +/- 40

HCl only

Stronger candidate





		T < 80%	
NO	3850	+/- 60	OK
NO	3350	+/- 50	OK
2425-2400	2350	+/- 40	OK
	2000	+/- 30	NO
	1820		
	1550		
	1300		
	1000		
	700		

2300 2380
2380 2300

C_6H_5BrFN is given by <60% T +/- 50

~~3350~~ ~~3460, 36 - 3342, 32~~
2350 ~2400 small ~~trou~~ dip
2050 ~2050 small peak
1820 ~1820 small peaks
1550 ~1500 - 4 strong peak
1300 1308 - 38
1050 1030 - 34
700 674 - 32

Spectral Database for Organic Compounds SDBS

u	cm^{-1}
2	5000
3	3333
4	2500
5	2000
6	1667
7	1428
8	1250
9	1111
10	1000
11	909
12	833
13	769
14	714
15	666

You are finding things in the books & the database search that you are not finding in the lab.

Instance: Analysis of benzene in plant Million book.

Now what does IR Pal say?

~3850 Nothing shown

2300 2440 – 2280 P–H Phosphine
2350

2080 R–N=C=S (2140 – 1990)

1820 Anhydrides

1530 Carboxylic Acids
Amides
N=O
N–O

1300 Many Choices

1000 Lots of Choices

700 Lots of Choices

You are really starting to learn things now. You take your time and you start poking through each section and what it really means. You have seen that a 700 peak can vary from 671 to 815 ($\Delta = 184$) just by variations in Hydrogen attached to a very structure.

And yet look @ the case of benzene around 671 on p 140 Nelson. What a perfect match with what you show. Also you learn that the solvent a preparation can affect magnitudes greatly. Also you see that benzene stretches between p 140 & p 112.

You have to see the big picture first.

In our spectrum the biggest factor after polyethylene is the peak @ 700. The polyethylene is that of alkanes. Alkanes are the simplest. C_nH_{2n+2}

alkanes - single
alkene - double
alkyne - triple

Now what does 700 mean?
It can mean:

~~(single)~~ Alkenes (double)
~~(double)~~ Alkynes (triple) Not a good fit.
Aromatics
MISC Not a good fit
Amines Not a good fit.

Now, with 700 peak; we are left with

Alkenes 725-675

Aromatics 730 - 675

With alkene we should be looking for

alkenes are a possibility:
Peaks at

3140 - 3040 doesn't look like it

* 1675 ~~1600~~ - 1560 possible on lower end
* 990 possible on upper end
910 doesn't look likely

Now look @ aromatics

* 885 - 675 Strong indication
1400 - 1500 Not usable
* 1585 - 1600 good indication

3000 - 3100 not usable.

$cm^{-1} = \frac{1}{\lambda_\mu \cdot 1E-4}$ so $\lambda_\mu = \frac{1}{cm^{-1} \cdot 1E-4}$

Now the overtone region of aromatics
2000 - 1600
shows a prominent peak @ 1820.

There is right in the middle of the overtone region. The appear to be a ~~strong~~ strong candidate therefore and

overtone patterns are characteristic of the ring substitution.

What exactly is ring substitution.

It is called electrophilic aromatic substitution.
The hydrogen atom on a ring is replaced.
As a result of an electrophilic attack
on the aromatic

Consider the real prospect that you have
both alkenes & aromatics. The
would certainly be possible. Do not
eliminate the alkene unnecessarily.

So the peaks @ 722 & 1820 along w/ fuzziness
of the spectrum indicate the possibility of both
aromatics & alkenes, & there is no reason you
can not have both. The 1820 however makes a fairly
strong case for a substitution on the benzene ring.

So now you have peaks @ 2380, 2300, 2080 &
1020 to help settle the scene.

The Remaining Problem:

Also notice IR Pal does not give an alkene @ 1820.
(But notice it also does not give an aromatic, but remember there is an overtone, so it is a mix of other frequencies)

IR gives an anhydride which I have no idea what it is yet.

Lets look @ 2380, 2300, 2080 & 1020

The remaining problem

~~Not a good match~~

2440 - 2280	P-H Phosphine	Stronger
2360 - 2100	Si-H silane	
2080 (2140 - 1990)	MISC	R-N=C=S
1020 ±		
Alkyl Halides	1350 - 1000	R-F
Misc (Phosphine)	1250 - 950	P-H
MISC	1050 - 900	P-OR
MISC	1110 - 1000	~~RCO-OH~~ Si-OR
Carboxylic Acids	1320 - 1000	RCO-OH
Esters	1320 - 1000	RCOOR

Lots of choices here @ 1020.

An alkyl-halide is a Carbon-halogen bond
It is also a
"halogen substituted alkane"

TP
Internet
Temp

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A phosphine is a "hydride" of phosphorus, PH_3

spontaneous flammable gas, fishy odor, diffuses rapidly and penetrates deeply into molecules, toxic very

These alkyl + aryl derivatives of phosphine are analogous to organic amines.

They can also form metal complexes.

Organophosphines are important in ~~complexes~~ catalysts when they complex to various metal ions.

It can also have a tie-in w/ bromine

Now with C_6H_5BrFN

		My Peaks	$C_6H_6BrNOHCl$
2380	2340 ~~2380~~ 2300	2350 - 2380	~~2350~~ 2450-2400
2080		2080	2080
1850		1820	1830
1300		1300	1290
1020		1020	1040
680		690	690
		Has distinct overtone peaks	many more peaks

Toxic
Methemoglobinemia
Complexes w/ Thyroid
Electron stealing

2440 - 2350 Mine IS 2380
PH T12 -(1)

Nakanishi Table

PH is indeed a Candidate

But my shaped peak is @ 2300

T4 C≡

IR table Pal

2340 IS a very strong Candidate for a phosphine

Phosphines form metal complexes & readily react w/ Bromine

The latest search is @

2340
2080
1850
1300
1020
600

+/- 50 includes C_6H_5BrFN

but +/- 40

reduces it to

benzyl thiocyanate

C_8H_7NS

We also have a match

Benzyl thiocyanate +/- 30 fails all

Colorless crystalline water insoluble
used as an insecticide

Saturated or unsaturated amine is a strong candidate!

2340 Phosphine is a strong candidate
study organophosphine
Can easily be a saturated amine! also p 39 Nakanishi

~~2080~~
Still need
2080 IR Pal gives one candidate ~~R~~N=C=S
1310 1250-1360 Aromatic ~~Amines~~Amines T CN Stretch (aryl)
~~1000~~ 1250-1370 various NO_2 T9

1000 P-O-C T12

12/30/31

We have a pretty strong case for the benzene ring. 700 cm^{-1} drop and the 1600 - 2000 overtones.

We also have a strong peak match out of ~30k spectra w/ C_6H_nBrF compound.

Now we have additional peaks @
2340
2080
1310
1000

We got a value 2340.
We see from IR Pal it could be P-H (2440 - 2280)
but we also learn from Nakanishi that it could equally be a saturated or unsaturated amine. : p39
@ 2250 - 2700 (saturated)
2500 - 2300 (unsaturated amine) (Stronger C and O)

~~Next we look~~ @ # SDBS
for 2340, 2080, 1310, 1000

SDBS Search

C_6H_5BrFN 2-bromo-4fluoroaniline
C_5H_4BrNO 2-bromopyridine-N-oxide-hydrochloride

Using all peaks below 2400

Matches with:
3350
2350
2080
1820
1550
1300
1000
700

3825 3350 2350 gives C_5H_4BrNO HCl
alone
2-bromopyridine N-oxide hydrochloride
3825
3350 +/- 40
2350
2000
1820
1300
1000
700

add 1550.!

~~SBD~~ SDBS is not available right now in Carlsbad.
Nakanishi is a good alternative.

Nakanishi:
2340 $C{=}N^{+}{-}H$ (T1) but also saturated amine
PH (T12)
2250-3000 Ammonium Compounds (T1) p 39
This sector is very important because aromatic amines are from 2300-2500 which is right on our range because we are actually seeing from about 2300 to 2400
He also says broad or a group of sharp bands. This fits our spectrum.

But now notice what is coming from the 1310 series.
We also are given on p 38 from 1250-1360

$Ar-N(R)(H)$ which also is saying an aromatic amine!

also IR Pal shows the same thing
An aromatic amine from 1250-1360.

So the case for an aromatic alkyl amine is now very strong.
700 drop off
1600 overtones
2340 peak group
1310 peak
} the set strongly indicate an aromatic amine.

Now we also have a strong match occurring w/ SDBS with halogen - C6 Compounds.

Since benzene rings are so flexible we must consider both of these combinations equally likely now.

	Based upon
We are likely to have	2340
1. Aromatic amine	1600
2. Halogenated Aromatic	1310
	700

We still have 2 more peaks to consider
2030
& 1000.

But before we go there search SDBS
2340, 1600, 1310 & 700

+/- 70 88 hits
60 31 hits N, H, O compounds dominate

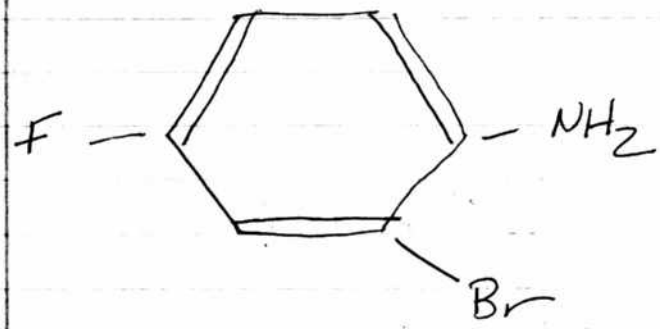
Now restrict to Br:
One alternate shows up C_5H_4BrNO HCl

Now boost back up to +/- 70% w/ Br:
This leads to adding:
$C_{11}H_{10}BrNO$ HCl

Now back up to +/-80%
This is where we pick up C_6H_5BrFN

Clearly we have compounds in the overall range that are comprised of C, H, N & O

but compounds such a Br, F, S can also emerge. We have already notched up the peaks across the board.



this is clearly an aromatic ~~acid~~ - amine.

We also must consider physical symptoms. Low temperature, methoglobenemia as characteristic of halogenated (aromatic amines)

" halogenated aromatic amines " metallo (Fe)

Many patents exist here.

Toxicity issues Come up:

Now we have work of interpretation of

2340 aromatic amine
1600 aromatic overtone
1310 aromatic amine
700 aromatic

Combined of SDBS.
What is left?

2080 ~~1000~~ also $C{=}N^{+}{-}H$ (T1)

also a whole slew of N, C, O, S Compounds (T4)

and @ 1000:
Quite a few options here. of Nakonishi

also IR Pal has quite a few options also.

Why halogens?

1. Methanogloberine
2. Toxic
3. Electron Stealing

* Toxic effects of halogenated Aromatic Amines,

Halogen bonds / Argument:

1. Observed health effects
2. Methemoglobinemia
3. Ease of chemical reaction (Hitchhiker)
4. Interaction w/ Iron

A powerful statement of fact.

undergo Google, Electrophilic halogenation

"If the ring contains a strongly activating substituent such as -OH, -OR or amines (!!!!)

a Catalyst is not necessary.

However, if a catalyst is used w/ excess bromine "tetra a tribromide" will be formed.

N is forced into the search now.
It has an aromatic amine:

Now we are making a search w/

2340 +/- 50
1600
1310
700

C=6

It must also be an aromatic ring w/ Nitrogen forced into the equation

We set 10 hits.

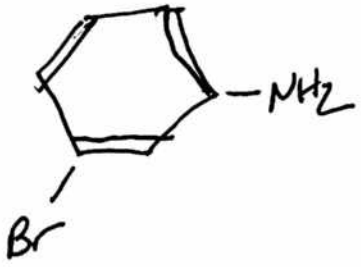
General Core structure is 25 Hits -

If Br is forced into the picture
We have only have 3:

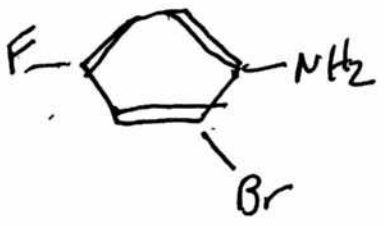
#		
1272	C_6H_6BrN	p-~~bromoaniline~~ bromoaniline
4872	C_6H_6BrN	m-bromoaniline
16606	C_6H_5BrFN	2-bromo-4-fluoroaniline

1272

4872



16606



Now, back off w/ Br and add in next peaks of

2080
1000

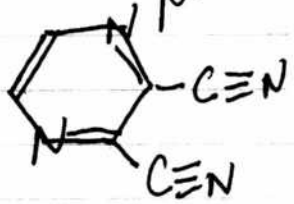
Sorry the search is CHN +/-50 with Conditional

2340
2080
1600
1310
1000
700

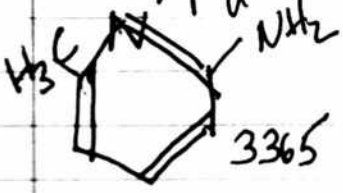
Now we get a total of four hits:

3365 $C_6H_8N_2$
18133 $C_6H_2N_4$
13771 $C_6H_8N_4$ 4HC
16606 C_6H_5BrFN

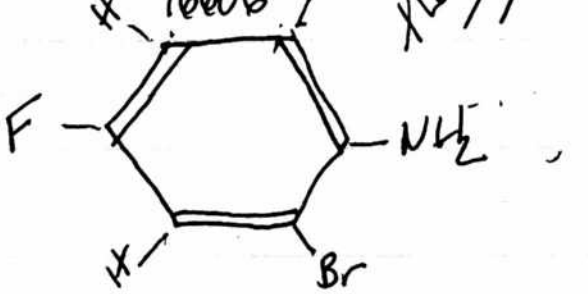
18133 Not aromatic!



13771
$H_2N-(CH_2)\ldots$
No Ring!



16606 H only one Not fits!



2-bromo-4fluoroaniline

Clem took box, also shown some analyses of gazelle. This will be in addition to Milo's chapter on aromatics.

IR Sample Preparation.

For liquid samples, you have a method.
Evaporate on a watch glass
Scrape to a powder.
Add Ethanol
Grind to a fine powder.
Let alcohol evaporate
Place dried finely ground powder within
a polyethylene sandwich.

You have also learned the sample does not
have to be dense. Sparse is sometimes
even better. It is all related to getting
the transmission of the sample, relative
to the reference, of 80-90%.

Ascorbic acid gave a messy and confusing
result.

The question now is, if something is insoluble
does it always give the same spectrum?

* It looks like ascorbic ~~not~~ acid (in alcohol & dried) gave the same general spectrum as the culture in alcohol dried

There is the critical and represents the potential for error!!!

Also, the oral sample did not repeat itself when it dried overnight!

What does this mean?

Problem appears to be:

If you have an insoluble material

You may be getting the same spectrum all the time???!!!

Keratin Studies

You are using drain cleaner (CLOG Remover)

(1) You are mixing mahogany w/ hair exposed to clog remover.

(2) You saw traces of blue. When you heated it, it turned yellow & yellow green.

(3) Now you do it again w/ no heat and it turns blue & purple, & am not blue yet.

(4) Then you take clog remover alone and combine it w/ mahogany. It does turn blue but it is a much different shade of blue. It is much lighter in color & it is not the same.

Now you are heating both (3) & (4) @ the same time. What is going to happen.

The resulting colors are entirely different. Hair turns green. Clogs by itself stays the same blue color.

We need to control better
Q. Hair in watchglass
1. 1 Pipette Caustic
2. 2 " Water
3. Time 2105-2145
4. 2 ml extract, 1 ml HCl = 3 ml total
5. Heat first the time to @ 90°
6. Add 3 drops Ninhydrin

It is light yellow before you ever start!
after heat, so that is some color.

Turns blue instantly but then clears up.

Add 3 more drops. Turns blue
Shake. Now turns yellow again
3 More: yellow again after blue
3 More: blue & then yellow;

This is the final color.

Now back into heated water.

Clogger Control

1 ml Clogger + 2 ml H_2O = 3 Total

Back to Hair
We added 1 ml H_2O] due to
1 ml Clogger] evaporation.
Back to heated test tube.
Color fixed as a burnt yellow

Clogger Control.
Hair is clear.
3 drops Ninhydrin - blue
3 more drops clear blue
It remains blue under heat.
3 more drops
Back to Hair
You centrifuge it.
There is a very slight yellow tint to
the hair treated w/ clogger & ground
up & centrifuged. But it is very
slight. Not nearly the same as w/ Ninhydrin

Spectrum remains blue. It is different

Back to Hair.
Use treated solution straight: Very light yellow.
6 Ninhydrin.
+3 Ninhydrin
I do not get the same color. Now I get a
dark blue green. Repeat. You left the hair
in the clogger a long time.

Repeat.
Hair again
1 ml Clogger + 2 ml H_2O
Time 2210
Grind & add 1 ml H_2O & 1 ml Clogger.
Ile heated ninhydrin solution
Should approx 15 min to turn
yellow green.
A coat is Changing from strong green
to yellow green.

Indeed the more it is heated the
more yellow it is turning
It is a slow process.

---

* Ok, you have repeated the process
Two identical spectra of hair now.
& it is nothing like the Clogger solution

You get a set of peaks between 400 & 450
that are unique. GOOD WORK.

You are optimizing if you can duplicate the
up the EPA filament.

Now centrifuge
Separate. (~ 3 ml)
Slight yellow color (OK)
Heat to 90°C
9 drops Ninhydrin - Deep Dark Blue

When you boil it it leads toward green.

When you let it sit long enough it turns darker yellow as a distinctive spectrum.

You do not know whether it is boiling or time that turns it yellow.

EPA filament.

A major finding

NaOH + KOH Strong solution & heat

is slowly dissolving the filament.

A dark ~~black~~ brown is separating

IR Spectrometer
Feb 28 2013

Culture Spec Analysis on KCl Plate

Three Strong Peaks

3300.
1600 - 1620
Broad Peak from 1025 - 1100

Secondary Peaks

~ 2950

3300 Alkynes
Carboxylic Acids
Phenols

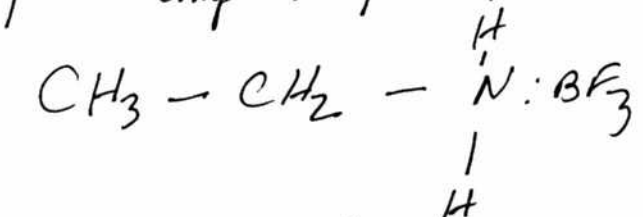
1600 - 1620
Alkenes 5 Ring
Carboxylic Acids
Amides
Amines

2950 Alkanes
Carboxylic Acids

Page 177B

~~1060~~ 1025–1100
Alkyl Halides
Amines
Carboxylic Acids
Misc

In SDBS we seem to have a fairly strong match in the Ethylamine Compound w/ Trifluoroborane



It is the organometallics class

Make sure search is on IR

Parameters of search are ±40 cm^{-1} & 0% T < 50%

Scale to 2225 Vertically.

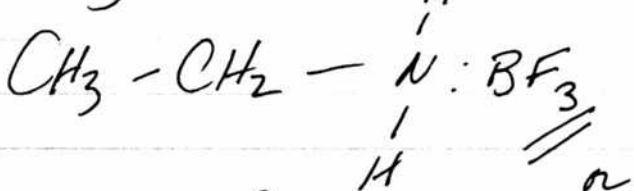
Organohalogens are almost all synthetic.

We know:

Organic
Amine Acids
Iron
Halogens very likely.

Possible Aromatic Compound.

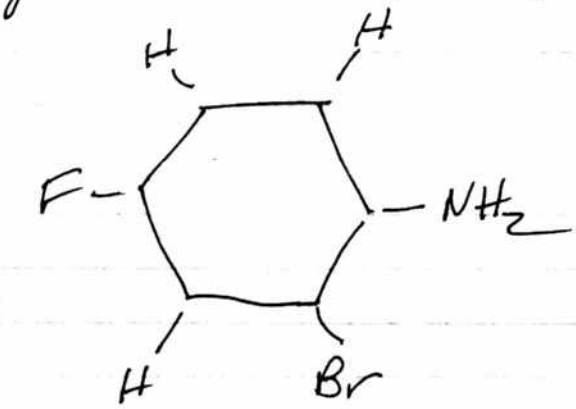
Strong Candidates are



Ethylamine Compound w/ Trifluoroborane

"Organometallic Halogen Compound"

or



2 bromo-4 fluoroaniline

Halogenated Aromatic Amine (also prone to tribromide)

Stronger Weight w/ KCl Plate

More uncertainties w/ polyethylene

Time to interpret the next Spectrum
Direct Oral Sample.

3400 ~~3300~~ Very broad. Alcohols, Carboxylic Acids
Amines, Amides, Phenols

2106, 2080

~~1600~~ 1620

1520 ~~1520~~ (small)

1435 ~~1435~~

1050

~600 (+/- 50)

There is something quite happening
~2950 both in Cultured
& Oral Samples

SDBS Results

March 00, 2013

1. ~~Want~~ Want really dry Lettuce

2. Separation is questionable — we may need to grind finer.

3. Culture & oral are really the same.

4. Raw filaments are a bust

5. KCl Card is beautiful

What is the Commonality between Culture & oral sample?

~~3300~~ 3400

2100

1600

1050

600

There is the possibility that water is slowly clouding the crystal & making it less sensitive.

Lets get a spectrum of water. GOT IT. Great

I think we see what is happening.
We have a spectrum of water. This was invaluable.

Mar 8 2013

A huge complication has developed.

Nujol on the pure KCl crystal came out perfect w/ almost no interference.

Nujol on a real crystal KCl came out almost identical to the sample. The means the sample spectrum is not accurate!!!

~~A Something that has been oxidized has lost electrons stolen~~ An oxidizer (oxidizer) agent (oxidant) steals electrons.

Reducing agents gain electrons ~~A Something that has been reduced has gained electrons.~~ A reducing agent gives electrons. Reducing agents become oxidized

So in the case of an electrophile (electron deficient, it accepts electrons) We should say that an electrophile is something that has been is reduced.

Now what does the dictionary say? Electrophiles are "reducing agents"!

May 10 2013

A huge issue has developed in examining the Magellan a Thesis paper:

Histidine!!!

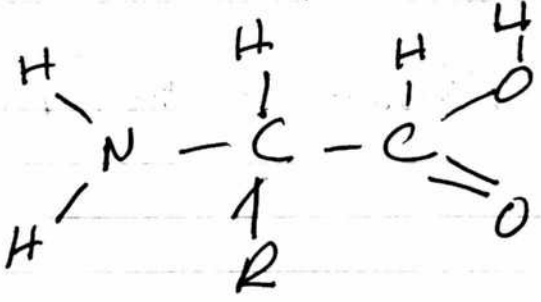
It is being found in the biological filament

But histidine is a part of blood also!

Get it!

What is the structure of histidine?

We know an amino acid has the structure:



R here is

Activator – Deactivator

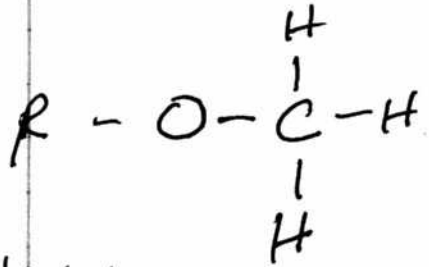
We have a great example here. The likely presence of OCH_3 (methoxy) group in an anthocyanin reactor.

Is it an activator
Is it an electrophile?

Electrophiles love electrons, they are withdrawers of electrons.

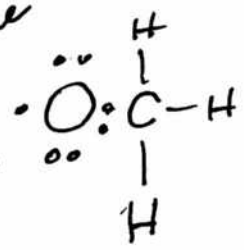
O has electron Configuration $1s^2 2s^2 2p^4$

So, it has 6 electrons in the outer shell



This is a methoxy group

How it looks like an electrophile



This has lots of free electrons.

This also looks like a free radical to me:

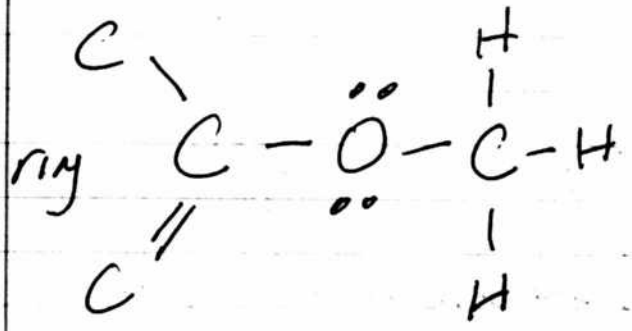
This group has a negative charge attached to it.

An electrophile seeks electrons

An electrophile seeks a pair or the completion of a pair of electrons

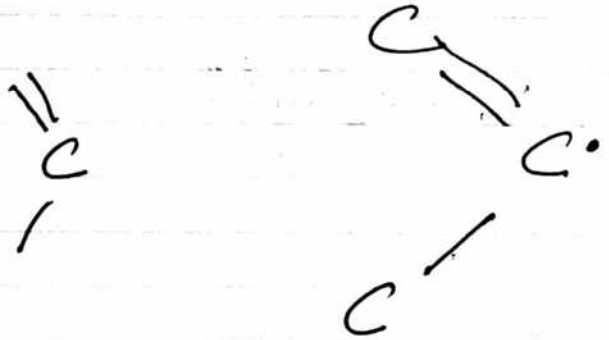
But if you have an R group attached, then what?

It also seems it can act as a nucleophile after the R group is attached because then it would have an excess of electrons.



Now it look like an nucleophile to me.

So what what was the aromatic ring like?
We know it is unsaturated

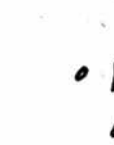


Benzene is a Nucleophile, it has electrons to share.
Nucleophiles have a pair of electrons they can share.

NH_2 OH

N is $1s^2 2s^2 2p^3$

This is why



This seems like it should be an electrophile too.

yes.

OH →  yes

make it an electrophile also.

But they are called activators a electron donating groups

Benzene is a nucleophile

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Note!!!

Find out what book this came from

This is wrong.

Electrophiles can accept either a pair or a single electron

Nucleophiles only have pairs that they can share

Why is benzene an a nucleophile?
Benzene is a nucleophile because of the excess of electrons above & below.

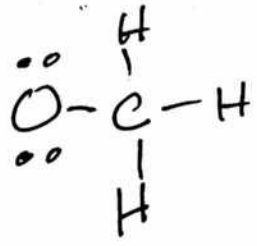
You know

Electrophiles are electron deficient, they want electron, they ~~lose~~ electrons.

Nucleophile are electron generous, they want to give pairs of electron.

We should be in a position to determine if OCH_3 is an electrophile or a nucleophile

So it should be



It should be an electrophile.

It should react as an aromatic ring which is an nucleophile.

Yes good it make methoxybenzene

or anisole

[redacted]

Page 190

Problems:

(1) What is a aryl hydroxylamine
vs an aromatic hydroxylamine

(2) what was the anti dopamine ga found?
And in SDBS?

Tyrosine is an amino acid.
It is metabolized to dopamine.

(3) ~~I~~ Cannot find my entry of dopamine

GOT IT!

Very hard to find.

It is oxydopamine . . .

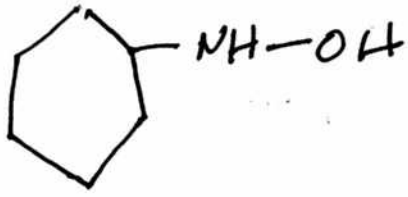
only one OH added
but a whole different story

Found it with the search
trihydroxy benzene dopamine
GOOD WORK.

An aryl group is obtained by removing a hydrogen from an aromatic g

phenyl group $C_6H_5^-$
derived from benzene

So keep an eye on term phenyl



06/27/13

Lets repeat the SDSS stack, this time with the restricted set that is actualy used:

3390 , 3150

2880-2950

2730

1730

1625

1430

1360

1140

920

710

We have a rather interesting metal taking place

With all peaks except 1-3 ± 40 @ 70°

With SOBS 13294

3390
3150
2950 - 2850
2730
2660
2360
1625
1430
1360
1140
1025
920
710

Crystal sample sent.

We now know it dissolves in water.

How about acidic or basic?
We know that it is alkaline ~8.0

Acids are electron acceptors etc
Bases are electron donors

Actually it appears slightly acidic.

~6.5

Boger also says ~6.5
pH meter confirms that mildly acidic.

Conductivity is SEG or TDS meter.
Tehre is very high

uS meter overloaded.
So it is highly ionized.

Test strip 5 in 1 gives general hardness at about 30
but not much else besides, pH ~6.5

Nitrates & Nitrites are nothing

General Hardness is about 30 ppm
Alkalinity is about 80 ppm
pH is indeed about 6.0 - 6.5

07/05/2013

A big night tonight.

I have a clean spectrum of the EPA filament. in Nujol

The spectrum is essentially identical to the biological filament sample.

Gain is ~250
The sample is actually of medium density. Substantial enough to see on the KCl plate w/the KCl cover slide.
It is not a dainty sample.

Microscope measurement:

Our microscope can measure to:

12.3 cm = 20 microns.

I can easily measure to Ø.5 cm.

$\frac{Ø.5}{12.3}(20) =$ Ø.8 microns

Magnification $= \frac{12.3E-2\,m}{20E-6\,m} = \underline{6150}$

Very good work.

IR Sample Prep

You are now ready to prep of liquid IR samples. Evaporate under low heat (if what is looks viable! Scrape & mix w/ Nujol.

Oxidation is the loss of electron

If something loses electrons, it becomes more positive eg Fe+3 vs Fe+2

Fe+3 is therefore more highly oxidized than Fe 2+ is.

Fe 3+ has therefore lost more of its internal energy than Fe+2.

If something has been oxidized the energy has been stolen from it.

Think of rust, what has happened? The metal has decayed.

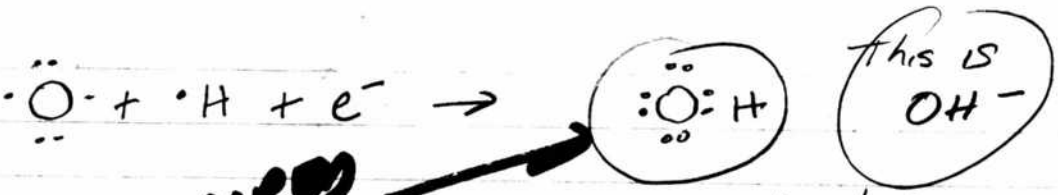
Most of free iron in the body is in the ferric state, i.e. more highly oxidized. Iron in the Fe+2 state is more available for use available.

"OH" variations

Page 198

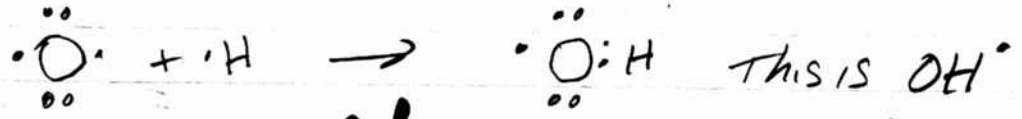
We have some things to get clear on

Hydroxyl ion, or hydroxide ion, are the same thing



NO UNPAIRED ELECTRONS NOT A RADICAL! an extra electron

Hydroxyl radical is different



The most powerful oxidant A Radical!

Hydroxl "group" (functional group is different)

R — O — H

Seems to me to draw a Lewis Structure for an organic compound you must know what the R is. The functional group designation is only that you have an OH. It does not tell you what the electron configuration actually is.

AN OXIDANT IS A FREE RADICAL!

What we do know is that OH in its neutral form is the most powerful oxidant so no wonder it is so reactive. If something is an oxidant it wants to

A free Radical (or Oxidant) they are the same thing by definition

has 1 or more unpaired electrons

in an outer shell.

An Oxidizer is the same thing as an oxidant.

It means it has an unpaired electron. This is why O_2 itself is an oxidizer it has two unpaired electrons

This point might be important to make.

Now is an oxidizer (or an oxidant) a source of electrons or does it steal. Seems to me it is a source since it is unpaired.

No it actually is the stealer of the electrons

Page 200

Identical terms from Wikipedia:

Oxidizing agent

Oxidant (also Free Radical!)

Oxidize

are all the same thing.

The oxidizing agent, the oxidant, the free radical, the oxidize is taking the electrons upon itself and ~~the~~ oxidizing agent is therefore reduced (if something is ~~reduced~~ it gains an electron).

Lets look @ O_2

The term is lone electron, or unpaired electron.
There is no such thing as a lone electron pair!! NO!

Conductivity of Urine 08/05/13

an interesting idea.

TDS 940 x 10

uS meter is overdrawn.

Mean TDS is 7012
I have 9400
range is 3028 to 18480

TDS has relevance in the process of Stone formation.

TDS > 12,000 has a statistical significance
"Urinary Stone patients, 2000 individuals.

NH_4 Test for Ammonia in Urine
5ml 1 drop = 4 ppm

NO_3^- Nitrates 10 drops up to 5ml H_2O = 5ppm

Nitrite is Zero

10ul urine in 5ml water = 2ppm !!!
perfect

$\frac{138 \text{ drops}}{5 \text{ ml}} = \frac{1}{X} \quad X = .036 \text{ ml} = 1 \text{ drop}$

10 ul = 10E-6 l = 10E3 ml

= ~~10000 ml~~ = pipette

$10 \text{ ul} = \frac{10}{1000} \text{ ml} = .01 \text{ ml}$

1 drop = 3.6 micro pipettes

So the micro pipette drops it down to 1/4

So our urine concentration of ammonia is

$\frac{.01 \text{ ml}}{5 \text{ ml}} = 2 \text{ ppm}$

but we diluted it by a factor of

$\frac{5 \text{ ml}}{.01 \text{ ml}} = 500$

So the actual concentration is $\frac{2}{500} = \frac{1E6}{500} = 4 \text{ ppb}$

fascinating

Incredible sensitivity.

Urine
1. Conductivity – TDS
2. pH
3. Ammonia levels
4. Nitrate levels
.. are all interrelated ~~tests~~

Biomedix has conductivity as being one of the most important parameters to measure.

Frequently, but not always, conductivity is directly proportional to ion concentration.

Took lots of vitamins

My distilled water has a conductivity of 6 uS, quite good. −12

Diluted by a factor of 3 (50ml → 150ml at a stall) too high.

by 6 Still too high
by 12 Still too high
By 24 OL measurable. = ~~1380~~ 1360 uS
meter goes to 2000.

So our Urine is 16,320 uS after 650 vitamins.

TDS is ~~6475~~ (24) = 15,600
VS 9400 on previous runs.

The suggests the vitamins have a strong impact.

My Concentration, normally, may be in the order of

$$\left(\frac{9400}{15600}\right) * 16320\,\mu S = 9835\,\mu S$$

Is this good a bad?
What does it mean.

Range of ammonia NH_3 given in urine is from ~~19~~ 200 to 730 mg/liter.

1 mg per litter is $\frac{1E-3\,gms}{1000\,gms} = \frac{1}{1E6}$ a 'ppm

So if we have
200 mg/liter we have 200 ppm to 730 ppm is the range.

Now revisit our study.

We added 10 ul of urine
to 5 ml of water.

this meant we diluted our sample by a factor of

$$\frac{5 \text{ ml}}{10E^{-6}\text{ ml}} = \frac{5E-3l}{10E^{-6}\text{ ml}} = 500$$

so we measured 2 ppm.

Since it was diluted by a factor of 500
We estimate 1000 ppm in our sample
with an expected range of 200 - 730 ppm

Since ours is a bit on the high side
It would seem.

This is valuable info.

Conductivity is directly proportional

Nov 15 2013 Page 207

Phenomenal Work today w the scope.

I have made it up to ~ 20K mag
w/ the new Camera & 100 objective lens.

Settings are:

1. Image is Close to lens
2. Light on scope is full
3 Condenser is full down
4. Exposure is @ 223
5. Gain is @ 60
6. Gamma is @ 10
7. Saturation is @ 5

You will have to learn how
to transition to this from 40x lens

Nov 16 2013 [redacted]

Urine +0.15 ORP
5.75 pH
71.0 uS
815 TDS

Tea pH 6.9

A big question:

How did I measure TDS of urine when my TDS mete should only go to 999?

I diluted it! Very Smart.

eg if something measures 50 ml and you had 100 ml how much is the ~~original~~ revised concentration?

$\frac{50}{150} = \frac{1}{3}$. Means dilution by a factor of 3

So we need to dilute Urine by a factor of 10.
No, by 20

Urine TDS Test Nov 16 2013

10 ml x20 = 200 ml.

$\frac{10}{250+10} = \frac{10}{260} =$ 26 times

TDS = 395

395(26) = 10,270 ppm

ppm

Critical Value seems to be on order of 12,000

Range is from 3000 to 18,500.

He measures

- Electrical Conductivity
- TDS
- Ph
- Specific gravity
- Redox

TDS has relevance in the process of Stone formation

Urinary Stone formation

Conductivity is 830 uS (26) = 21.58 mS

right on target!

This is exactly in the mean range

Range = 1.1 mS to 33.9 mS. Mean is 21.5 mS

Page 210

PubMed

Display Settings: Abstract

Urol Res. 2010 Aug;38(4):233-5. doi: 10.1007/s00240-009-0228-y. Epub 2009 Nov 17.

Electrical conductivity and total dissolved solids in urine.

Fazil Marickar YM.

Department of Surgery, Zensa Hospital, Trivandrum 695009, India. fazilmarickar@hotmail.com

Abstract

The objective of this paper is to study the relevance of electrical conductivity (EC) and total dissolved solids (TDS) in early morning and random samples of urine of urinary stone patients; 2,000 urine samples were studied. The two parameters were correlated with the extent of various urinary concrements. The early morning urine (EMU) and random samples of the patients who attended the urinary stone clinic were analysed routinely. The pH, specific gravity, EC, TDS, redox potential, albumin, sugar and microscopic study of the urinary sediments including red blood cells (RBC), pus cells (PC), crystals, namely calcium oxalate monohydrate (COM), calcium oxalate dihydrate (COD), uric acid (UA), and phosphates and epithelial cells were assessed. The extent of RBC, PC, COM, COD, UA and phosphates was correlated with EC and TDS. The values of EC ranged from 1.1 to 33.9 mS, the mean value being 21.5 mS. TDS ranged from 3,028 to 18,480 ppm, the mean value being 7,012 ppm. The TDS levels corresponded with EC of urine. Both values were significantly higher ($P < 0.05$) in the EMU samples than the random samples. There was a statistically significant correlation between the level of abnormality in the urinary deposits ($r = +0.27$, $P < 0.05$). In samples, where the TDS were more than 12,000 ppm, there were more crystals than those samples containing TDS less than 12,000 ppm. However, there were certain urine samples, where the TDS were over 12,000, which did not contain any urinary crystals. It is concluded that the value of TDS has relevance in the process of stone formation.

PMID: 19921168 [PubMed - indexed for MEDLINE]

MeSH Terms

LinkOut - more resources

Nov 21 2013

1. How adrenals Co. work hand in hand trace source

2. Note blood pressure test tying down

3. Remove halogens?

4. Increase tyroxine - iron problem in body

5. Iodine patch test

6. Iodine Hearing

Acids & Bases Nov 22 2013

We have a sense of acids & bases in terms of electrons.

We know that acids attract electrons & that they are electron acceptors.

Bases, in contrast, donate electrons.

Now we are going to look at it from the other side that a base is a "hydrogen ion" or a "proton" acceptor

& this has now twisted your mind since you are no longer talking about electrons.

So let's learn why:
What is ~~frustrating~~ hydrogen

1s¹ so there is one electron in the outer shell. If we strip off that electron we have an hydrogen ion

H^+ now something that attracts these is a base — why?

a base donates electrons
a base accepts protons
] These are like 2 sides of the same coin.

What happens if something donates electrons?

It becomes more positively charged

What happens if something accepts protons

It becomes more positively charged

Notice in both cases it becomes more positively charged. This seems to be what truly defines a base.

In contrast,
an acid steals electrons
so it becomes more negatively charged
An acid must donate protons.
so it becomes more negatively charged.

So in both cases, an acid becomes more negatively charged.
So this seems to be what defines an acid.

Fe^{+2} than this
Fe^{+3} more positively charged.

So why is Fe^{+3} more basic than Fe^{+2}???

Okay, I have found the answer.

$FeCl_3$ by itself is not either an acid or a base. It is an ion. It is only by combining with water that it becomes acidic.

The reaction is:

$$Fe(H_2O)_6^{3+}(aq) + H_2O(l) \rightarrow Fe(H_2O)_5(OH)^{2+}(aq) + H_3O^+(aq)$$

The Fe^{3+} act as a proton donor

a complex ion.

This is really interesting.

12/05/13

We are approaching the end of the page.
We know that we are working w/ Cognitive functioning.
Let's check that we have covered our list.

- ✓ iron
- ✓ amino acid - protein
- ✓ acidity
- ✓ oxidative stress
- ✓ oxygen availability
- ✓ thyroid + metabolism
- ✓ halogen toxicity
- ✓ joint + skeletal integrity
- ✓ blood + cellular integrity
- ✓ potential neural disruption — iodine connection

p31 Part I introduces the phenol group - good

p12 In Part II we have introduced a very important connection between

tyrosine

makes w/ Iodine → thyroxine

makes w/ phenol addition → dopamine

p13 We have already established a case for interference w/ Tyrosine

p21 Phenol in its pure form is a neurotoxin

Part II p25

We have the extensive discussion on dopamine - oxydopamine

We have done a superb job at presenting the neuro disruptive cause

Glutathion (ie, NAC)
Iodine

P17 Part III

Dopamine issues

12/14/13

1. Let's look @ pH & ORP of cultures
2. DNA of patch

pH of culture media:

1. 35 ml distilled H_2O
2. level teaspoon of fructose
3. 10 drops $FeSO_4$ (.5M)

pH = 3.50 reference
Oral Culture = 3.8 (but this is after replenishing fructose + $FeSO_4$)

mV = 415 reference
Oral Culture 315 mV

Projects on Tap 12/14/13

1. Finish the Paper
2. Add the Thesis section
3. Food Supply paper Liver, Carrot, Potato?
4. Learn how to reliably extract DNA
5. Extract DNA from hair?
6. Dissect a pig
7. Look @ old Organs - dissection
8. Environmental filament project
9. IR of blood?
10. Culture inhibition trials
11. Develop cultures under control
12. Gel electrophoresis?
13. Microdistillation
14. PCR equipment acquisition
15. Chromatography equipment - enhanced procedures?
16. Strach + DNA tests.
17. DRP - Weatherby pursuits? Functional testing
18. IR of tissue samples as signature?
19. Bio fuel demo
20. Enzyme testing

Since I have not been able to find a list of research projects in need and delayed I thought I would generate one again and get in the forum so that I do not lose it. This is all work which could be going on (or will take place eventually) if the means, time and staff permit:

1. Finish the paper
2. Add the liver section
3. Food supply paper, liver, carrot, potato?
4. Learn how to reliably extract DNA
5. Extract DNA from hair (hard to do, but needed)
6. Dissect a feral pig (tissue slide prep and recording of reference)
7. Tissue slide prep of older organs (liver, heart, kidney, etc).
8. Environmental filament project
9. IR of blood?
10. Culture inhibition trials
11. Develop cultures under greater control
12. Gel electrophoresis study - tied in w/DNA results
13. Develop microsdistillation techniques
14 PCR equipment acquisition and develop methods
15. Develop more sophisticated chromatography methods/equipment
16. Straight forward DNA testing on culture results with external lab
17. ORP - Functional Medicine tests for home use - Weatherby - BioTerrain analysis
18. IR of tissue samples (older vs reference comparison) - IR signature?
19. Bio-fuel demo
20. Enzyme testing demo

There are more, but this will get a dozen or two of us working. i.e,
Clone everyone on the staff x 3.

Best, from Clifford

Calibration of Buret Tube

0 ml. C10's

IIII IIII +5

$\frac{55 \text{ drops}}{5 \text{ ml}} = \frac{1 \text{ drop}}{X}$

$X = .091 \text{ ml / drop}$

10 — 160

200 @ 14.5 ml

$\frac{255 \text{ drops}}{14.5 \text{ ml}} = \frac{1 \text{ drop}}{X}$ $\quad \frac{+ \ 55 \text{ drops}}{= 255 \text{ drops}}$

$X = .057 \text{ ml}$??? looks better

-- This is what we used.

Again:

$\frac{300 \text{ drops}}{14.23 \text{ ml}} = \frac{1 \text{ drop}}{X}$

$X = .0474$

Again:

$\frac{300 \text{ drops}}{14.32 \text{ ml}} = \frac{1 \text{ drop}}{X}$

Very Good

$X = .0477$

Average $= .04755$ ml per drop

$= 47.5$ ul

Priorities:

1. Bette just finish the paper.

Today 12/18/13

1. We got the distiller working. Micro! Good work
2. We got the Kodak web cam working.
   It has a focal point that no one else has.
3. We got the Buchner funnel working.
   & the vacuum pump
4. We removed the large distillation
   equipment.
5. We did not work on the paper—

[redacted]

12/19/13

You have completely dissolved hair for the first time! It may have been a factor of time and well as concentration of NaOH & KOH.

The method achieved is hair clg maybe 10 drops in ~ 60 ml at 37° overnight and then heating in 98° water bath (97°) for 1/2 hour or so, then setting it @ 40° for another hour, it is completely dissolved.

The enzyme at soap @ 37° did not exactly do much. You can still ~~investigate~~ this. You now have a concentrated solution.

3 drops in 5 ml H_2O + Ninhydrin as already produces a nice color. After heating in 96° water bath for 5-10 times there is now color at all without ninhydrin. Now what is the pH? (should be alkaline) Yes, highly alkaline. It would be great to neutralize it.

You have pipettes that will do this.

50 ml of H_2O + 3 drop 4.5 M H_2SO_4

pH of this is: about 3.
We can measure with pool meter.

20 drops of HCl

30 drops it is finally neutralized.
So we could have made our neutralizing solution about 6 times stronger. (use ~5 drops instead)

~~20 ml~~

$\frac{3 \text{ drops } 4.5 \text{ M } H_2O_4}{50 \text{ ml}} = \frac{X}{20 \text{ ml}}$ $X = 1.2$ drops

but we want it 6 times stronger.
So a good neutralizing solution is

3(1.2) = 3.6 drops in 20 ml or 7 drops in 40 ml

So a good neutralizing solution is

3 drops Hair Solution in 5 ml H_2O $\simeq$ pH neutral $\simeq$ 7 drops 4.5 M H_2SO_4 in 40 ml H_2O

I think what it tells us is that it absolutely changes w/ pH

And by the way, notice that the color of the ninhydrin solution did change from orange-yellow to light green.

So Ninhydrin does react completely pH dependent.

Spectrometer is useful, & pH meter is useful.

Therefore, you must know what the pH is before you do any thing & it must be held at a constant.

For our hair extract we are going to use 5 drops in 5 mL of water.

This means $\frac{5 \text{ drops}}{5 \text{ mL}} = \frac{20 \text{ drops}}{20 \text{ mL}}$ Test this pH

The pH of this solution is ~ 12.7 (12.70) exactly as we would expect.

Now prepare your reference solution of acid ie 7 drops 4.5M H_2SO_4 / 10 mL H_2O pH ≈ 1.5

& how many drops it takes to neutralize the hair solution.

So now figure out how many drops of my H_2SO_4 solution to neutralize my Lair solution:

A test tube of mine is just slightly over half full. About 9/10 RIP.

So procedure is

1. Fill a test tube ½ full of H_2O.
2. add 5 drops of Lair extract reference solution. (pH ≥ 12)
3. Add 3 drops Ninhydrin
4. Heat s/b boiling for ~~10~~ min.
5. Take Vis spec.
6. Now neutralize with acid.
   . Drops: ~~1~~ & 20 25

It takes 100 drops of the reference solution to neutralize the Lair solution.

This is too much.

This means it will take about 25 drops to neutralize 5 ml of base solution in the test tube.

This means double the reference acid solution strength from

7 drops 4.5 M H_2SO_4 / 40 ml ⟹ TO! ⟹ 14 drops 4.5 M H_2SO_4 / 40 ml.

Then it will only take about 12 drops to drops which is just about right. neutralize.

DONE!

Beautiful Ninhidrin Test. Result. Looks great.

The pH does indeed make a difference in the visible light spectrum.
* pH 12.7 is giving you more data than pH 7.0 for Hair in Ninhydrin.

The lesson here is that the pH of a solution must be known before accepting its as a reference visible light spectra. This important.

Page 228

My report that you learn a little about

60 ml H_2O
10 drops ~~KOH~~ Caustic NaOH + KOH
3 hrs of boily water

take down the ham pretty good

Might be that 24 hrs @ 40°F
& 1 hr of boily water also take
care of it.

The 2nd ham extract was subject to high heat the entire time. Does it make a difference?

I believe that you are ready to start the environmental falandat project

EFP.

You have the vis spectrometer working again.

You can extract Kleratyn from hair but you sure dont know how to extract DNA from it. Many other people do not know as well.

You can extract DNA from bananas but not from a carrot. why?

What you have done is set the lab running in full operation now. This is good work.

1. Make spray ninhydrin
2. ORP measurements
3. Bleach label, pH, pH tests

12/20/13

This is lowest - indicates greatest charge

ORP of Potato Culture is +285 !

This is quite high, what does it mean? Does it conduct a current?

ORP of oral culture is +320 high

Siga-Gold is +410 so in a relative sense yes it is actually ~~+100~~ -90 to -125

This means it is a taker of energy not a provider of energy.

Let's check the other

[redacted] Culture ORP is +360

Culture Solution Broth is +360

Transfer Culture is +377 from Oral

In terms of Charge from reference Siga-Gold + Iron, from most active to least:

-125 1. Potato culture
-90 2. Oral culture
-50 3. [redacted] Culture & Culture Solution tied
-43 4. Transfer Culture from Oral

Culture Surface Area $\simeq -.89(ORP_{culture} - ORP_{ref}) - 28$

Culture Activity vs ORP relationship established.

This is showing a direct correlation between the activity of the culture and the ORP.

Activity of Culture ~~ORP~~ $= K(ORP_{ref} - ORP_{culture})$

Name	Surface Area of Culture	ORP Ref	ΔRef	ORP culture
	4			X
1	75			-125
2	65			-90
3a	15			-50
3b	20			-50
4	2			-43

$y = -.89x - 28.2$ $r^2 = .92$ Not bad

Quite Good.

~~$ORP_{cul} - ORP_{ref}$~~
~~$ORP_{ref} - ORP_{cul}$~~
~~$ORP_{cul} - ORP_{ref}$~~

Culture Surface Area $\simeq -.89(ORP_{ref} - ORP_{culture}) - 28$

ORP ref of 2 flat scaps sugar + 10 drops $FeSO_4$ = +410

good

So eq for $ORP_{cul} = +285$, Culture Surface Area = ~~129~~ 83

vs meas. ~~100~~ 75

Very good. 10 2

For oral transfer, CSA = ~~84~~ vs meas ~~45~~

good

pH testing

		Estimated	Actual
+.06	Baking Soda	8.2	8.26 Great
+.64	Bleach	12.75	12.11
−.48	White Vinegar	2.95	2.47
+.64	Ammonia	11.5	12.14
talks	~~+.05~~ Distilled Water	7.0	

~~X = +.10~~
~~+.10~~ This is superb.
~~+.07~~ −.05

~~Error~~ .05
~~+.04~~ 0 ≈ .52
5

What we see is that the closer it is to 7.0 the longer it takes to stabilize & apparently the more error there is in the process. It is taking a long time for water to stabilize.

With the closer to water I would say we are getting more error.

With water, it is actually perfect = +.01 You could not get any better than this.

It means your actual readings are probably good to ± .0.5 but your average is quite good.

Your baking soda - Washing soda buffer is quite good.

Your buffer is quite good.

Equal volume of baking soda & washing soda in water gives a pH of 10.0 at room temperature very closely.

So your average error is only ±.05

This means if you really want to you can calibrate your buffer to 10.05 if you want to.

I don't think you can get any more accurate when you consider temperature variation

Cysteine Verification & pH Calibration

1. We need to check pH – spectral analysis with ~~the color before the~~ acid ~~to~~ added before the color forms.

2. Check Cysteine on a separate test from hair

5 drops hair extract in 5 ml H_2O
11 ~~12~~ drops reference H_2SO_4
3 drops Ninhydrin
10 min heat
Spectra

NO, NO, NO it is way too acidic!

Trial 5, pm checking again, it was not bad.
11 drops neutralized it so it was actually close

Cysteine Ammonia has been verified in NaOH estimate pH 12.5

12/21/13

We now have the electronic test equipment available!

The Oscilloscope
The Multimeter
The new logging multimeter
Two freq Generators, 1 low range & high range.

You also have the environmental test equipment set.
Water Pollution
Chemical Composition of the soil
Nutrient Testing of the soil.

What have we accomplished over last few weeks?

1. Lab is really coming alive, many things in storage are coming out.
2. Incubator is now in place
3. A radically simplified & accelerated culture growth.
4. Relationship between OR/Redox potential & culture growth has been established
5. Research paper is done
6. Examination of soil supply
7. DNA extraction of plant has taken place
8. Method to extract Cytokine & positive identification method of cytokine has been established
9. Importance of pH in any Viable spectral
10. Microscope advanced analysis has been established

Oral & Potato Analysis 12/21/13

We are getting an identical reaction ~~better~~ between the oral culture and the potato culture.

First reaction is a green color

(Method is culture is placed in 6 mL H_2O w/ 2 drops NaOH - KOH added)

just like you use to get w/ wine & alkali.

Next is that we get a deep yellow color & then rust color.

pH is 13.1

Ops need to increase strength to get good results w/ Ninhydrin.

Need 15 drops of both oral & potato extract in about 4 mL of H_2O and 5 drops of Ninhydrin.

This is producing visible color.

The visible light spectrum of the oral culture extract @ pH 13.1

is absolutely identical to the vis. light potato culture spectrum @ pH 13.1

w/ Nitrogen used in both cases.

Conclusion: "Magellons is not a "human" condition by any means, it is a planetary condition.

You have distilled alcohol.
Looks quite good. It should be
~~82.6°~~ boily point. We are setty 72°
82.3.

Ah, are we setty 72°
Seems to be stabilizing @ 74°

The measured density is .821 gms/ml
Isopropanol
Wikipedia Boily Point 82.6°
Density .79 gm/cm3

More

I set Boily Poin 74°C
Density .829m/cm3

Ethanol Boily Point 78.4°C
Density .79 gms/cm3

80°C
-70°C

Maybe your thermometer is cooler up top?

Yes this was a problem. Direct measurement of temp was 78°C.

So you set a result very close to Ethanol.
Not sure why temp/density do not match.

My temperatures are 80.6 lower

Distillation

You want to be very careful when heating any old solution.
Lite
Bleach
Ammonia
] these are quite dangerous when heated.

So you must measure the solution before you so heat it up.

Bleach BP = 101° very high

You have separated a concentrated ammonia solution BP = 95°C.
Then water has come afterward @ = 100°F

water testing equipment (9)
But guess what. You can do it with pH!
pH is 12.21 1st fraction
pH is 11.48 2nd fraction

It is amazing that you can determine pH & Ammonia concentration from pH.
This assumes that you know it is ammonia to begin with.

The bottle says it contains ammonium hydroxide.

Formula? NH_4OH

You obviously need to know about dissociation

K_b:

K_b is a " base dissociation constant
This is amazing.

$K = \frac{\text{Products}}{\text{Reactants}}$

K_a is for acid.
First you need the reaction
showing dissociation:

$NH_4OH \rightleftharpoons NH_4^+ + OH^-$

K_b can be looked up in a table.
$K_b = 1.8E-5$

If pH = 12.2 then $pOH = 14 - 12.2 = 1.8$

$[OH^-] = 10^{-pOH}$

$[OH^-] = 10^{-1.8} = 0.158$ or $1.58E-2$

$1.8E-5$ $K_b = \frac{[1.58E-2][1.58E-2]}{NH_4OH}$

$NH_4OH = 13.87\,M$!!!

Wow, it worked perfectly.

This is 13.87M Ammonia.

Highly Concentrated.

Concentrated Ammonia is 14.8M!
I almost have commercial level Ammonia!

You are doing very good work!

Your 2nd fraction is

$1.0E^{-5} =$

$pOH = 14 - 11.46 = 14 - 11.46 = 2.54$

$[OH^-] = 10^{-pOH} = 10^{-2.54} = .0029$

$= 2.9E^{-3}$

$K_b = \dfrac{[NH_4^+][OH^-]}{NH_4OH}$

$NH_4OH = \dfrac{[2.9E^{-3}]^2}{1.0E^{-5}} = .4672M$

And our 3rd fraction.

pH is headed toward 7.
Take 8 for now

$pOH = 14 - 8 = 6$

$OH^- = 10^{-pOH} = 10^{-6} =$ a very small number

$$NH_4OH = \frac{[10^{-6}]^2}{1.8E\text{-}5} = .0000 M$$

Fascinating. My third fraction is almost complete water.

This was amazing. All that you actually needed to know was the pH & Kb.

K is a very important number.

Culture Comparison ~ Dec 24 2013

Sequence 5 800, 2000, 8000

M
P
OLD

	X	
15	2000	M
15.1	"	P
15.2	"	OLD
3	8000	M
4	"	"
5		
6		
7		
8		
9	BL	Potato
10		
11		
12		
13		OLD
14		
15		

Separation Skills

Dec 26.

Some Some Preparatory work & Skills Going on
w/r Separation techniques & equilibrium study

You have 4 methods of separation in place now.

1. Fractional Distillation (very good results)

2. Centrifuge

3. Extraction w/an understanding of polar vs non polar solvents

4. Chromatography is getting better w/ also an understanding of polar vs non polar solvents.

5. Equilibrium Constant is rather amazing You can determine Concentration sometimes from pH alone.

6. You also set two Columns on one rod.

7. You also improvised a separatory funnel w/ a Chromatography Column & a Cork!

The fact that you have a solution now w/ acetone & water means that IR work might proceed differently now.

also, what happens w/ ninhydrin?

A stirring rod as how you pour solvents into the Chromic Columns w/ out dexterity etc used!

Polar & non polar transitions are fantastic in Chromo. You just progress through to figure out what works & then you back out gradually in reverse to get out of trouble.

The Chromo List:

1. Water
2. Vinegar (acetic acid - white)
3. Ethanol - Isopropyl
4. MEK
5. Acetone
6. Xylene

Polar ↕ Non-Polar

You need more clamps - Hoffman & Bürett

There is indeed an ammonia
odor with the acetone based
potato culture Nash extract.

The question is whether
a hot acetone based solution
can be more effectively used
in the IR spectroscopy?

I have isolated the cross-backed

Jan 01 2014 Welcome to 2014!

We learned some very important lessons tonight

We did get the Spectra-Chrom CE [redacted] to collect tonight

First it worked, then it all jammed & was hopeless, then I stripped it down & learned to use it in a partially automated mode. Which in the end will be just perfect for us. It is really the most practical & economical piece for [redacted]. We are only limited now by the # of test tubes & we can easily acquire 3 dozen more & keep our labels full.

The other thing we have learned (and we actually learned this before) is that acids & bases can be very important solvents in Column Chromatography. The red food dye was locked up no matter what solvent was used all the way from water to xylene. & never could free it up.

You add H_2SO_4 & it completely freed up & isolated the dye into yellow, pink & red orange components. You simply never saw anything like this

The question is, why are acids &

[redacted] pH issue

not discussed w.r.t. Chromatography because we can see how important it is.

pH monitoring will be another important variable to monitor

You saw the chips when we used ammonia & it freed up the amino acids.

So the lesson is if you are getting stuck after the progression of solvents then alter the pH.

Have we really said lessons tonight?

I bet we could isolate carotenoid next.

The amount of yellow pigment you see here is fascinating.

You have many separations today, plus you must have had over a 100 feet tube of yellow alone.

The  like the world of what is called

"ion exchange Chromatography"

and

"affinity Chromatography".

Ends up the plt is still only ~6 when the H2SO4 works but something is obviously important here.

01/03/14

We have now set up the 250 ml Chromatography Column. Looks like it will be a dream. 5" of Column is plenty. If it is too dry it gets too hard to push the material through.

We have learned once again that acid for some reason, is required to get the Column to flow. It immediately speeds up the flow. This Column you might indeed be able to run overnight.

You had a very uneven flow before you started with straight water.

You wonder if oxidation potential as well as pH might be factor to measure

You have taken 2-3 drops of Post Colony as a test. You have separated into into 3 sections

Blue (approx 10 ml)

Green (approx 40 ml)

Red-Pink (approx 100 ml).

Then a rabbit emerges

Jan 05 2014

Goals Today:

1. Refractometer & UV detector on their way!

2. Fraction collector & Chromatography System is working great.

~~3. Liver culture~~

✓ ~~4. Algae potato culture~~

~~5. Concentrate rutidge~~

~~6. Person food color w/ fraction collector~~

7. Extract Carrot pigments - & Collect?

8. You also have a great distillation unit

9. Also a sep funnel improv!

~~10. Clean the Chromo column~~

~~11. Transfer to 46 mg now.~~

12. Concentrate & Collect the bacterial extract

13. Paper on cross domain bacteria.

~~14. Stains page up~~

~~15. Micro distillation Cooks to be in place now.~~

Alcohol & H_2SO_4 pumped the red color right on through. - Very quickly. It was an alcohol-water mix.

Will need

1. Lots of test tubes
2. Rod & 2 more clamps (3)
3. 60 ml sep funnel?

01/06/14

Something I did not expect to happened w/ the culture.

When you tried to distill it (micro) nothing really happened. It appear to be homogeneous But then you cooled it all down & noticed it was not cloudy even though originally it was a clear & filtered solution. Ya now have a "precipitate" form of the Centrifuge which looks remarkably similar to the sheet form which sometimes shown up in the culture.

You also notice "surfactant" behavior during the extensive boiling (micro distillation) process.

How can you get a "precipitate" from boiling (240°C sand), cooling & then centrifuge?

The chroms column is only orange
not beige even though original
solution is a nice bold orange color

C Column is highly acidic right now
(pH 3.0) with the red color that is
being pushed out by the NaOH culture
extract.

Have added NH4 Thio highly diluted
also in addition to the NaOH.

In theory that it is beige. Also
as column is that column was
completely white after the alcohol
& acid it ran clear.
So now why it is red?

It appears in our first C rxn w/ the NaBH + [redacted] is added (both basic)
We do have an acetic acid.
Our last [redacted] cooled gave a yellow color.

Column is now filled w/ alcohol & acid

01/02/14

1. Get alley image up
2. Extract under scope in H_2O vs acetone
3. Question of precipitate and centrifuge?
4. Look @ living under the scope!
5. Chromatography
   - Alcohol + Acid — Check pH!
   - Alcohol + Base
6. Check on shipment of ~~odium~~
   - Biuret test - standardize
7. Cross-domain paper
8. Extract Carrot Pigments?
9. Condense + Extract Cultures
10. Gram Stain.

You have a very curious appearing [illegible]
You have what appear to be cellular structures (large) in large vacuoles that are packed w/ the bacteria

Just it is not the bacteria alone - why?

In the centrifuge you have a major separation take place

Why?

01/07/14 Chroma : Alcohol, Water Mix w/ Acid

	Set	pH	Ninhydrin Test	ORP	TDS
Clr Brown	1	11.6 (Still highly alkaline!)	None	-44	240
	2	11.8		-48	107
	3	12.2	yellow	-47	565
	4	11.6	ever slight yellow	+16	261
*	5	4.1	White, Cloudy	+266	273
*	6	4.2	light white cloudy	+324	213
	7	4.1	Clear	+311	175

Wells A1-A6 in Culture Well plate have 01/07/14
1. 2 drops NaOH water based extract
2. Pinch of spatula sugar
3. 1 drop of iron

Set 5 is important.
We have a new precipitate!

Detection!

We already know, even without our UV detector and spectrometer, that we have a major difference occuring in solutions, even though they look appear clear to the eye. We have done this through a Combination of ORP & pH. You also know that the Columned speed slowed down Considerably & that happens to be another Clue but not nearly so clear cut.

Sure enough, w/ ninhydrin, there is a difference. What does ninhydrin react w/ to produce a white or cloudy solution? It is forming a visible white precipitate!

Do lietuon tubes 3, 4, 5
there are important differences.

We would what they are
by reading from

Solution #5 fails the biuret test.
The meaning the white precipitate
apparently is not a protein? So.
What is the white precipitate?

#6 also has a trace of the precipitate
however. There is something there.
What is it?

Set	pH	ORP	TDS	Notes
8	3.8	+410	415	Alcohol, Water, Acid Mix
* 9	1.76	+485	104	Tail End of Column, Clear Tan Color
* 10	1.5	+516	241	Slight tan color
11	1.6	+573	179	
12	1.9	523	102	

The looks like the column end has been leached. We have 4 separations of some kind that show some potential, more likely 3.

We know that we have an amino acid in sets 1 & 2

We have an unexpected precipitate in set 5 and set 9-10 is different in some way but we do not know how.

Let's do the Biuret test.

Set
1
2
5
9

} Biuret tests Dark on all accounts.

The proteins we know that we have an amino acid but not necessarily a protein structure?

01/08

We already have good, known separation taking place.

1	Colored	Colored
2-3-4	NiN yellow	
5-6	NiN - white precip	
9-10		Slight to color

Set	NiN	Biuret
1	Yellow	
2-3-4	Yellow-Orange w/ some precip	
5-6	White Precip	
9-10	No reaction	

Positive test on the ~~bacteria~~ solution also fails. We know therefore that ~~that~~ we have an amino acid in the bacterial form but there is nothing to say a ~~bacterial~~ bio protein structure. The protein can come from the keratin horns.

Nitric Acid Test!

Sets 1 & 2 do indeed form a white precipitate.

Especially set #1 & very weak in set 2.

Set 1 is giving a clear precipitate w/ Nitric Acid.

Fascinating that the Nitric Acid does not cause a precipitate reaction w/ the original NaOH deep extract. It is only after the Chromo Column under condition of Set 1.

There is a very pronounced reaction that occurs only after Chromo separation.

In your chromatography work from the past you have used KOH to break down the culture only NaOH. We can see that KOH - NaOH combination is far superior.

Lets do the Gram Negative Test!

01/08

(1) You have some strange things happening. You have made a very dark solution. Your method was NaOH first, then KOH – NaOH. You also took a large volume of culture material to work with. So you now have a lot to write up.

(2) When you prepared slides, you encounter something very strange. It appears the filaments do stick somewhat intact in one case, and they seem to align themselves in a certain direction. You have had a separate slide that does this.

3. On your 2nd column run you are not at all duplicating the first run. Your 1st tube has

pH = 1.3
and ORP = 435

So it is like run #8, hardly like set #1.

So you have to set to where you can duplicate a run.

01/10/14

(1) Milk & Egg fail to Nitric Acid Test

So it looks to be very specific.
This is important.

(2) Sugar Lvg looks Satisfactory

(3) Swine culture is in place.

4. Liver culture has some nuts very quickly.

01/10/19

We have a very interesting situation developing.
We [illegible] to duplicate the
Xanthoproteic protein detection reaction.

We can not do it yet.
Your solute that turns ammoniacal.
Not the same as when you used
NaOH but you are getting a similar color
coming out quickly on the column.

What is especially interesting is that
Nitric acid is having a direct visible
reaction on the culture extract - immediately!
It is dramatically clearing up the solution.

Secondly, the same reaction is not
occurring w/ the 1st (set 1 eluate) with
NH_4OH as the solute. It remains
slightly colored but it is not producing
a directly visible precipitate.

So we have some important differences.

Problems
1. Reproduce the precipitate HNO_3 test.
2. Find out why HNO_3 turns the extract clear
3. Identify the nature of the set 1 eluate.

No precipitate forms even upon heating but NIN is indeed giving a reaction so SET 1 in NH_4OH does have some type of amino acid in it, but it apparently is not ~~the~~ proteic.

Question, does NaOH + NaOH with alcohol + NaOH w/ alcohol + acid make a difference?

HNO_3, an increased concentration with set 1 (the NH_4OH) is indeed clearing up the SET 1 eluate. so it is behaving like the culture.

But the NIN test is giving a more reddish tint indicating some kind of change.

Set 1 Colored portion:

pH:	ORP:	NIN	TDS:
	vs -44	vs NONE	vs 240
* 11.4	-62	Positive (reddish)	+404

So difference on NIN + positive + TDS is higher so it is not the same as set 1 of 01/07/14 but it does have some relationship.

Doing a more reddish NIN test is occurring here on the ~~the~~ as a different reaction than before. This may be the histidine reaction? NH_4OH may also be involved in this.

01/11/14 · Sat

Today

1. We want to prepare an extract in a water bath that is heated only to the point of Cola.

2. We want to reproduce the results of the xanthoprotein test - this is very important.

3. We have results of nitric acid test which many questions.

4. We have indication of a amino acid that is more red than was produced by ammonia & water.

5. Can we get a fluid to go through the UV detector?

6. Bacteria paper

7. Burn the extract!
   Spectroscope

Need:

1. Power Cord
2. Power Strip
3. Ammonia
4. Distilled Water

The column pretty much locked up on us today. We had a strongly alkaline solution flowed upon ammonia & alcohol. We could also see the residual black ring @ the top.

We added H_2SO_4 + HNO_3 & et let broke the column free again w/ the dark ring now starting to move down slowly.

We definately have amino acid(s) coming out of sets 1-3 today.

Set	pH	ORP	NIN	UV	Nitric
1	10.0	+5	Red Orange	940	None
2	11.2	-30	Red Orange	1965	None
3	11.4	-38	Red Orange	1324	"
4	11.6	-47	Red Orange	969	"
5	11.6	-49	Yellow	911	"
6	11.5	-41	Weak Yellow	1065	"

Peak * * Column Locks Up

What we learn here is that they are all one set w/ no significant variation. It probably reaches its peak in tube 3 (set 3).

7	11.2	-20	Yellow	1240	None
8	9.6	+66	None	>2000	None
9	9.6	54	None	>2000	1940 nms None

Solution		pH	ORP	NIN	TDS	Nitrite
	~~10~~ 10	10.9	17			
Cl-12 Clear Colored	1	11.10	+36 wk yel		420	none
	2	10.12	+74 wk yel		200	"
	3	3.4	+353 clear		1810	clear

Notice this; even though eluate is colored there is no NIN - HNO_3 reaction but ORP is high & eluate switched to acid

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Control Flow Solvents: Great!

01/14

We are constructing a milde extract

less KOH
less time
more color

Make flow meter tests on NaOH, NH_4 & H_2SO_4 & water

FLOW CONTROL TESTS

	pH	ORP	NiN	TDS	Nitric
Tap Water	8.4	0		48	
H_2SO_4	1.5	+200		4960	
HNO_3	2.3	+200		1170	
NaOH	12.0	-62		335	
Alcohol H_2O	8.3	+115		30	
Hydrous Alcohol + H_2SO_4	2.7	+220		1100	
H_2O + Alcohol + HNO_3	2.2	+237		640	
H_2O + Alcohol + NaOH	12.2	-6		170	
H_2O + NH_4	11.5	-31		850	
H_2O + NH_4 + Alcohol				350	

This table is both interesting & valuable. It can be used to help you determine what the nature of your solvent & elevation. For example our nitric acid positive test come with..

11.6 -44 240

We can see that is closest to NaOH w/ the likely addition of a little alcohol.

01/14

Sets 1 & 2 at 01/07
have now both become cloudy.
So something has changed here & you
are almost out of sample.

I am reproducing the precipitate
formation w/ set 1 of 01/07. The
cloudy solution is now becoming
transparent & back to the original
tan color.

It did not form in the dilute solution
it must be full strength elevate!

We have a very important discover w/the
* extract solution.
Nitric Acid clears the solution up.
NaOH darkens it again!

Does this happen w/ H_2SO_4 also?

yes H_2SO_4 works fine also. So
what does this mean?

This result is produced with
IRON(III) Chloride!
Also a precipitate forms!
Maybe this is all it is — maybe
not a protein.
Remember the failure of the NIN test
w/set 1??!

No, the iron reaction is not the same reaction.

The precipitate w/ the set 1 eluate was WHITE @ first, then turns orange exactly as described in the Xanthoprotein reaction

When we have our pneumatic iron based extract, the solution turns clear & does not produce a precipitate.

The orange precipitate appears in the iron reaction only after reversing the acid reaction (by clearing by adding NaOH - then the orange precipitate appear & ultimately dissolves. These reactions are not the same.

Next thing we learn is that our concentrated extracts are essentially the same. Test this w/ the flow meter process.

	pH	ORP	TDS
Set 1	10.7	-130	4290
Set 2 (2 Stage)	10.6	-20	3000 ~~42~~

Somewhat different, but close enough to mix.

01/14

Today

1. Get a reproducing Xan the results

2. Gram Stain is needed!

3. Bacteria paper

4. Iron cultures - what is happening?

5. Perform spectrum of cysteine requires Confirmation. Human hair up to 25% cysteine.

Compare hair & commercial cysteine in KOH & the culture extract. Hair has a reddish tinge to it.

You must equate pH!

Variables here include the
1. pH
2. the conc of the amino acid
3. the conc. of NIN is also important.

If the culture produces such a strong Ninhydrin reaction in KOH why can't we use that to our advantage in the chromo column?

We have proven positively today that the extract culture has cysteine in it in high concentrations. We have a match w/ hair. We also know of the iron in significant number.

1. Let's try to reproduce Xantho

2. Nin reaction is strong - Can we use this to our advantage,

3. We see that acid really does clean out the column. This is why HNO_3 is used to clean out the UV detector.

4. Heating the diluted extract w/ NIN does indeed produce a very nice more reddish color.

01/14/14 Tues

Liquid Iron appears to highly favor culture growth.

pH is now alkaline but ORP is still high. Assume more NaOH must flow.

We have need to duplicate the Xantho test today & we have failed.

	pH	ORP	TDS	NIN	NIT
Set 1	13.7	-110	4860	BROWN!	NO
2	11.9	-12	460	Orange	"
3	12.9	-56	710	Yellow	"
4.	13.1	-75	1110	Weak Yellow	"
5	13.0 ~~+2.4~~	-61	970	~~weak~~ Yellow	

This is 4 set

We are using NaOH (strong) + alcohol in water as the solvent.

The question now is whether acetone was used in the first sample or not. Something still seems to be different & you cannot reproduce the results.

Discussion:

1. Cross domain backbone

2 Potential Xanth Reaction.

3 Separation seems to be in 2 main stages so far:
  1. immediate effluation (NaOH, alcohol)
  2. stubborn. might be taken down w/ acid.

3 We see that variables are
* 1. pH
  2. Concentration
  3. amt of ninhydrin used

01/15/14

We have another important discovery

1. Cross bacteria isolated

2. Gram Stain negative

3. Xantho reaction a single positive need to replicate

Isolated Case

4. We have a strong brown precipitate w/ NaOH set 1 w/ ammonium hydroxide + $CuSO_4$ in heated water bath

Not abnormal NaOH + $CuSO_4$ + heat = dark precipitate (cupric)

We also have a strong NIN reaction w/ alt #1. You should demonstrate this also

What we learn is that Ammonia is not required.

Cu alone is sufficient w/ set 1 eluate

We are also getting a brown precip w/ the extract alone but it is fairly more $CuSO_4$.

You seem to be getting a precipitate from the extract by itself but it does not seem as pronounced as the set 1 eluate

Now looking @ $FeCl_3$ w/ $CuSO_4$ heated
It is turning from yellow to orange.
There is indeed a precipitate forming.

$FeCl_3$ by itself, with no Cu after heating is turning a bright orange & red however. quite pretty.

What we have done by heating $FeCl_3$ is to create colloidal iron.
It is a cherry red color. Quite pretty and it is used in certain histological stain applications.

We have also seen that $FeCl_3$ with $CuSO_4$ produces a strong orange precipitate.

There two reactions are of interest but I do not think that they have immediate relevance to ours.

We also see that the extract by itself, also produces the dark brown precipitate.
So I guess the big question is, what is the precipitate? What is it in? How about the other test?

You are clearly showing the existence of iron oxide & an amino acid within the extract, cysteine almost certainly.

You also have a one time reaction of xanthro.
this indicates an aromatic amino acid but you have not been able to replicate it.

Try ~~diamyl~~ ethanol extract with acetone?

It appears to me that "acid" is primarily a combination of an amino acid & iron oxide. i.e.
This is parallel to the NIN test w/ set 2 and the tri $CuSO_4$ precipitate reaction.

We also believe we have an aromatic amino acid but we cannot duplicate it yet.

Set 2 stays at the top of the column. Let's go after it. We know that acid wash

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Set 2 - Ring @ top w H_2SO_4 to move out

1. Fails to NIN test
2. However $CuSO_4$ may be produce a precipitate. While to blue white in color.
It seems to be only after extensive heating

Crazy Here!

$CuSO_4$ + NaOH produces a blue precipitate
But when you heat it up it turns brown!
Just like what we have.

And we indeed have $CuSO_4$ + NaOH
in our solutions
This looks to be no good.
There may be some iron in it, but notice
the H_2O_2 reaction failed on the second test.

Our dark precipitate w/ $CuSO_4$ & NaOH
means nothing!

From like
$CuSO_4$ + NaOH + heat → thermal decomposition to
Copper oxide
Sulfur dioxide
NaOH goes to Sodium oxide

this reaction is primarily our
dark precipitate. It has no use here

In summary.

1. We cannot get replicate the Xanth reaction

2. We can reliably produce an amino acid, presumably cysteine in Set 2.

3. We can do nothing w/ set 2 other than to know that it is there. Acids remove it.

4. We can isolate the bacteria w/ Gram Stain negative

5. But ~~remember that we get the dark precipitate from the~~ ~~organic extract w/ $CaSO_4$~~ — not true without ~~that~~ heat.

6. You have to by acetonequation.

We may, but at best only a very minor may, be on the track towards duplicating the Xanth reaction. With acetone, you are getting a slight cloudy reaction w/ HNO_3. You cannot however, separate or identify a precipitate

01/17/14

Today we have a regression w/ the cough we need to take care of that,
Iodine, vitamins, antioxidants, alka seltzer

---

1. The distilling process is in full order, we have macro to micro now in 4 stages. Very custom. We have also contained the process in a pan in case something were to heat or catch fire. - also in sand.

2. We want to test the acetone problem and ingredients sulfate + water + acetone. We must get our compounds into an acetone base to get into the IR machine.

3. We have lobby discussion to write about
   1. Lab tops of cross domain hackers -
   2. Culture temperament - how much to divulge?
   3. Gram Stain result
   4. Xantho test result
   5. We need to show expecting
      1. oral filament
      2. Culture + based on food + oral sample
      3. environmental samples
   to show how similar they are

5. We have Contained the concentrator process - Bacteria fumes likely is not as smart.

4/12/14

Today we have a Ninhydrin based column. Therefore a more red solution than normal.

We had a very quick colored result last night before sending that had a colored test. It tested negative for both NH & HNO_3, i.e., amino acid test. It was highly acidic.

You still had a rather wide ring remaining @ top. The solution was very acidic @ the time. You may also have started adding some alcohol.

Today you have continued to add alcohol but you are turning it basic. The wide ring has now split into 2 bands narrow (looks like a yellowish tint) and the tan band (wider). It is very slow moving but the yellow band is very slowly moving down.

The current pH is: 7.0
It has turned neutral & it will become more alkaline.

Very successfully now we see that $MgSO_4$ dissolves in water. But in acetone it does not but it should be soaking up some or much of the water.
We now test the of evaporation tests.

The acetone evaporation test was a complete success. If you remove as much of the water as possible and then mix it into acetone & add $MgSO_4$ to absorb much of the remaining H_2O you have a solution that is mostly acetone & it will evaporate quickly.

Acetone (liq) 1 drop evaporate in about 6 minutes
½ H_2O - ½ Acetone " " 20 min +
H_2O based " indefinite - maybe up to an hour.

So we now have a way of getting the compound into acetone

There are many ways to run a plate now.
1. Deal w/ large quantity on watch glass & evaporate over slow heat - mix w/ mineral oil

2. Build up drop by drop on a Cardboard no overlap.

3. Let's try #1

Jan 18 2014

Goals:

1. Run an acetone test w/ and $MgSO_4$.

2. Does oral sample produce a different Chrom result?

3. You are after spectra of:

   1. Oral Sample (have it, but can repeat + refine)

   2. Potato Culture

   3. Oral Culture

   4. Lava culture

4. You are after breaking down the oral culture into fewer parts, determining their nature (ie by chromic & not?) & then their spectra.

5. You are after DNA of bacteria.

6. Put in a long term goal.

1. You are after replicating the Xanthan reaction

8. Paper on trace minerals
9. Paper on Xanth reaction.
10. What does HNO_3 clearing up the solution mean? Maybe it did rx to the Cl_3?
11. H_2SO_4 seems to combine w/ the concentrated extract?

A major accomplishment tonight.

I have extracted DNA from the culture 3 times out of about 12 attempts.

1. Concentrated culture extract
2. Place in ice water
3. Add salt (seems like very little)
4. Stir slowly moderately in the cold water.
5. Add the soap while it continues to cool down and let set for 10 minutes
I used my enzyme soap!
6. Pour into test tubes 1/3 high
7. add alcohol to 2/3
8. Insert very very very carefully w/ my glass dispenser can happen up to 10 to 15 min later.
9. Extract w/ pipette
10. Place under scope & photograph
11. I repeated & photod twice & still have a mg of sample in the test tube

01/19/14

Producing DNA as a major accomplishment.

How about if we also get better @ clean + do it with a potato?

Dropbox

MRP

1. Patient Questionnaire
   Asana
2. Patient Photos
3. Samples

Buffers are a very interesting topic

Jan 20 2014

Now that we can produce DNA we have many options open to us.

1. UV work
2. IR work
3. Gel Electrophoresis
4. PCR

With extra time we have

1. Mayan [illegible] work
2. Xantho reproduction

1. Let's learn about buffers
2. You want to extract DNA from something else to compare
3. [illegible] question
4. [illegible] Organic Chemistry

Jan 20 2017

The DNA extract is positive again!
This time 3 for 3!
The method was:

1. Ice in a measuring cup.
2. 50 ml Deet in ice.
3. add 15 ml of culture concentrate
4. add only a small scalpel pinch of salt - not very much was needed.
5. Stir intermittently over next 5-10 minutes while solution cools down. Stored @ 2°C.
6. Now add 2 drops of enzyme based detergent.
7. Swirl very gently & place it back in the ice
8. Let it sit approximately 15 minutes
9. Pour it into test tubes. ~ 3-4 ml
10. Drop ethanol very carefully along edge to 2/3 full. It is absolutely crucial for the Ethanol should be chilled & not. This ethanol was approximately 14°C.

11. Watch and study carefully over the next 10 minutes w/ a magnifying glass as you see bubbles forming. Ethanol should be forming.
12. Extract DNA/pipette to store in w/acetone

01/21/14

Having DNA available changes the ball game. It opens the door to genetic research!

Questions:

1. Does DNA IR spectra vary?

2. What will it take to get electrophoresis working?

3. Chromatography expectations?

You has succeeded w/ ONION DNA as well with the same method w/ 2 variations

1. Alcohol was chilled this time

2. Alcohol was dispensed using a pipette. Strangely, the less a little more coarse, actually seemed to work better than less very sensitive w/ an eyedropper. The tube w a slightly more disturbed alcohol-water interface seemed to have been more successful. The DNA does indeed eventually float to the top.

3. Letting it warm up over 10-15 min does seem to help.

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01/22/14

DNA test: A less concentrated solution being used. 10 pipettes of solute = 6 test tubes

The time:

0. You are not adding soap until salt solution reaches 2°C.
1. I let the soap solutions stand longer, up to about 20 min.

2. I put the enzyme in as powder before the alcohol w/ large pipette, and then added the alcohol w/out shaking. I do see bubbles right away & watery goo

Yes we are positively successful. Lots of bubbles means lots of activity. One grape is already visible.

3. You let the DNA form in tubes taken out of the ice water.

4. This time you chilled the ethanol in ice water for the 15-20 min along w/ detergent.

The amount of DNA produced is low (solution concentration is low) but it is visible in all 6 tubes. Next time it immediately flows to the top.

02/14

DNA Trial #2

Looks very good. 7 tubes

Need
1. Tryptophan
2. Tyrosine
3. Power Cord

01/23/14

Important things are happening.

1. Need papers on
   1. metals
   2. tryptophan - xantho - serotonin
   3. different amino acids depending upon nutrients
   4. Bacteria Isolation - Gram negative
2. Arana Study
3. Gel Electro
4. Chemistry Course Study
5. Need Chip In!

Jan 24 2014

Another very interesting day.

1. Control tests are in place of
   1. Sugar, NaOH & big iron & heat

IT IS TURNING BLACK! You are indeed getting an amber color that is darkening quickly w/ heat.

2. The Columbian pH is approaching Xanthe Condition. What exactly to put in the Column?

3. Now that we see it, what is this reaction w/ sugar, NaOH, & heat a run? So the black portion means nothing! This, then, means nothing.

4. Start Gel Electro

5. Sugar + Heat + Lye + Iron = Black — why? In the future we must clean the culture

The boiling temperature of this is > 100 °C. That is why it was having a hard time boiling yesterday, it is more like

Sugar + heat + lye + amino acid = Maillard Reaction.

Maillard

No wonder it gets heavy each
a last time boiling.
BP looks to be ~ 104°
Lighten up now.
Sugar + Lye + heat only, no iron

Light Sugar + 1 dp NaOH

Light Sugar + High NaOH

Fructose
Sugar + KOH + heat = orange very pretty

It is the liquid iron that is turning it
from Caramel/amber to black!

This is telling us that there is most likely
a great deal of iron in the culture!
which is what the test is to be the case

So Sugar + Heat is Clear!!
Sugar + Lye + Heat is Caramel
Sugar + Lye + Heat + IRON is BLACK!

This is why your culture is black.
Some of it may be contaminated but
some is not.

In the future, we will centrifuge all cultures & rinse them.

HNO_3 turns the solution clear. So it very much has to do w/ an alkaline base. This is why it is black.

So we could try DNA w/ acid added to the culture extract.

This is very interesting. Acid does not turn the culture clear after it has been heated?

1. Acid does turn the oral culture clear before any heating.
2. It also turns the black potato culture clear after heating.
3. It also does after adding salt. Why did our DNA experiments (only added salt) not turn clear after adding large amts of acid (HNO_3 & H_2SO_4) and driving pH to ~ 3.0?

??
!!

This is a mystery.

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Ok, Time to regroup.

What do we want to accomplish today?

1. ~~Do centrifuged culture balance the same way?~~ & raged!

~~2. Start Gel Electro~~

* 3. ~~Our DNA test is about as positive as possible~~ so we do not need ~~to worry about~~ that.

4. What caused the Xantho result?

~~5. Write to Kate.~~

6. Get the other paper titles up
   Metals
   Xantho

7. Get the fundraise link on the home page

8. Study Arsenic

9. Study chemistry.

The DNA test remained highly successful today. Even when I treated the extract w/ high levels of acid & drove pH to 3.0.

There change appeared to result from the acid:

1. The DNA filaments are more clear
2. The culture has turned a lighter color @ the bottom of the tube.
3. There appear to be a crystal type formation that has occurred & is visible under the scope ~ 240X easy to see.

You are working on purifying the culture final material w/ Centrifuge. This is going to be very important to remove the phenol & chloroform solutions.

Looks like about 3 centrifuge iterations are needed.

Do not heat up the medium test tube!

Purification of Bacteria

You have done some amaya purification of the bacteria today.

Let's recall how:

1. Grow the culture.
2. Centrifuge & rinse the culture several times, looks like about 3 times to get rid of sugar & broth solution.
3. Heat the final extract in: KOH - NaOH solution. A minimal reaction will be visible here, even after 30 - 40 minutes.
4. Add 1 drop of chelated (liquid) iron. A profound reaction will take place.
5. Continue to heat 5 - 10 minutes
6. Centrifuge the iron-filament precipitate & pipette the final solution, which is purified & concentrated bacteria. Examine this on slide
7. Now see if you can get DNA. No, the ~~please~~ clear solution is not where the bacteria are. They are attracted to & react with the iron.

This DNA test did not work.

You need more culture material.

1. Does it matter when the iron is put in to the purified cultures being heated up?

2. Is the color of big iron cultures going to be the same as the $FeSO_4$ cultures?

3. How are you going to use the mass iron reaction information - what can you do with it?

4. Everything says we are dealing w a bacterial form.

5. How about we show how we inhibit the growth of it?

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Jan 25 2014

1. Lets learn how to make a proper buffer.
   borax & boric acid.

2. How does pH affect gel electrophoresis

3. Extract DNA from banana, mung, potato?
   human?

4. The culture - precipitate vs filaments
   Test the hypothesis further.

5. Gel electrophoresis of proteins?
   BSA, milk?
   amino acids?

6. Chemistry Study!

Loading Dyes:

It looks to me like Glost Blue Food Dye is easy to work.
Glycerine should be at least 5 times volume of dye + DNA combined in my gues. We do not want too [illegible].

Methylene Blue looks like it will also work but you need to use a Concentrated form.

You can indeed make a mixed loading dye made of red, blue & yellow food color. Jet will work.
Estimate
1 drop of each color
15 drops of Glycerine
5 drops of buffer??

You will end up with

Yellow — Fastest
Red
Pink
Blue — Slowest

01/20/14

You have a wonderful method of accumulating & purifying Isle culture.

1. ~~Take~~ an existing culture & pour off, ~~potato~~ & all growth into a 50 ml ~~tube~~.
2. Pour 50 ml solution into 2 med. test tubes (only set available) and centrifuge
3. Draw off sugar/iron solution w/ pipette
4. Add water to test tubes
5. Centrifuge
6. Repeat 3-5 until all collected & clear.

The solution is tan colored and does not react so strongly to KOH - NaOH

Jan 29 2014

Back from Missoula.

We have our refractometer!
But we must be careful, it says that it is not made of organic solvents.

But I wonder if that is mostly because of the plastic casing.

I think alcohols are going to be fine, especially short term measurements. Maybe acetone, or at least dilute acetone?

Lets start calibrating.

How about distilled water vs. tap water

Distilled	0.0
Tap	0.0 Great
Isoprop	24.2 (Assume 91% for now)
Ethanol	10.0
1M NaOH	7.0
Very Weak Salt Water	1.1
Strong Salt Water	3.4
1 Molar H_2SO_4	0.2
25% Isoprop	10.7
12.5% Isoprop	3.7
6.25% Isoprop	1.8
Weak Acetone	0.1

You have a very powerful way of distinguishing!!! Clear Blue

THIS IS great!!!

We know now that with

1. Index of Refraction
2. pH
3. ORP
4. Conductivity if need be

That we can positively identify a uniqueness of solution.
Even if it for clear.

You probably need to start to listen up on your controls or your solvents
& measure that not from now on.
Then is very cool.

Fuse 305

The lab is really coming together now.

Tonight I would like to finish with

1. Determine if broken end is indeed my clasp.

2. Can we do separate the culture into acetone?
(Non-polar solvent)

3. When we work w/ Ninhydrin we want to start controlling the pH better

4. Chromatography solvents are also going to be measured better.

5. We can also do pure IR work with this new sample.

6. Enzymes & KOH - NaOH may have broken down the culture.
It does not dissolve it, but it does seem to be precipitating it.

Urine:
Brix 2.7 @ 16.6°

Major NIN reaction w/ culture.

Recall what you did:

1. added some acetone to the culture
2. added some enzyme to the culture
3. added some KOH-NaOH to culture, extracted solution.
4. added NIN & heat - we set a major reaction

It was pure acetone!

1. Acetone + NIN no Culture + Enz + NIN no
2. Acetone + Cult + Acetone + Enz + NIN

Got it add time here

1. Cult + Pure Acetone + Enz + KOH + NaOH + NIN

is giving a huge reaction!

Looks catalytic!

Formed precipitate

Remember we pulled off extract after precip

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GOT IT

1. Acetone to culture
   Shake vigorously.

2. Add enzymes to culture
   Shake vigorously & likely allow some time (Seems like this forms the precipitate)

3. Add KOH + NaOH seems like it forms a precipitate — Is this necessary? yes!!!

4. Withdraw the acetone!

5. Add water to the culture

6. Add NIN - major reaction purple
   NO heat required.

Question - does this happen if enzymes alone?

Enzymes by themselves do not even dissolve in acetone so there is no precipitation reaction take place.

Yes it does happen big time the enzymes KOH + NaOH is what is breaking down the enzymes.

It does not appear to be needed in the culture

Enzymes + NaOH + NIN produces the reaction

Jan 30 2014

So many questions:

1. How can you break down cultures?
2. Is potato culture different in any way than oral cultures?
3. How do you explain a repeat the xanthe results?
4. What is the Flow Process Results (FP) for the potato culture
5. What do different solvents do to the culture
6. Why does the pot culture not separate even though you had pot iron in it?
7. Does big iron react w/ the culture ie potato, yellow
8. Why was one extract brown, why did it centrifuge?
9. Then large upshot question again w/ the potato culture + the oral culture react differently to liquid iron.
10. Upon what bases are you going to separate Chromo - what are you really seeking here?
11. Can you advance gel electrophoresis Closer to real life?
12. Can you extract Human DNA, banana tomato, potato, carrot
13. Why does the type of purified culture look like - is it any different?
14. Are you ready for the inhibitor threads

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Reactions w/ Potato Culture

KOH - NaOH lyse, release some color

Carbol - major purple color reaction

It looks like Sodium thiosilicate (ST) completely dissolves the filament w/out heat.

It also seems to interact w/ the filaments & absorbs the purple color

The solution of

Culture (Med Density)
Sugar
Liq Iron

eventually turns from purple to clear.

ST is reacting w/ the IRON not the sugar
Proven by reaction w/ Liq Iron alone.
However the purple complex is formed from the combination of sugar & Iron.

Result:
The presence of ferric iron in the culture can now be directly demonstrated by NaThio Complex.

Jan 31 2014

A Map achievement today.
The Exhibition paper has been posted today.
The entire process from start to end
was 24 hours.

We added to our Electra spreadsheet
today w/ the culture + culture medium.

Main topics:

1. DNA
2. Gel Electro
3. Concentration of cultures
4. Chromatography

ORP pH Brix

C/N ratio 1.4

medium (Sugar + Lignin)

.130

$Ca(NO_3)_2$ 2 3

5 mols

45 * 962 = 43290.
1280 · 260 4.9231 332800
3 2

= 87.93.24

8.8E3

multiplication - least significant
division least significant

8.79324E3
to two = 8.8E3

4.5E1 * 9.62E2 = 43.29E3
1.28E3 · 2.6E2 3.328E5

= 43.29E3 = 4.329E4 = 1.3008E-1
3.328E5

= 1.3E-1

$$\begin{array}{r} 264.3 \\ -\ 258 \\ \hline = 6.3000 \end{array} \qquad = 2643E3 - 2.58E2$$

$$= .0630\ E2$$

(3) on numerator
2 on bottom

$$\frac{6.3000}{65} = .0969$$

$$= 9.7e-2$$

$$\frac{3 \text{ on top}}{2 \text{ on bottom}} = \frac{6.30}{65} =$$

$$9.7e-2$$

$$1.834E5 * \frac{1E3}{1E3} =$$

1 liter = 1000 cm3

$$1.834E0$$

Benedict's Solution
Make It

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Benedicts Solution — Formula

2.06 M $Na_2CO_3 \cdot 7H_2O$ 232.1
.94M Sodium Carbonate ~~106.0~~ ~~258.06~~
258.01? .59 M Sodium Citrate 294.1 ~~294.10~~ $Na_3C_6H_5O_7$
.069 M $CuSO_4 \cdot 5H_2O$ 260.4

.94 M Sodium Carbonate = X M Na_2CO_3

Na_2CO_3 is 106 g

$Na_2CO_3 \cdot 7H_2O$ is 232.1 gm

.94 Mz = 99.64 gms

to get the 99.64 gm need to increase it by the ratio of $\frac{232.1}{106} = 2.19$

so you actually need

2.19 (.94M Na_2CO_3) = 2.06 M $Na_2CO_3 \cdot 7H_2O$

Ratio	Compound	Molecular	actual GMS / 1 Liter
So			
2.06M	$Na_2CO_3 \cdot 7H_2O$	232.1 gms	478.1
.59	Sodium Citrate Dihydrate	294.1 gms	173.5
.069	$CuSO_4 \cdot 5H_2O$	260.4	18.0

or in 100ml

48 gms $Na_2CO_3 \cdot 7H_2O$
17.3 gms Sodium Citrate Dihydrate
1.8 gms $CuSO_4 \cdot 5H_2O$

& heat to dissolve stuff

Feb 02 2014

Benedict Testing for Sugars in Culture

1. We have a very small reaction w/ Ben reagent & fructose. Extremely sensitive
2. We put a little sugar in water & a test tube & heat it up & we set no color. The was not expected
3. W/ Culture clear solution we are getting an olive color w/ Benedict.

Fructose + Heat = Clear
Fructose + Heat + KOH-NaOH = Orange
Fructose + Heat + NaOH = Yellow
Fructose + Heat + Liq Iron + NaOH =
Fructose + Heat + Liq Iron + KOH - NaOH =
Culture + Benedicts + ~~Olive~~ ! Heat = Olive
No alkaline added!
Benedicts + Fructose = Brown Precipitate
Heat + Culture + $CuSO_4$ = no major reaction
Dilute Culture + 1 drop Benedict + Heat =
* Dilute Culture + 5 drops Ben + Heat = Major Precipitate
Ben + Heat = Light Blue =
Small - Fructose + Heat + 6 drops Ben =

pH of Benedicts is 11.5
that is how!

Pase 315

ORP ≤ −150

The precipitate from
Culture (maybe only from) + Ben + Head
More
seems to be heavily laden w/ bacteria.

Should be easy to extract DNA from.

The precipitate & sugar solution
seem to go away w/ continued heating

* 7 drops Ben, 1/3 Test tube w/ clear culture

It appears to be true that Benedicts
coagulates the bacteria out of
solution.

We have a very dark precipitate
w/ no lye added straight from
the culture w/ a pipette clear
extract added.

Long term heating. Also filaments?

Very extended heating seems to destroy
the malt
Advantage — No lye required

pH becomes 10.0
& ORP −500
after extended heating!!
How?

Notice this. It was highly acid

	pH	ORP	Color
10ml Clear Culture Extract	6.5	−35	Amber
Clear Culture Extract + 10 drops Ben	11.1	< −150	Milky yellow
Clear Culture + 10 drops Ben + heat 1 min			Milky Brown
6 min			Looks just like the sig a ~~test~~

5
~~3~~ 4 drops Forms a strongly precipitate

Time exposed to heat & Ben Concentration seem to be making a difference

What is different over Kott's that a precipitate form & that reacts something glued stays

Feb 02 2014

~~Feb 03 2014~~

~~Water evaporation project~~

(1) 1000 ml → 140 ml

(2) add to make 1000 ml → 140

A big question I have is how did the pH of the clear extract approach spectrally?

pH w/ Supra-alae ~ 8 a.m.
but the liq iron drops at immediately to highly acidic = 3.6

So here is how the pH can go up — by using up the iron!

Thick Culture pH 3.5

Moderate Culture pH 3.2

Clear Culture pH 3.9 actually still very acidic

buffer 10.2 still accurate

So indeed it is not neutral, it is still highly acidic

Culture 1/3 TSB w/ Clear, Add Water to 1/2
7 drops Benedicts
Heat 90 - 95°C
Brown Precipitate Starts to form @ bottom w/in 3 minutes
Solution turn Olive-brown above

7 minutes: Solution up top turns more clear w/ olive color. Tan precip settles @ bottom.

3 drops more 12 minutes in.
15 minutes, precip starts rising

Next Case is

1. Clear Extract
2. No Dilutions
3. 10 drops

Question: Are all Chemicals needed on Benedicts or only some for precipitate to form

Precipitate is a rust color.

Feb 03 2014

Questions for Today:

1. IR spectra of precipitate?
2. Can we get DNA from thick culture extract?
   Do we need to blend it? If so, how?
3. Can we get lots of precipitate from thick culture? What if we add alcohol to separate?
4. DNA from banana, etc?
5. Exactly what chemicals are required to produce the Benedict precipitate?
   Cu, Washing Soda, Sodium Citrate?
6. On the precip - Ben reaction today, will have added alcohol to the solution, in an attempt to get the precip to float above.

Very peculiar reaction
alcohol is reacting just like DNA does

7. Very strange repeat - it really does look like DNA?

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No it is a protein precipitate, not likely DNA

Feb 04 2014

1. DNA is produced by alcohol & heat alone. This is amazing. But we also learn ~~that~~ it is not pure.
2. Filter medicine extract
3. Cu - ~~to~~ question — OK
4. Your course?

Testing which chemicals cause the precipitate to form.

1. Iron filings + $CuSO_4$ pellet cause the iron to become coated w/ Copper (via your tube demo) and they also floated to the top w/ air bubbles. Solution turns from blue to green.
2. Clear Culture + Ben + heat causes an orange precipitate (looks like rust) to float to top.
3. Clear + $CuSO_4$ pellets + heat

Conclusion: all chemicals in Ben are required to produce the brown floating precipitate. *

$CuSO_4$ + Washing soda alone produce a major volume of precipitate. Just what this has to do with the brown floaty precipitate.

The snow water, after concentrating by a factor of roughly 10X still shows excellent results.
TDS is 54 This is very good.
pH = 7.8 not bad at all.

Feb 05

KO A(solid) ~~134~~ 254nm ~~260~~
~~37~~ ~~200~~nm 130

Cuttm 1.72/ 254
1.721 200

Water ∅
∅

Ethanol 207/80

1.722/1.360 CuSO4 .5M

We Love a UV detector workey!!!

Feb 06 2014 Page 324

1. We have a need to concentrate - does heat destroy? Can you set into acetone?
2. Build a connect to UV detector
3. Stay the course

I am heating up clear solution.
We heat to 80°C & then cool slow.
Looks like it works like a champ
& does not decrease DNA products
when alcohol is added.

Now I am not really sure it has any DNA in it.
When it floats after adding alcohol &
you extract it & transfer it to water
We cannot really say it dissolves.
It forms a precipitate. It is
a very easy way of pulling a precip
from the clear solution.

Remember that your DNA is an entirely
different process using salt detergent
enzymes & cold alcohol & never
any heat.
You also need to boil material, not the
clear extract.

254 260 ratio
~~460/340~~ = 1.41

Culture
diluted
approx 10.1

We have a 254/200 ratio of
1.41

This leads to 260/200 ratio
on Protein % Nucleic acid

100	0	.57
95	5	1.06
90	10	1.32
70	30	1.73

$$\frac{1.41-1.32}{1.73-1.32} = .2195$$

$$.2195(30-10) = 4.4$$

$10 + 4.4 = 14.4\%$ $\quad 90 - 4.4 = 85.6\%$

So we estimate at

85.6% Protein + 14.4% Nucleic Acids

There is more than uncertainty.

the means that the clear extract has max absorbance @ DNA & Protein wavelengths and that it easily exceeds theoretically of the instrument & that [illegible] a dilution of approx 10:1 fall w/ the midrange.

It certainly seems like we have some.

What would happen for example, if you used a banana extract?

Our first estimate is..

95.6% Protein
14.4% Nucleic Acids
} of the constituents which absorb UV energy @ 284/260.

This does not mean that there could not be other things

Second Trial 610/440 → Ratio ≈ 1.39
Same result, re [illegible]

Egg Yolk

960/770

Estimated concentration =
1.55 (g/dl) - .76(.77) = .90 mg/ml

This means your protein concentration actually relatively high.

260/280 Ratio ≅ 1.125

This says about 91% Protein, & about 9% Nucleic Acids

When you heat up egg white you get a precipitate!!! ie a solid.

So you are most likely getting a protein precipitate when you heat up the extract that is combined w/ alcohol

Try it w/ egg white like I did & you will see!

- You can transfer the precipitate to water!

Feb 07 2014

We have the Gilson 112 working!
Styrofoam holder & plastic cuvettes
are working fine!!!

We set a ratio on Clean extract of

Set 1 $\frac{117.5\%}{94\%} = \underline{\underline{1.25}}$ probably more realistic

$\frac{1.25 - 1.06}{1.32 - 1.06} = .73$ = 91.3% Protein
= 86% Nucleic Acid

This looks very reasonable.

Eq. for Gilson 11215

$$Abs = Sens.\left(\frac{Readout}{100}\right) * \frac{10}{Pathlength.}$$

So for us, Path length of cuvettes = 10 mm 50

$$Abs = Sens.\left(\frac{Meter}{100}\right)$$

We are now using Sens. of .12
So our actual reading is

$.1175(.2) = 235$ or on 11b = .023

Either way, our instrument is very sensitive.

Looks Good. 9o

We set time constants to 0.1 and it worked better. We zero @ a higher sensitivity

Water = 0.0 @ .12 AUFS
Isopropyl (254) = 18.1 @ .12 AUFS
(280) = -12.1 "
(254) = 20.0
-11.3

Culture
Seems to matter if it has settled or not.

TC=.1

254 = 148 @ .05
280 went negative ???

TC=1

254 122
280

You must autozero 254 & 280 one at a time!!!
So you measure 254 first for everything
then come back to 280
Autozero every time.

You are getting some stray light error, Add lightflop

Ok, with new autozero info.
.2 AUFS TC=1.0
254 Water 0.0
Iso 14.5
14.0

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Culture
Maybe you have to autozero selective?
Water did not hold autozero.

You are getting way too much stray light in
Results are not reliable.
To mice.
It did not work.
We use the old machine

Chemistry Course

Ex Lowest energy —
Y
X

X
Y
Z

The shells want to be filled
they want to seek out the lowest
Energy State
Good

Y
Z
X

Oxidation States:
(An electronic accounting system)

Oxidation state that all bonds are ionic
(They are not really)

Oxidation State Rules

Rules must be followed in this order!

1. Pure elements are all 0.
2. F^{-1} (Fluorine always ends up -1)
3. Metals:
   - IA = +1
   - IIA = +2
   - Al = +3
4. H ~~can be~~ is usually +1, but it can be -1 w/a metal
5. O is usually -2 (can be -1)
6. Halogen are usually -1

Prac 332

+1 −1

~~No~~ Cl (−4) ∅

~~Fe~~ O_2 ~~no~~

$H^{+1}(OH)^{-1}$ ← you missed that O_2 (times 2) = −4 !!!

Fe is +4

O^{-2} } leads to −1
H^{+1} }

I would rather think these through rather than memorize a set formula

F_2 ~~0~~ ~~Wrong~~ It is zero, elemental state

+5
RO_4^{3-} O_2 is normally −2
you have O_4 so this is −8
but ends up −3 so P must be +5

+3
PF_3 F is −1, so F_3 is −3, so P must be +3

+2
$Pb(OH)_2$

Al should be zero! SO_4 is −2

~~+4~~ +8
$Fe(SO_4)_2$ ~~0~~
SO_4

+4 −4

So why is a SO_4 equal to -2?

~~Sign~~ S can either gain 2 or lose 6 +6 −8
O apparently always loses so $SO_4 = -2$