CARNICOM INSTITUTE LEGACY PROJECT

A Release of Internal Original Research Documents

Authored
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5 SUBJECT

180 Sheets

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Chemistry - Lab Notes

Sep 2016

Vol XVII

Sept 1 2016

Good Morning!

Today we plan to:

1. Investigate alkynes more thoroughly w/ the polarimeter
2. Analyze the racemate w/ the polarimeter.

3. The invitation requires revision (CHPN)
4. [redacted] book to be downloaded + keys exported.
5. Repeat my ham analyses.

We learn w/ polarimetry that the solution must remain clear to transparent. A dense solution will block the light too much.

Trying again w/ a diluted COB red layer:

0.00	+.10	$\bar{x} = +.03$
+.15	0.00	
0.00	+.10	This is within experimental
0.00	−.05	error. No chirality evident.
0.00	0.00	

Let's run water by itself. ~~Do not~~, Yes, let's.

This also means, however, that the sugar is completely consumed. What about $FeSO_4$? Is it chiral? Apparently not!

6. Chiral analyses for alkynes?

14.44 and H_2O gas 14.5

but we measure 14.41
so this shows it may more dense than water.

You can, therefore not assume that it is more dense than water, eg an alcohol?

Barnwater Chirality Test Use both versions!

-.05 No chirality is evident
0.00 in racemate samples.
0.00
+.05
0.00

Chiral means Asymmetric

AChiral means Symmetric

And that means completely asymmetric in both structure & position (ie, fore and aft)

Then a very interesting. you are not picky up the car CO @ 120°C, only CO_2 & O_2 ??
How is this possible?

We are getting some ray Coyne results here with the water background and ATR Controls.

Something is out of control here.

If we just look @ water,

OK, found an error w/ Xylene background

The wrong background was picked. You picked Xylene by mistake.

OK, you are getting there now, but you still show some hydrocarbon activity in distilled water which should not be there.
Run all Controls again.

We are now looking @ ATR Air

vs ATR Air w/ Glass Slide!

There is some difference in the hydrocarbon area. why?
Also Co2 area is different.

So we do have some differences

The background should be recorded w/ the glass slide!

Write both air and water

We want
1. Hair
2. Urine
3. Red Test layer
4. Saliva
w/ proper ATR controls.

We want CO from car verified.

Blood in Polarimeter?
Saliva in Polarimeter
Urine in Polarimeter?

Conclusion:
Air ATR is identical w/
Air ATR w/ Glass Slide. This is good

Water with Glass Slide ATR is different Good.

Ok, we are getting a much better plot of the CO3 red layer now.

A Current clean ATR w/ destilled water seems to be important.

You definitely had an error of the red test tube layer. There may not be an alcohol there are definitely alkenes but alkynes are not known now.

Alkynes do not show up in red layer but alkenes do.

The ether functional test is having some real problems. Cripper p195 Iodine Test Lets repeat & keep Closer track

In addition Carbon Disulphide is bad news!

Be Very Careful!
No sparks, no shaking towards bag

On Chirality, a Carbon attached to four different groups, is Chiral

First Lesson:
Real Iodine is Completely Different than bottle Iodine.
Real Iodine is purple in Carbon Disulphide.
A brilliant purple.

—

We do have a successful test for ethers with the red layer.

The method is tricky.
The proportions of the Cripper test on p195 are important to match. The test is designed for a fuel strength ether.

If your ether is very dilute, or none at, you should scale the iodine CS_2 solution way back
Eg 1 ml of my red layer solution will only required 10-20 uL of Iodine - CS_2 solution

With the proper proportions, we as you will recall has an shift in color.

The test for ether is successful but we will repeat it when we reproduce the red layer with other cultures.

We have a very nice match in color taly place w/ Ethylene Glycol Monoethyl Ether

There was a shift towards from the red hue toward a tan hue that matches exactly with the ether defined above.

It is also called methoxyethanol
(also called methyl cellosive)

The initial assessment of the "red layer" is that it likely contains

1. alkene IR & Baeye test
2. alcohol (IR) & density determination
3. Ether (IR & Copper test)
4. Nitrogen (SOBS
4. Transition metal, most certainly iron

Possible CDB Butane Production.

By GC w/ a isothermal run have some evidence to indicate that butane might be produced by the CDB culture.

Conditions are
120° Isothermal
Retention Time = 4.44 min

	Model Prediction	Butane
MW	55	58
VP	-15	-.8
DP	Ø.18	.13

Assuming a hydrocarbon.
The following to be on the lookout for butane production by the CDB test tube culture.

Retention time @ 80°C is estimated to be: 5.8 min
Retention time @ 150°C is estimated to be: 3.5 min

Be on the lookout for these.

We are now learning that all of the following have either alkenes or alkynes in them.

1. Blood (alkynes known to be present)
2. Hair
3. Urine
4. Red CDB layer
5. Saliva?

Sep 18 2016 Sunday.

On tap today:

1. I would like to explore the polarometer a bit.
2. I would like to investigate saliva's by the difficulty?
3. I would like to reexamine the Env. Filament Proj
4. I would like to explore the potential butane production by COB.
5. I would like to assess the Concentration of
   alkene ether
   alcohol Nitrogen (SOBS)
   iron
   and Construct a tentative model.

   EMNO4 in acid test
   Alkenes: IR & ~~Baeyer Test (CS2 & Iodine)~~
   Ether IR & Baeyer Test (CS2 & Iodine)
   Nitrogen SOBS only (amines w/ Nitrogen?)
   Alcohol IR, NIR and density
   Iron Color and availability.

   Urea need has an alkene group so it could be resident.

6. We are evaluating Lair Blood Urine Red Layer Saliva for a Improvate assessment

We have just discovered another important reaction the CoB red layer produces a blue green precipitate in alkaline solution.
It is a significant reaction

It is insoluble in water. It is a blue green color. I believe that it is our protein.

Bradford test should work.

Fascinating. We now have a way of growing the protein, not just separating it.

Growing the isolated protein.

The protein is red and soluble in acid.
It is blue green and insoluble in base.

The entire Bradford test is in question. We know we have protein, now by IR, but what has happened w/ Bradford?

We add Bradford to water alone! no protein added and we get the color change to blue.
What does that mean?

The Bradford test in general test it is not what you [illegible] thought

The Bradford Test worked extremely well.

The trick is to acidify the solution w/ conc acid & then use that as the reference. You will have a double peak.

When there is protein there become a single peak which shifts to the left.

You definitely have created the protein

We know now that we have an original protein.

You are on track w/ a Biuret Protein. Now you need to learn how to identify a specific amino acid.

Also, how do you test for sulfur?

Sep 19 2016

Today the objective are

in progress 1. Continue GC investigation of COB both gas & red layer forms.

done 2. [redacted] hair

3 Saliva

4. What about the Protein labs?

Still to do 5. [redacted] Book

Done 6. ATR Noise Test is in progress.
The lesson from the ATR Noise Test is that all signals less than 3% must be disregarded with special attention to false signal @ 3275 & 1056

3275 =

1056 =

DONE 7. COB IR Gas Analysis? - No Signal - 24hr active

In Progress 8. Rainfall sampler Controller Continued.

First lesson for today:

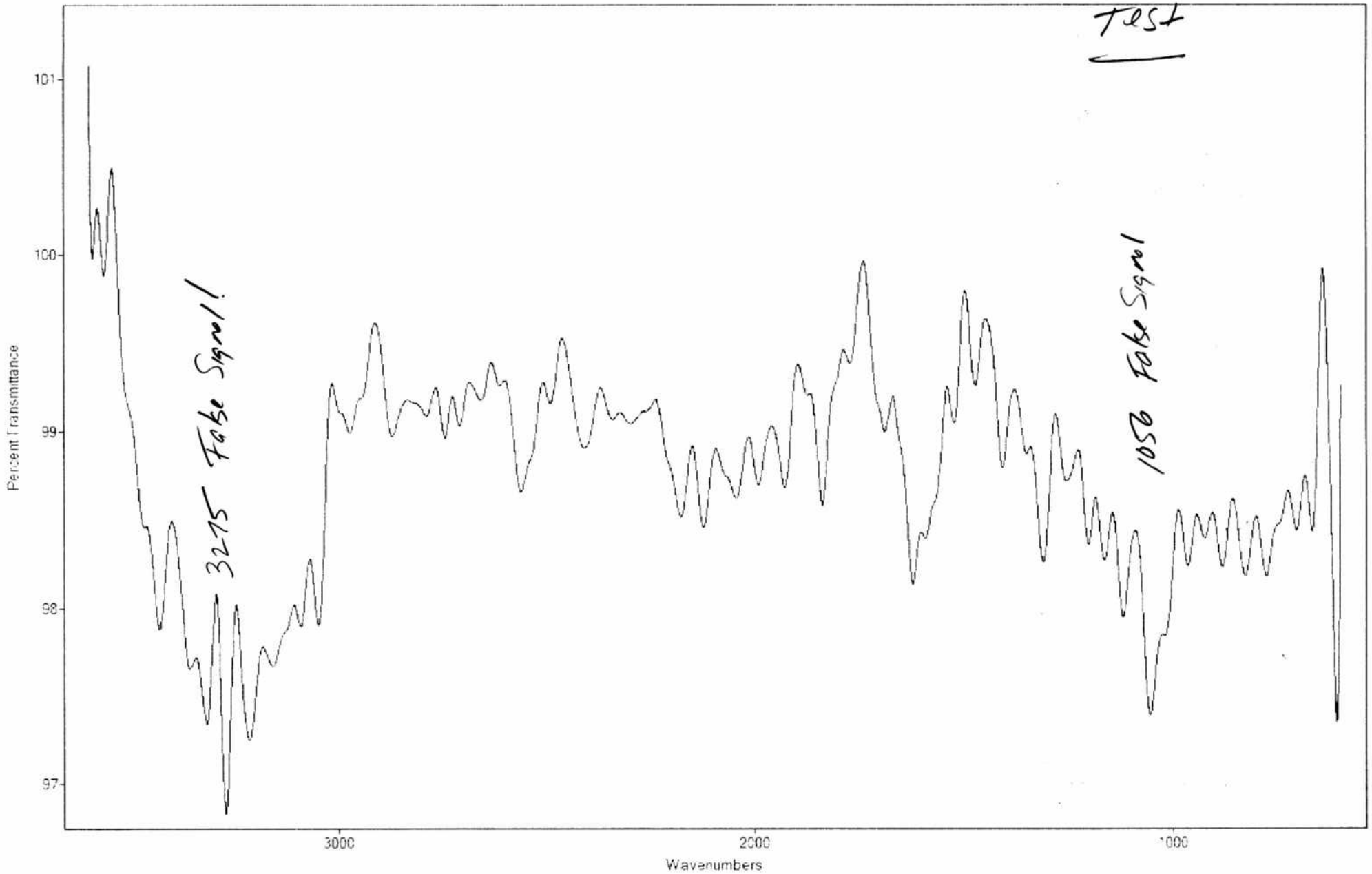
There is no IR Rainfall ATR Concentrate signal.
The noise test does affect the interpretation
Low Signal @ 3230 is ≤ 3% so we disregard it.

There is no signal!

9. [redacted] fund widget.

ATR Noise Test
Disregard all Signals < 3%

ATR Noise Test



eFTIR

The interpretation of this is that there are no significant organics dissolved in the rainfall Condensate.

This does not mean to insoluble materials have no inorganics, only they says that they do. If soluble the signal is too weak to detect.

Ok, a good chromatographic plot of GC COB Gas @ 120°C Isothermal. Only N_2, O_2 & CO_2 but still a nice stable plot.

Actually we do have something @ 16 min @ 120°C. Could be CO?

Now we have 2 peaks. Another @ 16.5 min @ 120°C Very Good. Both appear to be non-polar. Use the Car for Comparison.

It does not look like CO.
We have CO projected @ 40 min @ 60°C

We estimate 2.9" for CO @ 120°C so we expect something different here @ 16 & 16.5 min

Look for repetition Tomorrow. We have another w/ yeast @ 3.93 min @ 40°C ??? Not carbon dioxide

Now, reviewing Car Extract we have a good plot of CO @ 4.27 min @ 80°C.

Own two peaks cannot be CO.

Flow marked!

Notice we may have an agreement

Rainwater 220°C 64.6 min

CDB Red Layer 220°C 55.6 min

We have an unknown peak here that may be methyl.

What we found tonight was that the 24 hr CDB test tube culture, which we know produces CO_2, occasional CO & maybe some HC's Produced No Signal.

Try again when the culture is more developed.

Now, revisit our agreed list:

1. We have saliva — Polarometry?
2. We have the Protein Labs
3. We have [redacted]
4. GC Peak at 50 min for red layer & Rain water requires 10

We are or have worked on

In Process — 1. [redacted] found widget
Repeated OK — 2. [redacted] has repeated
Will Result — 3. CDB 24 hr culture IR Gas Analyzer (null)
Important Result — 4. ATR Noise test (Use 73% only)
In Process — 5. Reducing rainfall samples

to
Saliva remains of interest.
Polarimetry of urine seem to be a question
Protein Calc Conc.?

HEPA filter repeat.

My urine, when fresh, is not optically active.
(older urine is)
Thus probably a good thing because if it were
active it would indicate the presence sugars,
proteins etc and thus is not something that
we want.

With GC, you are getting defenite separation
to take place.
Five components, evidently water, determined within
the first 7 minutes,

Sep 20 2016
Today:

1. It can be seen that polarimetry is the perfect tool to monitor the metabolism of the culture as sugar is converted to protein. Let's test this idea along w/ citrate addition.

2. Saliva

3. Env. Analyzer

[redacted]

5. Hepa filter yeast analogy.

6. CDB Red Layer GC Analyzer. Zero In.

7. The Protein Labs
8. HEPA Inorganic

Well, we obviously have protonation material on the HEPA filter. KCl disks may amplify high end of the band w/ amines.

KCl disc indeed is what picks up signal 73000 which is important to detect amines.

ATR Can and does miss the high end signal!

Ok, you have some great work falling into place.

1.15 $\bar{x} = 1.175$
1.20

1.5 $\bar{x}$ 1.55
1.6

1.3 $\bar{x} = 1.325$
1.35

Ø.8 $\bar{x} = Ø.85$
Ø.9

.45 $\bar{x} = .475$
.5

.55 $\bar{x} = .575$
.6

$\bar{x} = .9925$
$\sigma_s = Ø.440$

Aza a Control Culture Solution
0.2 ml in tube

.95 $\bar{x} = .975$
1.0

Ø.2 $\bar{x} = Ø.25$
Ø.3

1.25 $\bar{x} = 1.275$
1.30

1.45 1.475
1.5

Not the best, but it is what we have.

Now, to other approach is that you can leave out the iron and use 1/2 ml of solution. If the well shows a high soya content.

Now just use soya water 1/2 ml in tubes. Test tube 1/2 full, 3rd spoon cup w/soya

.65, .70
.75, .85 $\quad X = .745$
.7, .75 $\quad \sigma_S = .012$
.8, .85
.65, .75 $\quad$ Si a clear solution gives a much cleaner readout.

Caltan 48 hrs.

0.5, 0.6
0.3, 0.4 $\quad X = 0.365$
0.2, 0.25
0.2, 0.25 $\quad \sigma = .143$
.95, .5

The indicate about 1/2 of the soya is consumed after 48 hrs.

The original concentration should be ~

$$C = \frac{d_{68}}{2.l} = \frac{.745}{64.5} = .0115 \frac{gms}{ml} = 1.15 \frac{gms}{100ml}$$

We estimate the concentration to culture is now $\frac{.365}{69.5\left(\frac{10}{10}\right)} = .0056 \frac{gms}{ml} = \frac{0.56 gms}{100 ml}$

So far, it has been cut in half in 48hrs.

Then a small practical unit of polarimetry. To set this experiment otherwise would be very difficult.
Then a very practical. You can do then or forward.

Refractive index may be a lot lower.

	B-1X
Refractometry	~~14.7~~
Control	14.7
Culture 48hrs	19.5 increasing!
Mature Red Layer	11.1 ?

Does this mean that a peak is reached?

Sep 21 2016

Active dots:
.70 .75
.50 .50
.25 .30
.15 .20
.4 .45

X = .42
O = .20

[redacted]

3. We have made some good progress w/ paper electrophoresis and now we see paper chromatography is also valuable! So our field methods have expanded considerably.
We do need, however, better paper!
It is on the way!
Art supply paper might work but we do not have access to that right now.
We could try a glass rod method.

Drawing Paper will work! Maybe?
It worked for good coloring but blood is not working @ all today. I do not know why.

You had a perfect separation take place yesterday w/ blood. Today you have none.

I really do not know why. I added salt yesterday and I have today. I have used two different papers and I see no change from that.
I have placed the spot in the center and no change. I have no idea why this is happening.

Maybe the pH was too high - it was @ 11.8 and I have dropped it to about 10.0

Also I had the extra tray in yesterday maybe it does need to be above the water or there is no net charge ∴?? It so if it is under solution there can be no difference in charge.

The main thing we need to do today still is measure Brix and optical rotation of culture.

09/20 We have: Control Culture, No Iron

$\alpha_{obs} = .745 \quad \sigma = .012$

Brix = 14.7

Culture 48hrs

$\alpha_{obs} = 0.365 \quad \sigma = .143$

Brix = 19.5

Mature Red Layer α

$\alpha_{obs} = 0.00$

Brix = 11.1

~~09/21~~
09/21 Culture 3 days.

$\alpha_{obs} = 0.42 \quad \sigma = .20$

Brix = 21.8

This shows no statistical diff. from 48 hrs @ this time.

How could this happen?

We put 6 drops of blood on the sheet.
Used regular toilet paper.
Use mold to stuff?
Borax?

Yes it is Borax & Boric Acid.
Last night you had lots of blood.
Today you produce no blood, very unusual.
Remember the three in the center worked
perfectly. The other @ the end did
not seem to work so well.
Did you add salt? I don't think so
@ what time.
You had your tray and the strips above
the water.

You had a wider strip so you have replicated
that.

Remember that you were not sure if it was
even food Coloring because it worked so well
so you replaced it with one that had been
sitting a days for some time

Maybe it should be Coagulated?
Maybe it should not be?
What happened yesterday that worked so well?

Apparently it is not going to work.
It is not reproducible.
The pits a slow change on the paper
electrophoresis idea.

Somehow it seems like only blood is useful
for the method as I tried any way.

I wonder what the factor was? Food color
worked great.

Food Colors are made from petroleum
and they are small molecules that bind to
proteins. They are not proteins
so the fact that they worked has nothing to
do with blood.

Phosphate Citrate or another type of buffer.

09/22 Ø.3, Ø.35   Ø.25, Ø.3   X=
Ø.1, 0.1   Ø.1, Ø.15 σ_{SJ}
Ø.15, Ø.25

10ml / 1/2ml sample
Remember the actual concentration is 20 times greater

Sep 22 2016

1. We are running our first SDS Page vertical electrophoresis trial w/ food Colors. We are getting a response.

2. We see the first hint of the red layer (very weak yellow at this point) on the COB cultures. 4 days in right now.

Culture Time | We want to measure: Culture | Notes Control

		αobs	σobs	Brix	
Ø hrs	9-18	.745	.072	14.7	(Control)
48 48hrs	9-20	.365	.143	19.5	
72 3 days	9-21	Ø.42	Ø.20	21.8	
96 4 days	9-22	Ø.205	0.10	25.5	First hint of yellow layer
120 5 days	9-23	Ø.270	Ø.17	23.5	
144 6	9-24			23.2	
7	9-25			24.0	
192 8	9-26	Ø.03	Ø.07	27.3	This says almost no yellow layer

(y=.2043 + Ø.530e$^{-.02049 hrs}$) r^2=0.95
,30 .50 .15 0.0 .35
,30 .50 .20 0.05 .35

~~(y= -2.08 + 2.62e -.0011 hrs) r=.95~~

αobs y = 1.071e$^{-.0172hrs}$ r^2=.97
Brix y = 28.04/(1+.919e$^{-.0168 hrs}$) Mse=1.14

		αobs	σobs	Brix	
240 10	9-28			24.6	
288 12	9-30			22.5	Red is
15	10-3	.018	0.084	22.4	developing
18	10-6			22.5	slowly.

abs all available $\hat{x}$=15.2   18.1 & 12.3 are two long term end product measurements.

Exponential Decay fit to sour

For 2065 $-.011 \cdot hrs$

$2065 \approx 0.736 e$ $\quad r^2 = 0.99$

for Brix:

$$Brix \approx \frac{25.62}{1 + .754 e^{-.014 \cdot hours}} \quad MSE = 0.25$$

Never forget to neutralize the lye or a strong acid before you put in in the ATR!!!

You will destroy the ATR if you do this $1500!!!
Pretty much irreplaceable. And it will set Cl back considerably and unnecessarily stain for the crystal in the Polarimeter.

Now we are titrating the Lye.

We have assessed the Brix.

We have mobile one using the CDS GC.

We have a 220 peak after a ramp up using 80-220 30 m @ 26 min.
Interesting. Is this real?

Sep 23 2016 Page 27

1. Good work w/ the SDS Page Food Colony trial. You see that it did get worse by the 3 days. You also see that you were able to break the gel Carey easily. You can also save your buffer.

2. We are trying to dissolve rubber and plastic in the microwave. HCl is very toxic w/ Chlorine gas. Hydroxides are not such a problem.

3 We know enough to proceed w/ the next layer of protein gel analysis.
Another lab also uses Vertical electrophoresis so we really only have 2 available + 1 backup.
This mean that our next run should be the real lab.

4. The lye does seem to be breaking down the plastic & rubber very slowly.

5. There is no real change in the cultured "red layer". It is still very weak yellow. Index of refraction meter may be sufficient @ this time.

Sep 24 2016

Many exciting projects going on now.

1. You must run a series of citrate cultures for Comparison
2. The protein run must take place today.
3. Collect new data on the current culture set.
4. What is the white crystal material that we have made?
5. Env. Filament Study
6. DNA Study?

For our position sensor we must
load in order

A	B	C	D	E	F	G	H
						Red Layer	

Leads are

A	B	C	D	E	Blank	Test Wire	F	G
1	2	3	4	5	6	7	8	9
Red	White	Blue	Green	Yellow			Red Wire	

Starter
1600
49.4V
.04Amps

Blood 9, 10

The protein runs in Mr. Neal McCoy.
#3 & #5 have entered the gel.
The reference dye does not seem to be working.

#3 is screen
#5 is spinach leaf.

1. We have measured the Brix on cultures.
   No surprises.
2. Protein cultures are frying.

800 ml H_2O
100 ml methanol
50 ml acetic acid @ 40%

Glacial acetic acid
is 17.5M
$= \frac{17.5M(60gms/mol)}{1000ml}$

$= \frac{1050gms}{liter}$

We have 5% solution with Vinegar = $\frac{5gms}{100gm}$
$= \frac{50gms}{1000ml}$ but we need 1050 so $\frac{1050}{50} = 21$ times

and 21(50) = 1050 ml
So use
fact. 900 ml tap Vinegar & 100 ml the methanol
Vinegar is cheap

I am very pleased. I now have the SDS page protein separation working for the very first time ever.

You had two major problems..

1. The cassette was facing the wrong direction (The writing needs to be facing toward the cathode so that you can read it as it is running.

2. You had the cartridge misspositioned so that the slit was covered up and it could not complete the circuit.

3. Also on previous runs you did not realize that you need to unwind the slit to begin with by removing the tape.. You should be in much better position now.

4. Next you need to learn how to prepare the proteins so they are in solution.

Now being able to conduct paper electrophoresis in the field would be very useful.

We do not know why it succeeded once and then it failed with blood. Why?

Are there any references on this?

Ok, Returning to Paper Electrophoresis also I see signs of progress again.
We have:

1. Switched to SDS buffer!

2. Add salt to ensure some current flow

3. Add just the right amount of water. Sections must be mostly separate but not quite separated. It is a delicate balance.

4. No air bubbles beneath the paper.

5. Closer to the electrode seems to be working better.

I started out @ 0.4 mA w/ Paper. I have added salt. I am up to 6 mA now.

The blood is moving but very slowly. Maybe it has coagulated from the finger source? Is there something we can add to keep it from Coagulating?

Let's drop Voltage to 15V overnight.

I now have the voltage up to 55V 58V
58V and the current up to 120 mA in SDS

I have 8.5 mA on paper now. Better.

—

You have made a mixture of blood, glycerine & a couple of the grams of salt.

In an attempt to prevent Coagulation
The mixture is already mixed in SDS.
This should be a good stacky channel dye.

Sep 25 2016

1. The third gel is now in place and running. We are learning. The gel looks much cleaner because you have resolved some problems. We have:
   1. Higher voltage 58V now
   2. Step removed
   3. Proper buffer
   4. Good loading
   5. Cartridge facing the correct way, with the grating facing us
   6. known submerged channels.
   7. Alkaline conditions are precipitating the CDB red layer protein.
   8. We now recognize the parameters of current flow & voltage on the activation, including paper electrophoresis. Our power supply that shows both current and voltage is daily helpful.

   9. I can see now that you are going to need an acidic buffer for the red layer protein CDB. Else it will precipitate in an alkaline buffer.

If something is acidic, it is more likely to dissolve or react w/ a base. Apparently this is called deprotonation.

This is all going to tie in with the Hasselbach equation.

Amino acids maybe positive, negative, neutral or polar in charge.

At @ pH below their PI proteins carry a net positive charge.

at a pH above their PI they carry a net negative charge.

Since most of our proteins are moving on our gel (SDS buffer has a pH of ~8.0) this means that they have a net negative charge since they are moving towards the positive terminal.

This means that the PI of these moving proteins must be less than ~8.0, the pH of the buffer.

Look up Albumin, it has a PI in water of ~5.0. This matches fine.

If the CDB red layer protein does not migrate [illegible] it indicates that the protein pI might be greater than 8.0, or the pH of the buffer.

But we also have a statement from Biochemistry for D. Moore, p 79 that [illegible] many proteins precipitate from solution.

We know this happens sometimes when pH > 7.0 from observation.

This does indicate that the pI of this protein may indeed be alkaline.
What does this mean?

PS It looks like blood may have an optical rotation.

For a protein with many basic amino acids, the pI will be high.

For an acidic protein, the pI will be lower than 7 are Critical statements.

Another important statement:

At a low pH, most proteins have a positive charge.
At high pH, most proteins have a negative charge.

Therefore most proteins @ a high pH (eg buffer = 8.6 SDS) will tend to migrate towards the positive terminal i.e., because they have a net negative charge.

This mean that our protein is likely not like "most proteins".

What are these side chain amino acids?
Well, guess what, there are three:

- Histidine
- Lysine
- Arginine

This means that our protein is almost certain to have one or more of these groups.

We also know that our protein has alkene within it.

Only one of these three has an alkene group within it, and this is histidine.

Histidine therefore is @ the top of our list as a fundamental amino acid of the protein.

We now know that the following attributes would appear to characterize the protein:

(Notice that all three have an alkene group (1 amine).

Attributes of our red layer protein:
Ø. Isoelectric Point $\geq$ 7.0, maybe $\geq$ 8.8 SOS?
1. Alkenes & Alkanes (IR & $KMnO_4$ test)
2. Ether (IR & Baeyer Test (CS_2 & Iodine)
3 Nitrogen SDBS, Amines in IR(?)
4. Alcohol IR, NIR & Density
5. Iron Color & Availability
6. Protein Bradford IR - Amines.
7. Likely a basic amino acid w/ a basic side chain
Most likely Candidate is Histidine NOT PROVEN
(via precipitation & IR)

↑ You can't say this! yet.

IR Plot available
GC - Some info available

	PI
Histidine	7.6
Arginine	10.8
Lysine	9.7

are all candidates

The pH of the protein red Complex is highly acidic.
2.5 ± 4

We must Calibrate our pH meter since the value is important for Isoelectric point determination.

We measure red laser protein @ 2.57

The polarometer sets @ zero.

Calibrate it w/ a known sugar solution

I have calibrated pH meter to Baking Soda @ 8.4 (It was @ ~ 7.8)

This now places the pH of the COB red laser @ 3.45

By precipitation w/ NaOH, it seems as though isoelectric point is in the neighborhood of 6.5 to 7.0

This places it as neutral as it opens up the amino acids considerably.

Hydrolysis is hardly proven.
You need to see how it behaves in SDS Page gel.

You would like to have 5% gels SDS 12% is too slow.

Evolution 652 gels are stated to be 9x10 cm.
We measure: 10.3 cm wide x 9.5 cm high as in the casing

$\frac{74}{6}$ = 12.33 apiece

Lonza has 10 for $116 = 11.60 apiece.
Genscript has a 20 pack for $140 this is much better

Genscript says only the 8x10 series will work w/ Mini Protean II
They have both gradient & ISO types
Gradient runs from 4-12 but the 8% would be just fine.

Choose the 10 well version.
Genscript is going to be your company.
$7 each instead of $12

They also have a protein standard 250 ul $135 also good

Range from 30 to 200 kDA.

Another important thing that you have learned from this latest SDS run:

Your raw proteins (blood in glycerol and CDB Red Layer) were shearable.

So obviously you need to learn a lot about sample preparation of proteins,

you need to heat them up in a sample buffer.

The sample buffer has in it: 0.5M Tris-HCl (pH 6.8)
4.4% SDS
300mM MercaptoEthanol
10mg/ml Bromethol Blue
mixed 50-50 w/ sample, bring to 95°C for 10 minutes and cool before loading. Centrifuge if particulate material is visible

You can just buy it.
NationalDiagnostics.com
It is called 2x Protein Loading Buffer.

It says 10 x 1ml (10 Containers?) $29.00

So it will cost about $200 for these supplies.

If a gel gets too hot it gets destroyed.

You are out of time to order new equipment. Rene will continue next year.

Let's test + calibrate a sugar solution for the polarimeter.

137 gms H_2O
5 gms Sugar

by weight we have $\frac{5\text{ gms}}{142\text{ gms solution}} = 3.52\%$

We measure 2.80°, 2.85°, 2.90, 2.95, 2.75, 2.60, 2.75, 2.80

$\bar{X} = 2.825°$
$\sigma = .071°$

It is actually much easier to see w/ the flashlight. It is much brighter

$$\text{Conc} = \frac{\alpha_{obs}}{l \cdot c} = \frac{2.825°}{64.5° \cdot (1^0/_{10})} = .044 \frac{\text{gms}}{\text{ml}}$$

$\frac{5\text{ gms}}{142\text{ ml}} = \frac{X}{1\text{ ml}}$ $X = .035$ This is good.

Both solutions round out to 40 mg/ml

So it works. LED flashlight works great.

The plasmids

Tomorrow we want to:

1. Prepare a Citrate reference culture
2. Env. Filament

[redacted]

4. Another SDS –
   We cannot denature the proteins?

5. Another LaS?
   Yes can do the other labs as they represent good training ground but you will apparently not be able to work on ~~regional~~ proteins, at least if they have disulfide bonds.
   How many do?
   Yes they do.
   What about powdered milk (Casein?)
   80% Casein, 20% whey
   It has very few disulfide bonds? (little to none)
   Whey has lots of them.

6. The Problem by JR

So Conceivably you could work w/ electrophoresis in the field & make some progress with those proteins that do not contain disulfide bonds but the question is, do you really want to do this. You need power, Chemicals, table space, still Condition, limited sample type.

I do not think so.

You would only learn the general molecular weight of the protein. How would you use the information for an immediate sense?

Protein purification & identification

Sept 26 2016

We are on the countdown starting today.
About 10 days left.

Our priorities need to be shifting toward:

1. Collecting as much COB protein information as possible that is dependent upon lab resources.

2. Collecting as much env. filament data as is possible.

3. Refining the packing list & methods for a field trip to be carried.

4. You have made some progress with SDS page, even have w/ paper electrophoresis, and more recent w/ agarose. You need to find out if you should be taking these with you on the boat or not.

5. Robert Bruce Thompson has some real gems in his book esp on problems in Biology.

It looks like we have improved the Protein Capture on IR.

Current method is to

1. Isolate the protein w/ nodes & extract w/ some water
2. Add dilute (1-2 drops most!) HCl
   Strong acid will destroy the ATR
3. Evaporate most but not all the water.
4. Add methanol
5. Evaporate again almost to completion
6. Place drop on ATR & evaporate w/ air flow to make a film.

This is a new layer

Consider Combination of ATR & KCl since ATR is very weak @ the high end.

For KCl & ATR use micropipette & evaporate. There will be no water when you are done w/ the method.

It makes a nice looking film on ATR. There is only alcohol (methanol) to boil you are done. There is no water!

Watch your blanks towards when you combine ATR & KCl

Everything in the IR Plot says that we have caused a "Fischer Esterification" to occur.

We appear to be on definitely to both purification methods & Stoke extraction procedures.

We:

1. Precipitate out the red layer protein via pH control. pH 7 ~ 6.5 will precipitate the protein.

2. Rinse and centrifuge until the solution (water is clear).

3. Remove almost all water.

4. Add to approx 2 ml of cytochrome complex in water 2 drops 1M HCl

5. The red color will reappear.

The is rather pure protein.

6. Add methanol & slowly evaporate to under mild heat film quality material

7. ATR & KCl plates can be combined
Apply w/ micropipette
Swap out the water & the alcohol.
Air dry the film on ATR

Our signal with the precipitation method looks to be very weak. It's use look very questionable.

It is not all that clear exactly was made like on it is not dying with the same. It's similar but the absorption in the water region is too broad & deep.

It is the same material. It is just not quite as pure it seems.

It is the same problem, however.

From CDB GC Analysis, we know that the CDB are producing O_2 and/or N_2 but we cannot separate yet.

We do see the CO production again. It is very small.

Notice culture has
Alcohol + Acid Environment and we find ester. This looks like another step for the Fischer esterification.

09-26 Delaware Rodij

~~0.0~~ 0.0
~~0.5~~ .05 ~~1.0~~ .1 x = ~~0.5~~ .03 σ = ~~0.60~~ .01
~~0.0~~ 0.0
~~1.0~~ .1 ~~1.5~~ .15
~~1.0~~ .1 0.0

Sep 27 2016

We have some good success now. Our third SDS gel experiment has provided useful results and they have been recorded by photograph. The is despite the case that separation was slow and minimal. We need a less dense gel.

You are running out of time to take this on as a major project. You have, however, made significant headway.

You have learned that you need to:

1. Learn how to prepare protein samples (ie, denaturing) with the use of specific sample buffer. This can be easily purchased along w/ stain gels @ a reduced price from Edvotek.

   Our companies to consider for supplies are:
   1. Genscript.com (gels)
   2. National Diagnostics.com (Sample buffer)

2. That you need a less dense gel.
3. Higher voltage might help but we need to get by w/ 60V for now. It is sufficient to generate heat - which is a problem.

We need to continue to prioritize our items.

The reflectance spectrometer has come in today. I am very pleased with this relatively inexpensive addition to the monitoring arsenal. We want to think about how we are going to use this.

I just found my Chromatography paper.

Alta Standards measurements (on Chrom Paper)

Wavelength	Dark Voltage	Reflectance Value
470	~~72~~ 69	1095
525		1150
560		1231
585		1090
600		1069
645		1040
700		1059
735		930
810		1058
880		1126
940		1092

Eq. will be:

$$\% \text{ Reflectance} = \frac{\text{Sample Voltage} - \text{Dark Voltage}}{\text{Standard Voltage} - \text{Dark Voltage}}$$

Since we already know the standard voltage & the dark voltage we can reverse our equation to:

$$\%\ \text{Reflectance} = \frac{\text{Sample Voltage} - 69}{V_\lambda - 69} \quad \text{or} \quad \frac{V_S - 69}{V_\lambda - 69}(100)$$

and V_λ is available in the table.

Example, for blue cover on book

	λ	V_S	% R	% Absorbance
1	410	469	39%	61%
2	525	263	10%	82
3	560	243	15%	85
4	585	187	12%	88
5	600	186	12%	88
6	645	275	21%	79
7	700	331	27%	73
8	735	222	18%	82
9	810	900	85%	15
10	880	991	97%	13
11	940	977	89%	11

If we would like an absorbance plot we can use

$$\%\ \text{Absorbance} = 100 - \left(\frac{V_S - 69}{V\lambda - 69}\right) \cdot 100$$

Not True

This is better for VIS work

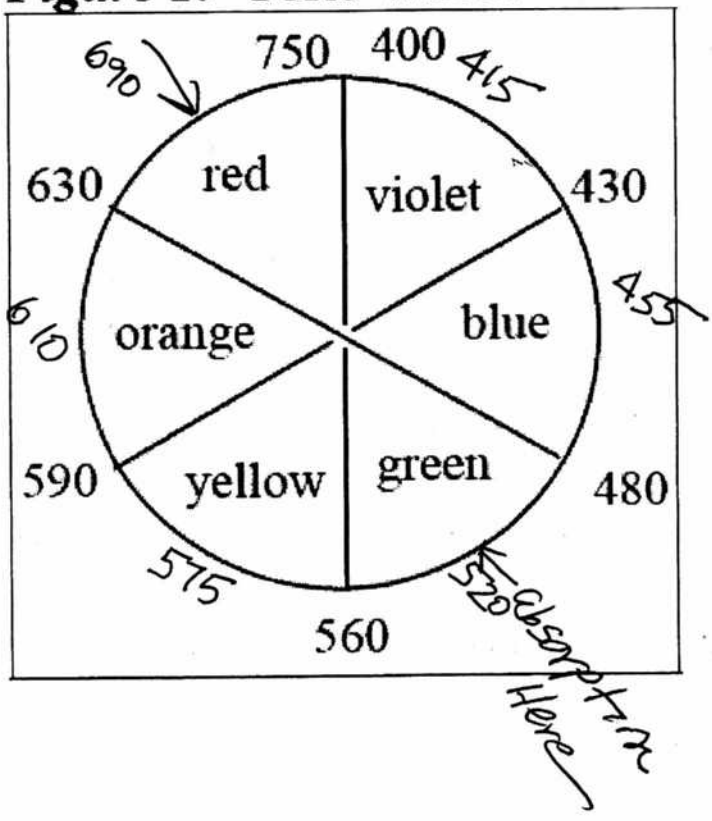
V_λ on table to left

A high no. means low absorpance
A low no means high absorpance

complementary color means opposite

makes it look this color here

Figure 1: Color Wheel



We have already learned of a problem.

The dark voltage of the sample is <u>not</u> a constant! It depends upon the sample! I measured a hot brick building & it was about 1500 – almost maxing at the meter.

Our adjusted equation is therefore:

$$\text{Absorbance} = 100 - \left[\frac{(V_S - DV_S)}{(V_{\lambda ref} - DV_{ref})}\right] \cdot 100$$

variable V_S = Meter reading of sample

constant DV_S = Dark voltage reading of the sample

constants $V_{\lambda ref}$ = Meter reading for each wavelength on the reference source (eg Chromatography Paper)

constant DV_{ref} = Dark voltage reading for the reference surface

This must be set up on a spreadsheet. It is the only practical solution.

Sep 28 2016

1. Bleach + Coomassie Blue stain have an interesting reaction w/ one another. It produces a white gelatinous precipitated white mass. I wonder what is in Coomassie Blue?
   It is a triphenyl methane dye
   A phenyl group is benzene minus one hydrogen. There is a paper on this reaction

2. Final cell preparation?

3. Can we concentrate nitric acid to test for tryptophan?

4. The env. filament: you are running out of time.

5. Do you want to run electrophoresis with gas? If no, paper only? Horizontal?
   TLC & paper chromatography may be enough.

6. Culture measurements made.

This is from the jar protein

An aromatic amino acid has now been confirmed w/ the xanthoproteic test. This restricts this aspect to either tryptophan or tyrosine.
Tryptophan is non polar
Tyrosine is polar

Hopkins Cole Test can be used to isolate tryptophan
This test has failed for both Tryptophan & Tyrosine
Can we differentiate by IR?

Indole can be produced by a variety of bacteria.
The indole group constitutes the side chain of Tryptophan.

A dedicated spectrum does exist

IR spectrum clearly says that it is tyrosine from the OH group.

Wait, what if it is phenylalanine? Take IR.

Phenylalanine does not dissolve in methanol.
It is <u>non-polar</u>!

We know now that we have tyrosine.

We know now that we have tyrosine.

The methods of identification includes:

1. pI is 5.67 (Tyrosine is 5.66
Phenylalanine is 5.48
you can not use this to separate
but it is still useful.)

This is for sample

2. Xanthoproteic test
Conc. nitric acid turns yellow

This confirm that an aromatic is involved.
The reagent eliminate to
Phenylalanine
Tryptophan
Tyrosine.
~~IR Plots of Tyrosine & Tryptophan and made.~~
Only Tyrosine has the dominant OH group Aromatic of group of all three amino acids as IR plot reveals.

In addition, direct comparison of Tryptophan w/ Tyrosine eliminates Tryptophan due to lack of OH group within IR Plot.

2. Solubility test eliminates Phenylalanine which is non-polar.

3. Color compound supports aromatics

4. Back to appearance company product tyrosine

We now know that our protein contains:

Protein	
Alkenes	IR & $KMnO_4$ in acid test
~~Ether~~ Ester	IR & Baeyer Test (CS_2 & I)
Alkanes	IR
Nitrogen	Amines
Alcohol	IR, NIR & density
Iron	Color
Tyrosine	~~IR, Xan~~ Xanthoproteic Test, IR, & Solubility.

& Bacteria

majority of the work in the Working Hypothesis Part I

The work is confirmed at a higher level of confidence.

We now know many components of the protein.

From GC we know we have @ least 3 components.
Alcohol & Ester could be separate
Protein could be separate

A search for:
alkenes ester alkanes nitrogen alcohol iron tyrosine bacteria
Brings up our paper at the top of the list.

Through research on tyrosine is now required.

Realize also that the protein reacts w/ acid & denatures. This suggests a basic group. It does [illegible] to mind histidine.

We have three ideas of immediate investigation

1. Heme binding

2. Dopamine disruption

3. Thyroid disruption.

We can also see now that fatty amino acid here is a over destructive IR Plot. This means that it can be used for identification.

Sep 29 2016

The countdown is in full swing. Likely to have only one n two full days in the lab left, today & tomorrow, maybe sat.

All the final runs and decisions must be made now. We lost 2 weeks of IR data, only paper copies left now.

1. Can we recover a good protein IR spectrum?

2. Env Filament is now becoming more critical.

3. You need to be able to conduct electrochemistry work on the proteins, both oxidized and the red layer - maybe for the field will be adequate.

4. Then you can start packing containers & work out the container arrangement & capability & the methods & resources to support the task

That a good list for today.

Film Preparation
Important Knowledge:

We made very good progress and I have acquired a good reference protein plot and I have it digitized. It is a weighted average of 4 ATR plots with 1 KCl Plot.

I have learned how to prepare better films now for IR ATR + even IR KCl.

1. Firstly get the material dissolved in one way or another.
2. Plan ahead for eliminating excessive acid, base or oxidation.
3. Evaporate the water (mostly) and transfer the material into any volatile solvent.
4. Evaporate w/ air flow assistance on the ATR - mild hair dryer would be helpful.

Last day in the lab. Maybe some nights left.

The phenol test w/ $FeCl_3$ does not work in methanol or even MEK. It is only works in WATER.

Phenol test positive does not produce an orange precipitate ($Fe^{+3}OH_3$). Primary phenol test color is purple.

Acidity & Base does have influence over $FeCl_3$ tests. Be careful.

Test alkaline water w/ phenol & $FeCl_3$ FAILS!
Test acid water w/ phenol & $FeCl_3$ FAILS!
Neutral water w/ $FeCl_3$ & phenol does produce a purple complex

Conclusion: The PHENOL TEST IS VERY SENSITIVE TO pH

You are learning over and over that many reactions may not work or are sensitive, and often very sensitive to pH. pH Condition must be at your mind w/ all investigative reagent reactions.

Now, what if methanol is neutralized?
NO! IT STILL FAILS. Must be water.

The phenol test is, for now, only good in neutral water.

The plant stat on the env. [illegible]
project [illegible] [illegible].

We learned something really interesting today.

Water absorbs NIR @ ~~940~~ 970 nm.

A negative absorbance means the absence of something, eg water!

Ferrous sulfate is supposed to be melty @ 70°C but it is not!

$FeSO_4$ did not melt up to 150°C.!
It is burning some but did not melt!

Simple thermometer only goes up to 200°C.

There are interesting NIR comparisons taking place. We have to

H_2O water : 970 nm

Rolls COB Red Layer Tube @ 932 nm

H_2O Env Filament Project - KOH - Methanol Extractio ~~965~~ 960-967nm
XF 963nm Is a very strong peak

This could absorb towards AsOH.

	ASD	Y
ROH is about 930	930	930
ArOH is about 955	950	952
H2O is about 970	960	965

from ASD

This means that the Env. Filament Project ROH - Methanol says that it could be water.

It says that our red layer tube is Alcohol or ROH

You have some conflicting results.

1. You have a
   1. IR Plot that show ArOH
   2. You have a Nitric Acid Test
   3. Direct CD3 Protein we have a positive Xantho protein test.

5. It might be w/ our red layer we are only confirming an alcohol by VIS spec.

Have enough that CD3 Red layer by IR shows an alcohol and an ester, not a protein.

The CO_2 Jar sample is what shows a definite protein.

But we also have the confirmation of protein from the Bradford test of the red layer.

Then all gets a bit confusing.

We positively know that we have a protein from alkaline precipitation of the red layer along w/ the Bradford test.

We actually do know that it is the same protein as the jar.

We seem to have a weakness w/ the CDB Red-
Large precipitated protein. I know that we
have a protein but there is no evidence of
Very [illegible] in tyrosine w/ the nitric acid test.

We have a very important finding here today.
We know that the CDB Red layer has a protein
in it because of the Bradford test.
However we know that the protein does NOT
contain an aromatic amine. This says
that the protein is different from the
protein extracted until so far.
This says that you have 2 variations of
a protein now, Not one.

There is very important in terms of
interpretation of the spectrum.

The ground protein IS NOT the same
as the extracted protein.

Sodium Carbonate – Sodium Bicarbonate Buffer Solutions, pH 9.2–10.8[1]

$Na_2CO_3 \cdot 10H_2O$, M. wt. 286.2; 0.1M-solution contains 28.62 g/l. $NaHCO_3$, M. wt. 84.0; 0.1M-solution contains 8.40 g/l.

x ml 0.1M-Na_2CO_3 and y ml 0.1M-Na_2HCO_3 mixed.

pH		x ml 0.1M-Na_2CO_3	y ml 0.1M-$NaHCO_3$
20 °C	37 °C		
9.2	8.8	10	90
9.4	9.1	20	80
9.5	9.4	30	70
9.8	9.5	40	60
9.9	9.7	50	50
10.1	9.9	60	40
10.3	10.1	70	30
10.5	10.3	80	20
10.8	10.6	90	10

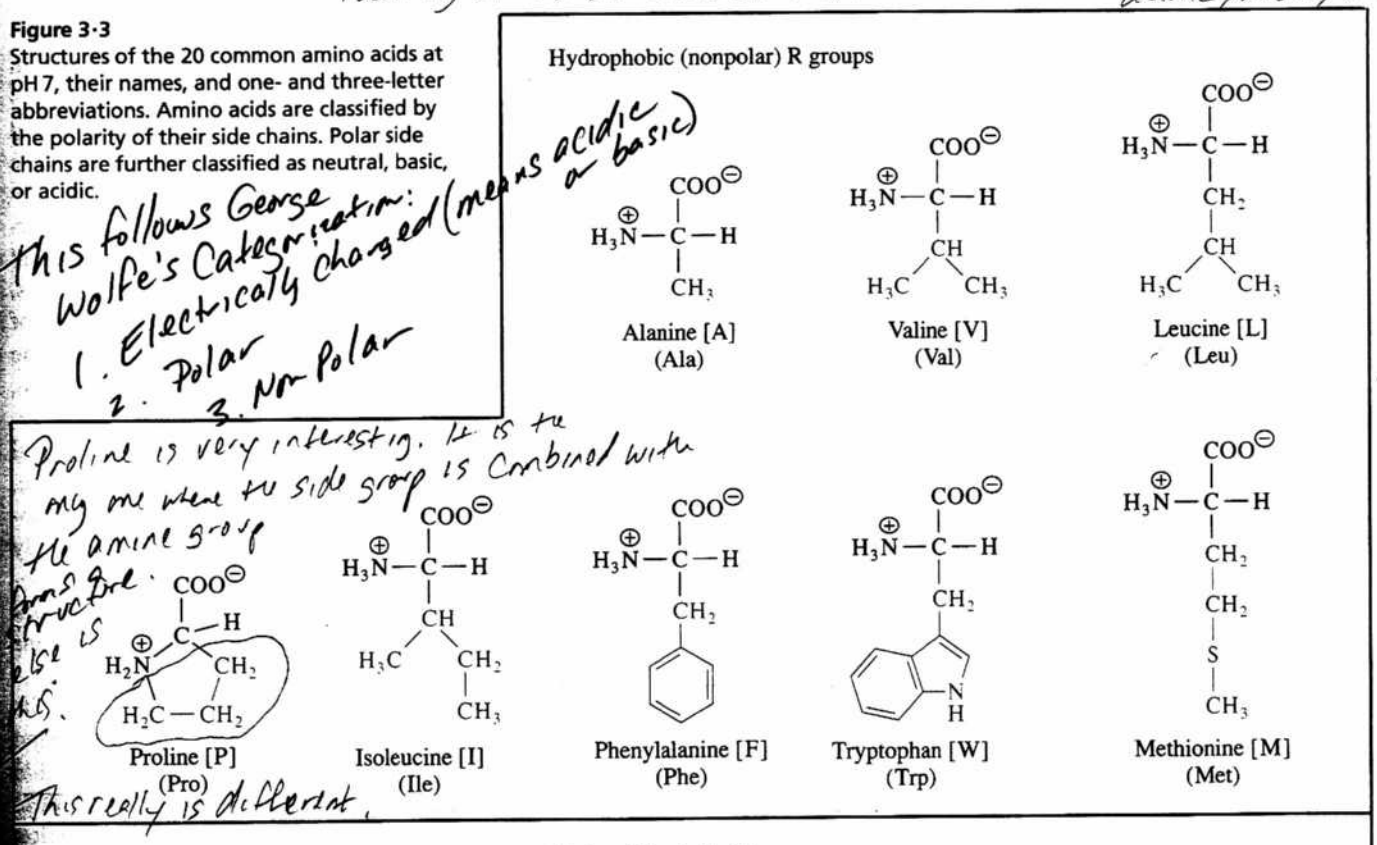
Helpful Formulas

Percentage by weight (w/v)

Solubility tests alone can likely tell you something about the identity of an amino acid - think about it! Polar, non polar, acidic, basic, etc.

Figure 3·3
Structures of the 20 common amino acids at pH 7, their names, and one- and three-letter abbreviations. Amino acids are classified by the polarity of their side chains. Polar side chains are further classified as neutral, basic, or acidic.

This follows George Wolfe's Categorization:
1. Electrically charged (means acidic or basic)
2. Polar
3. Non Polar



Proline is very interesting. It is the only one where the side group is combined with the amine group and structure else is as is. This really is different.

Notice the dashed line!

Oct 03 2016

We are packing up now for the trip.

Susan-culture meant:

Polarmeta

~~0.0~~, ~~0.05~~ 0.10		$X = .018$
.05 , .10		$\sigma_S = .084$
0.0	0.1	
-.1	-.1	

[redacted]

/

Oct 06 2016

Lets try to get density, & BP and IAR of red COB layer:

Density: Weigh boat = 3.32 gms

w/2.5 ml added 6.37 gms

6.37
- 3.32
3.05

3.32 start
6.23 end
Δ = 2.91 gms

2.91 gms / 2.5 ml = X / 1.0

X = 1.16 gms / ml

Then is really interesting because it implies that it is more dense than water but get it still rises to the top.

What is the density of water a control and also the Coupe flavol?

Water Test
start 3.30 gms weigh boat
5.90 gms end
Δ = 2.60 gms

2.60 gms / 2.5 ml = X / 1.0

X = 1.04 gms / ml quite good

CDB Red Layer Primary Physical Characterization

Lets go again..

Start 3.30 gms weigh boat
3.28

end 5.89 — 2.61 gms = 1.04 gms/ml
Δ = 2.61 — 2.5 ml

Quick good. Now for lower solution of red layer (clear)

	3.28	3.28	Start
	6.22	6.27	end
Δ	2.964	2.99	$\bar{x}$ = 2.96 gms

So we have $\frac{2.96 \text{ gms}}{2.5 \text{ ml}} = \frac{1.18 \text{ gms}}{\text{ml}}$

So we may have a slight difference between the two layer which make sense. The clear layer may be slightly heavier, but barely detectable. We now have results of

Polarimetry		σ_{obs}	Density	Brix	IOR	BP
.1, .2	Clear Layer	0.20	1.18 gms/ml	24.6	1.366	
.2, .3		0.27				
-.2, -.2	Red Layer	-.01	1.16 gms/ml	22.6	1.370	~~99.4~~ 101.4
.55, .65		σ = .05				
.2, .25						
0.0, 0.0						
-.05, 0.0						
0.0, 0.1						
-.10, 0.0						
0.1, -.05						

Now lets
So of to Polarimetry Rotation.

The table is that the clear layer still has substantial sugar in it, the red layer does not. So only the red layer counts.

We notice now that even the clear layer has turned red when it was exposed to air. This indicates that some type of oxidation has taken place. Effort has turned it red. Even though it still also contains some significant sugar. ie

$$C = \frac{\Delta 065}{\Delta \cdot C} = \frac{0.20}{64.5\left(\frac{10}{70}\right)} = .003 \frac{gms}{ml}$$

And since we use 0.5 ml in a 10 ml dilution the actual concentration is

$$.003(20) = .06 \frac{gms}{ml} = 60 \frac{mg}{ml}$$

when it was originally $\frac{.745}{69.5} = .011 \frac{gms}{ml} =$

and $.011 \frac{gms}{ml}(20) = .220 \frac{gms}{ml} = 220 \frac{gms}{ml}$

So the sugar reduction is $\frac{220 mg - 60 mg}{220 mg} = 73\%$

reduction or

$\sim 27\%$ of the sugar is left in the lower layer.

Very interesting results with the BP test.

We seem to boil off the color right about $98° \pm 2° = 100°C$ and we do see evidence of water.

The final BP sets up w/ a more clear solution of $99.4 \pm 2° = 101.4°$, slightly higher than water.

We have already shown that there is no significant oligomer here.

How does IR of this resonance distillate compare w/ the IR of water?

We get no match w/ Colby. edu w/ IR = 1.37 and BP = 101.5

We need to test the BP of water and look @ this compared to IR.

We have purified the protein today.
that is produced by the bacteria.
This was done by dialysis of the
CDB red layer. Some water loss
of the remaining material appears to be
almost all if not all pure protein

It forms a thin clear layer on a slide
under evaporation & it remains VISCOUS.

IR analysis matches the ATR CDB protein
in a year.

It is highly acidic.
Apparently contains bromine.
We lost it.

Frequency (cm^{-1}), intensity & shape are ALL important!

Oct 09 2016 Petty Creek - Alberton

We have found a great chapter on IR Analysis & Free Zone Method that does not presume availability of NMR. This is a very practical chapter. The book is not just a table; it is a logical approach to IR.

The 5 zones are: (alcohol → polarity here)

Zone I	3700 - 3200	OH, NH, sp C-H	aldehyde
II	3200 - 2800	sp^2 C-H (acyl or vinyl), sp^3 CH (alkyl), sp^2 CH, OH (COOH)	
III	2400 - 2100	C≡C (alkyne), C≡N (nitrile)	
IV	1850 - 1650	C=O (various functional groups)	highly polar
V	1650 - 1450	C=C (alkene) C=C (benzene ring)	

and this is even before the fingerprint region. This is less picture IR work. Multiple groups are quite likely.

We have three different versions of the protein to look at.
1. The jar version
2. The red layer version
3. The "purified" or "distillate" version.

The emphasis will be upon the jar ATR-KCl version but we will compare to all.

Start w/ zone I: 3700 - 3200

We had 3 peaks showing up here on ATR-KCl:

3432, 3332, & 3212

The new ways we are looking @
OH
NH
& Sp CH

Now we also compare this to the distillate form.
We also see a three peak subdivision like within a main peak.

Zone I Lets study this zone further between 3700 - 3200

Knowing nothing from measurements we can already predict frequencies based upon Hooke's spring model.

Pavia also covers this on p 38 and simplifies the model to

$$\bar{V} = 4.12\left(\frac{K}{u}\right)^{1/2} \quad \text{but actually} \quad K \cong n \cdot 5E5 \text{ dynes/cm}$$

where n = the no of bonds in the molecule.

$$\text{So } \tilde{V} \cong 4.12\left(n \cdot \frac{5E5 \text{ dynes/cm}}{u}\right)^{1/2}$$

$$\text{or } \tilde{V} \cong 2913.3\left(\frac{n}{u}\right)^{1/2} \qquad U = \frac{M_1 M_2}{M_1 + M_2}$$

for OH $U = 0.94$
$M_O = 16$
$M_H = 1$

$$\bar{V} \cong 2913\left(\frac{1}{.94}\right)^{1/2} = \cancel{2738}\ \cancel{2824}\ 3004$$

for CC: $\nu = 2913\left(\frac{2}{\left(\frac{12.12}{24}\right)}\right)^{1/2} = 1682$ Good

Actual is 1650 not too bad.

C-H: $\nu = 2913\left(\frac{1}{\left(\frac{12.1}{12+1}\right)}\right)^{1/2} = 3032$ Actual = 3000.

So your model is correct:

$$\bar{\nu}_m^{-1} \cong 2913\left(\frac{n}{\frac{m_1 \cdot m_2}{m_1+m_2}}\right)^{1/2} = 2913\left(\frac{n}{1}\cdot\frac{(m_1+m_2)}{m_1\cdot m_2}\right)^{1/2}$$

$$\boxed{\bar{\nu}_m^{-1} \cong 2913\left(\frac{n(m_1+m_2)}{m_1\cdot m_2}\right)^{1/2}} \quad \text{This is my preferred version}$$

for C≡C: $2913\left(\frac{2(24)}{144}\right)^{1/2} = 1682$ OK

So now for NH

NH: $\bar{\nu}_m^{-1} \cong 2913\left(\frac{(1)(15)}{14}\right)^{1/2} = 3015$

The range NH should be slightly higher than alcohol.

Polarity: Δ OH $\cong$ 3.44 − 2.2 $\cong$ 1.24
Δ NH $\cong$ 3.04 − 2.2 $\cong$ 0.84

The analysis tells you that OH should be lower Cm-1 than NH and that OH should be a higher absorption band.

Let's see where the centers of the band are:

OH : 3550 - 3250 $\bar{x}$ = 3400
NH 3460 - 3280 $\bar{x}$ = 3370

This did not exactly matter. They are so close that the distinction may not be detectable.

The model is good for qualitative values.

Aromatic peaks are sometimes confused w/ amide N-H bending peaks.

Hydrogen bonding broadens a peak.

Some Good Candidates of
Alcohol
Amines
Carboxylic acid
Amide

Benzoic acid is broad centered around 3000
Amines should shift it higher.

The C=O stretch for a ketone conjugated with a benzene ring is from 1700 - 1680.

Oct 01 2016

Website issues today for 2-3 hrs.
Time to move on.

Melting Point of Env. Fil. is useful.
We also intend to test for aromatic amino acid w/in env. sample using Conc. HNO_3.

Calorimeter water temp is ~~11.8°C~~, ~~18.0~~, 18.1

—

It is not true that the env. foil has a very high melting point. It burns cleanly w/a match.

Fe SO4 temp rose to ~~22.8°C~~ 22.5°C and it still is not melted.

Now let's compute

$$MP = \frac{m_w C_p \Delta T_w}{m_p C_p} + T_{water\ final\ in\ Calorimeter}$$

$$MP = \frac{150\,g}{36.0\ g\ m(.50)} (4.184)(22.5 - 18.1) + 22.5$$

$$> = 153^\circ ???$$

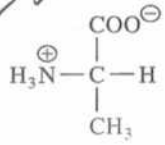
It says 70°C, - This means you only had iron left! Interesting, repeat.

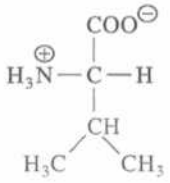
Solubility tests alone can likely tell you something about the identity of an amino acid - think about it! polar, non polar acidic, basic, etc

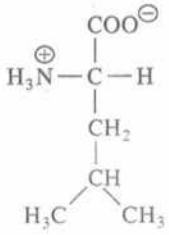
Figure 3·3
Structures of the 20 common amino acids at pH 7, their names, and one- and three-letter abbreviations. Amino acids are classified by the polarity of their side chains. Polar side chains are further classified as neutral, basic, or acidic.

This follows George Wolfe's Categorization:
1. Electrically Charged (means acidic or basic)
2. Polar
3. Non Polar

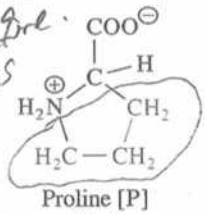
Hydrophobic (nonpolar) R groups


Alanine [A] (Ala)

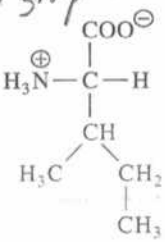

Valine [V] (Val)

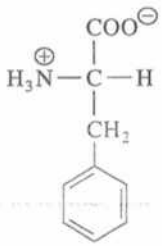

Leucine [L] (Leu)

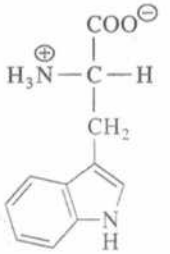
Proline is very interesting. It is the only one where the side group is combined with the amine group from the structure. Else is this.

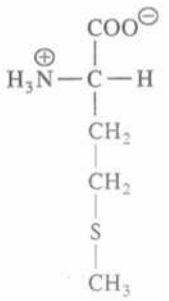

Proline [P] (Pro)

This really is different.


Isoleucine [I] (Ile)

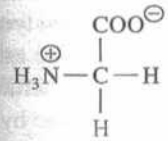

Phenylalanine [F] (Phe)

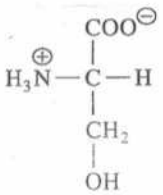

Tryptophan [W] (Trp)

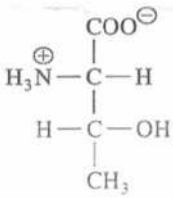

Methionine [M] (Met)

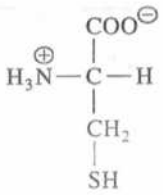
Hydrophilic (polar) R groups

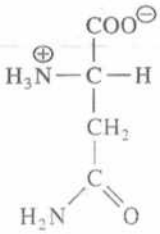
Neutral R groups

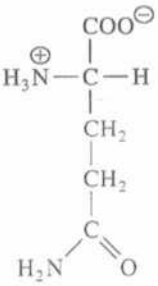

Glycine [G] (Gly)

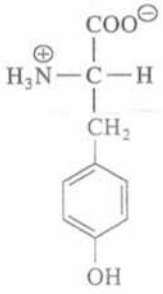

Serine [S] (Ser)


Threonine [T] (Thr)


Cysteine [C] (Cys)

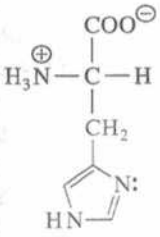

Asparagine [N] (Asn)

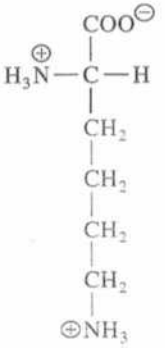

Glutamine [Q] (Gln)

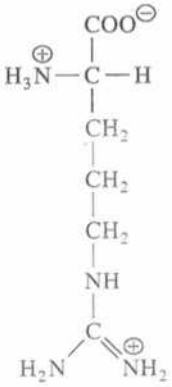

Tyrosine [Y] (Tyr)

Notice the dashed line.

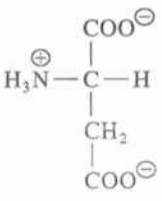
Basic R groups
(Cationic conjugate acids)

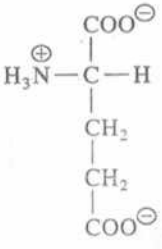

Histidine [H] (His)


Lysine [K] (Lys)


Arginine [R] (Arg)

Acidic R groups
(Anionic conjugate bases)


Aspartate [D] (Asp)


Glutamate [E] (Glu)

With the distillate from we have a broad peak centered @ ~ 3200 - 3300. $\bar{X}$ = 3250

IR spec provides us with an alcohol and an amine. Since we see multiple small peak, we believe we have a primary amine.

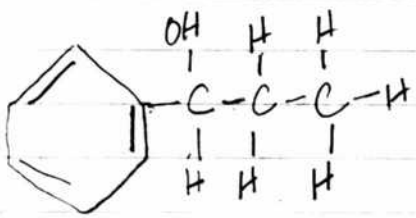
Now IR Pal gives us Ar-OH
So if we have an aromatic, what are its characteristics?
Actually our broad peak range from 3200-3400.
This places center about 3300 but the distillate is 3250
So well well indeed Clone ~ 3250.

Koji says polymeric OH.

Avram says that intermolecular hydrogen bonds give rise to broad bands. Intra molecular hydrogen bonds are sharp and well defined.

We know, though, that we have intermolecular hydrogen bonds.

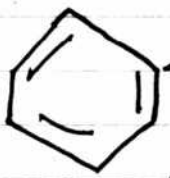
Avram p 260 has an extremely taller. This suggests 1040



Koji p 30 also distinguishes intermolecular hydrogen bonds w/ the polymeric form.

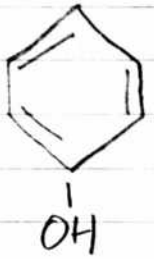
We have as a topic

intermolecular hydrogen bonding
polymeric alcohol
~~R-C-H-OH~~ R-CHOH-R'
Avram R Phenyl
1940 R' Ethyl


R this is a phenyl.

We also have an amino acid of tyrosine

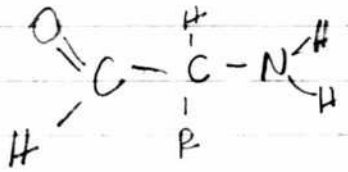
Indira Murray p448
Shown aromatic w/ 1500 & 1600 peaks
& also mentioned strong @ near 1050.
We have these. CO yes, this is fine, we have 1040.

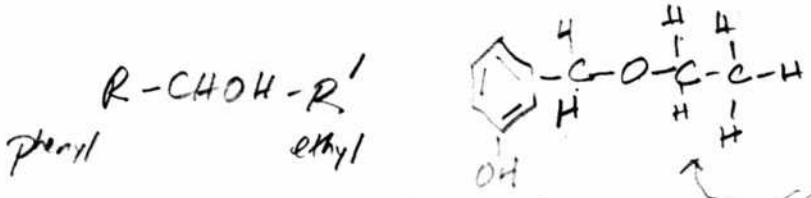


We also know that we have amine.

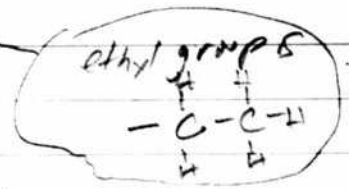
We also know we have tyrosine and a highly acidic protein.

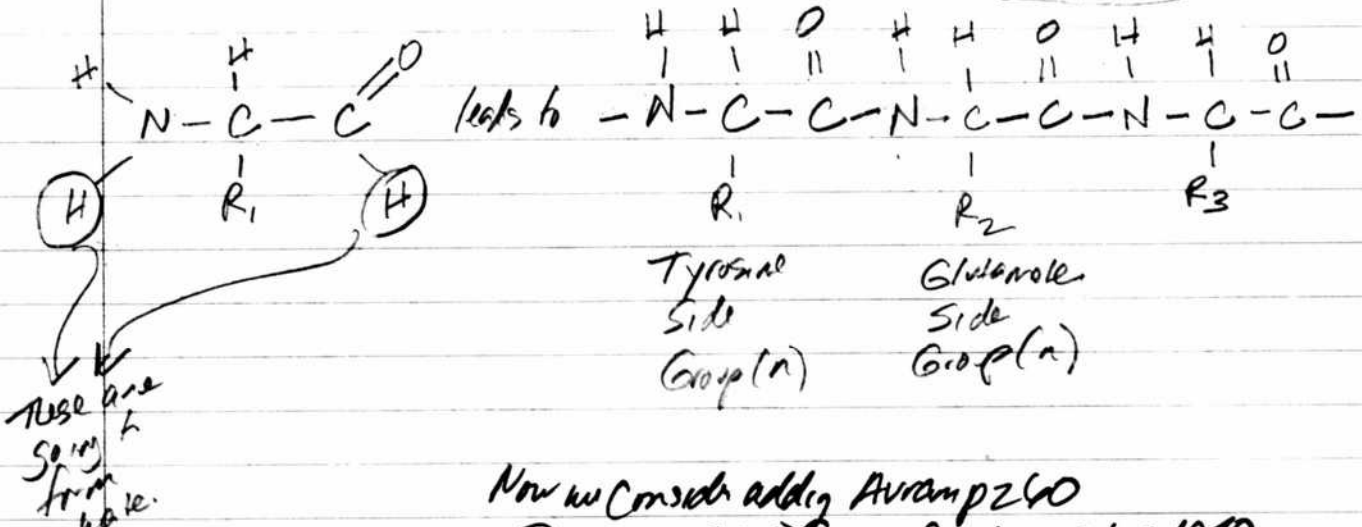
This strongly suggests



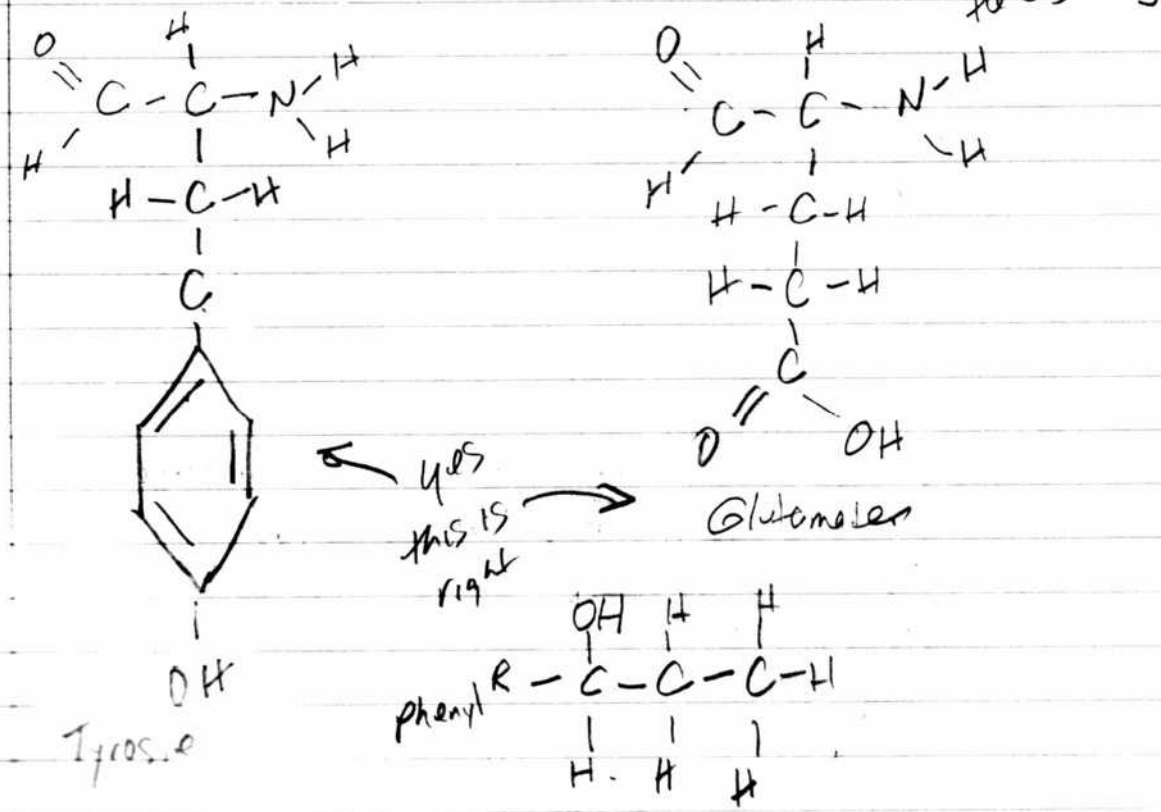


And the polypeptide looks like this:

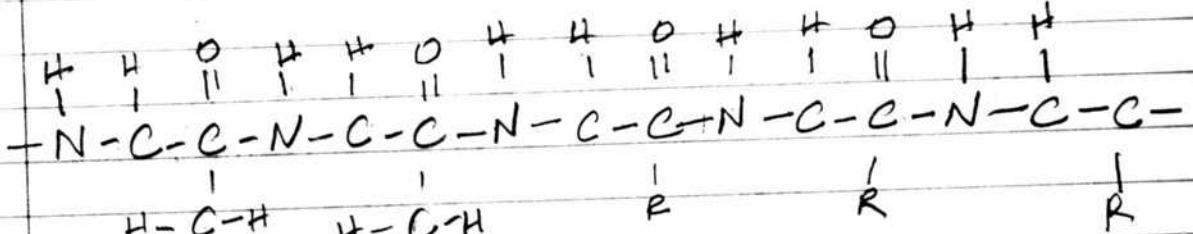


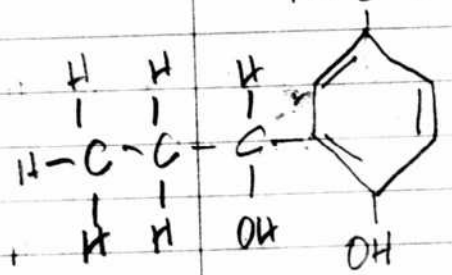


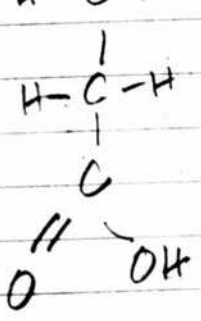
Now we consider adding Avramp260 Phenyl-Ethyl Group for V C-OH = 1040 w/ the beginning



So we now have the prospect of







Tyrosine (n)

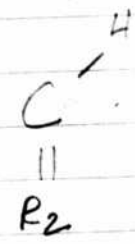
Glutamic Acid (n)

Need Iron

2934 is also amine

2890 is alkane (methine)

methine is



A methine group is also used for each carbon-hydrogen subunit of an aromatic compound although the latter does not have discrete single & double bonds.

There a perfect. Methane groups correspond to each corner of the benzene ring.

The 2934 may belong to a polycyclic compound
2935

2934 Can be methylene asymmetric stretch
or it can be (maybe not likely) an overtone of
the PO_2 asymmetric stretch.

Methylene is $=CH_2$

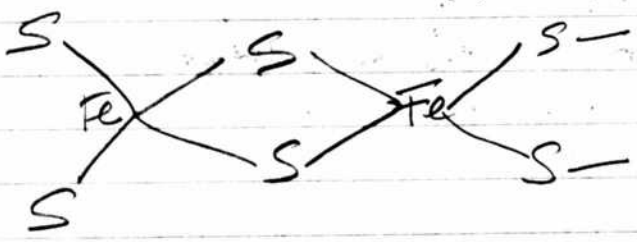
Do we have a $=CH_2$? yes, all over the place within
the aromatic ring.

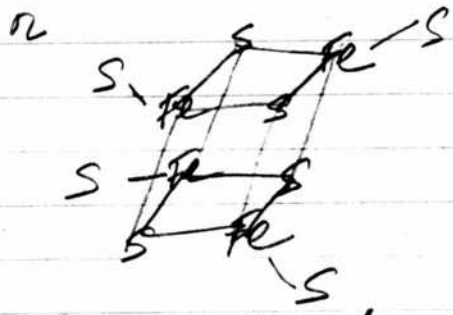
Then take care of both zones I & II.

Now we have both an entry between zone II & III.
@ 2557 and 2556 reliable.

We seem to have a definite entry in here from koji
for -SH on TII. There seem to be
free completion. There is a sulfur group.
Remember that we are using $FeSO_4$
What is the geometry/name of the group?
How many bonds does sulfur make? 2
Sometimes it makes 6.

Iron - sulfur proteins





Iron - sulfur clusters
Iron - sulfur proteins

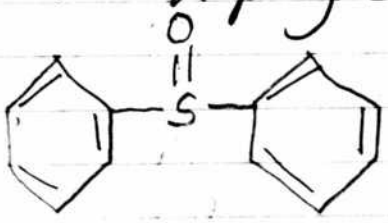
"The cluster is linked to the protein by the amino acids residues Cysteine & histidine".

From Avram, p 291 we have a very close match occurs with not dioxide! SH

Diphenylsulfoxide w/ 2537 and 3320

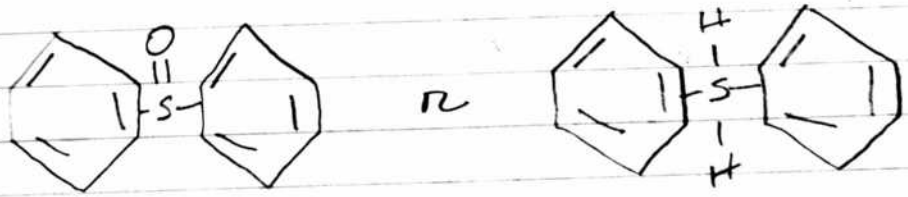
vs 2556 & 3318

What does diphenyl sulfoxide look like?



? Hydrogen? S-H?

Now Ko$_{II}$ says -SH
Aman suggests diphenyl sulfoxide
So gw will need to map gw to both,
S. this suggests either

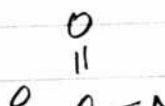


The could attack tyrosine. Read Chapter on amino acid reactions.

Now Zone III 2400-2100 Nothing. There are alkynes

Zone IV 1850-1650
1702 weak
1626 Strong
1558 Strong

Purified mg shows 1648 Alkene, Amide (1650)
We have an alkene.

Amide is  We have an amide. It forms the linkage between amino acids.

This means all corrected.

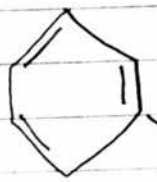
Zone V 1650 - 1450 We are reading this, now in the fingerprint region.

We are now with fingerprint region.
It is now necessary to start the mys components.

1. Poly peptide (Amino Acids + Amide)
2. Tyrosine & proposed Glutamic acid
3. 1040 Avram - Alcohol
4. Methylene
5. SH or dipolenyl sulfoxide or iron-sulfur polein
6. Methine group

Not in fingerprint; in 1350

Nitro : N-O aromatic!

1. 

Possibly amines, however.
Look @ shape of peak.
Amines may be more likely if NO is a sharp peak.

Next is 1046. This matches Avram p260 fairly well for unsaturated alcohols

8. Mys absorption fairly close ~ 628
Could be alkenes but it is strong.

some factors that affect IR Frequency

1. The masses involved in the bond
2. The order of the bond (single, double, triple, etc)
3. The polarity of the bond.

We also developed our own model for the base frequency:

$$cm^{-1} \cong 2913\left[\frac{n(m_1+m_2)}{m_1 \cdot m_2}\right]^{1/2} \qquad n = \text{bond order}$$

→ Polarity affects Intensity more than frequency.
Polar bonds (bond electronegativity) are more intense.

C-C bonds are not the same as C-H bonds!
No wonder C-C bonds are so weak, they are completely non-polar (eg alkenes, alkynes) will be even weaker than alkanes probably.
Anyway, the issue is symmetry vs polarity.

"yl" groups in organic chemistry are "R" groups (ie, "radical" groups)

They are NOT functional groups.
See Avram p 260.

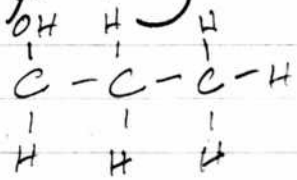
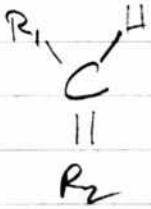
You can indeed learn quite a lot from the functional group region of IR, it is not only the fingerprint.

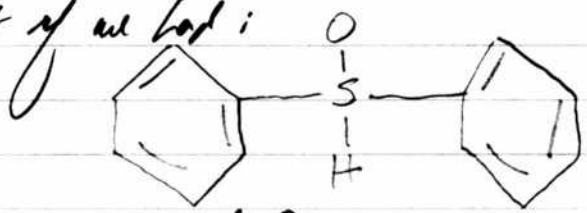
Remember also the absence tells you important things also.

We see we have nothing in Zone III. What is Zone III about?

Alkynes. Not as likely but remember they are also very weak.

Thus far for our protein we have identified the following groups & compounds.

1. Polypeptide
2. Amino acids
   Tyrosine
   Glutamic Acid
   are primary candidates also cysteine
3. Amines - primary
4. Phenyl -  alcohol group (+0.40) Avram p 260
5. Methine group  for junction of aromatic C
6. Aromatic alcohol w/ amine superimposed.
7. Methylene $=CH_2$ for aromatic ring
8. Sulfur Group | 1. Could be SH -(Koji)
   Has cysteine. 2. Could be diphenyl sulfoxide (Avram p 291)
   3. Could be iron-sulfur protein

What we had: 

This would allow easy bonding.

What is this called? It might allow for Avram p 291 slight shift? (2537 vs 2556?)

9. alkenes (1648)

10. Amide (1640) (vs 1650)

We have both of these and they are consistent w/ previous data

11. The aromatic nitro does become a prospect via IR spec @ 1348.
What does NMR say here?

The weight of evidence remains w/ the "aromatic amine"
but an aromatic nitro remains a possibility

12. $-CH_2-\overset{O}{\overset{\|}{C}}-R$ substantial glutamic acid and also a high correlation w/ amine alcohol assessment.
This was an excellent example of applying bending CH frequencies near 1400

per both Fig. 4 Chap 16 Five Zone method and IR spec crude alkene reference. Chap 16 reigns supreme here

The 1040 peak raises some interesting questions.

1. Alcohol is primary, strong, broad & notable all interpretations.

2. However it also has properties of amines AND Alkyl Halide.

We definitely have amines so this is fine.

Alkyl halides raises an interesting possibility.

Frequency, intensity & shape are all important. What does it mean to have intermolecular bonds vs intramolecular? How do you know what you have? Avram talks about this - broad peaks mean intermolecular, narrow sharp peaks mean intramolecular. See Chap 16 IR Fine Tone also.

IR Fine Tones is a really good chapter. It OC knowledge what can be done before NMR. Even a great deal can be learned about alkanes, including even the type of alkane group likely present (from the intensity of the peaks).

sp^3 C-H bonds are important to understand. Look @ Chemistry book for this.

Oct 11 2016

Question: Why does cyclo hexane have sp^3 bonds?

In alkanes apparently all the carbon atoms are sp^3 hybridized. Why?

It is because all of the carbons are tetrahedral This is why.

If you look @ the structure all carbon atoms are bonded to four other atoms. This make the structure tetrahedral. Tetrahedral is sp^3 (3 dimensional)

WebMO for PC seems to have incredible features such as IR Visualization!

It requires Apache & Perl but we are working on it

The complexity of sp^3 peaks on IR indicates information about the complexity of the bonds & Configuration.

Perl must be installed under advanced Configuration

MOPAC is the next step. $5000 software!

This was quite a little detour, but important, today with WebMol. We are headed towards some $5000 software which we hope to receive academically for free.

The motive for doing so is to be able to generate theoretical IR plots. This would be a huge plus. In the meantime, let's continue with study of IR basics from Chapter 16.

We also understand WebMol better now and the full version installed on the PC.

[redacted]

* The intensity of the SP^3 peaks (3000-2800) depends upon how many sp^3 CH bonds are present.

CH stretching is from 3000-2800
CH bending is about 1450.

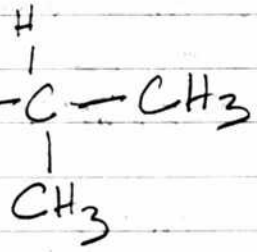
The bending peaks can tell about the "yl" or the "alkyl" groups in the molecule.
This method supercedes NMR, which we definitely do not have.

See example on the next page.

The bending peaks of ~1450 can tell us more about the nature of the CH Bending Character. Eg:

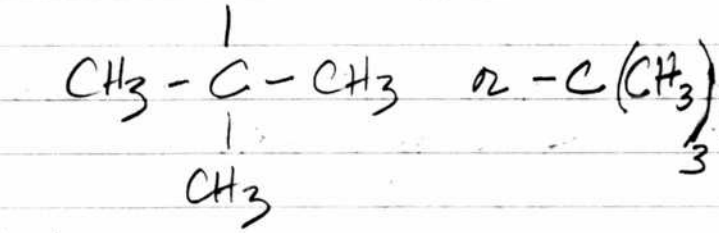
methyl) $-CH_3$

isopropyl)



tert butyl)

"tert" means tertiary



They are, therefore, specific variations of the alkyl groups

and the peaks @ ~1450 can start to tell us which ones are present.

We might have something @ ~1406. Does this apply?

First of all, the center is around 1400 not just 1450 and Koji has the full story available to us on p20-21.

We can see now that that is a tremendous amount of information available to us with alkanes & ONE! What you needed to do was really study the Koji Chart, not just assess that there are CH_2 & CH_3 groups present. There is so much more that you could have but now can learn from DETAILED examination of the alkane spectrum.

And it is indeed important. The hydrocarbons are usually going to form the backbone of the organic molecules.

So now we have seen some more information to be seen on the proton.

We carefully examine our distillate proton spectrum We find peak attributed @ 1424 & 1348.

We have already discussed the 1348 as a potential ~1/2 aromatic group per IR spec. The still holds. It seem to be fairly unique.

1348 - 1350  NO?

However, Koji shows @ least a couple more alternatives $-CH_2-CO$ but this is not great.

NO_2, SO_2 and other Candidates

Aromatic amines show up which does make sense!

@ 1340 an aromatic nitro is highly
Conforming w/ IR spec.

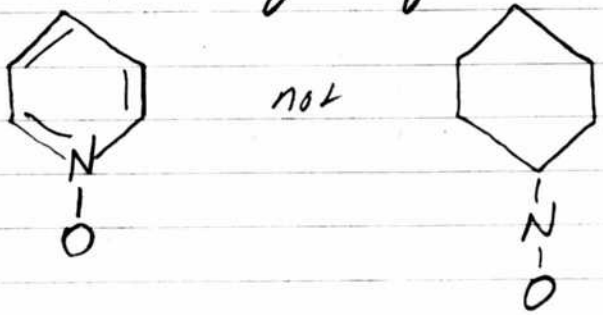
It is also indicated by Koji on p51
from 1200-1300 as an aromatic nitro
however it should have confirmation @ 970.

Notice that we do indeed have a disturbance
near 970 in the shape of the alcohol so
something is happening there.

Not true! The aliphatic is @ 970 but
the aromatic nitro is much higher (1200-1300)

However, Koji w/ an aromatic amine
as a whole is more straightforward
and is consistent w/ the ground chemical
test.

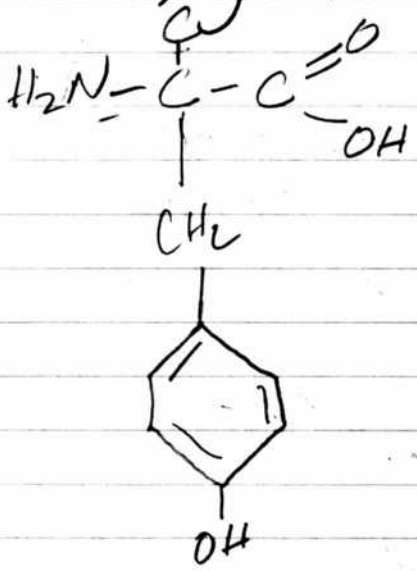
Incidentally the form of a nitro aromatic is:



Koji discusses aromatic amine on p 30.

This remains a debated possibility

However, tyrosine is:



Notice that even tyrosine does not exactly meet Koji aromatic amine because it actually has, on p 38 at the bottom:

And even this is not exactly what tyrosine is. The exactness of 1348 with the aromatic nitro does make it come relative to the non-noted w/ Koji in one exact sense as well as being (1348) on the extreme edge of the Koji aromatic amine band (1360-1250)

The prospect of an aromatic nitro group becomes stronger for we reiterate this.

For IR Pal w/ 348 we get exactly the same Candidates.

but aromatic nitro is the tightest range Candidate

1345-1355 is the aromatic nitro.

Ar-NH has a range of 1250-1360 which is much broader and does not match by more anyway.

the aromatic nitro would have another peak @ 1520. Do we have this?

$\frac{.4cm}{x} = \frac{2.8cm}{2000-1648cm}$ $x = 50$

1648 - 50 = 1598 No. We do not see it.

We do however, w/ the pyridine have a very strong reading @ ~~1548~~ 1558.

I would weight my two source @ $\frac{2(1558)+1(1598)}{3}$

$= \underline{\underline{1571}}$ So the does indicate a possible of 1570 for a nitro aromatic, but the does not match of nitroaromatic.

1510 Corresponds to amines so we should be OK there.

1510 corresponds to an aliphatic nitroso (N=O) so this does not provide confirmation for the nitro group.

The weight of evidence still resides for an "aromatic amine").

Ok, back we go to the heart of Chap 16 & the fine zone method. and therefore, back to the alkanes.

We head back to a 1424. Remember that there is very weak. IR spec gives two candidates

Alkanes & Nitro. We will start wide w/ the alkanes. Then a the bending CH to look for, Koji has it in detail.

Preferred Koji lists: $-CH_2CO-$ $-\overset{H}{\underset{H}{C}}-\overset{O}{C}-R$

No substitution CH_2-N

Notice how closely this matches our alcohol structure w/ Arom.

Notice also how well it matches glutamic acid if R equals OH.?

(1) We have some important questions brewing. What if the alcohol modification was not just an alcohol but a reflection of glutamic acid?

(2) Notice the very rough spectrum from 1700 to 1300. How does this compare w/ glutamic acid?

(3) What does an average of tyrosine & glutamic acid produce as far as an IR spectrum goes?

(4) Do amino acids react w/ other functional groups and if so how? See Chap 16 Fire Zone.

Notice in the spectrum for glutamic acid there are indeed peaks @ or near 1700 1600 & 1300. They are not out of bounds. It also has a sharp one in between. Notice also the sharp drop near 600, also not out of bounds. The main difference is the very strong absorption that corresponds to an aromatic alcohol. Look @ Tyrosine.

• Tyrosine does have very strong absorption @ or 1420. We have 1648 so this is too large.

Glutamic acid also has a peak @ ~ 1600 & 1300 so this is all reasonable.

A synthesis of tyrosine and glutamic acid is a very strong prospect.

7.05 um = 1418.44 cm^{-1}

So

$\frac{7.05\ um}{1418.44\ cm^{-1}} = \frac{1}{x}$ $x = 201.20$

So $cm^{-1} = um\ (201.20)$

You have done some quite amazing work:

1. You have learned how to grow the protein
2. You have identified the primary constituents of the protein (amino acids & metal)
3. You have created a theoretical IR spectrum that approximates quite well the measured protein.

Primary Constituents are Glutamic Acid & Tyrosine
We presume Iron & Sulfur to be additional inorganic constituents.

Possible ratio 4 Tyr to 1 Glu

All evidence points to a genetically engineered bacterium

MOPAC Software License Acquired

MOPAC Computational Chemistry has provided Carnicom Institute with a site license.

This is $ 5000 software.

Our site number is [redacted]
(I believe for their reference)

[redacted]

I am not sure exactly what version I download but the software is called

MOPAC2016

[redacted]

I have successfully installed MOPAC2016 and I have successful heal runs taky place.

Energy of formation seems to be a primary output.

Avogadro seems to be highly successful in interfacing w/ MOPAC. WebMO does not seem to be so much the case because it only talks into MOPAC 2007 instead of MOPAC2016? It seems like it is 9 years behind the trends.

Avogadro & MOPAC may end up being the better combination

There seems to be some difficulty however in computing vibrational energies no matter what files I have used thus far. Gradient errors are occurring. We can look @ this as we go along.

It will be of interest to see what the output from the program actually means.

Sent email of thanks to [redacted]

You apparently need to renew the program in 10 months as the site license may expire.

This is the field of computational chemistry.

Oct 12 2016

We can see that the functional group method is a very viable and systematic fashion to investigate the functional group portion of the IR spectrum.

It really is right to try and get the big picture correct first.

The absence is also correct.

IR Pal combined w/ Functional group method seems to be a good start.

Location
Intensity
Shape
} are all factors to consider @ a high level.

Koji is probably next along w/ IR Spec.

Avram kicks in @ the latter stages

Oct 13 2016

Back to Chap 6 - Five Types. We see that there is actually a wealth of information from hydrocarbons alone. Koji actually does quite well with these differences to an intermediate level. Arram dives to the final detail as needed.

All of this revelation has taken place in pB. Let's move on, P12 is where we are now.

We are now generalizing into additional aliphatic Compounds, such as alkynes. What we see is that the distinctions occurring in IR w.r.t. CH is not the fact that it is CH, but because it is: IS a big factor here

C-C
C=C
C≡C

- sp^3
- sp^2
- & sp] Bonding that is taking place.

Alkynes have a different type of bonding structure than sp3 so it is causing change in the IR spectrum, with new and different frequencies showing up, even though it is still generally CH.

You do not have hybridization taking place w/ diatomic molecules, such as C-C, C≡C, etc.

The Five Zone table, in detail is given on the last page of the Chap 6 document.

This is actually a very valuable resource and I recommend that it be used as an overview for functional group region analysis (4000-1500)

The chart can be used with IR Spec, IR Pal & Koji in the primary functional group analysis.

Chap 6 is a treasure trove of the big picture of IR analysis.

So the original Arsenal is:

1. Chap 16 - Five Zone Analysis & Pavia & Chemistry Toolbox is also quite good P 47
2. IR Pal support & Collaboration
3. IR Spec support & collaboration
4. Koji support & collaboration.

Save Avram until the portion is complete. Pavia may have something to offer here as well.

Pavia's Book is extremely valuable also as a reference.

Pavia has a full 7 page correlation table which should actually supercede or @ least complement Koji's Chart! This is Appendix 1 in Pavia & it starts on p691 of 745.

It is true that Pavia is devoted primarily to NMR but he does not abbreviate the IR material; in fact he is more elaborate than most and he probably ranks close to Avram in terms of detail.

With all of these resources, theoretically we have no excuse for failure. There are two approaches we have offered that seem to be equally at the level of Avram or Pavia yet. IR Dat is our best.

Pavia p 47 Starts a very systematic approach to IR interpretation w/ successive detailed examples. He is also superb.

Using Proton distillate from
a Chemistry Toolbox
We see some major patterns right away.

We seem to have an alcohol, a carboxylic
acid, Amide & Amine right away.

Why?

2500 - ~~3000~~ 3300 very broad peak
Characteristic of Carboxylic acid.

Alcohol is very broad also, from 3250 - 3550.

We see that we have both these combined.
This leads to an alcohol-carboxylic acid
Combination from 3550 - 2500
which is true. Two very broad peaks
Combined.

Next we have super positions on this broad peak
from 3200 - 3400 small peak
This is the amine & amide category.

We then show an alkane @ 2935
& 2900.

We also show the possibility of an alkene @ 3020.

Now as we look at these groups in Chem Toolbox we also have Collaboration.

Alcohols.

3550-3250	We have this
1430-1320	We have this
1160-1030	We have this
770-620	We have this.

There is no doubt then that we have an alcohol.
We also know that we have a protein from Bradford.

There are only two amino acids w/ an alcohol groups.
Serine & Tyrosine.
Notice that only Tyrosine has an alkene group (weak).
due to resonance.
The already tips the scale to Tyrosine.

Now for Carboxylic acid, we also show the very broad absorption from 2500-3300. We also know the pH of the protein is highly acidic. Only two amino acids are acidic, Glutamic Acid & Aspartate. ~~Aspartame~~. They only differ by one CH_2 group.

Before we discuss of the acid, we have another route of confirmation of Tyrosine.
Avram

We already have the prospect of a phenol showing up in ~~Raw~~ Pavia p47 because of broad absorption 3400-3300. We already know that we have an acid-alcohol combined.

From Pavia, we also have indications of alkene, but contained to support the existence of the phenol group.

From Pavia, Nitro groups are not indicated. Triple bonds are not indicated.

But also definitely something 2556.

Thiol group seems to be definite to strong candidate
2556 meas Thiol group is 2600-2540
Nothing else near it. S-H

We are leaning toward

alcohol
Carboxylic acids
Amide
Amine
Aromatic alcohol
Strong acid
Thiol group.

Guess What: There is only one amino acid w/ SH and it is Cysteine

We now have three amino acids Comprising the protein.

may be most dominant.

Tyrosine
Glutamic Acid
Cysteine

We expect interference w/ these three amino acids

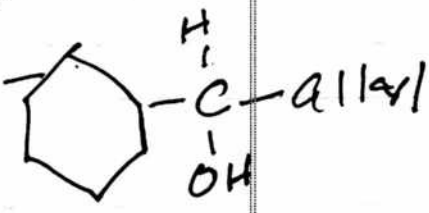
There is strong data now.
You can form a conjugated system.

Chap 16
alcohol 16.6E
Phenol

We are @ 1040.
Keji has a primary alcohol @ 1050.
Something is wrong w/ the alcohol.

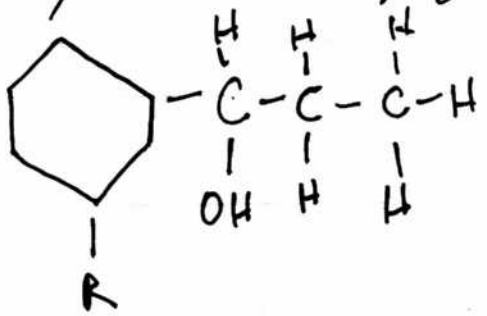
Chemical Test Confirms Tyrosine.
Serine is never confirmed @ this stage

From Pavia, @ 1040, we show to have either a

$-CH_2-OH$ or a 

This means again that
~~we have either serine or tyrosine.~~
We perform a chemical test & we know that we have tyrosine. Conc. nitric acid

Now, we can also prove this @ tyrosine.



Oct 17 2016

Additional Notes on Chap 16 - Five Zone Method

On pp 37-38 a mid level summary of the five zone is presented.
This is a useful adjunct.

We see therefore that each of the following sections has value to combine & interpretations

p 35-36 The short version of Five Zone

p 37-38 The mid level but expanded five zones

p 46 An ordered & tabulated Five Zone Condensed Chart.

All of these offer distinct values.

Our combined analysis of the purified protein indicate a combination of three amino acids:

1. Tyrosine
2. Glutamic Acid
3. Cysteine

] These are ultimately our interpretation of the spectra.

You can go two ways:

1. Claim the amino acids & then justify their existence by peaks & additional tests. (this is preferred)
2. Analyze individual peaks, suggest amino acid candidates, and then by intersecting peak sets reduce the candidate list to the three mentioned (more difficult and awkward).

Oct 30 2016 Horseshoe Thief Campground UT

We have had a break, a interruptions, dependent upon circumstances. Quite a lot of moving lately - we should be able to settle for the next two weeks.

We should be able to get organized.

1. No internet here of substance, maybe some critical text for o
2. [redacted]
3. We have good text capability
4. Email is highly marginal.
4. Power available is relatively stable and you can resort to tablet, phone & over hotspot as required. Hotspot is marginally effective. No real net.

That being said, let's outline our projects and topics.

1. The spectrometer (VIS) is now available. The does present some interesting prospects. Urine alone already is a very interesting topic.

2. You will probably need to use your computer extensively, at least with the spectrometer.

3. It would be to your benefit to study VIS spectrometry to the degree that it can be exploited. You just never know what color into the picture somehow

10. Electrochemistry and Impedance spectroscopy - there are many more possibilities.

11. IR is more powerful qualitatively UV-VIS is more powerful quantitatively but they still both have value both directions.

12. The "Supplemental Discussion" paper

13. The Density of ranges paper

14. You have numerous tools available for physical property analyses
    1. Calorimetry - melting point
    2. Index of refraction
    3. Density
    4. pH
    5. Conductivity & ORP
    6. Reflectance spectrometry. (PASCO AND VERNIER)

15. A rather Complete Chemistry set

16. Fuel Cell Analysis

17. Biology lab kit - STEM projects

18. You really need to study VIS spectrometry. What are antibonds?

19. What about the anthocyanin reaction? Can you determine what is happening and why? How might the developed protein be related?

20. Your best supplemental reading device is the tablet. You have a library close of close to 100 books.

21. We have a lot of video coursework available now. Two Chemistry courses

22. Molecular modeling software and Chemistry Computational software; very interesting.

23. We need the Pasco software on the tablet, it should use longer power.

24. We have many good books with us and a great library on the tablet. Books are no problem. Power is necessary for many projects but not necessarily all of them.

25. Thin Layer Chromatography!

26. Paper Electrophoresis

27. Paper Chromatography!

So now we have a good summary of some of the projects in top.

Now lets choose which we would like to go under current conditions of low power.

Now studying Thermo Scientific UV-Vis pdf.

Electronic state energy levels are widely spaced and it takes a relatively high energy to effect a transition.

Vibrational energy levels (IE, IR) are more closely spaced & therefore require less energy.

Rotational energy levels are even closer and only far infrared & microwave energies are required to excite.

You have noticed that the energy levels of IR, microwave & radio waves is even less than that of light. That is why the read from the top may be lower.

← MORE ENERGY — LESS ENERGY →

Cosmic, Gamma, X-Rays

Far UV, UV — Reference Point.

VISIBLE

Infrared, Microwaves, Radio waves

But microwaves do have more energy than radio and you know that RF can cause burns.

to the fact that it is higher than radio waves
lets go understand how it could still be
very harmful, depending upon the
photon involved.

More than one kind of energy change, π, electronic,
vibrational, rotational can take place
@ one time and can broaden out the spectrum
from being "line" oriented.

Solvents also cause the lines to smooth out
and create humps.

Ground state types

σ
π
n (non-bonding) this means a lone pair!

We also have two types of "anti-bonding" σ* & π*
What are these? What do they look like?

We need that "antibonding" is foreign to you as
because it comes from an alternate theory
to describe bonding.

We have
we know this sort. Valence bond theory
We do not know 2. Molecular Orbital Theory
this yet!

] 2 different explanations!

Chang P 321 Has the picture.

The description is on p 319.

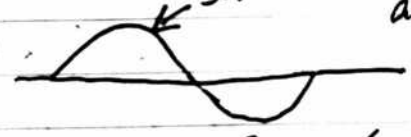
The Molecular Orbital Theory is its own topic. No wonder she did not know anything about it or what it mean to have "antibonding".

* The theory is based upon constructive and destructive interference of waves of electron densities

The theory applies to a molecule instead of an atom.

You finally have a picture of what this looks like.

Think of electron densities as wave functions



And then you have constructive and destructive interference of combining two waves.

Now it is physical, not just language

"Anti" Bonding means destructive interference

Bonding means constructive interference

Now we can go back to Thermo Scientific VIS Theory.

Not Fixed nm Common Ranges

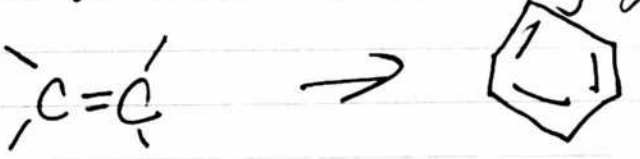
UV-VIS absorptions always involve a transition of an electron (σ, π, n) to an antibonding orbital. The possibilities are:

135-190 ~~180-240~~	$\sigma \rightarrow \sigma^*$ more energy	This is what happens during UV-VIS electronic transitions.
175-290	$\pi \rightarrow \pi^*$ less energy	
135-190 ~~180-240~~	$n \rightarrow \sigma^*$ more energy	
175-290	$n \rightarrow \pi^*$ less energy	

Notice that Molecular Orbital Theory is critical to UV-VIS spectrum interpretation.

Valence Bond Theory (geometric, limited to atoms) apparently can not suffice to explain UV-VIS spectroscopy.

Conjugation increases the wavelength of absorption and increases the intensity



~190nm ~250nm

The wavelength of absorption is a function of the molecule rather than of just the electron themselves. This is extension to Molecular Orbital Theory.

Two type of groups cause this.

Chromophores — N=O, N=N, C=O, C=S are common

Auxochromes — OH, NH_2, CH_3, NO_2 are common.

UV-VIS is used almost entirely for quantitative analysis.

(But not necessarily if you can produce a colored reaction)

Enzymes are Proteins!

An enzymatic rate reaction is a protein rate reaction.

Enzyme reactions are an important part of UV-VIS spectrometry.

There is an easier way to organize the energy relationships of the transitions:

$\sigma \rightarrow \sigma^*$
$n \rightarrow \sigma^*$
] more energy required (lower wavelength)

$\pi \rightarrow \pi^*$
$n \rightarrow \pi^*$
] less energy required (higher wavelength)

Methods to identify a substance or compound include:

1. Functional group testing
2. Chemical transformations
] Chemical methods

3. Physical properties
4. Spectroscopy
] Physical methods

Excited molecules are unstable and quickly drop down to ground state. Remember that "antibonds" are less stable, these are indeed σ^* & π^*.

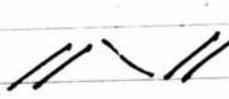
From Thermo Scientific:

"In general a compound will absorb in the visible region if it contains at least FIVE CONJUGATED CHROMOPHORIC & AUXOCHROMIC groups"

Chromophores (e.g.)	Auxochromes (eg)
$N=O$, $N=N$, $C=O$, $C=S$	OH, NH_2, CH_3, NO_2

A Conjugated Compound is a Compound with alternating double and single bonds.

 This would be one Conjugation

 This would be two Conjugations.

Conjugations (n)	λ
1	190
2	220
3	260
3	250
6	290
11	455

This is reasonably linearly Correlated.

$\lambda \cong 20 \cdot n + 184$ r = .94

or

$n \cong .046 \cdot \lambda - 8$

$n \cong .05\lambda - 10$

eg if $\lambda = 455$ (Carotene, we extremely $n \cong 13$

Actual no. is 11. Not bad @ all!!

The rule understandably.

This is only an ~~estimate~~ but it is still useful.

We actually get very good results:

$\lambda \cong 25 \cdot n + 170 \qquad r^2 = 0.97$

~~$A \cong 0.04\lambda - 6$~~ $n \cong .04\lambda - 6.5$

Example:
Carotene absorbs @ 455 nm
$n \cong$ ~~18~~ 11.7

Actual value is 11. This is really quite impressive. 700 nm estimates $n \cong 22$

How does metal complexing fit into this?

Well we do see that Chromophore + auxochrome radically alter & increase the wavelength. Methylene blue has only 3 conjugations. But about 4 chromophore/auxochromes
3 conjugations least to, $\lambda \cong 25(3) + 170 = 245$
but it is 660 nm!
Therefore
$k \cdot (CAC_n) = 660 - 245 = 415$

$k \cdot (4) = 415 \qquad k \cong 100$

So now we have
~~$A \cong .04$~~ $\lambda \cong 25 \cdot n + 100 \cdot (n\,CAC) + 170$ — maybe too high here. maybe it should be ~50.

So Chromophore + Auxochrome make a huge difference. This may not be too bad.

What this analysis shows us is that the conjugation aspect by itself seems reasonably predictable.

The Chromophore, auxo (CAC) aspect seems highly influential and likely much more difficult to predict.

Common spectrometric reagents are:

azo reagents (PAN, thoron, zincon)
dithizone
dithiocarbamate
8-hydroxyquinoline
formaldoxime
thiocyanate

These apparently form

Our latest VIS wavelength formula estimate is:

$$\lambda_{nm} \cong 25 \cdot n + \sum_{n k_1, k_2}^{15} (CAC) + 170\,nm$$

n = no. of conjugations

CAC = no. of chromophores + auxochromes

subject to modification.

HOMO means Highest Occupied Molecular Orbital
LUMO means Lowest Unoccupied Molecular Orbital

"The only molecular moieties that absorb light in the ~ 200 - 800 nm region are pi-electron functions and hetero atoms having non-bonding valence-shell electron pairs".

This is a crucial statement & it is the essence of UV-VIS Spectrometry.

This means that we are dealing primarily with:

1. $\pi \rightarrow \pi^*$ (200 - 700 nm)
2. $n \rightarrow \pi^*$ (Unsaturated compounds containing atoms w/ lone pairs show this transition)
3. Heteroatoms w/ lone pairs

What are example of heteroatoms with lone pairs?

Functional groups w/ lone pairs that have strong influence are: (Shift)

1. ketones
2. aldehydes — K for CAC $\overset{?}{\approx}$ 100
3. nitro
4. azo

Moderate influences come from (Shift)

1. acid
2. ester
3. amide
4. thiol

K for CAC $\overset{?}{\approx}$ 50

The presence of an absorbance band @ a particular wavelength usually indicates the presence of a chromophore.

It is also affected, however,

1. by the solvent
2. by the pH
3. by the temperature (remember your protein denaturation?)

Hamid in tablet pdf says that each conjugation shifts to the right about 30nm. We solved and got 25nm so we are definitely on the right track.

The more polar the solvent, the more the $n \rightarrow \pi^*$ transitions are shifted to lower wavelengths. (blue shift)

In a more polar solvent, the $\pi \rightarrow \pi^*$ are shifted more to the right (red shift).

Beer Law is only acceptable usually w/ concentrations $< .01$ M. We have seen this. A Power Law is much better.

There are lots of short rules that can be applied in UV-VIS but the fact is that you need to know the skeletal structure to be able to try them. This is why it is not a reliable method to determine structure. UV VIS is well suited to Concentration determinations and colorimetric verification.

Urochrome (or urobilin) is the chemical in urine that is primarily responsible for the yellow color.

Peak fluorescence: 420 ± 15
Peaks are determined by ammonium (NH_4^+)

Urochrome is rather complicated. There are 7 conjugations. This alone leads to $\lambda = 345$

But there are numerous heteroatoms. Which have a lone pair, however?

There are 2 oxygen that have lone pairs. C=O looks to be a chromophore. There are two there.

$345 + 2(75) = 495$?

$345 + 75 = \underline{\underline{420}}$ Interesting.

Comparison of Urine [redacted] Oct 31 2016
Carol's sample is now about 5 days old.
Results are radically different from first sample.

VIS

[redacted]	[redacted]
386	387
392	408
435	
450	448
709	709
904	905
931	932

We have a very close match here.

405 Fluorescence Ratio

[redacted]	[redacted]
520nm: $\frac{11.2}{18.4} = 0.61$	489: $\frac{11.8}{78.6} = 0.15$

What does the fluorescence mean?

Emit @ 405 absorb @ 520?
What is the phenomenon?

Do ammonium ions vaporize or dissipate over time?

Sunlight Maya Absorption Peaks 1615 on Oct 31 2016
Intensity
425 nm
554
744
765
828

Focus is here:

Absorption:

725	725.4 Intensity is 450	H_2O @ 720
755	No O_2 here ??	O_2 @ 760
810		H_2O @ 820

also

680	O_2 @ 690

This seems to be a very good match.
The absorption @ 430 remains unidentified.

~430 ??? NO_2? PPB 10 - 600

NOV
~~Oct~~ 03 2016

I have today considerably increased my power input into to RV. The controller needs to be by passed and replaced w/ a diode. The controller is placing the batteries into a float mode too early and reading and therefore they are never fully charging.

The controller is also not allowing me to overide the default settings.

It is charging the system very well now.
The power is doing much much better.
The batteries up to 13V now w/ a load attached.

We are now looking @ light thru the window.
It does not seem to be affecting the spectrum.

Spectrum Software

I

We have peaks @

			Nov 03		Oct 31	Nov 02
6	403	NO_2	427		425	~428 ok
			511	O_3?	~511 ok	~511 ok
methane?			618	O_2? - O_2 O_3?	~618 ok	~622 ok
8	681	O_2	682		~682 ok	~682 ok
	725	H_2O	724 - methane?		~724 ok	~725 ok
10	759	O_2	754		~754 ok	~754 ok

310 nm = 27027 cm^{-1}

950 nm = 10526 cm^{-1}

They are we get the same results through a window as we do outside.

We have identical results on all 3 days. Now we want to trace down the peaks.

We have extracted pigment from the juniper bush. A very strong singular peak @ 524 nm.

Our primary peak is @ 524 nm

Pasco says they are @:			
	430	662	Chlorophyll A
	453	642	B
	460	- 550	Carotenoids
	520		Anthocyanins

Who suggest carotenoids?
What are they?

Carotenoids are not water soluble. They are called accessory pigments. They are red, orange, or yellow.

Now what we did find is major fluorescence. This is characteristic of Phycobilins.

Do Carotenoids fluoresce?
Does Chlorophyll fluoresce?

Nov 04 2016

the juniper is absorbing @ 524 in isooctane.
We learn that manganate ion (VII) absorbs @ 524nm.

Carotenoids absorb between 460 & 550.
Carotenoids are fat soluble.
They are terpenoids. $C_{40}H_{56}$
Junipers are gymnosperms (cone bearing)
Junipers do indeed contain carotenoids.

One carotenoid associated w/ juniper is rhodoxanthin
Utah Juniper is the species.
The cones were eaten by Indians.

The berries are apparently the cones
Carotenoids can indeed fluoresce.
Violaxanthin is another carotenoid pigment

Power will be abnormal today. We need to adjust accordingly.

Because of power outtage today, it is more appropriate to.

1. Use books as much as possible
2. Then use the tablet as required
3. Use the computer as it is important.

Today we would like to:

over ✓ 1. Collect a spectrum of a cloudy day.

2. Construct a [illegible] raising widget for SGG

3. Study fluorescence - talk to them.

4. Can you extract a water soluble form of pigment from [illegible] leaves or [illegible]? What about [illegible] pine?

5. What are the additional spectrum peaks? Do we have ozone production taking place with NO_2 production?

6. The supplement paper is coming up as you get access to the net & [illegible].

7. Also the [illegible] paper needs to be developed.

[redacted]

✓

[redacted]

Spectrum acquired. Mostly Cloudy Today

More detail identified. We now have peaks @ 397

NO_2?	429	Weak	~~Methane~~ CH also is " " @ 431?
O_3?	512	Strong	Max Plank supports this This is weak in Max Plank
O_3	585	Weak	~~the lists possible~~ Max Plank supports this This is strong in Max Plank
~~O_3~~ ???	625	Moderate	~~Methane~~? This is unknown @ this time
O_2	684	Weak	
H_2O	723	Strong	
O_2	756	Strong	
	811	Weak	
	863	Weak	

NIR and visible light do pass thru glass.

CO_2 is infrared active
N_2 is not infrared active
] Remember your dipole moment?

Ordinary window glass passes about 90% of the light above 350nm

—

only a relatively small number of compounds can fluoresce. In general molecules that fluoresce have one or more aromatic groups in its structure.

—

You have clearly identified two different pigments in your spinach leaves, one non polar and one polar.

Isooctane (Non Polar)
Peaks @ 524, 680 & 949.
405 Fluorescence @ 442, 465 & 663
500 Fluorescence: None

Water (Polar)
Peaks @ 411, 558, 610, 709, 774 & 946
405 Fluorescence None
500 Fluorescence None

Two very distinct pigments identified here.

VIS-NIR Analysis of Juniper Leaves
The work is actually very good.

We have important form of separation that have taken place here.

One is polar and one is non polar.

The main peaks differ between 524 & 558.
But there are many other differences besides.

There is also a major difference in flourescence.

From PASCO

Chlorophyll A	430 & 662
Chlorophyll B	453 & 642
Carotenoids	460 - 550
Anthocyanins (PH DEPENDENT!)	520 @ pH 4.5

The is important of respect to pH

We all have that we have

No match for 430	Chlorophyll	A
No match for 662		A
No match for 453		B
No match for 642		B

We have a match for Carotenoids

$$460 < 524 < 550$$

but

$$558 > 550$$

but it is close.

This suggests that both of our peaks may be Carotenoids.

But Carotenoids do not photosynthesize directly so what does this mean?

The Carotenoid pigments are termed as "accessory pigments". P191 Chapman Tablet

Question: Do all plants have Chlorophyll A & B?

You could have done this by Chromatography but I have to read spectrometry as more capable and specific and definitely less work involved.

Question is, where is Chlorophyll in the sample? What if there were more than one pigment in a sample? Chromatography would detect this.

Recently you have learned a fair amount on the visible light atmospheric spectrum. You still have a problem with identification.

You have also made good inroads into the spectroscopy as well as fluorescence.

There are also some weaknesses here in identification but it is still a compliment to the picture.

Many many things have color to some degree and reactions can be learned which cause color to be formed. This is largely for concentration work.

However, if you can find a standard then it can be used for identification.

I wonder how Pinyon Pine compares.

You have worked on Pinyon Pine w/ Isooctane. You had mild contamination w/ water. Be cautious of reference spectra they give.

You have a fairly but interacted spectra for 400 - 525 nm.

You have 5 peaks shown up in this range. It is definitely a beneficial.

I have to wonder if there is a VIS database available somewhere.

If you have a reference spectrum you can determine
Identification uncertainties of the samples.

Nov
~~October~~ 5 2016

Some good accomplishments yesterday.

1. [redacted]
2.
3.
4. Solar spectrum collected & investigation extended
5. Some interesting work w/ VIS spectrometry
   You are still learning the capabilities of the system. It can act as an identifier if you have a suitable reference available. Are there any online databases?
5.5 We learned to demonstrate the Controller! No diode required.
6. The difference between Chlorophylls and Carotenoids is revealed. Many interesting questions here.
7. You have learned how to establish comparisons of spectra; they must all be collected within the same session.
8. Do all green plants have Chlorophylls? Or can they only have Carotenoids? Could one pigment actually be one or more pigments, esp. as far as the VIS spectra is concerned.

9. Continue to learn how to create colored reactions w/ organics. This can also serve as an identifier along w/ the spectrum.

10. Enzyme & Concentration reactions would also be of interest.
11. Max Planck is a good example of an atmospheric reference.

Today:

There is no sun so solar power looks to be nil.
This means that we are in ration mode.
If power was available, we would like to:
1. Post 720° Mirzellow paper on the site as well as FB.
[redacted]
3. Continued VIS work

But, WI do not seem to have power, so:
4. Fund raising widget for SGC
1. Env. Science book on tablet is good fodder for a couple of hours.
2. You have one spare battery for the laptop - you might be able to choose between VIS work and getting the paper posted, if you can find it.

Additional outstanding & high priority projects:
[redacted]
2. The rain paper is to be developed
3. The supplemental discussion paper is to be developed.
4. TLC & paper Chromatography are in the horizon.
5. Chemsky software, a/power demonstrating

Re-tabulate all methods & means available for winter research.
6. The math papers.
7. Carbon monoxide symptoms paper

On a cloudy day the spectrum is less well defined. We have peaks @:

Compared to Nov 04 Data:

467	weak	new	If the intensity decreases @ these points, then there is absorption. The intensity of infra red light is very low. The intensity of visible light is high.
509	weak	same	
540	weak	new	
615	weak	similar	
721	weak	same	

On a sunny day, there is a higher intensity of NIR.

Localized decrease of intensity means absorption So something is absorbing it. Since there is less intensity of NIR with the clouds it means the clouds are absorbing the NIR. This means that the resulting heat that is generated gets generated into the lower atmosphere, and it does not warm the surface of the earth. The net heat, however, should be similar within the troposphere. However, the heat would decrease in the atmosphere because the heat would radiate upward. A dense layer of clouds would reflect the heat upward and the moisture content would be higher.

In contrast, thin high clouds would not contain much moisture, they will also heat up, but the heat would dissipate vertically much more so because of mixing. The surface of the earth would continue to heat up.

Even with a heavy cloud cover we are still getting some power into the battery. Not a lot, but some, and enough to make a difference.

There is a fair chance that I can get the laptop charged, even under adverse conditions.

Let's go to local a tablet mode whilst at a Chargery. I think that we should save the tablet. Env. Chemistry book would have been useful.

Nowicki - Great Course - Biology is amazing, even though her style is somewhat confused. Photosynthesis is amazing.

We also learn that Carotenoids also photosynthesize but they do so @ different wavelengths (or equivalently) frequencies.

The color of younger leaves, therefore, is understandably different from full green plants.

The laptop is charged! Even under a cloudy sky!
It should be able to post the survey results.

I have been able to get it posted in a rudimentary
form. It will be employed upon.

Nov 06 2016 - Sunday.

All of the weekend campers have gone home.
Most of camp to myself again.
Today we have stood power again, recharging.

Another spectrum has been collected. Full sunshine pattern is now becoming apparent.
Cloudy spectrums need repetition.
Need all undefined peaks.

[redacted]

Attempted fundraisers sent not connection is insufficient.

[redacted]

Mozellons memmay page is also now on FB.

We certainly have many projects

Page 151

[redacted]

2. Rain paper completed
3. Co paper completed (Carbon Monoxide)
4. Supplement paper developed
5. TLC study, VIS Study, paper chromatography study
6. Chemistry software - always much to learn there
7. Env. Chemistry book - much to learn there
8. The two math papers developed
9. Ham study - major hamfests are coming
   yuma, tucson, quartzite
10. Fund raising widgets
   [redacted] expansion, rent, Preservation
   of paper
11. Math study in general?

But the question is what project really grabs your attention right now?

1. Soldering wires & fixing container interests me right now!

   and that is what I did!
Resonance on the antenna is very interesting to work with.

Nov 08 2016 Tuesday

I am able to get some things done on the net but the connection is highly limited & variable.
Remaining needs are

[redacted]

2. [redacted]
3. [redacted]
4. Ray - amend to Wallace
5. Set up fundraiser for
   1. cat & Utilities
   2. HES fundraiser
   3. NMR
   4. Distribution channel?

[redacted]

Resonance in antenna.
We can measure directly & interpolate

Full length $f_1 =$

NM ~~Nov 29 2016~~ Dec 05 2016 Percha Dam State Park

Testing urine for ammonia NH_3/NH_4^+

Initial readings w/ pure urine for both [redacted] & [redacted] per the meter test strips C ≥ 6 ~~ppb~~ PPM

I have now diluted the sample [redacted]

(actually drops vs ml)

30 ml H_2O distilled
15 ml urine
45 ml total.

My solution is therefore
33.3% by volume.

We estimate our readings now @ 3.0 PPM.
This indicates a reading of $\frac{3.0PM}{.333}$ = 9PPM
Etc

first estimate.

[redacted] sample under stem Condition I estimate @ ≥ 6PPM. This leads to estimate of ≥ 18PPM.

Now lets dilute further.

AS 30 drops H_2O
5 drops urine
~~35~~ 50 drops

leads to a ~~1/7~~ 1/10 solution

I set reading [redacted] ≥ 6PPM.
This leads to estimate of ≥ 60 PPM

Dilute further etc.

Lets drop to [redacted] = 2nd solution.
49 drops H_2O
1 drop Urine
50 drop

Now I think we have it @ ~ 0.5 PPM
This leads to 50 (.5) = 25 PPM

[redacted] sample: I get the same result w/ the same dilution, ie ~ 25 PPM.

The indicates no substantial difference between the Concentration in the urine.

You could run another set and dilute by a factor of 2.

Use glass vial, put 1 drop in ~~100~~ 99 drops H_2O. 100 I used
Then remove 50 into small vial for testing!

Not bad. I would estimate @ 0.3 PPM.
$\frac{1}{101 \text{ drops}}$ = ~~0.90~~ .990% solution

$\frac{0.3 \text{ PPM}}{9.90E-3}$ = 30.3 PPM ≅ 30 PPM GOOD.

Now for [redacted] I get 0.25 PPM

$\frac{0.25 \text{ PPM}}{9.90E-3}$ = 25 PPM

Both reasonably close to one another. What is the mean?

Lower Limit of reference range is 10-20 uMol/L
Upper is 35-65 umol/L

Odor can be detected @ >5PPM.

Molar mass of urea is 60.1gm/mol

1 umol therefore =

you can taste ammonia in water @ ~ 35PPM.

1st Molarity estimate of urea is 60,000 molarity.

NH_3 200-730 mg/liter from NASA

Urea is 9300-23,3000 mg/liter.

$\frac{1\ mg}{liter} = 1\ PPM$

Molarity = No. of Moles per liter

from 1 source $\frac{1\ mg}{liter}$ of NH_3 = 0.82PPM NH_3

Total solutes is 36,700 $\frac{mg}{liter}$ to 46,700 $\frac{mg}{liter}$.

Typical value of ammonium in urine is

$9.6 - 36.4 \frac{mg}{dLiter} = 100\text{-}360 \frac{mg}{liter}$.

Our result is 30. This sounds low.

NH_3 is a gas. It is not found in solution especially. What will be in solution is the ion NH_4^+. The terminology is often used loosely and incorrectly.

What is the molar weight of NH_4^+

N = 14

So the atomic weight is 18 gms/mole

So 30 micromoles is: $30E\text{-}6\ (18\ gms) = \frac{5.4E\text{-}4\ gms}{liter}$

$\frac{1\ mg}{liter} = \frac{1E\text{-}3\ gms}{1000\ gms} = \frac{1}{1E6} = 1PPM$

The Normal range is

$\frac{5.4E\text{-}4\ gms}{liter} = \frac{0.5\ mg}{liter} = 0.5PPM$???

This does not make any sense, but it does if it is referring to the gas form, which is what it says! So it is probably saying no gas, which is correct.

Now, with respect to NASA, they may be only the ones loosely & incorrectly.

It seems plausible they are referring to NH_4^+ instead of NH_3 @ 200 - 130 my taste.

The mean = 200 - 130 PPM.
But we estimate long 30 PPM.
The says that we label a low.

Now, the source says you can smell/taste it @ 35 PPM.

Dec 08 2016

Vis spectrometry work. Blue Food Dye as the sample.

Comparing + looking for any errors between glass test tube + plastic cuvette.

First lesson. Calibration for dark and reference look like they should be done with the reference solution + holder in position. This was a surprise w.r.t. the dark removal. False spikes were created using a glass test tube w/out including the dark removal w/ in the calibration. The result can easily be tested anytime.

We also see that the use of glass shows some important differences from the plastic. First the glass shows a much higher absorbance level which indicates that glass may be much more transparent than plastic. This was unexpected. In addition, the glass shows an entirely additional peak of significance that the plastic does not. Also the behavior in the NIR region is also different and the glass also appears more sensitive there.

In addition, we learn that smoothing can possibly introduce a false peak, at least with the reference solution. This also is a surprise.

The glass tube may therefore end up being superior by not be calibrated properly.

Let's test the glass calibration again.

First glass run:	380 nm
	405
absorbance Peaks	613
	694 dropoff
	948
2nd glass run:	402 nm
	480
	613
	724 dropoff
	950 appeared w but not peak

so we see definite peaks @ ~402 & 613.
The plastic did not pick up the 402 peak so there is an issue between glass & plastic. Proper calibration of the glass (or anything) is important. It appears that both Clark's reference removal is important regardless of what type of cuvette is used.

No Air Bubbles Allowed!

3rd Glass Run:

You must turn the recorder off when you are done with the Calibration! There is an essential requirement that is not obvious.

This time we noticed that removal of the air bubbles have a huge impact on the result. Now we get 3 very definite peaks:

382 nm
619
754
944

Notice the peak @ 754 is highly unique and significant now. It is a maya peak that was noticed. The remainder of the spectrum is flat as a pancake. This looks rather remarkable and clean.

Procedure.

1. Glass Tube
2. Dark & Reference both run w/ sample in
3. TURN OFF RECORDING, Check Reference
4. NO AIR BUBBLES in test solution
5. Look for a clean & repeatable spectrum.

!
*!

I will be able to use the BC300 for testing purposes w/ the isopropanol creosote extract combined w/ yellow food dye. We may also be able to explore redox reactions w/ the electrochemical interface. - Palmiens.

In addition to this, there has been some further exploration w/ paper chromatography now being applied to isooctane creosote extract. We are dealing w/ a solution that is primarily colorless. There is a great problem with respect to unknown separation effectiveness, solvent polarity & visualization. We may have movement, it may be more rapid than expected. We do have a level of visualization take place w/ iodine crystals. A good first start to colorless paper chromatography separation.

I believe that creosote has
1. Water soluble components
2. Isopropanol (alcohol) soluble components
3. Isooctane soluble (non polar) components.

I can already see that an emulsion is formed when water is added to both the isooctane and the alcohol extracted components. The extract is undoubtedly chemically rich.

Feb 09 2017 Biochemical Analyzer.

Adequate solar power & time today. Goal is exploration and familiarity w/ the BC analyzer. Power up & initialization successfully, and now we have the greater work.

When we boot up we are @ 37°C
We can turn the lamp on & off easily on the panel.
On requires a 1 min wait.
We have manual hold rinse OK, toggle.
Print
Aspiration, Cancel

Now I have a mouse (wireless) menu & better control over the keyboard.

TRIAL TEST IS

250 ml H_2O
30 ul yellow dye food.

This combined is the sample.

Kinetics @ 340nm
will be added ~~20 ml 20 ul of~~ 10 ul of blood.

This is the reagent

My first kinetics ~~test~~ was a total success.

At 340 nm it showed @ first a slight decrease in absorbance and then an increase.

One mistake you had was that you had the reagent volume incorrect @ 5 when it should be 10. The units or volume are uncertain.

What we need now is to double the bleach effect.

We had 10 ul Bleach in 230 ml H_2O w/ 30 ul dye.

So now we want 10 ul dye in 100 ml of H_2O, 20 ul bleach, and 10 minutes of time.

So now the ~~sample~~ blanks are compared to
1. 100 ml H_2O + 10 ul yellow dye.
2. Reagent 10 is 20 ul bleach
3. Time is 10 minutes.

Next time:
Double the dye, cut the bleach in half ie
~~1000~~
1. 100 ml H_2O w/ 20 ul dye (Blank)
2. 10 ul bleach
3. 10 minutes

I forgot to rinse.
Make sure you rinse when you are done

1. The reagent blank has an abs of ~ .085
2. When you blankout, the abs drops to zero.
3. As the reaction proceeds, the absorbance increases to reach the max of ~ .085.

The now means the dye is transparent. It is somewhat like a reaction in reverse.

We are definately seeing how things work now.

We could for instance, determine the concentration of bleach in solution by this reaction.

We should also be able to determine the concentration of a dye solution by regression.

We also need to know upon what method & base it determines concentration.

	Test 1 Reagent started as 5 after it was 10	Test 2	Test 3
Vol of Water	250ml	100ml	100ml
Vol of Dye	30 ml	10 ml	20 ml
Vol of Bleach	10 ml	20 ml	10 ml
Time	5 min	10 min	10 m
Result	.003 g/l	.008	.01

Now, if you think about it, Test 3 had twice the dye but half the bleach and the end result should therefore be the same, which it is.

Now you need to learn how Concentration is determined.

Assume Jewel abs. $\cong$.

$\frac{10}{100} = 0.1$

$0.1 ($

$\frac{10E-3^{6}}{100\,ml} = 1E-9$

$= \frac{10E-5\,ml}{1000\,ml} = \frac{.00001\,ml}{1000\,ml}$

= Not sure how.

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History

Print time: 02/09/2017

Date	Sample	Name	Test	Result
04-02-2017	1		ALB	0.082g/l
04-02-2017	2		ALB	0.000g/l
04-02-2017	3		ALB	0.001g/l
04-02-2017	4		ALB	0.085g/l

Test 1

Absorbance curve of samples

Test: [redacted] (Trial)

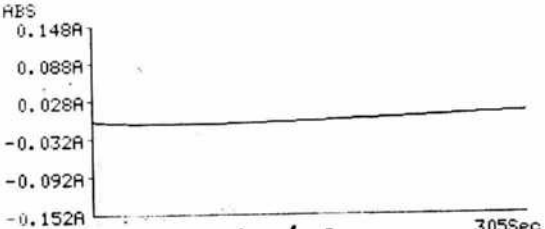
Factor: 1.000

Sample: 1

Result: 0.003(g/l)

Ref range: 0.000-100.[illegible] l)

Linearity range:0.000-1.000(g/l)



Still climbing

Test 2

Absorbance curve of samples

Test: [redacted] (Trial)

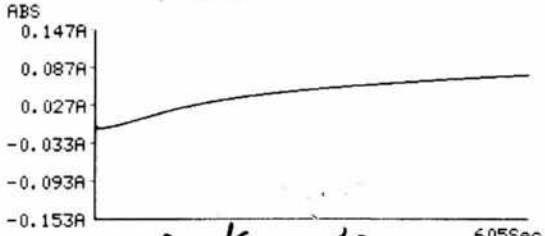
Factor: 1.000

Sample: 2

Result: 0.008(g/l)

Ref range: 0.000-100.000(g/l)

Linearity range:0.000-1.000(g/l)



peaking out

Test 3

Absorbance curve of samples

Test: [redacted] (Trial)

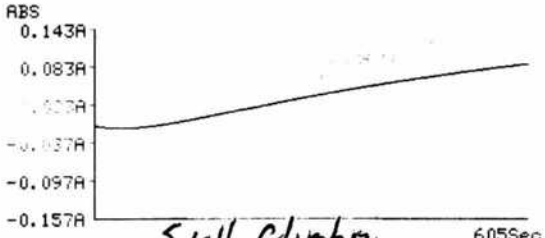
Factor: 1.000

Sample: 3

Result: 0.010(g/l)

Ref range: 0.000-100.000(g/l)

Linearity range:0.000-1.000(g/l)



Still climbing

We would do this w/ yellow dye alone.

10 ul	dye in	100 ml	.056 .055 gms/l
25 ul			.131 gms/l
50 ul			.269 gms/l.

$\frac{25}{10}(.055) = .137$ vs meas .131 gms/l

$\frac{50}{10}(.055) = .275$ vs meas .26 gm/l

Now our actual concentration is

$\frac{10 ul}{100 ml} = \frac{100 ul}{100 ml} = \frac{0.1 ml}{1000 ml}$

$= \frac{0.1 gms}{liter}$ but it says .055 gms liter

So I am not sure where the absolute concentration is coming from.

We now have reagent vol = 10
sample vol = 100
Lets change to 1 & 1 and see how it affects the test.

Relative concentrations are fine but how to get absolute concentration.

Now repeat w/ reagent vol = 1 & sample vol = 1

Dye	H_2O Volume	Abs	Conc
10 ul	100 ml	.049	.049 gms/l
25	100 ml	.116	.116
50	100 ml	.218	.218

2.5 (.049) = .122 vs meas .116
5 (.049) = .245 vs meas .218

Relative concentrations are certainly correct.
But absolute concentration is not calculated @ all.
Our actual concentration was

$\frac{10 \text{ ul dye}}{100 \text{ ml}} = \frac{100 \text{ ul}}{1000 \text{ ml}} = \frac{0.1 \text{ ml}}{1000 \text{ ml}} = \frac{0.1 \text{ gms}}{\text{liter}}$.

but this is not ~~what~~ right. It is .049 gms/liter
The mean absolute must be calculated or
you put in a factor.
We determine the concentration factor as 0.5
We used the concentration factor of 0.5
We did this wrong the factor is 2.0
~~Wrote Down~~ not 0.5. Let @ 2.0.

Ok we know this right.
Next adjustment $\frac{.5 \text{ gms/l}}{.438 \text{ gms/l}} = 1.14$ (2.0) = 2.28

This is the new factor.

Ok, then is one way to set a calibrated
Concentration. Calculate it!
Now the was single point calibration
you also needed to revise.

This works well.
You could adjust again if need be.

.519 gms/l = 1.038
.50 gms/l

and 2.380 (current factor) = 2.293
1.038 This would be new factor.

Ok, We now set .504 gms/liter!
Then is good.
Even though we do not know the molecular
weight of the material We know how
much material there is. The could
probably be used for titrations as well.

Now the next move would be to calibrate
a series of solution.

We also notice that tests were not saved.

So what is the diff. between control & calibrate?

Control is a Quality Control procedure w/ statistics on Tests.

Calibration is what you need.

10 ul	.059	= 100 ul/l = .1 ml/l
25 ul	.082	= 250 ul/l = .25 ml/l
50 ul		= 500 ul/l = .5 ml/l

Ok, we have some curious result now.

We put in 10 ul but I think we should put in
25 ul
100 ul

for Concentration

$$\frac{10 ul}{100 ml} = \frac{100 ul}{1000 ml} = \frac{.1 gms}{liter}$$

Our factor is way off.

OK, I am getting closer now!

I got Ø.40 gms/liter when it should be ~~Ø.5~~ Ø.25 gms so I am only off by a factor of 2 now.

[redacted]

It is computing a factor and the factor is off by close to a factor of 2.

OK! I have finally done it with a single point calibration.

It must have the calibration data active in memory to use it. If it is not there it needs to be recovered with a calibrator recovery during the actual test.

A single point recovery can be used if desired or needed but multiple point will give better results

Very good. You have done it now.

It should be easy to test if you keep at least one standard available.

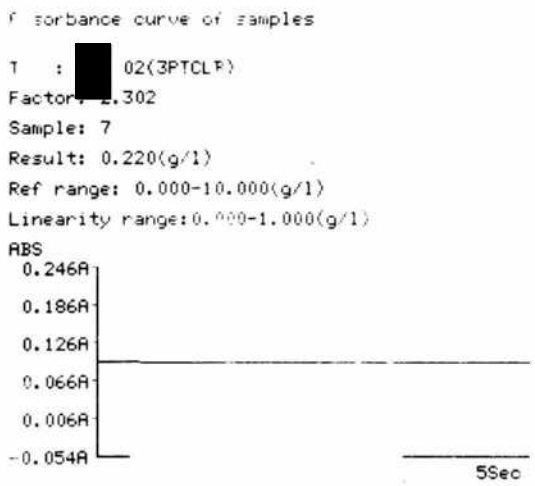
The problem I had was I set the aspiration volume too low and it could not take up enough liquid to perform the concentration or calibration test. Keep aspiration volume @ 500 ul.

It is looking better.

Calibrations seem to work w/ a repeat of 2, but 3 repeat seems problematic so far.

Yellow food dye unknown concentration determination.

This is the first determination of an unknown concentration using the BC300. Based upon 3 point regression with repeat msmts. This is good work.



You have also succeeded in monitoring an oxidation reaction (bleach on yellow food dye). This should be able to be used to find concentration on a functioning time in the future.

You also worked out quite a few issues, especially involving calculations. Even 1 pt calibration can be used if required; of course this assumes linearity.

Then for the sample volume and the reagent volume do not seem to be critical inputs

Feb 12 2017 Joshua Tree National Park

1. Ya should be able to separate blood (to some degree) w/ paper chromatography.

[Note: amino acids can be separated by TLC some of the solvents used are butanol, acetic acid, water and ethanol, ammonia & water. It is stained w/ ninhydrin.

Deproteinization must be performed (acid?) prior to TLC.]

For total protein we use blood serum rather than plasma.
Serum total protein is 6.5 - 8.3 g/dL
Methods include:
1. Refractometry!
2. Biuret (Ya have seen problems here w/ stability of the reagent)
3. Dye binding - Coomassie (Bradford method I like)

2. Seems as though it should be possible to determine Fe+2 & Fe+3.

3. Some progress today with:

Enzymatic reaction of starch, iodine and amylase.

also trials with
1. Amylase
2. Papain
3. Oxalate
4. Betaine HCl
5. Oxidase (green tea extract)

4. Food testing kit.

5. EW Environmental water pollution testing kit w Wallace & Tiernan colorimetric

6. Oxidation of urine vs ORP of urine

NADH absorbs light @ 340 nm
NAD does not.

NAD is a Coenzyme (a coenzyme is an organic cofactor).

A cofactor is a non protein molecule that may be required for enzyme activity.

NAD or NADH is often convenient as a reagent for a coupled enzyme assay (when neither NAD or NADH is a coenzyme for the reaction).

Commonly used enzymes include horseradish peroxidase, alkaline phosphatase, glucose-6-phosphate dehydrogenase and β galactosidase.

The enzyme in these assays functions as an indicator that reflects either the presence or the absence of the analyte.

For the Liver we want:

.98	ALT
.98	AST
1.31	ALP
2.20	GGT Gamma Glutamyl Transferase
1.64	Bilirubin
.52	Albumin
.52	Total Protein
?	Total Globulin

What is the sample material used?

Feb 17 2017 Biochemical Analyzer Explorations.

BC300

Blood Concentration might be a fairly good sample run.

gms/l

10 gms/l $\frac{10\,ul}{1\,ml} = \frac{10E^{-3}\,ml}{1\,ml} = \frac{10\,ml}{1000\,ml} =$

1 gm/l $\frac{10\,ul}{10\,ml} = \frac{10E^{-3}\,ml}{10\,ml} = \frac{10\,ml}{10{,}000\,ml} = \frac{1\,ml}{1000\,ml}$

0.2 $\frac{gms}{l}$ $\frac{10\,ul}{5\,ml} = \frac{x\,ul}{1000} = \frac{200\,ul}{1\,liter} = \frac{0.2\,ml}{1\,l} = 0.2\,gm$

Now reverse the seq from least conc. to most

(1) 0.2 gms/l

(2) 1.0 gms/l

(3) 10 gm/l

Wavelength Chosen will be 576 nm

We now have a [redacted] blood concentration calibration curve.

That which has been learned from today's exercise:

1. You must save the calibration scenario or it will default to 1 sample, one time, to determine the factor.

2. You must rinse out promptly after the sample injection or you will clog the cell and machine! Take blood for instance, even @ 10 gms/liter is enough to seriously impact the tubing & cell.

3. The wavelength chosen might be arbitrary but try to set it within a reasonable range.

4. You could now determine an arbitrary concentration of blood. 10 gms per liter is a lot, 1 gm/liter is still enough to affect a spectrum (unless hydrolyzed w/ acid) and 0.2 gms is weak but still definitely visible.

5. Our blood calibration factor is 8.375

We ran a sample check:

Mean Blood Concentration: 0.29 gms/l
Actual 0.20 quite good.
w/o rinse

Also distilled water came in @ .0019 gm/l: very good.

I think what we are after today is determining a blood protein level / concentration in comparison to something like whey.

What do we need?

1. A Bradford Calibration curve with out whey in it to set the reference blue green spectrum.

2. A Bradford Calibration curve w/ whey added to set a Calibration curve that accounts a shift in wavelength.

3. A ~~blood protein determination~~ via Bradford in comparison to the whey samples

Let's go after #1 first.

Then the Calibration factor is equal to

1/slope of the regression line.

This is actually equivalent to:

$$\text{Calibration factor} = \frac{\text{Conc}_2 - \text{Conc}_1}{\text{Abs}_2 - \text{Abs}_1} \text{ or } \frac{\Delta C}{\Delta A}$$

We have 0.20 gms of powdered milk added.
Lets make a reasonably concentrated solution

Let's place the w/s in 10 ml of dist. H_2O.

We now have a color chart spectrum on the phone.

Blood should absorb in the 490-500 region.
Appears red, absorbs blue green.

We chose 518 nm as our first trial.
We have available:
340 405 510 546 578 620

In retrospect we should have chosen 510 but 578 will suffice for now and we are getting good results.

For Protein, blue color should appear @ ~ 600 nm, actually about 580. (absorb yellow)
So we are perfectly activated when we are for Bradford.

We just need a Bradford reagent w HCl blank.

Bradford

Lets try
Control Solution
10 ml H_2O
100 ul 1M HCl
~~100 ul~~
100ul Bradford reagent.

Control Protein Solution
0.20 gms of powdered milk (Lowfat)
dissolved in 10ml H_2O.

We now have 2 calibration solutions:

Bradford Control	Protein Control
10ml H_2O	10 ml H_2O
(1) 100 ul 1M HCl	(1) 100 ul 1M HCl
100 ul Bradford Reagent	100 ul Bradford
	10 ul Protein Solution

There is definitely a shift in wavelength between the two.

There is definitely a shift in wavelength between the two.

Lets run 2 pt Calibration for both solutions.
Lets dilute both solutions by a factor of 10.

Dilute Solutions:

1 ml Bradford Control	1ml Protein Control
added to 10 ml H_2O	added to 10ml H_2O

Therefore the Concentration of the dilute solution is 1/11 of the Control solution

So our calibration curve Concentration for the Bradford Control will be

Bradford measurement

Full: 100ul in 10 ml H_2O

Dilute: $\frac{100E\text{-}3\ ml}{10\ ml} = \frac{X}{1000\ ml}$ $\quad X = 10\ gms/l$

Dilute $\frac{(100E\text{-}3/11)\ ml}{10\ ml} = \frac{X}{1000\ ml}$ $\quad X = 0.91\ gms/l$

Let's run 2 pt Calibration curve @ 570 nm
Reverse Concentration order on Calibration curve.
2 Standards, 2 repeats "BRD CTL"
$C_1 = 0.91$ gms/l
$C_2 = 10$ gms/l.

Look good. Calibration Factor = 48.376

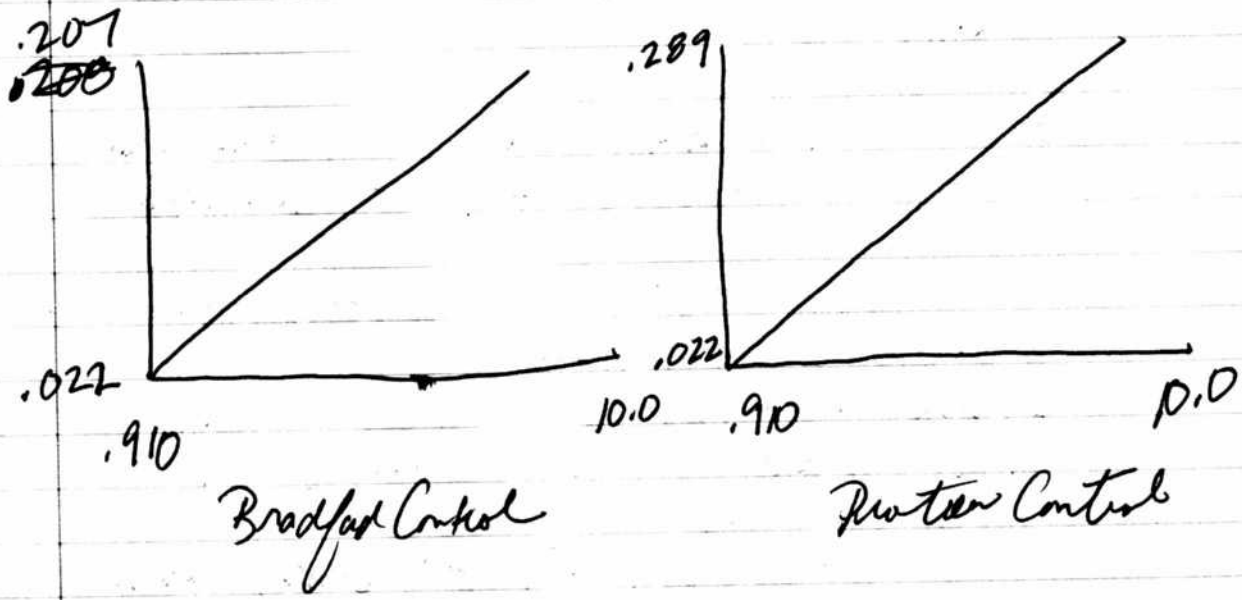
Now to Calibrate Protein A standard
2 Standards, 2 Repeats "~~BRD~~ CTL"
$C_1 = 0.91$ gms/l (Check meas: 1.04 gms/l-ok)
$C_2 = 10$ gms/l

Look good. Calibration factor = 34.616.

We now have 2 control solutions, one for the Bradford reagent, by itself in an acidic solution, & one w/ protein added.

We should now be able to test blood concentration compared to milk, as long as we are able to turn the blood color clear w/ 100 ul 1 M HCl.

The will be an arbitrary experiment. But before we do this, let interpret the magnitude of the calibration factor between the two control solutions.



This means that the Abs of the protein control is higher @ 516 nm than for the Bradford w/ Protein added Control.

Due to the reciprocal nature previously understood in the slope of the regression line, the Calibration factor will be at a lower magnitude for the protein solution. This is exactly what we found.

Bradford Control Factor = 48.376
Protein Control Factor = 34.616

Now lets try blood. We already, and still have available a blood Concentration sample(s) prepared. Let's use the mid sample of 10gms/dl Concentration.

Lets prepare a Bradford Control solution and add 100 ul of 10 gms/l blood solution to the 9 rem the protein test that has been developed

I have succeeded!
We get a result of 11.243 gm/l !!!
A very realistic value. The shift in wavelength was also quite visible when I added the blood

Second test result 11.383 gms/liter.

So our average value is 11.3 gms/liter

Now we need to interpret the value. This is a good time to shut down the instrument and analyze the results.

You have done good work today. You have developed a method and procedure to determine the amount of protein in blood relative to that of dried milk. When you can get an absolute on dried milk you will have an absolute in blood.

~~In now, assume the conc. of milk standard is 1 gm/liter.~~

~~In our dilution~~
Look @ our milk solution first.
We have a Conc. of 0.20 gms / 10 ml H_2O

$$\frac{0.20 \text{ gms}}{10 \text{ ml } H_2O} = \frac{x}{1000} \qquad x = 20 \text{ gms/liter}.$$

Original milk solution.
Now, we took 10 ul of this, and added it to 10 ml + 100 ul HCl + 100 ul Buffered.
Our total solution volume is therefore

$$\frac{20 \text{ gms}}{1 \text{ liter}} (10E\text{-}6 \text{ liters}) = 2E\text{-}4 \text{ gms}$$

This is in a total of
100 ul = ~~100E-3~~ 100E-6 l
+ 100 ul = 100E-6 l
10 ul = 10E-6 l
10 ml = 10E-3 l
$\Sigma = .0102$ l

We therefore have a Conc of milk being tested as

$$\frac{2E-4 \text{ gms}}{.0102 \text{ l}} = \frac{x}{1 \text{ liter}} \qquad x = .0196 \frac{\text{gms}}{\text{liter}}$$

We notice this is a very weak solution.

Now, with blood, we took a solution (the most concentrated), which measured $10 \frac{\text{gms}}{\text{liter}}$.

We then took 10 ml of that and added it to: 10 ml H_2O
100 ml HCl
100 ml Bradford reagent.

Our Conc. of blood is therefore

$$\frac{10 \text{ gms}}{\text{liter}} (10E-6 \text{ liter}) = \frac{1E-4 \text{ gms}}{\text{liter}}.$$

This was added to 10E-3 l
100E-6 l
100E-6 l
10 E-6 l
= .01021 liter

Our blood concentration is therefore

$$\frac{1E-4 \text{ gms}}{.01021 \text{ liter}} = \frac{x}{1 \text{ liter}} \qquad x = 9.79\text{E-}3 \frac{\text{gms}}{\text{liter}}$$

Which we see is ~~almost the same~~ about 50% as the milk. $= .00979 \frac{\text{gms}}{\text{liter}}$

$$\frac{.00979}{.0196} = 49.95\%$$

Next, the Conc. of the blood was determined as 11.3 gms/liter.

The assume, however, that the blood is milk.

Notice that our original blood sample is 10 gms/liter

Notice that our original milk sample is 20 gms/liter

Notice this ratio = 50%.
But the ration of $\frac{\text{protein}}{\text{blood volume}}$ is not necessary the same as $\frac{\text{protein}}{\text{milk volume}}$.

Lets think this through.

What we are clearly w/ therefore is

11.3 gms/liter of protein in blood

Compared to 20 gms/liter of milk

$\frac{11.3}{20} = .565$ is the ratio.

So if we have 100 gms of total protein in ~~blood~~ dried milk per liter, we have 56.5 gms of protein in blood per liter.

Check all of these.

I have acquired some numbers on blood & dried milk).

Blood: Total Protein is 60-80 gms/liter (Plasma)

Dried Non Fat Milk is 26.5% Protein
Therefore if we have 0.2 gms then .265 (.2 gms) = .053 gms Protein / should be our reference value.
We therefore $\frac{.053 \text{ gms}}{10 \text{ ml}} = \frac{X}{1000 \text{ ml}}$ $X = 5.3 \frac{\text{gms}}{\text{liter}}$

This should be our initial concentration.
We then take ~~20 ml~~ 10 ml

$10 \frac{20E-6}{1000. \text{ l}} (5.3 \text{ gms}) = \frac{5.3E-5 \text{ gms}}{10E-3 + 100E-6 + 100E-6 + 10E-6 \text{ l}}$

1. $= \frac{5.3E-5 \text{ gms}}{.01021 \text{ l}} = \frac{X}{1 \text{ liter}}$ $X = 5.191E-3 \frac{\text{gms}}{\text{liter}}$

is our anticipated solution of protein control.

Again: We took 10 ml of a $5.3 \frac{\text{gms}}{\text{liter}}$ solution.

This is a ratio of $\frac{10E-6}{1} * 5.3 \text{ gms} = 5.3E-5 \text{ gms}$

This was placed in $10E-3 + 100E-6 + 100E-6 + 10E-6$ l
or .01021 l

Therefore $\frac{5.3E-5 \text{ gms}}{.01021 \text{ l}} = \frac{X}{1 \text{ liter}} = 5.19E-3 \frac{\text{gms}}{\text{liter}}$

is our concentration of milk protein in the test = $.00519 \frac{\text{gms}}{\text{liter}}$
= 5.19 mg/liter. This is very small.

We designated this value an 10 gms / liter as a totally arbitrary value.

The actual value of protein is .00519 $\frac{gms}{l}$

Ratio = $\frac{10}{.00519}$ = 1926.78 times too high.

Now we measured an concentration of blood in Bradford, under identical volume circumstances as 11.3 gms liter

This ratio is $\frac{11.3}{10}$ = 1.13

This means that our expected concentration of protein in blood is $1.13(.00519 \frac{gms}{liter}) = .00586 \frac{gms}{liter}$ in the diluted state.

But the volume was diluted by a factor of $\frac{.01021\ l}{10E\text{-}6\ l}$ = 1021

This would lead to an initial sample concentration of $1021(.00586 \frac{gms}{liter}) = 5.983 \frac{gms}{liter}$

But recall that this sample itself was diluted by a factor of $\frac{1ml}{10ul} = \frac{1E3\ l}{10E\text{-}6\ l}$ = 100

Dr therefore means that the original blood sample has a concentration of

$10(5.983 \frac{gms}{liter}) = 59.8 \frac{gms}{liter}$.

The compare w/ reference value of $60-80 \frac{gms}{liter}$.

The appear to be very good ones.

These results are very intriguing. They will need to be repeated several times. Understand all this is based on an assumption of dried milk. Let us use albumin (egg white) next trial.

Out of curiosity what is the impact of low blood plasma proteins?

Methemoglobin! What a surprise ...

Nitrates & Nitrites are toxins that cause this.
p 597-598 Harrison

See p 600 also. Carbonoxygenic
Acquired

Feb 14 2017 Valentine's Day - A Reasonable Scenario Emerging

There are some very strong connections that exist between nitrate production and methemoglobin. Certain patterns may well be in place that are highly consistent w/ the research

1. Nitrite production in the urine
2. Intermittent cloudy urine
3. Increased ear wax production
4. Strong evidence for an enteric bacterial form (Gram negative, nitrite production) (rod shaped is uncharacteristic)
5. Possible low protein (globin) production w/in the blood - requires verification.
6. Acquired methemoglobin is a strong possibility then that is consistent w/ nitrate production
7. Carbon monoxide toxicity also leads to acquired methemoglobin - evidence for this exposure has also been acquired.
8. Fermentation of the culture & gas ratio production is currently under process. This will serve as an further classifying factor within the enteric bacterial form.
7. Possible increase in fatigue levels is also consistent w/ the scenario.

Total protein determination is a priority.
Also ketosis production after the blood is a serious prospect.
Urine test required of cloudy urine sample.

Albumin from egg white will be used for next comparison.
Also labeled protein powder

Blood Total Protein Reference Range 60-80 gms/l
Dry milk nonfat powder with Indian reference value is 26.5% protein.

Separate blood w/ paper chromatography

Enzyme reaction exploration:
Iodine
Amylase, Papain, Ox Bile, Betaine HCl
& Oxidase (green tea)

Urine oxidation - ORP
Bromcresol

Clardy Urine Test Feb 17 2017

URO	Norm	
BLD	—	
BIL	—	
KET	—	
GLU	—	
PRO	—	
PH	6	
NIT	+	Again, Again & Again.
LEU	—	
SG	1.025	
VC	3+	

Notice the incredibly consistent results on urine testing. All signs normal except for repeated existence of nitrites.

The scenario presented is consistent.

A reasonable hypothesis is that the blood will also show nitrites.

This now produces consistent results three times over a five week period.

We also notice that a blood sample in water (distilled? (not likely) produces a fibrous mass after incubation for a few days. This may be a clotting predecessor - fibrinogen formation but it requires investigation under the scope.

0.42 gms albumin
21.58 gms Total

We know that the water added is
21.58
− 0.42
21.16 ml or 21.16 gms

We therefore have a

$$\frac{0.42\text{ gms}}{21.58\text{ ml}} = \frac{X}{1000\text{ ml}} = \frac{19.46\text{ gms}}{\text{liter}}$$

The will be our standard protein concentration.
We will create a Bradford reagent with:
10 ml H_2O
100 ul HCl (1M)
100 ul Bradford
10 ul Protein Standard.

~~We can actually use our existing Standard~~. Not true. We need to make another ~~RBC~~ EGG STD.

The egg white does not completely dissolve in water. You must therefore add the acid to the alb-H_2O solution @ a conc. level of 100 ul HCl per 10 ml H_2O. Therefore you must add

$$\left(\frac{21.58\text{ ml}}{10\text{ ml}}\right)(100\text{ ul HCl}) = 215.8\text{ ul HCl to add.}$$

We could do this for the entire solution.

Our protein standard well therefore be comprised of:

Protein @ a concentration of (albumin - egg white)

(1) @ 19.46 gms / liter

(2) 21.58 ml

(3) 215.6 ul HCl

(4) 215.6 Bradford

A problem. I made the blood solution far too concentrated for testing.

It is a 2 step process.

Step 1. 30 ul blood in 3 ml H_2O
No additions
(Equivalent to 10 ul per ml).

Step 2. Extract only 10 ul of this solution and add to 10 ml of H_2O

!! then 100 ul HCl
100 ul Bradford

Now get absorbance.

Ok, we learned of several problems here. Your blood solution was way way too concentrated.

With your diluted blood sample, ya get
Abs = Ø.442
& Conc = 19.562 g/l This does not make sense.

Our factor is calc @ 44.043

With our albumin sample, we are getting the same result, which makes no sense.

We get Abs = Ø.447
Conc = 19.103

Interesting, now that ya look @ it closer, the two samples may not be as different as you think they are.

~~Test this by using~~ Test your solution by using 100 ul of your blood solution diluted w/ 10 ml H_2O
100 ul HCl
100 ul Bradford.

Now we get
Abs = Ø.457
Conc = 20.144 gms/liter

[redacted]

So obviously we have a problem that the concentration is not being recorded properly.

We now test the Abs. range in the Parco. We get identical results:

$Abs = 0.447 @ \lambda = 578 nm$

There is a spot on result. In addition we note that the peak absorbance occurs @ 593 nm so we also made a good pick within the bc 300 filter set.

Maybe the one point calibration is not sufficient? Let's go back and dilute it by a factor of 10. and at least see what happens to a 2 pt calibration.

Now for the protein standard we have two controls

$C_1 = 1.95$ gms/l
$C_2 = 19.46$ gms/l

Our calibration is similar in the result.
Our factor is 46.2

Now to 10ul blood sample Abs=.471 C=21.9 gms/dl
100ul blood sample. Abs=.623 C=28.7 gms/dl

It seems like the concentration result of your blood samples is way too high.

Let's test standards again.

Control 1 Conc = 0.90 gms meas vs 1.95 theoretical
Control 2 Conc = 18.0 gms vs 19.46

These are acceptable results.

Question: does this process actually obey Beers Law?

There are some serious questions here.
The dilution factor of the 10ul blood sample is $\frac{10E-3 \text{ ml}}{10E-6 \text{ l}}$

Dilution factor = 1000

The Dilution factor for the 100ul blood sample is $\frac{10E-3 \text{ l}}{100E-6 \text{ l}}$

Dilution factor = 100.
And then produces impossible results.

What has actually happened here?

What we have found here today is that the Bradford test is very reliable for detecting minute levels of protein via a wavelength shift to 592 nm but that it is not at all following Beer Laws for Concentration determination.

This is a very interesting result. We also learned that overload on Bradford is very easy. The actual protein isolation must be absolutely clear before you can add the Bradford reagent.

Your only predicament out of this is to prepare at 3 or 4 pt calibration with very weak protein solutions and see if you can confuse the Beers range more properly.

They must be independently prepared standards, not a diluted single standard.

0.275 gms albumin / 20 ml of H_2O = x / 1000

We can see that this does not dissolve.
5 drops 1 M HCl = 5 (.06) ml = .30 ml
+ 500 ul = .50 ml
Σ = .8 ml
+ .2 ml
1.0 ml

We need to go back to powdered milk.
The egg white is not dissolving properly, even w/ HCl added.

0.80 gms / 10 gms H_2O

adding HCl to this curdles the milk!
Do not do this!

0.26 gms / 10.06 ml = x / 1000 = 25.84 gms/l

Dilution factor
1095 Choose 10 ul + 10.85 ml + 100 ul HCl = .0236 gms/l
meas 0.130

215 50 ul + 10.62 ml + 100 ul HCl = .1202 gms/l
meas .138

105 100 ul + 10.36 ml + 100 ul HCl = .2461 gms/l
meas .148

And here is our problem. The solutions are not different

1984

The regression line has been computed incorrectly by the BC 300.

~~Slope = 2.97~~ No it is correct.

BC 300 Calculated .315

	Abs	Conc
S1	.395	.024
S2	.436	.120
S3	.469	.246

Ok, we have proven that Bradford reagent does not follow Beers Law.

I can not determine protein Concentration w/ Bradford reagent only the existence of protein

and for that it is very sensitive

ie 24 milligrams per liter can easily be detected. That is very sensitive.

Maybe Biuret method is better for this.

Feb 18 2017

I have therefore successfully returned to the Biuret reaction and reagent. I have some concern that it will not be as sensitive as Bradford but we shall see. The reaction is more difficult to replicate as you recall.

First combination of success
1. Five drops of diluted milk protein solution
2. You need more 1M NaOH than you would like (about 4-5 drops)
3. 1 ml of solution is adequate
4. About 2 drops $CuSO_4$ seems to be enough.

The shift is from a more opaque aqua to a more transparent blue shade

You will have to carefully control concentrations.

The copper chelated solution does not work. Chelation is tying up the copper.

You could use some more milk and protein powder - calibrated?

You have been quite successful in bringing the biuret method back to the forefront

We now have several protein test methods available to us:

1. Milk (Calibrated & stated protein amount)
2. Whey (previously calibrated powder)
3. Bone meal dissolved in acid
   (Completely un calibrated then you must ~~you~~ could calibrate with
   1. Specific volume of water
   2. Specific amount of HCl
   3. Specific amount of time
   4. Ideally a reasonable temperature.

The advantage of bone meal is that it avoids ~~danger~~ precleaning until required.

4. Whole blood.

The entire point of the project is to determine the protein concentration relative to a calibrated solution (eg milk).

After the project, we began looking @ how to determine the concentration of nitrates in the blood.

We have some sodium nitrate ~~coming~~ from ebay. We may also need to speculate some $CuSO_4$.

Until we have calibrated sodium nitrite
Our only source will be urine.

Our goal is to come up with a method of
converting nitrate to nitrite via oxidation
and then determine (somehow?) the concentration
of nitrites from the concentration of nitrates.

Feb 20 2017

Good work in place.

1. Ultrasound study goes well. Introduction to heart, liver, thyroid(?), bladder, kidney upcoming. Intro to software features, video capability, image storage & measurement. Atlas of Ultrasound & WHO textbook on ultrasound are in place on tablet.

2. Protein Calculation via Biuret is upcoming and on tap.
   - Milk standard
   - Blood Meal (P.S)
   - Whey Powder
   - Whole Blood.

3. Nitrite - Nitrate oxidation and Concentration studies are planned.

4. We also have UV-Vis air pollution studies w/ preliminary work is done.

5. Revisions on WP is a prospect
   Nitrite-methemoglobin - CO poisoning relationships via Harrison Internal Medicine
   Use Uri, Orem grape suggestions
   Counter bacterial stat prospects
   Nitrosomas eg.

6. Paper Chromatography projects

You do not want the temperature of any tissue to rise more than 1° w/ ultrasound.

Bone and air are the exceptions to ultrasound (US).
US highly valuable for:

1. presence, position, size & shape of organs
2. dysfunction of organs
3. tumors & lesions
4. inflammatory diseases
5. abnormal fluid collection
6. transplants
7. congenital defects and malformations
8. Management of chronic disease
9. Essentially risk free (temp & Cavitation) & low cost

You can evaluate the chest & lungs, even w/ bone and air impedances.

P124
Investigate transudate ascites ~~Fig 6.21 in WHO (I)~~
w/ floating small bowel loops Fig 6.21
that are w/in the abdominal cavity in WHO (I)

P122 WHO (I) There are benign ascites caused by portal hypertension

for beam. There are three disorders listed w/ transudate ascites:
The closed gallbladder/ 1. Portal Hypertension (liver, spleen, gallbladder)
wall 2. Venous Congestion (heart failure)
Echo Poss. 3. Nephrotic syndrome (kidneys)

Ascites are a common symptom.

Ascites is a common ambiguous symptom of many diseases. p136 Who I.

Both benign (thickened gallbladder wall) & malignant

This is a topic for investigation.

Lyles p143 shows measurement.

Mar 08 2017

Controls: The Ultrasound Atlas is organized into 12 sections

pp			Anticipated No. Series	
14-71	1	Vessels	1-19	
72-117	2	Liver ×	20-29	
118-133	3	~~Gallbladder~~ ×	30-39	The
134-167	4	Pancreas ×	40-49	number
168-179	5	~~Spleen~~ ×	50-59	series is
180-201	6	Kidneys ×	60-69	only
202-217	7	Adrenal 69	~~70-79~~	approximate.
218-241	8	~~Stomach~~ 70-79	~~80-89~~	
242-249	9	Bladder 80-89	~~90-99~~	Page nos.
250-259	10	Prostate 83	100-109	Control
260-271	11	Uterus 85	110-119	OK now.
272	12	Thyroid 100	120-129	

Now we can understand the book more easily numbers 80, 85, 89 refer to the stomach, for example.

The last page, P 296 has the index for numbers. You can see why it is awkward.

You have a picture of the index on your phone. The index is ~~enlightened~~.

Notice the heart is not in the index!

Liver: subcostal or intercostal.
p139 in US Manual

In this manual we have

	pp
Cardiography	69-102
Liver	139-180
Gallbladder	181-203
Spleen	204-221
Kidney	222-242
Bladder	284-291

Mar 15 2017 Virgin River Gage

Today, the BC300 Comes to the fire again.

1. Protein Concentration Project.
2. Nitrate - nitrite Concentration project.
3. Iodine possibilities also

Protein:
1st stage is to Calibrate milk
& also standardize to Biuret reagent.

BC300 has integrated property
I think the Colorwheels used in the PC
which is not convenient.
Agilent UV-Vis on tablet has one Chart
that has been put onto phone.
It is OK, but not great.

Pavia has a Chart also:

Observed	Wavelength	Absorbed
yellow	400	Violet
Orange	450	Blue
Red	500	Blue-Green
Red-Violet	530	Yellow-Green
Violet	550	Yellow
Blue Green	600	Orange-Red
Green	700	Red

Vikki Color Chart

somewhere around 515 should be observed blue.

Vikki has an alternate chart: (p14)!

Observed	Wavelength	Absorbed
Yellow Green	400-435	Violet
Yellow	435-480	Blue
Orange	480-490	Blue-green
Red	490-500	Green-blue
Purple	500-560	Green
Violet	560-580	Yellow-Green
Blue	580-595	Yellow
Blue-green	595-650	Orange
Green-blue	650-750	Red

This looks like a more helpful chart. Biuret reagent will be blue-green w/out protein and will shift to more blue w/ protein.

Blue-green vis: 595-650
Blue vis: 580-595

This is saying Biuret should be shifting to the left w/ protein.

Now, the BC300 records @
340, 405, 510, 546, 578, 620

The tells us that we should be working @
~ 578 in the Biuret test.
This is what I needed.

Now the first step is to standardize a
Biuret Reagent.

Run some small tests to see what
works with milk, & then create a volume of it
in a container. Eyedropper volume would be
best.

The reagents can be arbitrary amounts as long
as we make it uniform.

20 ml distilled H_2O.
10 drops 1M (approx) NaOH (20.54 gms)
Ø.14 gms tartaric acid
10 drops $CuSO_4$ solution.

This does not work.
3 ml H_2O
2 "X drop" $CuSO_4$
"Pinch" tartaric acid (removes precipitate)
6 "X drops NaOH
+ Protein

This works. Solution is clarified

Biuret reagent appears to be more successful with

3 ml H_2O
5 drops NaOH (~ 1M)
4 & drops $CuSO_4$. (~ 0.5M)
"pinch" Tartaric

Look @ sensitivity to concentration.

Tartaric acid removable precipitate but the solution remains cloudy. It would be good to record the wavelength of max absorption.

ya apparently did not have enough copper. No protein remains cloudy & blue/green. Protein definitely shifts toward blue.

However! The test also does not appear to be affected by concentration as much as ya need it to be, so ya still have a major problem.

Test is sensitive to existence, but not concentration. This is the same problem that Bradford has.

It appears the first test of lower volume of reagent was satisfactory but it took a while to react, namely 10 min or so.

We may be able to get by w/ less reagent

Try
3 ml H_2O.
4 drops NaOH
2 drops $CuSO_4$
pinch tart.

3 different conc. levels added.
Then is certainly some sensitivity to concentration

I have added 1 drop NaOH since reaction does not seem complete.
It did improve the ideal protein solution some but not the other.

Now I have added 1 drop $CuSO_4$.
Adding more Tart acid the messed everything up. Keep this to a minimum. So

3ml H_2O
5 drops NaOH
2 & 3 drops $CuSO_4$
trace Tart

Weaker Conc. of protein this time

Weak to mid conc solution produce a discrete visible difference.

Mid to higher conc produces no discernable difference.

The is favorable in the sense that it would detect a distinguish between very low protein concentration but it will be difficult to calibrate.

2 drops $CuSO_4$ appear to be adequate but 3 could be used.

We also added another NaOH drop it clarified 1 & 3 further but not the middle protein.

The lesson at this time.
1. Little Copper is needed.
2. NaOH seems to help
3. Test seem useful only @ very low Concentrations.

Now we suggest:
1. 3 ml H_2O
2. 2 drops $CuSO_4$
3. Very trace test
4. 6 drops NaOH
5. Very low protein levels

OK, we have succeeded!

We have a test now which is very sensitive to VERY LOW Concentrations of protein. This is good as we can always reduce the concentration.

The successful Biuret reagent is:

1. 3 ml distilled H_2O
2. 2 drops $CuSO_4$ (~ 0.5M)
3. 6 drops NaOH (~ 1M)
4. Miniscule Cream of Tartar pinch (smallest visible on small spatula).

Now subject to very low protein concentrations similar to tartar amounts but increasing.

3 shades of increasing blue in a transparent solution can now be observed.
Let's calculate the assay
1, 2 & 3 units of protein in solution.

Set up BC300 Test @ 518 nm.

We now have a calibration test for low protein assaying concentration levels of 1, 2, 93.
The factor is 5.371

We have a very good result from our calibration.

We tested w/ the middle tube of assumed Concentration 2.0.

BC300 gives a mean result of 2.029 gms/liter
This is excellent.
On to others

Tube	Assumed Conc (gm%/liter)	Mean
1	1	1.700
2	2	2.029
3	3	2.24

So it really did not work all that well.

We get means of

Conc Assumed	Abs
1	.337
2	.393
3	.424

Casio gives a linear regression of:

$y = ax + b$

$abs = .0435 \cdot conc + .298$ $\quad r^2 = 0.97$
(looks correct)

$conc = 22.313 \cdot abs - 6.61$ $\quad r^2 = 0.97$

Assumed	Casio Regression
1	0.93
2	2.18
3	2.68

These are very good results.
The BC 300 regression is not at all OK.
Standards are recorded properly but regression is not right.

The regression and computed facts are not correct.

Ass. Conc (gms/l)	Meas Conc (gms/l)	
1	1.00	
2	1.90	Great Results here!
3	3.10	

OK. Wonderful results. This shows you that the linear regression is very dangerous & you must be cautious. The non linear regression picked up the activation immediately.

History

Print time: 03/15/2017

Date	Sample	Name	Test	Result
15-03-2017	7		BIUCLB	1.000g/l

Theoretical = 1.00

History

Print time: 03/15/2017

Date	Sample	Name	Test	Result
15-03-2017	8		BIUCLB	1.897g/l

Theoretical = 2.00

History

Print time: 03/15/2017 Theoretical = 3.00

Date	Sample	Name	Test	Result
15-03-2017	9		BIUCLB	3.100g/l

* The lesson here today is never assume the regression equation captures the standards & Controls unless it is tested against study.

Complicating factors:

1. The linear regression may assume the y intercept @ x=0 is zero and this may not be the case.

2. Your reference solution here of Brunt Isa not clear, it Colorless. Therefore if you use a linear regression you are almost certain to be required to remove the reagent blank.

3. There is more than one approach to solving the problem but you must always test & challenge your results. Do not just assume the regression captured the data, in the case of linear regression most definitely did not but the non linear regression captured it very well.

4. You now have a suitable & sensitive Biuret reagent standard that has been developed. Now you need to quantify the protein concentrations. You should a clear separation visually by three different concentration levels

Tint of blue	1 } Actual
Light Blue	2 } Concentrations
Pink and medium blue	5 } Ratios Estimated.

5. Next we work toward
   1. quantifying the protein concentrations
   2. Transferring the methods over to blood protein concentrations.

6. The Biuret reagent developed is:

   1. 3 ml H_2O
   2. 6 drops NaOH (~ 1M)
   3. 2 drops $CuSO_4$ (~ 0.5M)
   4. Absolute trace Cream of tartar

Add very low Protein Concentration & reagent will detect the color changes in the blue portion of the spectrum.

It looks like creating the reagent first and then adding protein is required.

Mar 16 2017

Today we head toward blood protein concentration.
First we need to prepare a standard set of known concentrations of protein
Let's work by color first & then pull on BC300.

We will start w/ 20ml distilled H_2O.

The milk protein concentration is $\frac{8\text{gm}}{23\text{gms}}$

= 34.8% protein

The is Great Value (walmart) dried milk.
It is not nonfat or low fat dried milk.
This is a high protein concentration - good.

Our low control protein solution is

$\frac{0.15\text{gms}}{20\text{ml}} = \frac{X}{1000\text{ml}}$ $X = \frac{7.5\text{gms}}{\text{liter}}$

Now lets look @ 50ul of this solution into 20ml H_2O.

$50E\text{-}6\text{ liter}\left(\frac{7.5\text{gms}}{1\text{ liter}}\right) = 3.75E\text{-}4\text{ gms}$

in micropipette.
Therefore our #1 dilute solution is

$\frac{3.75E\text{-}4\text{gms}}{20\text{ml}} = \frac{X}{1000\text{ml}}$ $X = \frac{.0188\text{gms}}{\text{liter}}$

$= 18.8 \frac{mg}{liter}$

Lets make 2 more solutions.
One 5 by a factor of ~~10~~ 5, one 7 by a factor of ~~11~~ 5.
The current solution will be called #2

So our solution concentrations will be

			abs
#1	3.75E-3 gms/l = .00375 gms/l		.359 .373
#2	.0180 gms/liter		.496 .529
#3	.094 gms/l	NO. VOID problem	.354
#4	.375 gms/liter		.633 .679

Now we run a Biuret solution reagent test on each of these. (calibration) using:

1. 3 ml ~~distilled H_2O~~ of each concentrate
2. 2 drops $CuSO_4$
3. 6 drops NaOH
4. trace tartar

Your suspicion is that you need to let the color develop over a period of time, possibly as high as 30 min.

#2 solution does not seem to be developing. I am not sure why.

I think we need to use a more concentrated solution.
Let's try #4

1000 ul in 20 ml of H_2O.
#4 has 10 min on the clock. Needs 30

The concentration is therefore

$$1000E\text{-}6\ l \left(\frac{7.5\ gms}{l}\right) = 7.5E\text{-}3\ gms$$

$$\frac{7.5E\text{-}3\ gms}{20ml} = \frac{X}{1000} = \frac{.375\ gms}{liter}$$

It really looks to me like the color takes a long time to develop, may be 60 min now. I am, however, with the exception of tube #2, seeing color develop properly.

Timer: 30 min on #1, 2, 3
10 min on #4
Reset to 30 min.

By Mosby, the Conc. of total protein in serum is 60-80 gm/liter

To be in range of our test standards, we would require a expected dilution of serum on the order of

$$\frac{10 \text{ gms/liter}}{0.1 \text{ gms/liter}} = 100$$

This means for a 20 ml solution we would add

$$\frac{20 \text{ ml}}{100} = .0286 \text{ ml} = 28.6 \text{ ul}$$

This is perfectly in range.
We therefore anticipate to add 30ul of blood (ideally serum) in 20 ml of H_2O to determine our protein concentration.

control standard

The color on the sample generally seems to be developing reasonably well. You did add more factor to tube #2 to see if you can bring out color. That does appear to be a range visible with tubes 1, 3 and 4.

We clearly have a non linear regression. Test the regression

	Conc (theoretical)	Conc (meas)	Std. Abs
#1	.004 gms/l	.004	.359
#2	.019 gms/l	.17	.499
#4	.375 gms/l	.211	.633

The regression is also pretty much weak.

Casio linear: $\text{Conc} = 1.345 \text{ Abs} - 0.54$ $r^2 = .77$

Power: $\text{Conc} = 8.79 \cdot \text{Abs}^{7.80}$ $r^2 = .93$

Lets try blood dry quickly.

Before we do that, try a linear regression.

Linear regression results

#1 .218
#2 .158
#4 .205

Thru the origin.
Power regression is the most useful.

I am not satisfied w/ the results of this trial
Now let @

#1 100ul = 100E-6l(7.5gms/l) = 7.5E-4gms
2 300ul 300E-6l(7.5 gms/l) = 225E-3 gms
3 500ul 500E-6l(7.5 gms/l) = 3.75E-3 gms
4 1000ul 1000E-6l 7.5 gms/l) = 7.5E-3 gms

100ul 1 $\frac{7.5E-4gms}{20ml} = \frac{X}{100}$ $X = .0375 gms/l$

300ul 2 .1125 gms/l

500ul 3 .1875 gms/l

1000ul 4 .375 gms/l

You had a problem getting reacts. to dissolve.

You added - 8 drops NaOH
2 drops $CuSO_4$
Triple the tartaric acid.

Your concentration is not nearly light enough.
Forget the intermediate concentration.
Take solution directly. Use 3 ml.

$$25\,ul = 25E\text{-}6\,l\,(7.5\,gms/l) = \frac{1.875E\text{-}4\,gms}{3\,ml} = \frac{X}{1000} \qquad X = .0625 \text{ gms/l}$$

50 ul $\qquad X = .125$

75 ul $\qquad X = .1875$

100 ul $\qquad X = 0.25$

You added 1 more drop $CuSO_4$
5 more drops NaOH
twice again the tartar.

The regression is not going well. I do not know why. It is not steady @ 516 nm?

Only #2 & #4 are transparent but they both have the same absorbance so they do not reflect a concentration difference.

We have a problem. We are not duplicating the results of yesterday where we could see a clear difference.

Try to repeat the first.

We are getting really strange results. I can see the color intensity by eye but the absorbance is not reflecting that. We must use Pasco to determine max wavelength.

We learn now from Pasco that the max absorption is occurring @ 635 nm. Not 518. (But this is blue green, not blue.) This indicates the reaction is not occurring properly. Let's adjust to this.

This is not recording the absorbance properly. Why?

@620. BC300

@620nm Pasco Abs:

1	.748	So the results are garbage here.	.687
2	.763		.664
3	1.163		.721
4	.676		.595

Why such a big difference between the two instruments? This also does not make sense.

Your Biuret protein solutions are worthless
I have no idea why @ this point.
You should have saved your other milk sample.

A major bust today. The Biuret test and reaction was not @ all reliable today, and no idea why yet. The only known variable of change was the dried milk product; in the sense that does not account for the problem in any way.

I will work only visually now.
Also we have the question of 578 vs 640 nm
640 nm will be in the blue green portion which is not where we should be.
We will run a visual test only here.

You know that Bradford did not work either but Biuret should.

Also why is distilled water shown such a high level of absorbance. It should be zero.

Research indicates max absorption should be @ 540 nm exactly as anticipated.
It should therefore be purple, and not blue and especially not blue green.
Therefore you do not have a valid Biuret reaction

Sensitivity of the test is also stated to be low, requiring 1 mg.
But 1 mg in how much solvent?
They did not say so other info is worthless.

300 ml of 10% NaOH to 500 ml of a solution containing 0.3% copper sulfate pentahydrate and 1.2% sodium potassium tartrate.

or

1.50 gms $CuSO_4$
6.0 gms Sodium potassium tartrate
Dissolve in 500 ml H_2O
Add 300 ml 10% NaOH
Make up volume to 1 liter.

Cream of tartar is potassium hydrogen tartrate.

One person used 2/3 of cream of tartar, or 4 gms but also said it did not work

We could reduce all by to 10%:

0.15 gm $CuSO_4$
0.60 gms tartrate → 0.4 gms?
Dissolve in 50 ml H_2O
add 30 ml 10% NaOH
Bring to 100 ml.

NaOH MW? 40 gms/mol
So a 1M solution is 40 gms/liter
a 10% solution is 100 gms/liter

So there is very little
a 1M solution would require $\frac{100}{40} = 2.5$ times as much.

So 30 ml of 10% NaOH would require 85 ml of 1M NaOH. This is a huge amount. We do not have this amount.

$\frac{30 ml}{1000 ml} = .03$ and .03 (100 gms) = 3 gms in 30 ml.
This is very high. There must be another problem.

So 0.15 gms $CuSO_4$
0.40 gm Cream of tartrate
dissolve in 50 ml H_2O
3 gms NaOH
Bring to 100 ml.

Projected Reagent.

This is quite different. Did you bring any? NaOH?

I have solved my problem.

see next page.

The problem was that the pH of the reagent made was not nearly high enough. 1M NaOH drops did not adequately cut it.

Fortunately I have one bottle of high-concentration KOH-NaOH solution and it is adequate to bring about the reaction.

You never did have the reaction proper, even w/ yesterday's work. The shift is from blue to violet. It is quite discernible and it is why the max is recorded @ 540 nm. 546 will be adequate, but see if there is a 540 in the BC300.

You are in business again, & you are ready to proceed w/ the calibration of standards once again.

A frustrating day w/ a good reward at the end & a little deeper research & luck enough to have brought along 1 bottle of high concentration hydroxide.

BC300 does indeed have a 546 nm so we are set.

Mar 17 2017

~~.0254 gms~~ 1.00 gms = X X = 49.26 gms / liter
20.30 ml 1000 ml

The actual protein concentration is .348 (49.26)

= 17.14 gms / 1000 ml

20 ml / 10 ml

Now prepare 34 standards in ~~20~~ ml H_2O

100 ul · 100E-6 l / ~~20~~ ml (17.14 gms/l = X / ~~1000~~ ml) = 1.714E-3 gm = X / 20 ml / 1000 ml X = .086 gms / liter

400 ul 400E-6 (17.14) = 6.856E-3 gms = X / 20 ml / 1000 X = .343 gms / liter

100 ul 100E-6 = .012 gms / 20 ml = X / 1000 X = 0.60 gms / l

1000 ul 1000E-6 = .017 gms / 20 ml = X / 1000 X = .857 gms / l

Use:
5 drops KOH-NaOH
2 drops $CuSO_4$
Visible pink tint

Definite shift taking place.
10 min wait.

Some problem w/ Calibration but it will work out because you can see the difference

First off we should see any 578 in our case because our concentrations are strong enough to cause a blue shift But NOT A shift to violet.

Our Control & tube 1 have a precipitate so there is not adequate concentration in either one to be testing.

Best approach seems to be using a

1. 4pt
2. 578nm
3. No blank
4. linear or non linear?

It looks to me like we need to consider a non linear approach

Lets go back to 3 point @ 546, non linear

What we have occurring is a reverse curve here. @ 546 nm, it is actually drawing away from violet (blue green control) and headed toward blue.

There is still the question of whether we have enough NaOH or not, or even tartar.

Sample 2 gives Conc of	.306 gms/l	vs	~~.343~~ 0.60
4 " "	.319	vs	.857

So we still have a problem.
If the control precipitates, you are not adding enough NaOH and/or tart.

We have increased
NaOH to 8 drops
Tartar more visible
2 drops $CuSO_4$

This is looking better. Still blue however but a definite reaction. Chose 578, 4 pt, nonlinear. The control is blue green, which is closer to 620. 546 is violet.

OK, we have our best results so far but it may be you should have chosen linear

Measured. This is BC300 nonlinear regression

	Meas	vs	Theoretical
#1	.083 g/l		.086
#2	~~.415~~ .690		.343
#3	.677		.600
	.778		.857

Now it seems as though linear may be better.
It looks like non linear created a 3rd order regression.

Collected regression data

Abs	Conc
.344	.086
.386	.343
.382	.60
.474	.857

Linear:
Conc = 5.46 · Abs − 1.69
r^2 = .82

2nd Order.

Not really justified. Quadratic: r^2 = .84

Test the linear regression..

We see that the BC300 assumes a zero line intercept. This can lead to a faulty regression until we learn how to intercept a blank w/ color.

We are learning, since we do not have purple in our solution and that our regression is marginal, that we do not really have enough protein in our solution.

Since we will be using distilled blood however, it may be a good idea to go ahead & set for now.

We have an interesting Case w/ blood.

I am only using BAL as my sample size in the tubes: 3 ml H_2O, 2 drops $CuSO_4$
8 drops NaOH, 9 VIS. tartaric acid.

The is turning the Control from the light blue + BLUE GREEN- NOT VIOLET !!
Why??

We measure abs. @ 518nm C .609

This leads to an expected conc of:
5.46(.609) - 1.69 = 1.635 gms/liter

But this was diluted by a factor of $\frac{3\text{ml}}{8\text{ml}}$ =
$\frac{3E-3\,l}{8E-6\,l} = 375$

So this would indicate a protein conc of 375 (1.635) =
613 gms/liter

But the expected value in serum is
60-80 g/liter.

But what about in whole blood?
BC 300 gives an elaborate
score of .764 g/liter.
But this is still way too high:
315(.764 g/l) = 286 gm/liter (about 1/2)

Why didn't turn blue green?
Is it because blood is red?
If anything, red blood would shift
the color lower in wavelength, toward 495,
not toward 650?

Reference
Each 10 ml whole blood produced
400-1500 ug protein

Therefore:
$\frac{1000}{10} = 100 \Rightarrow (100\ ml)\ 1500\ E\text{-}3\ \frac{}{ml} =$

$\frac{1500E\text{-}6\ gms}{10ml} = \frac{X\ gms}{1000ml}$ $X =$

Abs. for [redacted] w/ similar blood sample
was .706

This leads to 315(.706) = 265 vs 286

or

5.46(.706) − 1.69 =

& both samples produce a similar color and have correlated similar expected BC300 concentrations, even if they are higher than expected.

Your trial solutions are not concentrated enough.
I have now placed

$\frac{3.09\text{gms}}{20.3\text{ml}} = \frac{X}{1000\text{ml}}$ $X = \frac{152.2\text{gms}}{\text{liter}}$

and actual protein is. .348(152.2) = $\frac{52.97\text{gms}}{\text{liter}}$

~~Some~~ 200ul of this produce the new purple color with a

3ml H_2O
2 drops $CuSO_4$
8 drops NaOH
visible tarter

$200E\text{-}6\left(\frac{52.97}{1\text{ liter}}\right) = \frac{.0106\text{gms}}{2\text{ml}} = \frac{X}{1000\text{ml}}$ $X = \frac{3.53\text{gms}}{\text{l}}$

Now so for 125 ul $125E\text{-}6(52.97) = \frac{6.62E\text{-}3}{3\text{ml}} \Rightarrow 2.21\text{gms/l}$

Ok, I will finally have a visible gradation from control to blue & violet

75 ul $75E\text{-}6(52.97) \Rightarrow \frac{3.97E\text{-}3}{3\text{ml}} \Rightarrow 1.324\text{gms/l}$

40 ul $40E\text{-}6(52.97) \Rightarrow \frac{2.12E\text{-}3}{3\text{ml}} \Rightarrow .706\text{gms/l}$

We will set @ 546 nm again
since we now have purple
non linear regression, 4 standards

Good work. We finally have a monotonically
increasing curve. It is definitely non
linear, but this is just fine.

Now lets test the results.

	Meas Conc gms/l		vs Theoretical
#1	~~.383~~ 1.290	.44	.706
2	2.046	1.71	1.324
3	2.522	2.37	2.21
4	3.231	3.24	3.53
	TC non linear results	Casio Results Linear	Actual

Not bad.
Let's look @ our own regression.

Abs	Conc.
.384	.706
.628	1.324
.754	2.210
.920	3.530

Linear: @ 546 nm
Conc = 5.218Abs − 1.56 r^2 = .92
Note this is not too different from earlier equation w/ very weak samples @ 548 nm.

This regression is quite respectable.

Before we determine our own regression, measure the bilirubin.

My bilirubin comes out @ 1.1~~05~~ g/l.
But this is diluted by a factor of.

$\frac{3E-3\ l}{8E-6\ l}$ = 375 and 375(1.1~~05~~) = ~~619~~ gms/liter.

Seems incredibly high.
So there are many questions here.
Back to our regression
Why does our graph look different than BC360

One lesson is that the Casio will produce a much better regression result because you get an error estimate.

My blood absorbance is @.527 @ 546 nm
Therefore
5.21(.527) - 1.56 = 1.189 gms/l
& accounting for dilution:
375(1.189 gms/l) = 445 gms/liter

Now that still seems way too high but it is whole blood & there is some logic to it.

Today, therefore, I have repeatable results. Many problem areas were uncovered along the way, especially

1. pH of solution was not nearly alkaline enough
2. Tartaric acid was insufficient
3. Conc. levels of proteins was for too low.

You needed 1-4 gms/l of protein to detect properly vs your original plans & hopes for 0-1 gms/l.

You have also learned that the BC300 regressions are very questionable and uncertain since

1. The linear regressions assume a zero intercept which is not the case w/ a colored reagent blank. You should work w/ them further.

2. The form of non-linear regression is not stated therefore you do not know what you are working with.

3. There is no error analysis of the regression solutions; this is a major disadvantage

4. The BC300 does store the calibration results which make it very useful.

2 6 DA

From your own regression & linear analysis from the stored calibration data:

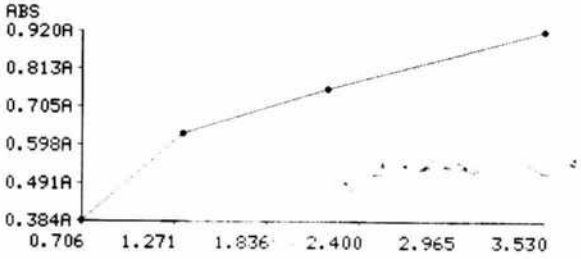
$g\ Conc = 5.21 \cdot ABS - 1.56$

Standard [redacted]e

Print time : 03/17/2017 15:17

Test: [redacted]BIUCLB)

Std.	ABS	C(g/l)
S1	0.384	0.706
S2	0.638	1.324
S3	0.754	2.210
S4	0.920	3.530



Let's see if we can learn to work w/ a colored reagent blank.

Reagent Control Abs = 0.217

Try to subtract reagent blank & now end up with

	Abs	Conc (calc)	Theoretical Conc
#1	.170	.427 g/L	.706
2	.443	1.040	1.324
3	.602	1.413	2.21

So it is subtracting the reagent blank, and there is little but still is weak. as I assume it still assume a zero intercept. The facto is 2.396. Yes it is straight multiplication w/ no intercept involved. Thus is not good. Our own regression from ~~Cano~~ Cano will always be superior, & w/ error analysis available.

BC 300 regression is
Conc = Facto x Abs (no intercept)

Cano regression is
Conc = 3.86 · Abs − .86 $r^2 = .92$
(Intercept & error analysis)

Since we know the linear regression is very weak, let's try the non linear w/ reagent blank.

Non linear regression w/ reagent blank

	Abs	Conc (meas)	Conc Theoretical
1	.204	.341	.706
2			
3	.580	1.347	2.21

So I should still check that the BC 300 regression are such standard.

But

1. Storing the results in very helpful
2. Subtracting a reagent blank is also useful.

and just maybe subtracting a sample blank (ie, distilled water) is also helpful

Prepare another volumetrical set.

(4) 200 ul
(3) 125
(2) 75
(1) 40

Now for the problem w/ both a sample blank and a reagent blank.

No, you cannot have both. Only a sample blank OR a reagent blank.
Not both.

	Conc
1	.706
2	1.324
3	2.210
4	3.530

What if you add water the control as a zero concentration

So	Conc	Std	Meas Abs	Calc Conc	Theoretical Conc	
0	Ø	(1)	-.302	.943	Ø	No good.
1	.706	(2)	.208	.651	.706	
2	1.324	(3)	.221	.690	1.324	
3	2.210	(4)	.413	1.292	2.210	
4	3.530	(5)	.630	1.985	3.530	

Then if you also add a reagent blank it should subtract it out and create the equivalent of an intercept.

Now look at raw regression data.

The zero value did not help anything at. Again the Casio regression superior. Based only upon tubes 1-4, no water control

Abs	Conc	Calc Conc
.706	.53	1.0
1.324	.545	1.12
2.21	.742	2.70
3.53	.84	3.48

Conc = 8.00 · Abs - 3.24 $r^2 = .93$

2nd Trial, 2nd regression w/ Casio

Conc = 4.69 · abs - 2.14 $r^2 = 0.95$

	Meas	Conc (calc)
1	.645	.88
2	.575	.56
3	.832	1.76
4	1.019	2.64

Up these we see the many pitfalls of the Biuret method. The Color also Change w/ time. So there are many variable here to produce Consistent results.

Can you test proteins w/ iodine?

Today I have made acceptable progress and some good work and learning along the way. Buret is best somewhat idiosyncratic and beneficial @ the same time.

Factors influencing success are

1. sensitivity of the test (very insensitive)
2. pH (strong alkaline reqmt)
3. Tartrate must be sufficient
4. Color shift as metal ion intensity is involved, and there is not exactly a linear process @ a single max frequency.
5. Time passage is affecting color development.
6. not reliable or useful for very low concentrations
7. Max absorption (where) selected therefore depends upon concentrations involved.

8. Blood complicates the color reaction tremendously and is apparently not very suitable for protein concentration. Blood (whole) shifts to blue green instead of violet.

9. Reagent blank & sample blanks present interfering possibilities.

10. Question: Can you solve the blood problem will bromocresol (sp?) work?
bromcresol green method

No sources describe a blue-green biuret reaction, blood or otherwise. $Ca(OH)_2$??

Mar 18 2017

Iodine looks to rely upon reacting w/ most organic molecules.

Iodine reacts w/ starch (Carbohydrates)

Iodine is also reacty well with proteins!
If you add
Protein (Milk) + Iodine you get a
cloudy yellow color
but if you add a little acid, the
yellow becomes transparent. If you
then add more Iodine and it turns
a nice red yellow.

This goes a long way.
Now I need to see how sensitive the
test is.

—

Ok, we have determined that Iodine acidified
can be a very sensitive indicator to
protein. There is definitely a shift in
wavelength from red range of acidified Iodine
to yellowish w/ protein added (eg 10 ml
of diluted blood).

It would be good to see what the
shift is w/ Pasco.

It should be going from around 490 to 460 nm.
We probably measure @ 405 then?

Reagent:
3 ml H_2O
2 drops 1M HCl
10 ul Betadine

$Abs_{405} \cong 1.22$
(It readily lower?)

Yellow color shift is detectable w/ dilute whole blood. (20 ul)
$Abs_{405} = 1.967$

Therefore, the shift is highly detectable w/ minute amounts of protein.

So now we calibrate with milk again.
It might also be good here to start learning to use a reagent blank? But not on the first round.

$\frac{0.53 gms}{10 ml}$ milk is the Control solution $= \frac{53 gms}{1000 ml}$
and $\frac{.384}{.348}(53) = \frac{18.44 gms}{liter}$ is the Control milk Solution

Choose Standard in 3 ml H_2O as:

6	200	$200E\text{-}6(18.44) = 3.68E\text{-}3 gms = \frac{3.68E\text{-}3 gms}{3ml} = \frac{x}{1000}$	$x = 1.229 gms/liter$	
5	150	Ok, the regression came out pretty screwy also. It is decreasing or yellow. It is.		.922 g/l
4	100			.615 g/l.
3	50	Conc = −.136 · Abs + .88		.307 g/l
2	20	$r^2 = 0.52$ Very Poor	Lets add a blood.	.123 g/l
1	10			.061 g/l

So what happened here?
Lets add the blood to the measurements.

Blood I got 1.710 abs.

vs 1.967 so they are similar.

So blood defenitely make a difference.
Now lets go back to milk and
increase standard concentration

need 1-6 gms protein.
This means 100 – 1000 ml of the milk solution
ie, 0.1 ml to 1 ml.
add the mixt to 3 ml of solution so changing the
Concentration volume too much.

So just add a given amt of dried milk
to a standard & see how it behaves.

We only need one sample to test the idea.
Control absorbance = 0.183
Notice it is decreasing rapidly.
This could be a time problem

Iodine
Standard
Reagent

t_1 .783 ⇒ therefore a change
t_2 .811 ↘ w/time also.
t_3 .546
t_4 .392

Lots of milk, 20 ml Benedicts
A_{405} = 2.089

So yes, plenty of milk definitely increases Iodine reagent absorbance @ 405 nm.
Thus good.

Iodine level should be higher, eg 20 ul.

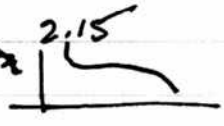
Ah w/ time, the reagent control is already even less yellow when a fear.
But w/ protein it is turning more yellow.

The next thing to learn is of the iodine reagent stabilizes w.r.t. time.

Reagent proposal:
3 ml H_2O
20 ul Betadine
2 drops 1M HCl

Test w/ kinetics
10 min interval.

Kinetic Test of reagent:
Initial Absorbance $_{405}$ = 2.15
It is definitely decreasing.
The decrease is not even linear!



After 10 min $Abs_{405} \cong 1.7$
2nd 10 min test: Abs_{405} Initial = 2.206
Final = 1.705

OK we learn from this
that the reagent is sensitive to 405 light!

Now test for 30 min. Does it become stable?

Ok, there creates an interesting artifact in as well as potential problem.

Now a 30 minute test:

0 min Initial Abs_{405} = 1.971

30 min Final Abs_{405} = ~~0.474~~ 0.2 Very significant reduction

The means that it is not stable initially. You would need to model the decay by the curve and then subtract it out. What is interesting is that w/o protein (ie Control) Abs_{405} is decreasing. However, when you add protein, the Abs increases, apparently to a point of stability. The appear to be the cells when the protein conc is of a sufficient level.

Low protein concentration tests would cause a problem because it might be hard to know who has the dominant influence.

There is a much slower decrease to occur from room light, but it also does occur over time.

A Lesson Here: The iodine might've have developed as sensitive to light, esp 405 nm

Now, you can also see how the reagent is behaving by setting over time. It's turning more orange.
The means the reagent wavelength is drifting towards 485nm, which is fine.
It is happening w/ room light also, it is just happening much faster in the BC30.

This is a very interesting topic.

OK, your answer to this problem is to make up this reagent ahead of time and expose it to the sunlight!

Large batch of reagent:

20ml of H_2O
6.5 ml 1M H_2O

200/3 = 67 Bethesda 67(20E-6 liter) = 1.34 ml

Now we expose them to sunlight.

OK, I can see the reagent is sensitive to 20ul minimum, maybe less which is 100 mg/liter.

$\frac{1E-3}{1000}$ $\frac{100E-3\ gms}{1000\ ml}$ = $\frac{X}{1,000,000}$ X = 100 ppm

The means our test is sensitive @ least down to 100 ppm.

Sensitive Time a light Protector method:

OK, I am developing in w/ the sensitive reagent. I suspect it will be sensitive down to 10 PPM.

Develop standard (still have some timing flaws)

	ml	gm/l (C)	Abs
1	10	.061	.696
2	20	.123	.881
3	50	.307	.905
4	100	.615	.944
5	200	.22[illegible] 1.229	.858
6	500	~~3.0~~ T 2.305	.699

The did not work @ all. We may have our wave spec. to 405 nm.
All of them came out to be the same. The seem to be a problem as soon as it is subjected to 405, ruined a sample either way.

The is somewhat bizarre. It looked a maximum.

This method is too complicated and cannot produce reliable methods. Iodine reaction is too volatile & complex

Go back to start w/ new reagent.
It has been sitting for ~ 30 min.
Kinetics show exactly what is expected.
Abs_{405} initial is now @ 0.340
and indeed it decreases as subjected to
405 nm wavelength. This is all as we
expect.

The question is when ya added protein why did
the Abs_{405} not increase dramatically as it
did w/ previous tests?

Sun reagent:
Abs_{405} Initial = 0.340
600 sec = 0.135

Now a reagent tube w/ 50 ul milk added
and kinetics applied.

NO significant difference in Abs, except
that it stabilizes.
So the did not work. The reagent is getting
brighter (ie more transparent) as time
passes and there is no reaction taking place.
Therefore to produce the reaction I believe
you need to create the reagent in a darkened
bottle

Now we are learning that light is destroying the iodine reagent, not helping it. This means that time sensitivity is an issue. Exposure to the instrument is also an issue.

So to create a standard you need to
1. minimize the time of exposure
2. Consider the use of either a reagent blank or a sample blank
3. Take the initial absorbance readings.

It was all a good try. Iodine is definitely responsive & sensitive to protein, even small amounts on order of PPM. The problem is that the acidified iodine reagent is also very sensitive to light and variable in reactions w.r.t. Concentration levels.

It will be good @ detection but not @ determining concentration. Biuret method remains superior but it is only moderate in its general capability & sensitivity.

Where to now?

1. Reproducibility of Barrett method is helpful
2. The question of the blood reaction w/ Barrett is a very interesting problem. Then is what motivated you to try iodine as an alternative.
3. What is the info on light sensitivity of iodine? Can it be stabilized?
4. Headed toward nitrate concentration determination
5. The oxidase test, can it be developed?

Mar 19 2017

Investigated elemental iodine crystals today. Have general result.

Conclusion: acidified iodine solution does produce a distinctive yellow color when exposed to protein, but it is not (ie, the color) sensitive to concentration in any linear or reliable manner. It is, as we have seen, also sensitive to light and under a continual state of change.

1. Repeating Biuret would be helpful

2. The blood color change to blue green in Biuret is intriguing & then fades unexplained. It suggests that a copper hydroxide solution is likely formed instead of reacting to protein. Why does this?

3. Starting to work with nitrate and nitrites will be useful. Somehow you will need to oxidize nitrate to nitrate and also see if you can reduce from nitrate to nitrites. Voltammetry test?

4. What is the basis of the oxidate test? Can it be replicated in some alternative form?

Nitrates & Nitrites by voltammetry should be doable!
Nitrite NO_2^-
Nitrate NO_3^-

Nitrite can be reduced & oxidized
Nitrite is known to bond to metal centers in @ least 5 ways

* Oxidation of the nitrite ion w/ permanganate ion can be used for the quantitative analysis of Nitrite by titration.

$$5NO_2^- + 2MnO_4^- + 6H^+ \xrightarrow{\text{warm, dilute } H_2SO_4} 5NO_3^- + 2Mn^{+2} + 3H_2O$$

Sodium nitrite is a reducing agent.
It is highly toxic @ higher levels.

Nitrite is detected by the Griess reaction a red dye compound, so there is a colorimetric method.

UV can be used @ 220 & 275 nm to estimate nitrate concentrations. This certainly seems the easiest.

Bleach and peroxide can indeed be used to oxidize nitrites to nitrates

$NO_2^{[-]} + H_2O_2 \rightarrow NO_3^{[-]} + H_2O$ (should have charges)

~

$NO_2^{[-]} + NaOCl \rightarrow NO_3^{[-]} + NaCl$

Theoretically it takes 5.3 lb of bleach or 2.3 lb of H_2O_2 to "treat" one lb of nitrites

1 mg/L of nitrite is converted to 1 mg/L of nitrate

Nitrite in blood oxidizes hemoglobin to methemoglobin
(So what you really would like to do is to test blood for methemoglobin)

Urine should not contain oxidizing agents such as bleach or hydrogen peroxide.

Drug tests will be helpful
"Klear"
"Whizzies"

The API test for nitrate is working perfectly. I must only presume that the test works by reducing the nitrate to nitrites. Which produces the Color Change.

Now when you add bleach (20 ml.) it turns the test solution back to the reference yellow. This means that it has oxidized the nitrate back to nitrate. I suspect there are such measurable reactions.

Next, the idea of oxidizing the urine to change nitrite to nitrate & then tests for nitrate did not seem to work. No evidence of nitrate in urine by API modified test. Now use the BC 400.

Ok, a fantastic result. The urine tests has come out, for the first time as negative w/ controls

Results:

URO	Norm	PH	6
BLD	—	NIT	—
BIL	—	LEU	—
KET	—	SG	1.03
GLU	—	BVC	3+
PRO	—		

OK, this is a huge deal.
What have I done?

One major change is salts. I have been taking electrolytes,

The list now includes: Antioxidants, of course + pressure

COQ10
Papaya
Fish Oil
Glucosamine - MSM
General Vitamins
Fish Oil
Calcium D Glucarate
Bone Strength - Phosphorus
Increase Calcium Citrate
Hair Skin + Nails
Vit B (Methyl)
Increase Magnesium
B Complex
New Cranberry
Gelatin
Enzymes (General)
Probiotics
Vit C
New Kosher Salt (Redmond Real Salt - Vitamin Cottage)

The negative test on nitrites is incredibly important. Now you will need to see if it can be maintained. This is your first normal urine test across the board.

The changes known are

1. Kosher, trace mineral salt intake
   This seemed to be beneficial in several aspects including reducing norms seen in the leg and foot, increased neural-synapse activity, & potentially less fatigue, and elimination of nausea, dizziness which was becoming more dangerous during driving.
2. Addition of cranberry
3. Increase in Calcium citrate
4. Increase in magnesium

This is a monumental change, lets see if we can hold it now.

Mar 21 2017

Metabolic enterference detection
vs Drug Test (12) tests.
Relion Home Drug Test (12)

My test results are negative on all accounts
Thus is highly favorable as it indicates
no metabolic interference is in place
here. Between urine test strip results
and the drug lab test all signs are
favorable toward normal urine metabolism.

The tests conducted today are:

		Result
1	Amphetamine	Negative
2	Barbiturates	Negative
3	Benzodiazepenes	Negative
4	Cocaine	Negative
5	Methamphetamines	Negative
6	Morphine (Opiates)	Negative
7	Methadone	Negative
8	Oxycodone	Negative
9	Phencyclidine	Negative
10	Tricytic Antidepressants	Negative
11	Marijuana	Negative
12	MDMA or Ecstasy	Negative

This is a fascinating, simple, efficient
insightful sophisticated test procedure.

1 Illegal Drugs (?) Marijuana not always illegal
5 Prescription Drugs

No drug illegal if prescribed —

Page 283


ReliOn
Home Drug Test
12
Drugs Tested
7 Illicit Drugs
Marijuana, Cocaine, Morphine (Opiates), Methamphetamine, Ecstasy, Amphetamine, Phencyclidine (PCP)
5 Prescription Drugs
Tricyclic Antidepressants, Barbiturates, Benzodiazepines, Methadone, Oxycodone
Results in minutes
Up to 99.8% accurate
FDA cleared
HOSPITAL QUALITY
Made in the USA
EN ESPAÑ

Page 283A

ReliOn

URINE SCREENING TEST

- Amphetamine
- Barbiturates
- Benzodiazepines
- Cocaine
- Methamphetamine
- Morphine (Opiates)
- Methadone
- Oxycodone
- Phencyclidine
- Tricyclic Antidepressants
- Marijuana
- MDMA or Ecstasy

INTENDED USE

The ReliOn® Home Drug Urine Cup Test is a rapid qualitative immunoassay. The device provides preliminary results for the detection of potential abuse of one or more drugs. See list below. This is not a screening device to monitor prescription medication. It is for Home use, not for Internal Use.

Abbreviation	Substance	Cut-off ng/ml
AMP	Amphetamine	1000
BAR	Barbiturates	200
BZD	Benzodiazepines	300
COC	Cocaine	300
MET	Methamphetamine	1000
MOR/OPI	Morphine (Opiates)	2000
MTD	Methadone	300
OXY	Oxycodone	100
PCP	Phencyclidine	25
TCA	Tricyclic Antidepressants	1000
THC	Marijuana	50
XTC	MDMA or Ecstasy	500

The device provides preliminary test results. Preliminary positive results are recommended to be confirmed by a more specific analytical method.

KIT CONTENTS

- 1 ReliOn® Home Drug Test Urine Cup, packed in a sealed pouch.
- 1 Instruction Sheet.
- 1 Confidential Confirmation Identification Label.
- 1 Plastic Sealable Bag.

⚠ IMPORTANT!

Do not open the pouch until ready to perform the test.

PRECAUTIONS

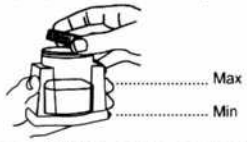
- Do not use expired cup.
- Do not reuse the cup.
- Do not use test if color-blind.
- Not for internal use.

STORAGE

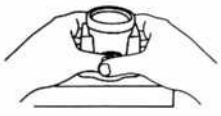
- Store the test cup in the sealed pouch until use.
- Store the test cup above 30oC may shorten its shelf life.
- Store the kit at room temperature 15-30oC (59-86oF).
- Do not freeze the test kit.
- Do not expose the test kit to temperatures over 30°C (86°F).

URINE SAMPLE COLLECTION AND TESTING

1. Remove the test cup from the pouch. Remove cap. Urinate directly into the cup. The urine level should be between Min and Max marks on the cup. **Do not over fill. Replace cap tightly to prevent leakage.**



2. Place cup on a flat surface. Make sure the cap is closed tightly on the cup. Push the activation knob all the way into the cup body.Use samples within 8 hours of urine collection.



3. Read the results between 4-7 minutes (Do not read results before 4 minutes or after 7 minutes.)

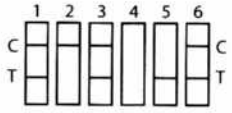
READING THE RESULTS

Each test strip is labeled with device code. For example, "THC" is for a Marijuana test. A complete list for each test can be found in the Questions & Answers section.

⚠ IMPORTANT!

Read each test independently. Do not compare color intensity of one test to another. Do not compare color intensity of the T line to the C line.

1. Check that the test is working properly. The area between the two letters C on each side of the window is called the Control Region. A color line in the control region is called a C line. The area between the two letters T is called the Test Region. A color line in the test region is called a T line. For the test to work properly, the C line must appear within 4 minutes. If the C line does not appear within 4 minutes on a test strip, the test is not working correctly. The result is invalid. In this case, repeat the test with a new cup. If the test is still invalid after using the second cup, contact the manufacturer. In the example below, because there is no color line in either control region or test region on test strip 4, test 4 is invalid. On test strip 5, a line appears in the test region only and there is no C line, therefore test 5 is invalid.

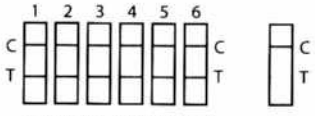


Invalid for test 4 and test 5

2. Read test only if the C line appears within 4 minutes.

Negative Results:

If both C line and T line appear on a test strip, the urine sample is negative for that drug. If both C line and T line appear for all the tests, the urine sample is negative for all the drugs tested. In this case, no further test is required.

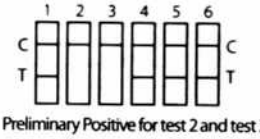


Negative for all drugs tested

Note: Even a very faint T line is a negative.

Preliminary Positive Results:

If a C line appears and there is no T line on a test strip, the urine sample may contain that particular drug. In this case, we recommend to send the sample to the lab for confirmation. Additional tests by a more accurate laboratory technique such as Gas Chromatography/Mass Spectroscopy (GC/MS) or High Performance Liquid Chromatography/Mass Spectroscopy (HPLC/MS) will be performed. In some cases, more than one test may be Preliminary Positive. In the example below, because there is only C line but no T line on test strip 2 and 3. the test result is preliminary positive for both drug 2 and drug 3.



Preliminary Positive for test 2 and test 3

A preliminary positive result does not always mean a person took illegal drugs. A negative test result does not always mean a person did not take illegal drugs. There are a number of factors that influence the reliability of the drug tests.

Please call us at toll free 855-776-0662 Monday-Friday 7am-7pm CST. Our staff is available to assist you.

SENDING THE SAMPLE FOR CONFIRMATION

We recommend sending all Preliminary Positive Result samples to our laboratory for free confirmatory testing. Please mail the sample to our laboratory as soon as possible for accurate analysis. If the urine sample is sent within 1 day, it can be kept at room temperature. If it is more than one day, the urine sample should be refrigerated and will be good up to 7 days. The urine sample can not be tested if the sample is more than 7 days old.

1. Please Check that the cup is tightly capped.

2. **VERY IMPORTANT**: Attach the portion of the confirmation security label (the part with the barcode) to the cup and place a check mark for the drug that was preliminary positive on your test. It is important that you indicate which drug or drugs were preliminary positive so that laboratory confirmation can be performed on the specific drug or drugs.

3. Please attach the other portion of the confirmation security label to the designated area below. You will need this information to receive your results. Please store this information in a safe place.

PLACE HERE ONE PORTION OF THE CONFIRMATION IDENTIFICATION LABEL

4. After attaching the confirmation label on the cup please place cup into plastic transportation bag. Seal the bag. Place the sealed plastic bag into a shipping box.

Mail to:
Confirm Biosciences
6370 Nancy Ridge Road, #104
San Diego, CA 92121

5. We recommend using next day delivery for timely delivery of results.

6. Test results will be kept on file for thirty (30) days. You must call within that thirty (30) day period to receive your test results. Remember to have your identification number handy when you call. Results will not be disclosed without an ID number.

RECEIVING RESULTS

Results will be ready in 5-6 business days after the laboratory receives the sample. Call **1-855-776-0662** to receive your results. You will be asked your Confirmation Identification Number that is on the security label so please have that information available when you call.

QUESTIONS AND ANSWERS

1. What does the ReliOn® Home Urine Drug Test Cup do?

The ReliOn® Home Drug Test cup test is a drug screen test. It provides preliminary results for the detection of one or more of the drugs at the cut-off level.

Abbreviation	Substance	Cut-off ng/ml
AMP	Amphetamine	1000
BAR	Barbiturates	200
BZD	Benzodiazepines	300
COC	Cocaine	300
MET	Methamphetamine	1000
MOR/OPI	Morphine (Opiates)	2000
MTD	Methadone	300
OXY	Oxycodone	100
PCP	Phencyclidine	25
TCA	Tricyclic Antidepressants	1000
THC	Marijuana	50
XTC	MDMA or Ecstasy	500

2. What is cut-off level?

The cut-off level is the concentration of the drug in urine above which a result is considered a preliminary positive, and below which it is considered negative.

3. If the test results are negative, can you be sure that the person did not take drugs?

No. There are several factors that can make the test results negative even though the person is using drugs.

- You may have tested for the wrong drugs.
- You may not have tested the urine when it contained drugs. It takes time for drugs to appear in the urine after a person takes them, and they do not stay in the urine indefinitely; you may have gotten the urine too soon or too late.
- The person knowingly added something to the urine to prevent it from reacting with the test chemicals.
- The chemicals in the test went bad because they were stored incorrectly or they passed their expiration date.
- If you get a negative result, but still suspect drug abuse, you can test again at a later time. You should also consider testing other types of drugs. Talk to your doctor if you need more help deciding what steps to take next.

4. What is false positive result?

A false positive result is a screening test that reads positive when the drug or drug metabolite is not present or its concentration is less than the detectable cutoff level.

5. When is the best time to take the test?

The best time is to use the first urine in the morning, as it would be the most concentrated one. However the ReliOn Home Drug Test can be used any time of the day.

6 Does a faint line indicate a negative result?

Yes, faint lines do indicate negative results. The presence of a test line, regardless of how light, indicates a negative result.

7. How accurate is the test?

The test is fairly sensitive to the presence of drugs in the urine. This means that if drugs are present, you will usually get a preliminary positive result. If you get a preliminary positive result, you should send the urine sample to the laboratory for a second, more accurate test. It is very important to send the urine sample to the lab, because the drug of abuse urine screening may give positive results when no drugs are actually present. Certain foods, food supplements, beverages, diet pills, or over-the-counter medicines can cause a reaction with the tests. Laboratories use a very reliable test, with very few errors, to determine whether or not your sample contains drugs. Many things can affect the accuracy of this test, including but not limited to:

- The way you did the test
- The way you stored the test or urine
- What the person ate or drank before taking the test
- Any prescription or over-the-counter drugs the person may have taken before the test

ADDITIONAL RESOURCES

National Institute on Drug Abuse
Phone: 301-443-1124
www.drugabuse.gov

Center for Substance Abuse Prevention
Substance Abuse and Mental Health Services Administration
Phone: 301-443-9110
www.prevention.samhsa.gov

Centers for Disease Control and Prevention
Phone: 404-639-3534 Phone: 800-311-3435 (toll-free)
www.cdc.gov

Safe and Drug-Free Schools Program U.S. Department of Education
Phone: 800-872-5327 (toll-free)
www.ed.gov/offices/OESE/SDFS

National Clearinghouse for Alcohol and Drug Information
Phone: 800-729-6686 (toll-free)
www.ncadi.samhsa.gov

National Council on Alcoholism and Drug Dependence
Phone: 800-622-2255 (toll-free)
www.ncadd.org

American Council for Drug Education
Phone: 301-443-3860
www.acde.org

Please call us at toll free 855-776-0662 Monday-Friday 7am-7pm CST. Our staff is available to assist you.

Lets figure out + Make a stock buret solution:

3 ml H_2O 200 ml H_2O

6 drops NaOH = 0.3 ml

visible trace tartaric

2 drops $CuSO_4$ (Lt. 0.5M)

$\frac{0.3\text{ ml NaOH-KOH Hair Unclogger}}{3\text{ ml}} = \frac{x}{200\text{ ml}}$ $x = 20\text{ ml}$ KOH-NaOH

66.7 (visible trace tartar) =

66.7 (0.10 ml) = 7 ml

So the Layer volume of reagents: Buret:

1. 200 ml H_2O
2. 20 ml KOH-NaOH (Hair Clog Remover)
   The is a Concentrated solution
3. 7 ml 0.5M $CuSO_4$
4. 0.75 gms Cream of Tartar

Mix thoroughly filter & seal in a dark bottle.

Let's repeat Biuret now w/ stock solution and previously use standard controls.
The preferred ~~violet~~ purple wavelength is 530 nm

We have 540 available.

Add 0.5gm to 1 ml H_2O for milk.

I would prefer to work with a small spatula but I do not have one.

3.96
3.05 ml H_2O
0.91gm milk

$\frac{0.91 gms}{305 ml H_2O} = \frac{X}{1000 ml H_2O}$ X 298.4

$X = \frac{298.4 gms}{1000 ml}$

Actual Conc	Extract	
3.46 ~~3.82~~	100 µl = 100E-6 (298.4) = .0298 gms/3ml = $\frac{X}{1000}$ = 9.95 gms/l	
7.64	200 µl	= 19.89
10.38	300 µl	29.84
13.85	400 µl	39.79

	g/l Conc	Abs
3.46	9.95	.562
7.64	19.89	.723
10.38	29.84	.801
13.85	39.79	.993

Ok, superb results here

Conc = ~~.0138~~ · Abs + ~~0.424~~ 70.91 − 29.71

$r^2 = .977$

y x

Conc = ~~8~~ 24.34 Abs − 9.90 $r^2 = .994$

Therefore, for the first time, you have a calibrated protein standard. w/ a good regression curve.

Given that dried milk is .384 actual protein
Our actual regression curve is

$$\text{Conc} \cong \frac{.348}{.384}(\frac{10.91}{.0138}\cdot \text{Abs} - \frac{29.71}{10.34}) \quad \text{or} \quad \frac{24.68}{24.23}\cdot \text{Abs} - \frac{11.41}{11.41}$$

$$\text{Conc} \cong 5.30E\text{-}3 \cdot \text{Abs} + .464 \quad \text{w/ } r^2 = .977$$

Always use Cavis regression curves.

Test insit:

$$\text{Abs} = 0.518 \qquad \text{Conc} = \frac{24.68}{24.23}(.518) - \frac{10.34}{11.41} = \frac{2.44}{2.70}\ \text{g/l}$$

vs $.384(9.95) = \frac{3.46}{3.82}$ theoretical

We now have a calibrated protein standard that is effective between 3 - 15 gms / liter.

There is on the order of $\frac{3\ 10}{1000\text{ml}} = 10{,}000\text{ PPM} = 1{,}000{,}000\text{ml}$

So it is hardly a sensitive test but it is useful and reliable.

Now for serum, can we try blood again?

10 ul of blood in 3 ml

Came out as Abs = 0.356

20 ul Came out as Abs = 0.619
The r is in range

So
Conc = 24.68(.619) − 10.34 = 4.94 gms/l

but we have a dilution factor of
3 ml / 20E-3 ml = 150

So once again the leach to a protein concentration of whole blood estimated to be

150(4.94) gms = 741 gms/liter
The # of concentration may high when we know that it should be about 70.

One final regression is

Conc = 24.34 · Abs − 9.90 r^2 = 0.984 superb

And we measured, w/20 ul of whole blood in 3ml H_2O reagent. Abs = 0.619

Conc = 24.34 (.619) - 9.90 = 5.17 gms/dl

But there is highly dilated but you still get strong absorbance and an unexplained shift in color to green. This indicates some other reaction has taken place, but we do not know what it is.

Our dilution factor is indeed

$$\frac{3ml}{20E\text{-}6l} = \frac{3E\text{-}3l}{20E\text{-}6l} = 150.$$

And $150 (5.17 \text{ gms/dl}) = 775 \frac{gms}{l}$

The would say that whole blood is 3/4 protein. Is such a thing possible?

Mar 22 2017

UV-Vis spec tests on Biuret reagent - Pasco

Max absorbance of Prepared Biuret Reagent

1. 200 ml H_2O
2. 20 ml Conc. KOH - NaOH (Hair Unclogger)
3. 7 ml 0.5 M $CuSO_4$
4. 0.15 gm Cream of Tartar

is @ 653 nm.
The matches blue green observed by Vikki Chart and red absorbed. (It's the blue tone)

Now add milk.
There is a fair amount that is sorry on like w/ the addition of milk.

The solution turn purple.
However, three peaks are created

λ	Abs	Obs	Abs
415 nm	0.58	Yellow Green	Violet
445	0.57	Yellow	Blue
574	0.43	Violet	Yellow-Green

It is not very interesting that the max absorbance is in the yellow & yellow green observed color area. And that violet is actually closer to 574 nm vs the assumed 546.

The full spectrum certainly reveals a lot of information, a lot more than the colorimeter @ specific wavelengths does. It actually says that the spectrometer is a very useful tool. Given that the BC is not completely regression especially well or they negate some of the presumed value of bc300 usage. But both well have their place. Pasco also uses less power w/ the netbook PC.

Now, the big mystery is, what is happening w/ blood added to Biuret?

This is very interesting. You still have the major peak of absorbance @ 513 nm so no real change there. But now you have a major increase of absorbance @ 400 nm. This is indeed yellow & yellow green, as Carl was suggesting.

So with blood what we have is:

	Visible:	Absorbed
~400	Yellow Green	Violet
~420	Yellow Green	Violet Blue
574	Violet	Yellow Green

It is also extremely interesting that these two colors (yellow green & violet blue) are the complements of each other. So why is this and what does this mean?

The spectrum has revealed to you things that you simply never could determine w/ the Colorimeter.

The Colorimeter is used when the problem has been fixed. It is not very useful for investigative matters.

This means that our blood protein concentration should, in theory, still be valid. But why is the number so astronomically high? And why does blood w/ Biuret absorb so highly in yellow green area?

What you are seeing is that blood does absorb highly in the yellow areas (~420) in its raw state. So this is where the yellow coloration is coming from.

Red blood cells are not a part of plasma. Red blood cells do contain protein, i.e. hemoglobin.

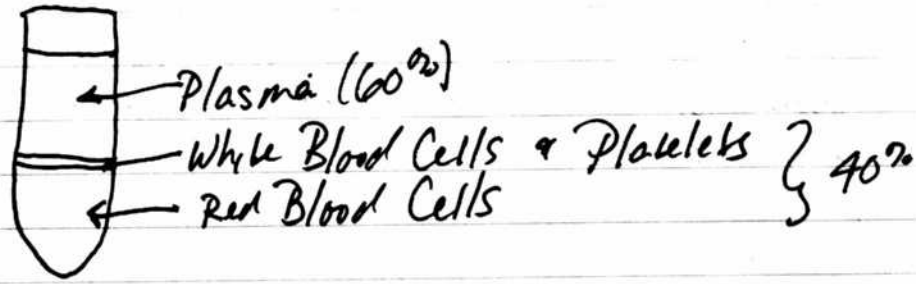
Hemoglobin is a metallo protein.

Whole blood protein averages 16.4% w/ a range from 12 to 19%.

(This is presumably dry weight)

Source: The Protein Content of Whole Blood & Plasma in Cancer, Ruth C Theis 1921.

The protein content of serum



Human blood serum (plasma) contains about 7% protein.

Whole blood contains on avg about 16.4% protein.

The mean of blood serum averages ~ 60-80 gms/liter of protein that whole blood should average about

$$\frac{16.4}{7} = 2.34\,(70\text{ gms/l}) = 164\text{ gms/l}$$

or a range of: 140 – 190 gms/l.

So our big question is how and why do we end up with a number like 775 gm/l. this is not possible.
We are off by a factor of ~ $\frac{775}{165} = 4.7$
or essentially ~ 5 times too high.
Why and how or this?

How can I have an error of ~~these~~ magnitude?

This would mean that our sample injected into 3 ml Biuret would need to be 100 ul instead of 20 ul

100 ul leads to a dilution factor of

$$\frac{3\,ml}{100E\text{-}6\,3\,ml} = 30 \quad * 30(4.94\,gms/l) = 1489\,\frac{gms}{l}$$

which would be right in range of what you expect. But we did not measure 100 ul, we measured 20 ul.

Since our standards are shown to only have values from approx ~~**~~ 3-15 gms / liter the most plausible explanation of our problem is that the test is not sensitive enough @ low concentrations to accurately measure.

We say that Biuret is only a mediocre test and it probably why Bromcresol may be better.

Read for a highly sensitive test it did not seem to follow Beer Law. It would be so much better if it did.

The other approach is to increase the concentration of blood or the order of 100 ul instead of 20 ul.

We already have our standards recorded so you do not need to redo everything. and you should be able to use Pasco even for the moment. Let's try it.

We have tried
1. Bradford
2. Folin
3. Biuret.

The Jo Biuret is the most stable test but it is not highly sensitive

Using Pasco:

Trying 30ul @ 546nm Abs = 1.15
10ul @ 546nm Abs = 0.62

But notice that ~~20ul on~~ 10ul on bc 300 came in at 0.36
and 20 ul came in at 0.62
so we are twice as high w/ Pasco?

I think you need to run the calibration on Pasco.
Assume values are low on bc by a factor of 2.
30ul @ 1.15 ⇒ 30 ul @ ⇒ Abs. 0.75
Regression Result: 3.85 gms/l $\frac{3 \text{ml}}{30E\text{-}3 \text{ml}} = 100$
and 100 (3.85) = 385 gms/l
where a more likely, but still off by a factor of $\frac{385}{190}$ = 2.6 times too high, but certainly better.

Let's run the test and calibration by Pasco: We have exactly the same Concentration a standards.

Y	X
Conc g/l	Abs
~~3.46~~	
7.64	.809
10.38	.852
13.65	.962

Pasco does solve for a good regression and does not assume a 0 intercept. Buret, again, however, gives less than desirable results. The 3.46 g/l solution has faded out and has changed color & the absorbance value has drifted completely out of range (much too high, no less) so this corroborates the problem we had before of highly variable absorbance.

We have a very good solution w/ the remaining standards 7.64, 10.38 & 13.65 g/l, however the slope is very flat meaning very poor dilutions or titrated concentrations

These tests w/ Buret also seem to be a problem.

With Pasco regression, we have $\lambda = 550$ nm

Abs = .0249 · Conc + .610 $r = .904$
but this is inverse so Casio is once again better

		Conc.
10 ul Blood	Abs = .829	8.86 gms/l
30 ul Blood	Abs = 1.245	25.01 gms/l

Casio Conc = 38.80(Abs) − 23.30 $r^2 = 0.91$

~~Therefore~~ Now, there is fascinating. Pasco comes up with a very reasonable correspondence:
$\left(\frac{30\ ul}{10\ ul}\right)(8.86) = 26.58$ vs 25.01 by regression

Then a very reasonable. However, your dilution factor of $\frac{3\ ml}{30E-3} = 100$ cannot be used again!
So obviously you cannot assume the linear regression holds for high concentrations.

Pasco is also picky worse with a non reversible regression equation and gave a graphical treatment of error solution of the concentration level.

The dilution factor cannot be applied in the manner that you are.

Protein Reagent Developed w/ Rit red dye

Exploring a color protein reaction
w/ Rite Red Dye

We may have one.

Control = 20 ml of 1/2 + 1/2 Rite Red dye in 3 ml H_2O

1. alkalize w/ NaOH (6 drops 1M?)
2. add 1 drop $CuSO_4$ (0.5M
3. add protein in variable amount

We appear to have a color reaction.
Purple - high protein concentration
Orange - low concentration

We have definitely produced a reaction and it is much more sensitive than the Biuret test.

Recipe:
3 ml H_2O
6 drops 1M NaOH
1 drop $CuSO_4$
Visible Tartrate added.

add protein. It is sensitive to 20 ml of dilute milk. It shifts to purple.

Protein Reagent & Test Developed

Now
50 ml H_2O
200 ~~100~~ ml dilute dye
1 drop conc. NaOH - KOH (this turns it purple)
4 drops $CuSO_4$ - this turns it orange (better)
but with precipitate
add tartar to remove precipitate
(nice cherry red)

Now add protein - shifts to purple, very sensitive

& new protein reagent!!!

3 ml H_2O
20 ml dilute 1/2 & 1/2 red dye
6 drops 1M NaOH
1 drop 0.5M $CuSO_4$
trace visible tartar

200 ml H_2O
20 ml 1/2 & 1/2
20 ml 1M = 2 ml 10M NaOH
3.5 ml $CuSO_4$ 1M
.15 gm Tartar

Final color is orange, not red, not purple.

This works! Can detect down to 40 ul protein.
This is 1.38 gms/liter of protein.
Appear to be a order of magnitude (~10) more sensitive than Burrell.
I suspect, therefore that I can detect to 1 gm/ml or ~ 1/1000.

This looks just right. You want the solution to be orange. If you add too much $CuSO_4$ it will below it. You have it very close

You might be able to dilute this by 50%.

BC300 closest is 510 nm

You can dilute this reagent 5q 100%
and still get reliable results,
sensitive to 20 ul = 0.75 gm/liter
That's great.

Maximum Absorbance @ ~~540~~ 514 nm. 488 nm.

Therefore the recipe is:

400 ml H_2O
2 ml 1/2 & 1/2 Ritz Cherry Red Dye
2 ml 10M NaOH
3.5 ml ~~Acetic~~ 0.5M $CuSO_4$
0.75 gms Cream of Tartar

Easily detectable to 1 gm protein / liter

Wavelength of max absorption =

With Protein the reagent also has one peak @ 517 but it has another @ 534 nm.

The point of reactivity appear to be @ ~~534~~ 532 nm.
This would be the preferred point of assay concentration.
(In the BC300, 546 nm is the closest but Purdy can be used @ 546)

If you look @ the spectra comparison the increase occurs definitely in the 530 region, which is in the middle of purple. In the reagent orange + purple are fairly evenly distributed. With protein the spectra definitely increases in the purple region.

March 23 2017

Modified Reagent Test.

1. Remember to dilute reagent by 100%!

2. We will use Concentration of ~~20, 50, 100ul~~
20, 40, 75, 100ul Of protein

Absorbance re to be tested @ 534nm

3. Control Absorbance = .584

Conc gm/l	Conc(ul)		Abs.	Abs-Control
.77	1	20	.658	.074
1.55	2	40	.736	.152
2.90	3	75	.786	.202
3.87	4	100	.963	.379

(ul) Conc = 262.8 · Abs − 5.72 r^2 = 0.91

This is a very repeatable solution.

Milk Conc is $\frac{1gm}{3ml} = \frac{x}{1000}$ x = 333.3gms/l
(.348) = 116gms/l

20ul = 20ul (116gms/l) = $\frac{2.32E-3gm}{3ml} = \frac{x}{1000}$ x = .773 $\frac{gms}{l}$

40ul = 1.55

75ul = 2.90

100ul = 3.87

Conc (gms/l) = 10.18 · Abs + 0.22 r^2 = .91

Random Test:

75 ul green Abs = 0.819

so Conc (gms/dl) = ~~10.18(.819) + .22~~
= 10.18(.819 − .584) + 0.22
= 2.61 gms/l

vs theoretical 2.90 gms/l
This is excellent. We have a reliable method.

Now try blood again for check.

50 ul blood in 3 ml reagent gave Abs = 2.314

Conc ≅ 10.18(2.314 − .584) + 0.22 = 17.83 gms/l

But the dilution factor is 3 ml / 50E-3 ml = 60

60(17.83) = 1070 gms/liter.

You have the same problem in terms of the dilution ratio. You cannot do this.

Somehow you would need calibrated blood.

The real peak absorbance @ 532 nm

Mar 24 2017

Today we work on the question of the background absorbance level for blood. The suspicion is that blood alone absorbs @ the frequency of interest so that this effect will need to be subtracted out.

We have seen the value of, but no requirement for, subtracting out the reagent reference on a blank. It will make the results easier to interpret. The same will be w/ blood.

Power is not adequate to:
1. Charge & run both laptops simultaneously
2. Run the BC concurrently.

Wait for both laptops to fully charge & then try again.

OK, Blood does indeed have significant absorbance @ 531 nm (as well as 563) so you have likely identified the source of a problem.

Questions: are absorbance additive? You can easily test this.

Page 304

Let's start addition of blood absorbance & reagent.

Max absorbance of the reagent is @ 488nm
Max absorbance of blood is @ (50ul in 3ml)

1.95 @ 534 nm
3.00 @ 567 nm

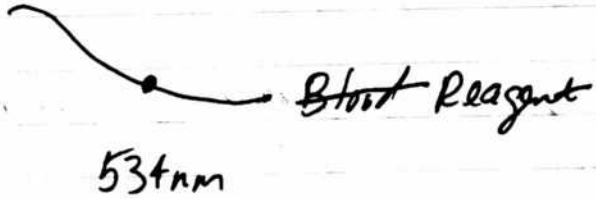
And now we see the nature of our problem.

And lastly, the absorbance of blood in the reagent is:

But, before we do this, the absorbance of the control reagent is:

0.83 @ 488 nm
0.62 @ 534 nm.

Therefore 1.95 A



It has peaked out, it is too strong for blood 50 ul & reagent to settle. No wonder we have a problem.

Dilute by 100 ml:
the least an absorbance of blood in reagent as 1.45 @ 534

So now we take into account the dilution ratio and explore the additive hypothesis

Reagent is 0.826 @ 534
Blood is 1.95 in H_2O (50 ul)
Blood is 1.45 (25 ul) in reagent.

Blood in water only shows absorbance of blood in general in water.

Now the blood in water @ 50 ul in 3 ml is 1.95
If it was 25 ul we anticipate Abs to be = .975

The reagent has a fixed reference absorbance of ~~0.826~~ 0.620

The means the expected contribution of the blood in the reagent is .975
$-.826$
$= 0.149$

Ours blood in reagent (25ul in 3ml measure @ 1.45)

We extrapolate that we subtract
0.826 effect of reagent
0.149 effect of blood alone

$= 1.45 - (.826 + .149) = 0.475$
is the expected contribution of the protein reaction w/25 ul

Conc = 10.18(.475) + 0.22 = 5.05 gms/dl
But the dilution ratio is
3ml / 25E-3 ml = 120 & 120(5.05) = 606 gms/l

Still way too high but better.
Let's keep dreaming then.
Your blood concentration was way too high in the reagent.

~~1.45 - .475 = .445~~
Try again w/ 25 ul blood.

Reagent @ 534 : .68 $\bar{X}$ = .66
.64

Blood (25 ul) @ 534 .908 in H_2O

(50 ul) @ 534 1.54 in H_2O

Blood in reagent (25ul) 1.433

.66 Look @ Δ: 1.57
+ .91 − 1.43
= 1.57 = .14

10.18(.14) + .22 = 1.64 gms/l
determined

But Dilution ratio is
120
120(1.64) = 197 gms/liter

vs an expected range of 140 - 190 gms/l

Then are great. I appear to finally be in range.

this indicates a protein range lower in blood which is on the lighter end of the range but within range.

The work repeatedly suggests that the absorbance of blood in reagent actually decreases relative to the absorbance of blood in water.

Reason: If we add the absorbance of the reagent plus that of blood in water, we get slightly more than that of the blood in the reagent alone. This has happened twice now. We can repeat this.

We also see, however that the absorbance of blood in water is not directly proportional to the concentration linearly.

25 ul Abs = .908
50 ul Abs = 1.54 (vs. 1.82)

This was a really well run test. It may be the best you can do now for us without a calibrated milk solution.

So the idea here is

~~$\Delta =$~~

$$\Delta = \left| \left[\underset{\text{Combined}}{Abs(\text{reagent} + \text{blood})} \right] - \left[\underset{\text{Uncombined}}{Abs(\text{Reagent}) + Abs(\text{Blood})} \right] \right|$$

and that we use the absolute value)

Now, there is an even more interesting question as to whether the intercept of the regression equation should be 0 or excluded since you are actually measuring a rate of change.

Notice: $10.18(0.14) = 1.42$

(no 0.22 term added)

and $1.42 \frac{gms}{l} (120) = 171 \frac{gms}{l}$

which is right in range.

Let's see if these results can be repeated by you, first by the Δ term development.

OK, here is an idea.

The color of blood interfere w/ the interpretation of absorbance @ 534 nm and makes use of the reagent much more difficult and complex.

So the idea is remove the color (or alter) the color of blood so that it does not interfere very, for example, bleach.

And so we ran the idea:
Bleached blood has an entirely different spectrum and the interfering absorption is removed. But the question is does it alter or destroy the protein interaction?

And so I have tested the idea by adding bleach to milk. It did not alter the shape of the spectrum after reaction w/ the reagent. Bleached milk produced the same spectrum w/ the reagent, it is only slightly more pale. So the idea definately has promise.

The procedure is now to bleach the blood samples.

.46 Bleached (50 ul) reagent
.57 Bleached (50 ul) blood
1.03

1.15 Bleached Blood (50 ul) and reagent.

1.15
-1.03
.12

10.18 (.12) +.22 = ~~2.29~~ 1.44

120 (1.44) = 173 gms/L

w/in expected range of 140 - 190 gms/L

The Blood Protein Method Appears to be in place. The looks very cool. It looks like I may have a method here.

The second method is much simpler. w/ only 3 curves required.

1. Calibrate w/ water, all means @ 534 nm.

2. Measure 3 ml reagent w/ 50 ul bleach (Abs = 0.46)

3. Measure 3 ml water w/ 25 ml of whole blood added and 50 ul bleach. (Abs = 0.57)

The sum of these two absorbances are a your reference calibration blank as it includes the effect of bleached reagent & bleached blood.
$\Sigma = 1.03$ @ 534 nm.

4. Now measure 25 ul blood in 3 ml reagent w/ 50 ul bleach added.
Abs = 1.15

$\Delta = 0.12$ This is the Δ absorbance due to the protein interaction.

5. Apply the regression & dilution factor.
eg $10.16(.12) + .22 = 1.44$ gms/liter

$\frac{3 \text{ ml}}{25\text{E-}3 \text{ ml}} = 120 \Rightarrow 120(1.44) = 173$ gms/l
Reference range 140 - 190 gms/l

Then appeared to have been a great strategy. If the color of the sample overlaps or coincides w/ that of the reagent, then remove that segment of color from the sample (i.e, bleach the blood).

Test appropriate controls, eg milk, to make sure that the reagent reaction has not been interfered with. It was not.

So the idea that emerged here is that if the color of the sample interferes w/ the color used in the reagent, then alter (bleach) the sample but you must also bleach the reagent to maintain similarity.

The fortunate advantage here is that the dye used (red 1 & 3) was generally very insensitive to the bleach & the reference reagent was therefore only marginally affected.

A very smart method. It is also evident that a significant difference in intensity exists between the bleached blood in water & the bleached blood exposed to the reagent. This makes sense.

This opens up the range of testing for proteins considerably now.

Mar 27 2017

Today the 4 dye are tested
The chemical composition of RIT dyes is
not specified on the MSDS sheets
The dye are listed as being proprietary
Dyes commonly react w/ proteins of fabrics
such as wool, etc.

Lets test acid & base reaction first
of each dye

Yellow Dye

Acid: Makes it clear
Base: Might make it a bottle darker
but not significant.

Yellow Dye w/
Acid w/ protein: protein insoluble, clear cloudy
Base w/ protein: darker yellow & clear

~~Green Dye~~
Yellow Dye w/ base w protein w/ $CuSO_4$
produce a strong Cobalt reaction.

No significant reaction w/ protein but
the yellow dye does react w/ $CuSO_4$
& NaOH to produce a green colored complex.

Green Dye (actually produces a blue green)

Acid turns it more blue
Base no change

now add protein. No major change
now add $CuSO_4$: to the base solution we have a deep blue that is formed.

OK, we have a successful reaction here also

3 ml H_2O
30 ml ½ & ½ emerald (green dye) (where is actually a blue green dye
1 drop conc. NaOH - KOH
visible tartaric acid

+ protein - Causes a shift to pure blue
sensitive to both 30 ml & 100 ml dehydrated milk.

Good, we now have two tests.

Purple: Acid & Base solutions both weaken the color.
$CuSO_4$ in Base Solution & purple causes a light to significant color change from ~~purple to blue~~ blue purple
No significant color change w/ protein added.

Conclusion: Red & Green RIT dye serve as sensitive indicators of protein. Purple dye can also be used as an additional if need be.

We now have 3 different methods to determine protein concentration

With Emerald (green but actually blue green)
Cherry Red
4 Purple Dyes

Reagent:
3 ml H_2O
1 drop Conc. NaOH - KOH
visible tartare
visible dye

+ 30 - 100 ul milk protein.

It is very good to have these options because if your sample (eg blood) is the same color you can use a reaction in a different portion of the spectrum in addition to bleaching the sample if need be.

We know that 10 does react w/ starch.
Do any of these dyes react w/ sugar?
Does 10 react w/ sugar?

We also have a reaction taking place with the emerald dye & sugar.

~~3 drops H2O~~
3 ml H_2O
4 drops conc NaOH
2 drops $CuSO_4$.
visible tartaric

sugar + Cu ion a mild shift from blue green control towards blue. Not as sensitive as the protein test but yet is doable.
So now the question comes up is the reaction unique enough & how much overlap is there w/ the protein reaction that you identified?

Notice that it requires considerably more NaOH. The higher conc. NaOH - KOH does produce a nice red blue color.

Sugar is not reacting w/ either yellow & red dye w/ H_2O, dye, NaOH & $CuSO_4$ & tartar

But the blue green did work.
(emerald)

We have methods now that should work for

1. proteins (dye methods, biuret) (including blood!)
2. sugar (emerald dye - blue green to blue) higher NaOH content.
3. Starches - Iodine should be easy enough
4. What would you do for lipids?
   Well the dye based w/ alcohol? dissolve?
   What is a lipid colorometric test?
5. How do you go about working w/ electrolytes?
   Tests for K, Ca, Mg Na, etc?
   Are there colorometric tests for them?

Salts do not seem to be producing any type of reaction w/ the dye thus far, yellow and emerald being tested.

Fuji Dri-Chem has Clinical Chemistry tests - w/ a Dri Chem NX 500 Analyzer

They include testing for Na, K, Cl, Ca, Mg

Doctor Foster & Smith has the electrolyte stabilizer we need.

Salifert looks even better.
Bulk Reef Supply.com
@ aquacave.com

[Ca, Mg, K
Nitrite, Nitrate
Phosphate
Cu, I, Sr, O_2 (!)
Silicate?]

I have ammonia & nitrate

There is a colorimetric test for lipids, it is a bit involved but it is there.
Lieberman - Burchard reaction for cholesterol ~~oils~~

RAU test? foods

Emulsion Test:
Adding alcohol (ethanol) to solution, then add water. Lipids cause it to turn cloudy white

Iodine does react differently w/ saturated and unsaturated fats.

Lipid peroxidation is a useful test. (MDA production)

Spectrophotometric methods for lipid peroxidation

1. Conjugated dienes (absorption in 230-235 nm)
   Serum lipoproteins

2. TBARS / MDA TBA Complex w/ MDA
   532 nm
   plasma, urine

3. Iodometric method

4. FOX test - ferrous oxidation in xylenol orange

Iodometric method - lipid peroxidation
- colorimetric

"Lipid peroxide number"

Mar 29 2017 Bryce Canyon Nat'l Park

I have come to an awareness that the chronic cough infection is almost certainly a result of biofilm production that lodges and seeds refuge in the lungs. Also has been found w/in the sinuses.

It is an additional marker beyond nitrate production. Now what is interesting is the composition of biofilm.

a self produced matrix of "extracellular polymeric substance (EPS)"

generally composed of DNA, proteins & polysaccharides

Polysaccharides matrices typically enclose bacterial biofilms, Quorum sensing is also at work here.

Enzymes may be a suitable means of dispersal.

Not just bacteria are involved. Bacteria, archaea, protozoa, fungi and algae,

Composition:

H_2O	97%
Microbial cells	2-5%
Polysaccharides	1-2%
Proteins	<1-2%
DNA & RNA	<1-2%
Ions	?

A major survival and growth defense mechanism.

" Sugar cutting enzymes" are known to be effective".

Alkalis & acids are effective but not ok as the study but good for protein test and possible sugar test method developed.

a Polysaccharide is a Carbohydrate Composed of long chains of monosaccharide.

or: a carbohydrate (eg starch, cellulose a glycogen) whose molecule consist of a number of sugar molecules bounds together.

glucose & fructose are monosaccharide.

Starch is a polymeric carbohydrate composed of a large number of glucose units.

Starch $(C_6H_{10}O_5)_n$

Glucose $C_6H_{12}O_6$

Iodine can be used as a chemical indicator in redox titrations

Sugar Detection Alternate!
Conc. H_2SO_4 + Phenol

An iodine reagent is potassium iodide and iodine in water. Both iodide & iodine are present.

Lugol's Iodine Reagent is this.

"An example, the reagent could be used when there is excessive concentrations of oxidizing agent or reducing agent. Excess oxidizing agent causes the complex to turn blue, whereas excess reducing agent causes the complex to turn colorless because the iodine and iodide ions break up and become separated. In other words, both iodine and iodide ions are required for the color change to occur."

1. Why isn't starch useful?
2. Iodine react w/ mono & disaccharides.

Proteins contain nitrogen.

We have an alternate sugar detection method:

1. Conc. H_2SO_4
2. Phenol

] + sugar produces an orange-yellow color

Simple sugars, oligosaccharides, and polysaccharides all work.
(iodine also?!)

I may have an alternate also w/ RR dye

April 03 2017

I do not have Rit dyes with me, tried to
run for repair. I do have food dyes.
Food dyes also apparently react w/ proteins.

Incidentally Cornmeal like may also be
* perfect for a dye enhancer to the Brunet
reagent.

Any food dye preferable?

[redacted]

Red -3 food dye (Carmoisine) is reactive with proteins via ~~Ojec~~ Czech J food Science 2010.

It is 2-(4-sulfo-1-napthol-4-sulfonic acid)

I will try this in combination w/ Biuret.

The test is highly successful and also appear to be reasonably sensitive, on par with that of PIT dye!

We now have another protein detection reagent.

3ml H_2O
1 drop Conc. NaOH-KOH
1 drop 0.5M $CuSO_4$
visible tartaric acid
30 ml Milk (~ 1 drop dye in 3 ml H_2O) Red Dye #3

+ milk. @ 60 ul it is highly significant as a Color reaction (Great purple)
@ 20 ul it appears to be marginally but nevertheless detectable.

This is therefore an increased sensitivity and improved reagent for protein testing.

What we need now is a slit of standards and a lysate sample.

We have now started our test w/ saliva. The first test was to determine if saliva had any proteins in it.

This test w/ the #3 reddye modified biuret reagent fails. The indicates that no active or water soluble & alkaline soluble proteins of significant concentration are to be found in saliva.

~~HOWEVER~~,

Looking at over time there may be a very slight or marginal color reaction taking place but it is very slight.

I would now modify the above statement to read that it appears there may be a VERY LOW concentration of water soluble & alkaline soluble proteins w/ in saliva. I can also tell that there is a clarification of the reagent taking place so there is a very subtle reaction taking place, but it is very subtle.

HOWEVER what is interesting is that a filamentous mass has precipitated out of the reagent. It is clearly the biuret component of the solution. The conclusion is that a copper complex filamentous precipitate is being formed

this may end up, after being subject to microscopy being identical to that of the urine precipitate
This is potentially important as a secondary method of producing filament mass.

There is indeed a color reaction indicative of protein being present but it is very slight. A fairly large amount of mass is being produced! It takes time to develop.

One question might involve the similarity of Carmoisine to anthocyanins.

Carmoisine (it is an azo dye)
$C_{20}H_{12}N_2Na_2O_7S_2$

We have a detectable mild color reaction w/ saliva.
It took about 20 min to develop fully.
Two reactions w/ saliva:
1. Mild purple development (ie protein detection)
2. Appearance of a blue filamentous precipitate complex

We have now extracted the biofilms filaments from the gums using the VHH/C method described earlier w/ in a paper.

The question now, IS, are these filamentous structures the same as semifibrin material?

(1) The oral keratin-vit C filament extract is now in Conc. NaOH - KOH, approx 1 ml.

(2) The reagent (buret + red dye #3) contains the saliva sample (no vit C). In case 2, the reagent produces & complexes the filaments in addition to producing a deep violet protein color reaction.

In case 1 only vit C is being used to pull the filaments + keratin from the gums.

(In Conc NaOH) Our primary question here is whether the keratin + vit C - gum extract contains a higher protein concentration level?

We are now testing the oral keratin extract in Conc NaOH w/ the Carmoisine modified reagent. First trial was 50 ml. There is definitely a reaction occurring however the keratin - vit C complex has high NaOH. This appears to be causing a shift towards green, where both extracts are reactive. Is almost certainly pH dependent and this variable will need to be controlled and accounted for.

I am going to mildly acidify the solution to attempt to compensate for the NaOH increase it should shift toward purple if correct,

ok, the absolute worked. In a strongly alkaline solution the color shift to the toward green (similar to wine, anthocyanins, and grapes) Toward neutrality, the reaction shift toward pink.

Our color reaction is certainly present and looks to be useful for detection of proteins.
Pink - acidic
Green - alkaline
Purple is presumably mildly alkaline? Test it

NaOH does not react w/ Red Dye 3 alone to produce green. It also does not produce it when the $CuSO_4$ & tartar is added. It does indeed seem to be tied into protein.

The purple complex with the Carmoisine reagent requires the alkaline solution. It does not occur in an acid environment.

With the Biuret extract we are definitely producing a green color, not purple.

A very strong & distinct reaction but it is not purple, it is green, or yellow green.

Big question: What type of compound produces a yellow green reaction w/ our Carnosine modified reagent?

1. Polysaccharides? (Starch?)
2. Complex sugar?

What common foods a substance contains polysaccharides?

What we see of value here is that our modified reagent produces

TWO separate color reactions. not just one w/ proteins. Now we need to learn what caused the second reaction (yellow green).

The color becomes more yellow as the hydrolysis is added ~~to~~ to higher concentration.

What has polysaccharides?
rice, potatoes, wheat

Cracker extract presumably polysaccharides IS NOT

produces a yellow reaction w/ the Carnosine modified reagent.

We therefore had an important mystery to solve.

What, in the biofilm (w/ NaOH concentrate) produces a yellow reactim?

It does not appear to be polysaccharides at this point.
Whatever it is, appears to be an important reactim.

Crackn extract (polysaccharide) definitely does not produce the reactim
What is it? The yellow, yellow green reactim.
All biomolecules are: Protein failing?
Carbohydrates failing?
Lipids ?
Nucleic acids ?

* NaOH has extracted something in the biofilm-Vit C extract. Remember that you

* heated the Biofilm-Vit C extract.

* = This could be an important part of the reactim.

The colored reactim discovered do take some time to develop, at least 15 mins although less if not a half hour.

Remember that we also have our Vit C solution added to our reagent. Does the produce yellow?

It looks like I have found the source of the yellow color!

Vit C with the Carmoisine reaction produces a bold yellow color. It is even opaque it is so strong. Let's try to dilute it.

Ok, the Carmoisine modified reagent is extremely sensitive to Vit C. It is producing a color change but it is NOT exactly the same by any means as the Riboflavin - Vit C reagent reaction for low Vit C concentration at produces a muddy brown color (it takes a while to develop) but it is not the same yellow of the Riboflavin - Vit C combined reaction.

The Riboflavin - Vit C in Conc NaOH contained therefore to produce a unique reaction.

Vit C in the reagent produces a muddy color. The Riboflavin - Vit C in Conc NaOH produces a strong yellow color. It remains

In acid medium as opposed to alkaline the Vit C solution produces a pink color that is transparent. May be good for a detection limit as not sensitive to concentration.

I am now back to ~~attempt~~ to reproduce the biofilm-Vit C reaction w/ the reagent. I am now using 50 ul added. No reaction is immediate. Note that the Biofilm-Vit C extract has been sitting in Conc NaOH for sometime now, approx 2 hrs. The Conc. NaOH solution has also turned pink to some extent so there has been a reaction that occurred.

Something important has happened here. The yellow reaction from before has been almost, if not completely degraded. There is only a very slight yellow tinge evident, but it is almost invisible. We are now @ 100 ul of the Biofilm-Vit C extract. Now up to 200 ul w/ another drop of NaOH added. It is actually closer to the muddy color which says you are likely picking up only traces of Vit C. Add acid, it should turn it pink.

And it does. This is saying that you are now only picking up Vit C.

This is all very intriguing.

We have an interesting situation.

1. We know that the Biofilm - Vit C oral extract produces an intensely and apparently unique reaction (strong yellow color) when combined w/ the Carnosine developed reagent. This also assumes that the extract has been placed in high conc. NaOH - KOH. The reaction occurs, therefore, only with a specific combination.

2. Over extended time (eg 2 hrs) the Biofilm extract solution turns pinkish. This solution no longer reacts with the Carnosine developed reaction.

3. The biofilm extract therefore contains "something" which produces a unique color reaction and also which degrades when subjected to conc. NaOH.

4. The Vit C from the extract is retained in the conc NaOH solution and it reacts accordingly & properly (depending on pH) of the Carnosine reagent.

At no time are we able to produce a purple protein complex color with the use of the extracted biofilm filament structure nor with the saliva produced filaments when placed into the Carmoisine reagent.

So the question is:

What is in the biofilm and extracted filaments that reacts with the Carmoisine reagent:

3 ml H_2O
1 drop Conc NaOH - KOH
Visible cream of tartar
30 ml dilute red dye #3.
1 drop 0.5 M $CuSO_4$

that degrades when exposed to NaOH.
Do not use NaOH next time!

No sign of protein at this time.

I still do not have a test for polysaccharides except how about iodine?

We have a control iodine test that worked very well: Cracker extract (starch ie polysaccharide) and iodine (Betadine) produces a strong purple color. Only H_2O added.

The iodine test fails for the Vit C - filament extract that has been in Conc NaOH for several hours.

This can not be extrapolated to a real oral sample or one that has not been subjected to NaOH.

Course of action

1. Aquire more oral extract material w/ Vit C

2. Test w/ saliva for the time being.

Both iodine & Carmoisine reagent tests are going to be required.

In the interim, a test w/ urine is also run w/ the Carmoisine reagent. No Color reaction (either purple (protein) or yellow (unknown) occurs with urine.

This is a good thing. You do not want protein for the unknown (from oral extract) in your urine!

Now let's run the test w/ raw saliva and then call it good until you produce additional oral extracts.

Good work today.

1. I have developed a very effective protein detection reagent, quite sensitive. An alternative to the use of Rit Dye methods.
For now, it will be called the Carmoisine – Biuret (C-B) reagent.

3 ml H_2O
1 drop Conc. NaOH-KOH
1 drop 0.5 M $CuSO_4$
30 ml dilute (1 drop/ml) Red Dye #3
Visible Cream of tartar

+ Protein. Gives a strong purple shift under weak protein concentrations.

2. Discovery of a reaction with the Oral extract sample and the C-B reagent. A significant yellow color produced. Fresh sample, but in Conc. NaOH. The nature of this reaction remains unknown @ this time.

3. A vitamin C reactor has also been uncovered w/ the C-B reagent but it produces a cloudy precipitate.

Question: What is an azo dye? It is

$$R-N=N-R'$$

R & R' can be aryl or alkyl.

The suggests the oral extract may be reacting w/ nitrogen.

What compounds produces a yellow color in combination w/ Copper & Nitrogen? in alkaline solution?

Remember the green also:

A copper chloro complex is green to yellow

Cu-thiosulfate produces a yellow precipitate

Another source:

$[Cu(OH_2)_6]^{2+}$ is blue.

Conc. HCl will turn it yellow green.

The yellow-green complex is $[CuCl_4]^{2-}$

This comes from Vg. Edw. Cu

A Copper Chloride complex is there is of highland interest w/ the oral extract. You need your Copper Container to work up this.

I have added Conc. HCl to a weak solution of $CuSO_4$. It did not turn it yellow at least not rapidly. It might have altered the blue color some but it definitely is not dramatic. More testing of $[CuCl_4]^{2-}$ complex is required.

There might be a tinge of yellow now visible (along w/ blue) but yes it is very weak & the spectrophotometer will be required. Also some heat added. I do believe that I see a tinge of yellow but it certainly is weak. Blue remains dominate

another question is how else could bromine be produced? I am fortunate to have come up w/ this method.

Are bromine filaments and the wire filaments the same thing? Notice they both produced a greenish color in an alkaline solution.

Is there a color reaction w anthocyanins? Certainly yes, w/ cabbage & pH.

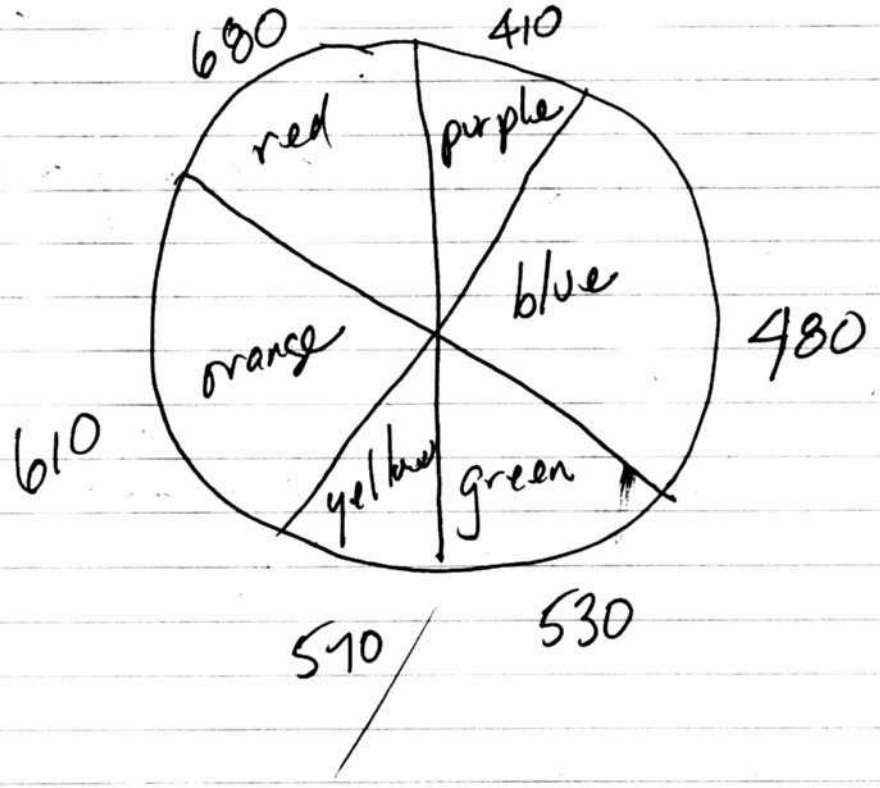
Anthocyanins do indeed appear to form numerous metal complexes
Complexin

1. lead (II)
2. Copper (II)
3. Iron (II)
4. Iron (III)
5. Cr (VI)
6. Cerium (IV)
7. hydrogen peroxide

alkaline anthocyanin yellow complex color Copper?

(Carotenoids & Flavonoids)?

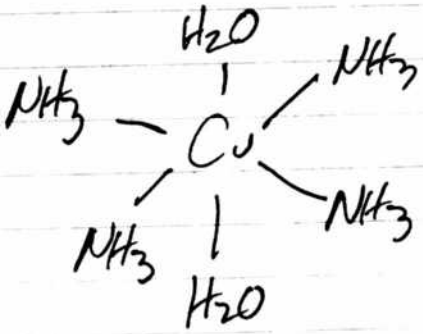
Absorbance Chart.



The color for a coordination complex can be predicted using the Crystal Field Theory.

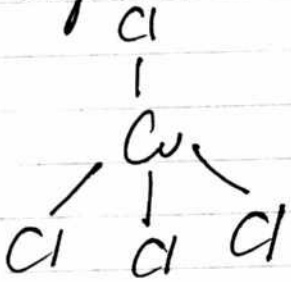
Cu forms octahedral complexes
(this means six ligands)

eg



$[Cu(NH_3)_4(H_2O)_2]^{2+}$

and
4 coordinated complexes (tetrahedral) (less common)



$[CuCl_4]^{2-}$

$Cu[H_2O_6]^{2+}$

The solutions of most Cu(II) octahedral complexes are blue.

You can, with the spectrochemical series sometimes be able to predict the color of a coordinated complex. If you know the metal involved you may be able to suggest the ligands involved in the complex, especially in a relative fashion.

See LibreTexts: Colors of Coordination Complexes.

Crystal Field Theory — Implications & Consequences

We know, therefore if our solution appear yellow, then it is absorbing a shorter wavelength, a a higher frequency.

Higher frequency mean higher energy

yellow light — Low spin mean large Δo & strong field ligand

green light — High spin mean small Δo & weak field ligand

(Higher Energy State)	(Lower Energy State)
Strong Field	Weak Field
Large Δo	Small Δo
Low Spin	High Spin
Yellow Light Appearance	Green Light Appearance
Violet Light Absorbance	Red Light Absorbance

CO^-, NO^- (acetate), $CN^- > NO_2^-$ (nitrite) $> en > py \cong NH_3 > EDTA^{4-} > SCN^- > H_2O >$

$ONO^- > O^{2-} > OH^- > F^- >$

$SCN^- > Cl^- > Br^- > I^-$

$Cu(NH_3)_2(NO_2)_2$ is green!

Cu - nitrite complexes are green.

~~might be brownish~~

tetracyanocuprate $(Cu(CN)_4)^{3-}$ (purple color?)

Now, the fact that the solution turned green initially & then went to yellow indicates it went from a lower energy state to a higher energy state.

$[Cu(H_2O)_4]^{+2}$ is light blue

Cl turns it green (went to a lower energy state)
NH_3 turns it dark blue, rich (went to a shorter, ie, higher frequency)

$NaNO_3$ sodium nitrate turns it green (went to a lower energy state)
Potassium Bromide turns it blue green.
This should be enough to evaluate the spectrochemical series.

The all match perfectly and your interpretation of a solution going from a lower energy state (green appearance) to a higher energy state (yellow appearance) is correct. The

therefore suggests potential ligands for the Copper Complex, eg Nitrite, CN, etc.

Which of these ligands is likely to form a yellow complex w/ Color.