CARNICOM INSTITUTE LEGACY PROJECT

A Release of Internal Original Research Documents

Authored
by
Clifford E Carnicom
President, Carnicom Institute

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CHEMISTRY VOL XVIII

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5 SUBJECT

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Chemistry – Lab Notes

Apr 2017

Vol XVIII

Apr 04 2017 Bryce Canyon Natl Park

Resuming testing from yesterday w/ Carnoisine reagent. There is no reaction of the reagent w/ anthocyanins extracted from Chard. Anthocyanin is solid orange in color.

This anthocyanin extract is not being responsive to pH. Why is this? Alkaline does turn the solution colorless but it is not significant. Apparently cabbage extracts are not the same therefore ~~the same~~ as Chard.

Lets go back to the oral extract. The first step is to see if we can repeat the reaction.

The relation $\Delta o = \frac{hc}{\lambda}$ in Joules/molecule

seems like it could be a very helpful relation. Each coordination complex should have a distinctive value? It is the "difference in energy". I wonder if there is a specific name for this term.

It is called the splitting energy

Yes there are some values calculated.

"Crystal Field Splitting Energy"

This time I have mixed the oral sample of the Vit C. The oral extract is in Conc. NaOH-KOH. At this point, 5 min in there is no distinctive reaction.

There is NO reaction occurring and therefore IT DOES NOT REPEAT.

The Carnoy's reagent is extremely valuable for both protein detection/concentration and Vit C detection (solution is opaque orange so I am not sure how valuable it is for Vit C concentration)

but there does not appear to be any reaction with the mixed oral sample.
You must have had contamination from the Vit C producing the yellow color.

However, we do have absolutely a positive Iodine test for polysaccharides (starch) Also, it take a great deal of acid to neutralize the color. The Conc. in NaOH is, however, low.

We have strong absorbance in the region of 550 - 600 nm. This means absorbance in yellow-orange region. The means appearance in the violet-blue region.

We also have absorbance in IR @ ~ 830 nm and this is strong.

This positive iodine test confirms polysaccharides.

The 830 absorption indicates $RNHR'$ Amine Group
We also have high absorption w/ a jump @ 705 nm

705 nm = CH_3

The suggests we should test for amino acids.
OK, yes we did w/ ninhydrin - a positive test.

Very important steps today:

1. Extract oral filaments w/ Vit C solution
2. Rinse thoroughly
3. Subject to conc NaOH - KOH & moderate heat
4. Perform iodine test for polysaccharides test result is positive.
5. Spectral analysis (VIS-NIR) also confirms positive iodine test and NIR indicate presence of amine group.
6. Ninhydrin test is highly positive for amines.

Conclusion:

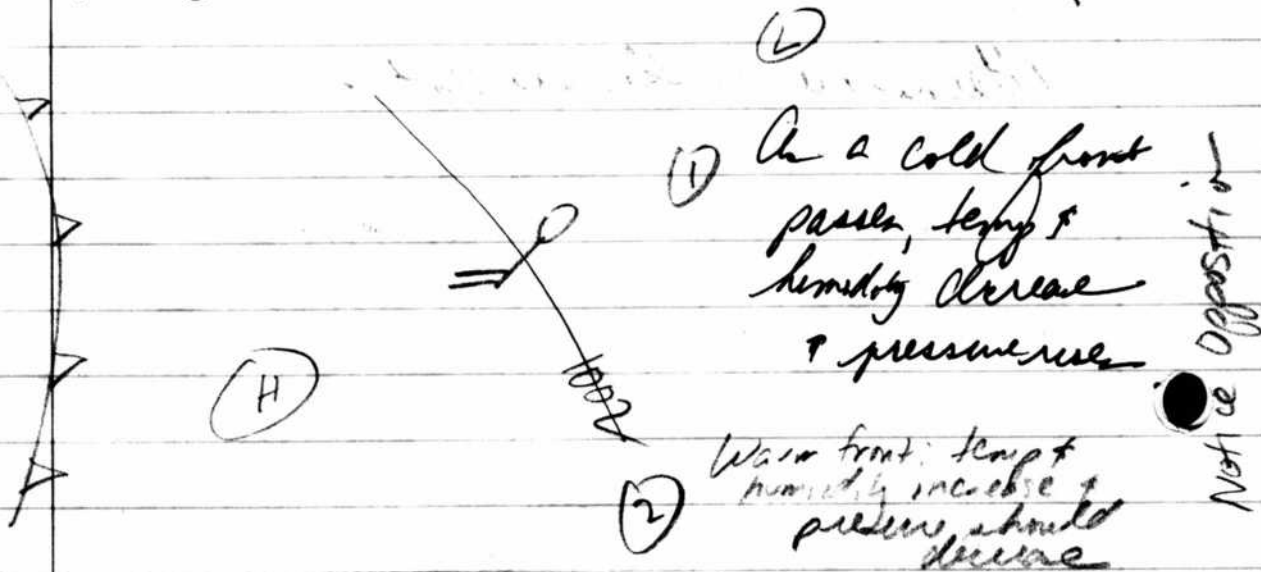
Oral extracted filament structure are composed of, in part, polysaccharides and amino acids.

Alternative to the new test.

April 0 2017

C strong interest in improving weather forecasts

Wind is blowing strong from SE to ~~SE~~
Humidity is very low. This indicates cold air is going to passing through. Pressure is low (1002 mb)



Most wx moves from west to east.
Temp is now very warm. The days we are currently under influence of a warm air mass under low pressure. Sky is mostly clear so the clouds contain limited moisture.
The high pressure will deflect the cold air around us to some degree. The moisture will tell us how likely we are to get rain

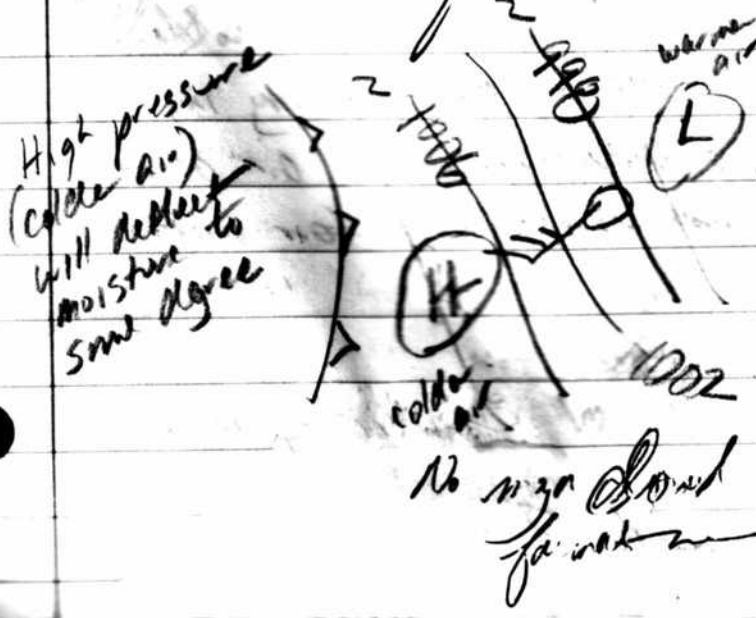
Temperature, pressure wind changes alone pose to a strong chance for wx prediction.
Humidity & Cloud types only assist.

In term of cold front & warm front humidity & temperature change are generally in opposition to pressure change the does not apply to stationary & occluded fronts.

Right now we have

Low Pressure & Falling — Cold Front
High Temp → Warm Front
Low Humidity → Cold Front

The scenario the tends toward a cold front approach. How else can you deduce this? We know our pressure gradient from the west but there is not a front or air mass We know and have a colder air mass to the SW (high pressure relative) & a low pressure to the NE (higher temp) the suggest the cold front is to the west of us.



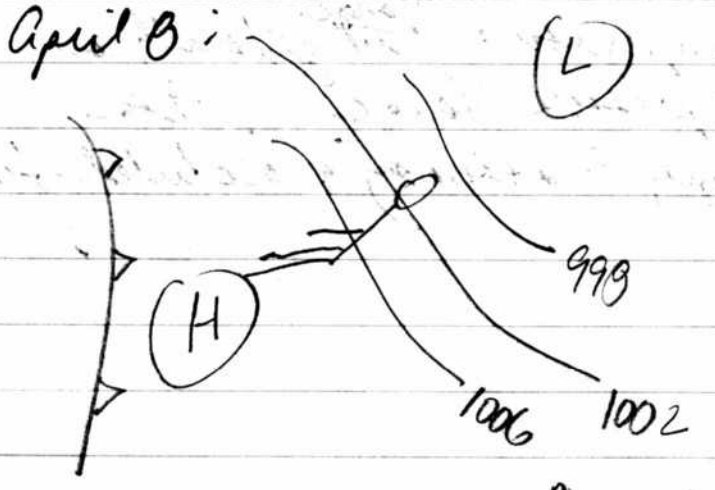
and the a
ended occlusion.
based upon current
surface analysis
map.

No warm front
for now.

Areas that should
not in should be
primarily to south

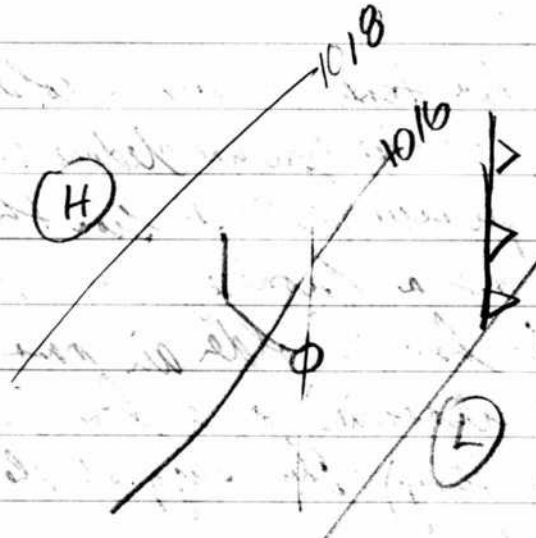
April 9 2017

April 8:



Temp warm
Humidity Low
Pressure Low
Wind Strong from S
Mostly Clear

April 9:



High Pressure
Low Humidity
Low Temp

Cold Front
Cold Front
Cold Front

Temp Low
Humidity Low
Pressure Rising
Wind West from NW
Clear

Assessment: Cold front is in place. Denser, colder air with less moisture capacity. High pressure moving in, this means warmer, > moisture capacity air ahead. Since no clouds visible, this signifies fair weather ahead.

This ~~notes~~ teaches barometric forecasting
1016 Rapid Rise, Wind to W, Storm ending, Clear & colder
Very Good Forecast Here

Front forecasting for today:

Pressure: Rises rapidly - cold front in place
Wind: Veers decreases a veer squall
Warm front in place, Cold front in place
Temp Sudden fall: Cold front in place
Clouds: Now, no match
Wx: Now, no match
Visib: Good, no match

Best match: Cold front in place.

Methods:
1. Temp, Humidity <> Pressure method
2. Barometric Forecasting
3. Front forecasting.
4. Wx map construction.
All 3 are Coincident

April 10 2017 [redacted]

Weather. Pressure = 1026 mb - very high. Temp 54 @ 1030
Clear Humidity 30%, fairly low. Pressure steady very slowly. Wind appears variable @ ≤ 5 MPH. (southerly?) SE?

Barometric: 1023 Steady = Fair WX
Front: Rises Slowly = Cold Front passed
Wind: no note
Temp: Sudden Fall (23°F last night) Cold Front in place
Clouds - No note.
WX: No note
Visibility: Good = Cold Front passed

Temp, Humidity <-> Pressure
Low, Low <-> High indicates Cold front

All methods are Consistent. Cold Front has passed through, fair WX in store. Look for pressure, wind, or cloud change.

(1) The movement of fronts depend upon the formation of pressure systems (ie, wind)

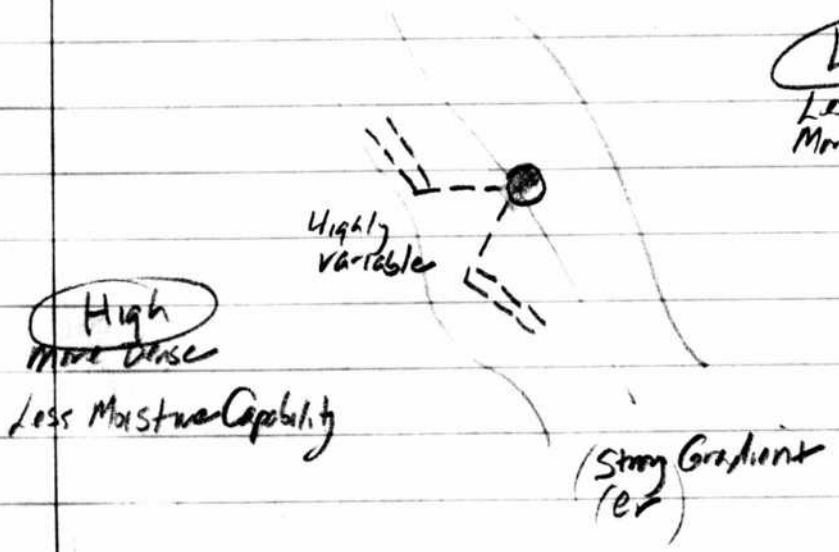
(2) Most changes in the weather occur along fronts.

Two very fundamental statements.
You should always apply these to your wx map.

April 11 2017 - Moab region.

Temp 76°F
Humid 4%
Pressure 1012? Dropping?

Had Cirrus, Increased Clouds Lower, Temp Rise
Pressure should fall
This corresponds to < Warm Front
Do not have barometer yet.



Increased clouds indicate more moisture
Best estimate is pre-warm front. Seek barometer as soon as possible. Reset to 1013 mb.

4% Humidity! Continental Tropical Air surmised.
This is a type of air mass. Very warm & usually Cloudless

Aerosol Operation - Desertification Documented

The "weather" yesterday was a bit absurd. The cirrus layer turned into a continuous sheet of mid-level "clouds" as the day progressed. At the same time, the humidity level remained between 10-14% all day and throughout the night w/ temps in the mid 70's. Barometric pressure remained flat all day. Significant aerosol operations were visible under full moonlight.

In the morning, the sky was clear and pressure has risen slightly after the "clouds" passed through.

These were not natural, water bearing clouds. It was clearly a large scale aerosol bank that locked up all available & minuscule water vapor in the desert air. The diffuse nature of the bank is a signature trademark of the aerosol operations as they continued to maintain and develop the bank through night hours. The erratic & gusty winds accompanied the conditions. The bank has now moved on to the east.

April 12 2017

0900

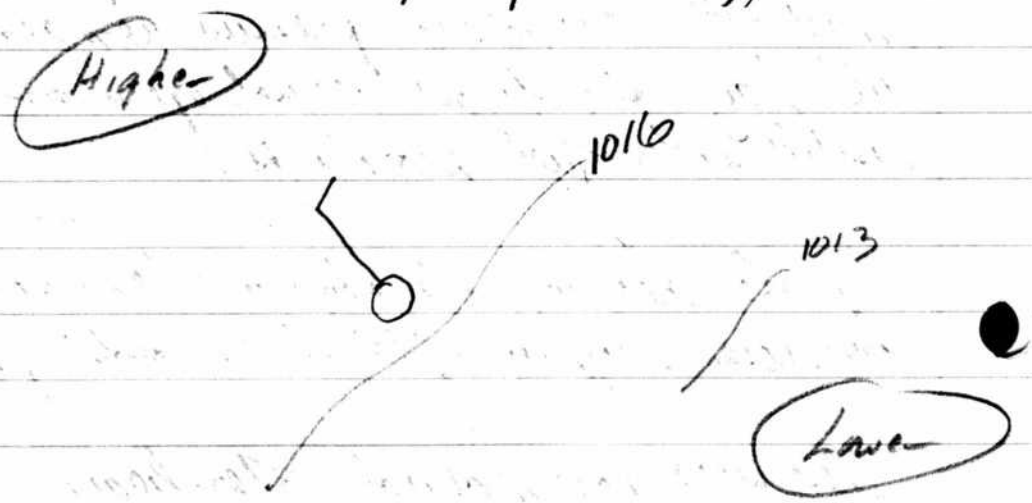
Pressure today 1016 rising sharply

Humidity 26% (~35% @ sunrise)

Temp 5°C

Wind NW 5 MPH

Conditions: clear (except sky is actually pale blue)



Rising pressure (mildly from 1013) indicates colder air w/ higher moisture capacity is coming in. A mild but discernible change which agrees w/ slightly higher humidity. Signs of a weak cold front.

Now for barometric & front & opposition forecasts:

Recall backing is counterclockwise

veering is clockwise

There we will have mildly veered & stabilized.

Opposition method:

Pressure up

[Humidity up
Temperature stable]

There is no pattern of opposition here so the method only suggests a weak cold front may be approaching.

Barometric forecast:

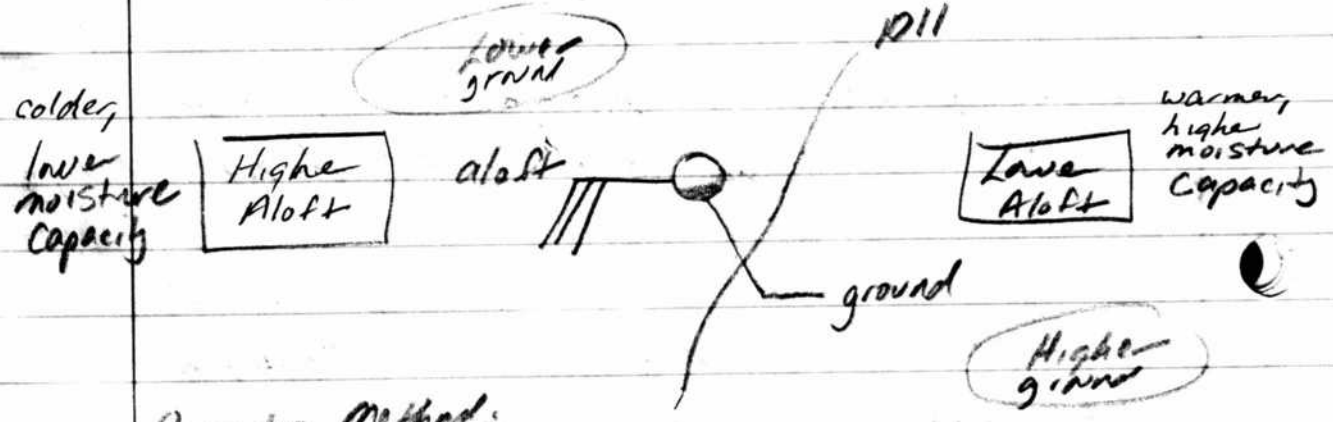
Best match is 1016 mb slow rise indicates clearing & long term fair wx. (winds SW to S do not match).

Fronts forecasting: We really only have pressure to work with. Mild rise does correlate w/ weak cold front.

[redacted]

April 13 2017 0900

Pressure 1011 - Steady but slightly fallen
Temp 61° but extremely warm overnight, low 57
Humidity 19%
Wind SE 5-10
Mostly clear, pale white sky, some aerosol banking to SE, some increasing "high clouds"



Opposite Method:
High Temp - warm front
Lower Humidity - cold front
Lower pressure - warm front

Very mild signals, but weight supports sustained warm front

[redacted]

Barometric method

Pressure slowly falling, wind ~~from~~ now to SE

Suggestion of some wx moving in

Front method

warm front passage

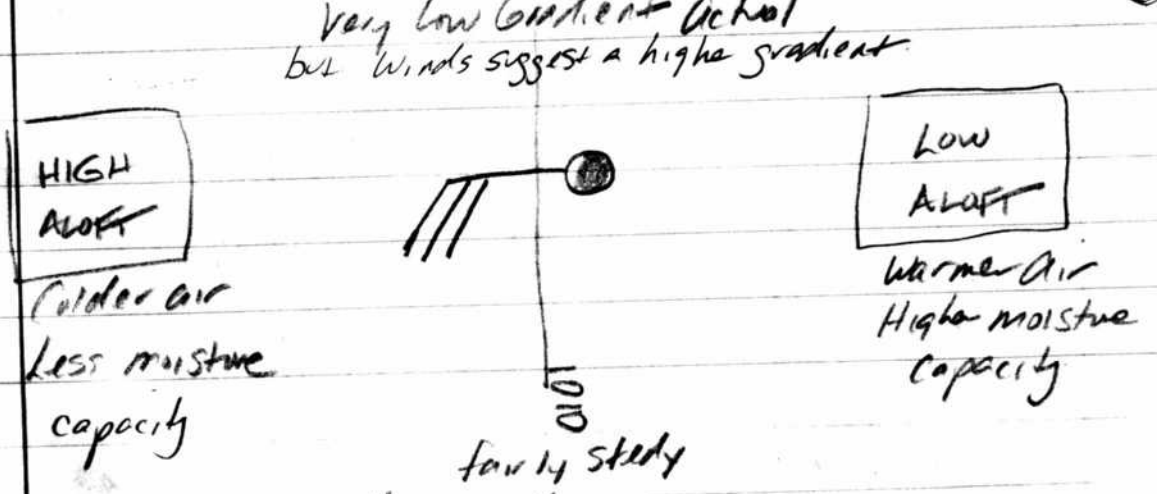
Temp - warm front in place

General forecast: In the midst of a warm front, potentially some wx developing w/ the low to the NW.

Some additional observations. Winds aloft are from W while ground winds are from SE. The ground winds are already shifting more southerly, so this is a veer. Humidity dropping from 20 to 15% w/ increasing "clouds" Aerosols again - desertification

So winds aloft diffe considerably from ground level winds. Winds aloft should be driving main weather

We have some conflicting scenarios because of winds aloft vs ground winds. There is a significant difference.
Winds aloft are from west.
Ground winds are from SE.
Winds aloft will dominate the weather scene.
Pressure is very slowly dropping. Now 1010.
Humidity down to 13%, but also increased "diffuse" stratus clouds increasing @ mid levels. So now.



Forecast would seem to be more of the same, tied in with the moisture interruption & variable gusty winds. Both are consistent w/ aerosol impacts:

1. Increased Cloud cover due to ionization & moisture absorption
2. Drier/Low Humidity even w/ "diffuse" "Cloud" development
3. Gusty & erratic winds
4. We also observe a strong difference between ground winds & winds aloft

1315 Humidity now down to 10%. Strong ground winds (gusty) remain from south 20mph

1515 Humidity is now 4% and pressure is now dropping and is 1006.
Now we know that lower pressure has come in. Lower pressure is usually warmer w/ greater moisture capacity. This is not happening.

So we have Wind to S, lower pressure, & very low humidity.

Return to BC 300

Where are we now? We have two alternative methods of sensitive protein detection & concentration that have been developed. One uses reddye #3 and the other uses RR dye (red).

We also have iodine for polysaccharide detection & concentration.

We may have one method for saccaride detection in place but this will need to be repeated.

We also have biofilm extraction via vitc and subsequent polysaccharide detection as well as protein detection.

It is a worthwhile question of biofilm extraction (filament network) by vitc and the network extracted via wine is the same material w.r.t. polysaccharides & protein. Do you have any grape juice?

You have a series of electrolyte reagents coming along w/ bromocresol green for albumin testing.

Ok, we have a little same here. NaOH & KOH w/ Iodine cause a negative halogenation reaction & turn the Iodine Colorless. This distorts the reaction.

We do have a strong reaction w/ cranberry supplement that has dissolved in water with the oral spit test. However, after centrifuge and running on numerous series & then filtering, we end up w/ a pink clear extract. The extract DOES NOT give a positive polysaccharide reaction w/ Iodine in the way that the oral vitc extract did. The suggest the two materials are distinct from one another. The is potentially important & it will require reconciliation.

But now you need to be careful...

Even the VIT C solution, itself, which tested highly positive for polysaccharides w/ iodine, now fails the test after sitting for several days. The original solution is stated to be in Conc. NaOH.

Question: does NaOH degrade polysaccharides?

A test w/ starch + iodine:

Iodine (Betadine, and you do not need much, 1 drop max in 3 ml H_2O) produces the nice purple color when starch is added. You do not need much starch also.

HOWEVER! NaOH added to the purple reaction completely turns it colorless. Therefore alkaline solution completely negates the iodine polysaccharide reaction. This is crucial to know.

You cannot use NaOH w/ the real extracts.

And

ADDING Conc. acid does NOT bring the purple back!

DO NOT ADD NaOH to the polysaccharide sample.

At this point the polysaccharide test applied to the real cranberry extracted filaments is NEGATIVE.

This was not the case w/ the VITC extracted sample (interestingly, what was subjected to NaOH). So this will need to be repeated. Next move is to try and culture the filaments.

April 14 2017

Repeat the Vit C and filament levoglucosan polysaccharide + protein test.

We now know that both NaOH + Vit C will both turn iodine colorless. You cannot have that. You have excellent microscopic and a macro photo of the extracted levoglucosan.

You must protect the levoglucosan sample.

Next add starch to NaOH + Vit C samples w iodine to see how much NaOH + Vit C can be accomodated and still produce the purple color.

You must keep the iodine color light to form a transparent purple. One drop of Vit C Concentrate solution in 2 ml of iodine reagent w/ starch negate the purple color. The Vit C must be rinsed out completely from the levoglucosan material

1 drop of 1.0M (!) NaOH in 3 ml of H_2O w/ Iodine & starch also neutralizes the purple color!

OK, so now we learn that the cranberry oral extract is entirely different from the Vit C oral extract. There is no similarity & relationship. We can repeat the test w/ Wine & grape juice someday but there is no further need to work w/ the cranberry extract. That sample is a non issue.

Our method of producing the polysaccharide issue must be to go ahead & dissolve the Vit C extract in conc. NaOH. There is also a time factor against us involved. The method must be to have as high a conc of Lactobacillus as possible, then take only 100 ul max of that concentrate into the 10 clear solutions.

And first, we must rinse the Lactobacillus sample of Vit C thoroughly before subjecting it to NaOH. Keep NaOH as low as possible.

Ok, remove the lucifer of Vit C
Ok, this is done.

Now, what we do is only add 1 drop of
conc. NaOH to the lucifer. It will be
definitely adequate.

Next we to add slight iodine to the water
to produce the light yellow. Now add
50 - 100 ul of the lucifer - NaOH
dissolved matrix. It will largely decolorize
the iodine but slight purple can be detected
by eye w/ careful observation.

To get this recorded on the spectrometer is
a challenge since it is so weak. The
Method is that you must use the iodine
solution AS THE REFERENCE TO SUBTRACT
OUT, NOT WATER!

Iodine absorbs @ ~450, the iodine -
polysaccharide reaction absorbs @ ~ 550 nm.

You can easily identify the broad subtle peak w/ max of ~550 nm after subtracting the iodine reference.

I have therefore proven the existence of the polysaccharides (i.e, biofilm) within the extracted sample orally with Vit C.
As you can see, the steps are several but the result is incontrovertible.

Now, the question is, can we once again detect the protein component.
Yes, we did it by two methods.

1. NIR @ ~825 nm
2. Ninhydrin test was highly positive before. Positive result again, but it is very weak this time.

Conclusive report: Polysaccharides & Amines

April 16 2017

You have developed live cultures from both the Cranberry nal sample & the Vit C sample. Both samples are producing viable cultures

What you would like is a way to verify the culture nature without having a suitable microscope available.

The fact that the culture grown is sufficient. But make a solution w/ only iron sulfate or H2O & visually compare.

Time & filament formation is an alternative confirmation.

~~Upon Con~~

April 18 2017

Several goals in mind.

1. We now have a nitrite form. Attempt to develop a nitrite colorimetric test.

2. Verify the culture development from the biofilm culture - a 2 step process

3. Ultrasound work now - Brooke has arrived.

4. WP paper up - health info suggestions

5. Impedance analysis w/ mini GP 9 software.

Seeing a colorimetric reaction w/ nitrates:

Purple dye + Conc. acid gives no change in color
" + Conc base shifts to purple
" + Conc acid + Nitrites gives bubbles
+ it weakens the color.

Bubbles are not colorimetric but they are a result you do not need to dye. Nitrites & Concentrated HCl gives bubbles. Dye makes it easier to see. Smells like it might be chlorine gas!

Hydrochloric acid + Nitrite Ions → Nitrous Acid, HNO_2

Nitrous acid is known only in solution and in the form of nitrite salts.

Very Cool, when $CuSO_4$ is added we get a nice light green color.

Therefore we have a Colorimetric reaction:

Nitrites + Conc. HCl + $CuSO_4$ → a green Complex.

It is definitely sensitive to Concentration of nitrites.
We have a good reaction.

You do not need Conc HCl. Dilute HCl is sufficient

Nitrites + H_2O + Dilute HCl + 2drops $CuSO_4$ → Green Complex

The color to be a manually sensitive test.

We may indeed have some nitrate production w/ the culture. If so, it is weak. Spectrometry is required. I can detect the green color by eye.

—

Test, some controls.
The Cranberry culture, w/out any reagent (acid or $CuSO_4$) added has a component @ 408nm (and also strong @ 950nm).
It has a yellowish tinge. This is 2ml H_2O, 1ml Cranberry culture.
Now 408nm = yellow green visible, violet absorbed

Now, acid + $CuSO_4$ produces a small peak @ ~430nm and a very strong peak @ 850nm.

Next, $NaNO_2$ in water w/ HCl + $CuSO_4$ produces an extremely broad & powerful peak @ 800nm. It also remains strong @ 750nm which is the green blue. So that is the difference

$NaNO_2$ increases absorbance @ both 380nm & @ 800nm. It definitely causes a shift from 850 to 800nm.

We can see what happens here:

We anticipate the $NaNO_2$ concentration to be quite weak. Therefore the peak @ 300 is not increased & thus is understandable.

The peak @ 430 is due to the reagent alone ($HCl + CuSO_4$) so it is dismissed.

The sharp rise @ 950 nm is also due to the natural culture solution w/ out reagents so it is also dismissed.

BUT there is indeed a relative rise in the range of 750 - 800 and a shift of the broad hole in that direction from 850 nm. This does indeed signal the existence of nitrite production within the oral cranberry extract culture.

This however is not the hydrogen culture but it is the cranberry oral culture.

3 ml H_2O
1 ml cranberry culture solution

Same concentration as control w/out reagent.

Now, let's think about what your client's reference of removal is.

It can be:

1. the Cranberry culture by itself.

Yes, you have it. The culture produces a very strong broad peak w/ max @ 600nm but extends from 750-850 strongly also detect in @ the tail of the blue green.

You have therefore confirmed nitrate production by the oral culture based on Cranberry (anthocyanin method).

A highly definite & positive result.

This particular culture was based upon

1. Cranberry oral rinse
2. Rinse filament network thoroughly.
3. Mg Lobe added 1 drop Conc NaOH.
4. Place in normal culture medium except a brown sugar was used.
5. Incubate for 3 days.

Then subject to nitrate colorimetric test developer.

Here is what we see next:

The Vit C based culture, even though it appear to be a perfectly viable culture

IS NOT PRODUCING NITRITES @ a significant level. The culture looks to be complete, pure and terminal in its development. A microscope will be required to verify the CDB formation, which does nevertheless seem to be highly probable.

The oral culture by cranberry is producing the traditional sheen and shows the prospect of developing further growth. Recall also that brown sedge was used for that culture.

You have done excellent work here. You have:

1. Developed a robust reliable colorimetric test for nitrite existence & concentration

2. You have verified nitrite production is by the polarized return of an anthocyanin linked culture.

We have now developed colorometric + tests for.

1. Protein detection & Concentration.
   Very sensitive methods. 2 different ways
   Red Dye/Biuret & Red Dye Food/Biuret.
2. Polysaccharide detection & Concentration
3. Vit C detection but not Concentration
4. Nitrite detection & Concentration.
5. Headed toward electrolytes w/ API.
   Also have ammonia and nitrate w/ API.

and we have proven nitrate nitrite production by the COB.
We have also learned that HCl + nitrites can be used to produce nitrous oxide NO_2 (?)

6. Arsenic & Niahy drin

Apr 19 2017

A very important discovery today.

The control culture, consisting only of

1. Water
2. Sugar
3. Ferrous sulfate
4. H_2O_2

produce a visually similar result to the actual cultures. This is important as it reveals a significant portion of the culture is composed of iron oxide alone. This explains our result of 70% of the mass of a weighed culture being iron oxide. The good microscope would be invaluable here but you will not have that until June.

Gleen production on the surface of the liquid culture, nitrate production and microscope examination @ high power, as well as glucose monitoring are all valuable confirmation methods in determining the existence of the culture.

Now it is interesting that the culture w/ highest sheen & highest nitrate production is the cranberry culture and also happens to be brown sugar medium.

The VITC culture might be more productive than jar 2

* If you inspect closely, however, there is a macro VISUAL DIFFERENCE between the control ~~cotton~~ solution & the culture that uses white sucrose sugar. The culture (in this case, VITC) is of a darker hue, it is more diffuse along the edges, and curiously enough it seeks the circumference of the bottom of the jar, leaving growth @ the bottom of the jar. The control solution is of a ~~gran~~ granular nature, a lighter hue, and covers the bottom surface of the jar.

There are, therefore, distinct differences in appearance. We must also recall, however, that the control jar is only 12 hrs old & the culture jar is ~ 4 days old.

If we run the nitrite test, we therefore anticipate to find some result of the culture jar but not of the control jar.

I run the nitrate test on 5 samples

1. Control medium
2. Vit C culture 12 hrs old
3. Cranberry culture in sucrose 2 days old
4. Vit C culture in sucrose 3 days old
5. Cranberry culture in brown sugar medium 5 days old

On the solutions #5 is the one that shows the most reaction, producing a transparent solution. The other solutions remain cloudy to the same extent. There is more visible material @ the bottom of the tube for sample #5 also. All solutions seem to have a greenish tint but only #5 is a transparent result.

Subjected to HCl (dilute) and ~~1 drop~~ $CoSO_4$.

Next a microscopic comparison, esp. cultures #1 & #5.

Question: Nitrites in blood or urine?

Ok, by low power microscopy we see that #5 is also the radically different culture. Brown sugar seems to be a much more favorable medium than white sugar.

Apr 23 2017

Today we have an important confirmation that has taken place.

1. Ability to produce the filamentous form of the culture on site (mobile)

2. Visual confirmation of filament production after approximately 6 days of incubation in the brown sugar modified culture. Approximately 6 different filament examples were recorded under the microscope @ ~800X.

3. Confirmation of ~~nitrate~~ nitrite! production via the developed colorometric test using HCl & $CuSO_4$ with absorbance taking place @ ~450 nm.

The sample origin on the case I believe to be the anthocyanin culture via Cranberry.

We still need to confirm the same results using the Vit C Mucofilm extraction. We still have some of the sample remaining.

Remember that I have also confirmed that the Vit C culture is partially in part composed of polysaccharide & protein.

I have now confirmed the production of nitrite by the Vit C mucofilm extract/culture. The mean means that CDB production is confirmed by:

1. Growth of the culture in a specific medium (sucrose medium)
2. Appearance of the culture (macro)
3. nitrate production & absorbance @ ~ 450 nm w/ developed colorimetric test ($HCl + CuSO_4$) in 3 ml H_2O Dilute 2 drops 0.5 M
   as well as bubble production.

Apr 24 2017

We are now applying the nitrite detection test to a urine sample (CEC)
1 ml urine + 2 ml H_2O = 3 ml urine sample

A visual shift of the urine sample towards green is visible. We therefore anticipate that some nitrites are within the urine sample.

We do, however, have a control absorbance of H_2O before & after the test that will need to be examined. Visually we know that we do have a color shift towards green.

All measurements @ 451 nm.
Reagent absorbance = .043
Urine sample absorbance = .084
(Urine absorbance @ 450 also completes the interpretation of the test)
Urine Sample + Reagent Absorbance = Ø.146
before mixing.
Theoretically, we have absorbance of the sample + reagent as ~ .084
+ .043
.127

(3) Prostate size reduction effects are also in play w/ Phytosterol Complex.

After mixing we have absorbance of 0.146
0.146
- 0.127
.019 Theoretical nitrite absorbance

This seems to be quite small and appears insignificant @ this time. In addition, we have a control absorbance of water @ the end of the test @ 0.053

The value drowns out the theoretical nitrate value, therefore no nitrates appear to be detected in the sample. We will now conduct a separate urine analyzer test.

Also note that yellow urine + blue reagent = green! So there is no surprise that a visually detectable shift toward green will occur. Green is @ ~530 for absorbance

(1) (2) At this pt, change is attributed to cranberry + electrolytes A very good report here.

Urine Test [redacted] Apr 24 2017

URO	Norm	PRO	—	A second validation
BLD	—	PH	6	No nitrites present.
BIL	—	LEU	—	Great progress here!
KET	—	SG	1.03	Specific gravity also
GLV	—	VC	+-	increased to a more normal value.

Prep of 0.5M $CuSO_4 \cdot 5H_2O$

MW $CuSO_4 \cdot 5H_2O$ = 260.4 gms

260.4 (0.5) = 130.2 gms / 1000 ml

$\frac{130.2 \text{ gms}}{1000 \text{ ml}} = \frac{X}{60 \text{ ml}}$ $\quad X = 7.81$ gms

---

Now we test blood for nitrites:

The test for nitrites in blood gives a negative result.

The solutions of comparison are

Water - Control
HCl + $CuSO_4$ - Reagent Control
Dilute Blood - Control
Dilute Blood + Reagent - Primary Sample
Dilute Blood + HCl - Control reference also

There is also a positive sign w.r.t. health issues + methemoglobin

Note: It is desirable to develop an oxidative / free radical test, similar to the Oxidase test.

The next test is to examine a set of ancillary a secondary cultures for nitrite production

(1) Abs 450	Culture #	Description	(2) Abs 450
0.035	1	~7 days Vit C culture, white sugar base	.027
.041	2	~7 days Vit C culture, white sugar base	.044
.068	3	~5 days Cranberry culture, sucrose base	.020
.056	4	~5 days Cranberry, sucrose, 2nd generation	.042

With 2 Controls, H_2O & reagent.

.036	5	Reagent	0.050 @ end of test .04
0	6	Water	0.004 @ end. 0

The test shows that there is minimal to no nitrate production. Lets repeat the test.

From test #2, there is no detectable or identifiable nitrate production w/ the sucrose secondary cultures. This is an important finding and it explains the lack of filament production & subsequent growth w/ the sucrose based cultures. Brown sugar changed the entirely.

Apr 30 2017

The cultures have several distinct properties:

1. Feathered growth on edge of oxide
2. An oil sheen upon more progressive development (filaments likely)
3. Brown sugar produces a much more developed culture than white sugar does
4. Nitrite production seems to correlate directly w/ the higher metabolic & physiological activity w/in the brown sugar culture.
5. Previous glucose measurement.

Projects on tap:

1. Visually inspect the cultures for stages of growth, with special interest in variation between the white sugar culture & the brown sugar culture.

2. What can we learn about the oil sheen on the top? Lipid Composition enzymatically? Iodine, acid? NIR?

3. We now have potentially numerous electrolyte tests that now can be conducted, both in body fluids as well as the culture.

4. We also have developed semi-quantitative tests for
Dextrin
Polysaccaride
Amines
Nitrites
We also have a glucose meter.

5. We now also have bromocresol, which can be used to test for albumin.

6. Our electrolyte test set now include
1. Ammonia
2. Nitrates
3. Nitrites (2 methods now, API & my own HCl - CuSO4 method)
4. Mg
5. Ca
6. Phosphate
7. Silicate
8. Iron
9. Iodine & Iodide
10. Oxygen
11. Potassium

There is a fairly high level of capability now.

What interests me the most right now is the nature of the oil sheen. NIR may be the best place to start.

Recall also that we have the biochemical analyzer in addition to the electrochemical interface - voltammetry.

NIR analysis of sheen (lipid) layer:

1. Some activity appears to be occurring @ ~ 710 nm. - 720 nm

2. From 800 - 900 we have gradually increasing absorption.

3. At 900 we appear to have a peak absorbance with a strong decline from 900 - 950

The sheen is most likely the biofilm itself.

This leads to an assessment of

1. CH_3 likely & CH_2
(710-720) (710-730)
(870-910) (910-920)

Declined absorbance @ 950 indicates less likelihood of ArOH or ROH.
Indicates lack of polarity.

We also have a possibility of amine RNHR' (800-850)

Therefore we now check for
1. polysaccharides
2. Amines

Both of these tests fail. This tells us that there IS NOT the protein.

HOWEVER, the added Iodine does turn clear. This is the important Iodine test for lipids. This tells us that we do indeed have lipid production taking place.
Let's research the Iodine test, a so called Iodine Number.

So now we want to know what exactly is going on with the Iodine reaction?
Is there any way you can discern the structure of the lipid? Saturated vs unsaturated?
Potassium permanganate reaction also?

So far 4 drops of Iodine bonds have decolorized with what we likely a weak concentration of the lipid. 1 ml diluted lipid in 2 ml of water = 3 ml of solution total.

Notice that we have some foam also has produced in the test tube w/ Iodine added.

We also suspect the emulsion test will succeed here.

—

Need betadine concentration.

Betadine solution is an aqueous solution of 10% providone Iodine.

Formula $C_6Hg I_2 NO$
MW = 364.95 gms/mol

The iodine value is the mass of iodine in grams that is consumed by 100 gms of a chemical substance.

Betadine has max absorbance peaks @
386 nm not visible
409 nm. Visible is yellow green
434 (small peak) Yellow.

w/ broad absorption from 400-530 nm.

With decolorized iodine (partial) we do still have absorbance @ 408 nm but a small peak @ 434 is not visible.

We are now running a Calibration Control w/ pure lemongrass essential oil. We have success here.

Control Solution:	Max. λ	Abs
3 ml Isopropanol	386 nm	1.042
20 ul Betadine	436 nm (small peak)	0.223
With Lemongrass (30 ul added)	Absorbance drops to:	
	383 nm	0.452
	434 nm.	0.009

Page 52

113
114.3? OK

Lemongrass has an iodine no of ~~200.9~~

The shows us that we have definite reduction of iodine absorbance w/ lemongrass oil added.

Now lets add 50ml oil to a (in stages?) Control solution that has 100 ml iodine added.

Ok, we have a control solution:

3ml Isopropanol
100ml Betadine

The yields a very strong absorption peal in the visible range @ 416 nm. The will be our actual point of reference.

	Δ		Total	
	Time elapse	Conc of Lemongrass		Abs
total	ΔT		0 ml	~~1.266~~ 1.312
5	5min	10 ml	10 ml	1.312
10	5min	20 ml	30 ml	1.305
15	5min	30 ml	60 ml	1.245
20	5min	50 ml	110 ml	1.102
25	5min	50 ml	160 ml	0.703
30	5min	25 ml	185 ml	0.259
35	5min	20 ml	205 ml	0.195

The appears to be near the lemit of the reaction. Let us try to determine the Iodene no. of lemongrass, which is known to be ~ 113.5 gms/

Now MW of Betadene is 364.95 gm/mol.
Iodine is 69.54% of the mass.
Therefore we have 253.19 gms of I per mol of Betadine
Now our solution is only 10% Providine - Iodine
so therefore will have 25.38 gms / mol
and a 1M solution of Providine - Iodine has
25.38 gms of Iodene in it.
liter

Note that we are using alcohol
Now we are only using 100 ul of Betadene in 3ml Isopropanol
Therefore we have $100E\text{-}6\,l \left(\frac{25.38\,gms}{1\,l}\right) = 2.54E\text{-}3\,gms$

so we are only actually putting 2.54E-3 gms of I into our test tube w/ Isopropanol.
Our reactor Completed with ~ 205 ul of lemongrass oil.
Now what is the density of lemongrass oil? .89 gms/cm³
Therefore $205E\text{-}6\,l \left(\frac{.89\,gms}{.1E\text{-}3\,l}\right) = .182\,gms$ lemongrass oil.

So we have $\frac{2.54E\text{-}3\,gms\ Iodine}{.182\,gms\ lemongrass\ oil} = \frac{X}{100}$

reacts with

$X = 1.39$ Iodine No. Actual Value $\tilde{=}$ 113-114

Ok, we have a problem by a factor of ~100.
Lets see if we can track that down.

We start w/ our determination that Betadine has ~ 25.38 gms I / liter

Now we are extracting 100 ul of the solution.

$100\ ul \left(\frac{25.38\ gms\ I}{liter}\right) = 100E\text{-}6\ l \left(\frac{25.38\ gms}{l}\right)$

$= 2.54^{E-3}\ gms\ I$ should be extracted and placed in our 3ml of Isopropanol.

Now we react this w/ 205 ul of benzoyl ~~ac~~ chl.
205 ul = 0.205 ml.
So what we actually have is

$\frac{2.54^{E-3}\ gms\ I}{3.205 - .205\ ml\ benzoyl} \left(\frac{.89\ gms}{ml}\right) \approx \frac{2.54^{E-3}\ gms\ I}{0.182\ gms\ benzoyl}$

Reacts w/

$\frac{2.54E\text{-}3\ gms\ I}{0.182\ gms\ benzoyl} = \frac{x}{100\ gms} \qquad x = 1.40\ \frac{I}{100\ gms\ benzoyl}$

Iodine No for Coconut oil is 9 Density = 0.92
The remain off by a factor of 100
but we can still proceed as the culture oil.
in a relative sense.

Now for coconut oil: @ 416 nm abs

Total Δt		0 ml	1.345
5 min	50 ml	50 ml	1.339
10 "	50 ml	100 ml	1.333
15 "	50 ml	150 ml	1.344
20 "	100 ml	250 ml	1.357
25	100 ml	350 ml	

Ok, a large difference. What we see here is that an unsaturated oil like coconut oil does not absorb iodine at all, it consume to clear. Lemongrass does very much.

Ok now we have reference points established.
Now we proceed to COB oil folm.

CDB Sheen: 416 nm

total	Time (min)	Δ	Total		Absorb	
0	0	0	0		1.368	
5	5	30 ul	30 ul		1.315	
10	5	0	30 ul		1.319	
15	5	30 ul	60 ul		1.401	
also 710 nm 20	5 strong CH3 detect	50 ul	110 ul		1.555	sharp peak @ 890
25	5	50 ul	160	!!!	1.814	
35	10	45	205		1.88	

Notice NIR peak shown up @ ~890.
There is CH_3 Hydrocarbon present.

Some very interesting reaction taking place.

1. We are definately picking up hydrocarbons as the concentration of the sheen layer is increased.

2. However, even though we know that there will @ some point be a reduction of the 10 dense Color, the absorbance @ 416 nm is actually increasing!

We have a very curious reaction here. The hydrocarbon is actually reacting with the iodine to produce a more intensely colored yellow complex.

But we also know that it decolorizes iodine in water. This means the alcohol is likely involved here in the reaction that is causing the change.

We are trying the reaction in water instead of alcohol for comparison. There is definitely an interesting reaction taking place here of some sort. Not only that, the reaction is taking place exactly @ the iodine peak frequency of 416 nm.

Ok, the nature of the problem has been discovered.

Iodine in water produces an ORANGE complex!
Iodine in Isopropanol produces a YELLOW color!

This is the heart of your problem.

We now understand the nature of the "problem" and the reaction better now.

What is happening is that the COB culture has produced an alcohol in addition to that of a hydrocarbon.

We know that we have a lipid because of the sheen and because of the NIR CH_3 definite peaks @ 1710 & 890.

But what we did not realize is that we also have alcohol production. The micro will be in sync w/ your extended incubation work w/ the culture, & believe you have both alcohol & protein production in that case.

So alcohol & iodine produce a yellow color. Increase in absorbance was telling you that you have alcohol production w/ the culture.

Now, the big question is, what does alcohol production mean w/in bacteria?

Alcohol & hydrocarbon production sound like huge fuel fuel contain production to me!

Production of alcohol is highly synergistic w/ respect to oxidative stress.
Alcohol produces reactive oxygen species.

So now we know the culture produces:
1. Alcohol
2. Lipid
3. Protein
4. Gases (CO_2, CO)

Look up the iodine reaction w/ alcohol to produce a yellow complex.

Organism appears to be facultative:
Alcohol → Fermentation - anaerobic
Oxidation - iron - oxygen utilization
Both aerobic & anaerobic

May 01 2017

Interesting discoveries yesterday.
Interactions occurred between iodine consumption and a iodine-alcohol colorimetric reaction.

Major differences between lemongrass & Coconut oil

Also CDB culture demonstrate presence of alcohol intensifying the yellow absorption instead of a expected decline during iodine consumption.

Water & alcohol vary in their color w/ iodine. 2ml H_2O + 1ml culture broth also did decolorize the iodine, so we also know that lipid is present along w/ NIR work.

Lets analyze the regression on lemongrass.

Note: It is desirable to develop an oxidation or free radical test, similar to the Oxidata test.

The next test is to examine a set of ancillary a secondary cultures for nitrite production

(1) Abs_{450}	Culture #	Description	(2) Abs_{450}
Ø.035	1	~7 days VitC culture, white sugar base	.027
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With 2 Controls, H_2O & reagent.

.036	5	Reagent	Ø.050 @ end of test .04
Ø	6	Water	0.004 @ end. Ø

The test shows that there is minimal to no nitrite production. Lets repeat the test.

From test #2, there is no detectable or identifiable nitrite production w/ the sucrose secondary cultures. This is an important finding and it explains the lack of filament production & subsequent growth w/ the sucrose based cultures. Brown sugar changed the entirely.

Iodine-Lipid Absorbance Reaction Regression.

Lemongrass data:

		y	x
t	Vol (ul)	Abs	t·v
0	0	1.312	0
5	10	1.312	50
10	30	1.305	300
15	60	1.245	900
20	110	1.102	2200
25	160	0.703	4000
30	185	0.259	5550
35	205	0.195	7175

$y = -1.711E-4 \cdot x + 1.361$ $\quad r^2 = 0.97$

Quite decent. A good approach to the reaction

Therefore

$$Abs \cong -1.711E-4(t_{min} \cdot Vol_{oil}) + 1.361$$

the reference solution here is 3 ml of isopropanol w/ 100 ul ~~and~~ betadine.

It could also be 3 ml H_2O + 100 ul betadine if oil was water soluble, but it is not!

So the general model is

$$Abs \cong a(t_{min} \cdot Volume_{lipid}) + b$$

$\cong$ oil

The slope of this line will relate to the iodine number

The slope of a lipid with iodine no = 0 is zero. the slope of a lipid with iodine no. 114 is -1.711E-4

x	y
Slope	Iodine No.
0	0
-1.711E-4	114

Therefore, iodine no = -666277 · Slope

or ~~Iodine~~ Slope ≅ $\frac{\text{Iodine No}}{-666277}$

Therefore

$$Abs \cong \frac{\text{Iodine No.}}{-666277}\left(t_{min} \cdot Vol_{oil(ul)}\right) + b$$

Therefore

$$\text{Iodine No} \cong \frac{(Abs - b)(-666277)}{t_{min} \cdot Vol(ul)_{oil}}$$

where b is the absorbance of the control solution.

Iodine No. Model based upon Δ Absorbance vs t & vol.

Lets test this.

@ t = 20 min, v = 110 ul, abs = 1.102

Abs. of Control = 1.312

Iodine no ≅ 66 a bit low it seems.

Lets adjust model to.

$$\text{Iodine no} \cong \frac{666 \cdot 2 \cdot 11 (Abs_{Control} - Abs_{meas})}{t_{min} \cdot Vol_{oil(ul)}}$$

w/ assumed Control Solution!

Now choose t = 35, vol = 205, $Abs_{Control}$ = 1.312 Abs_{meas} = 0.195

Iodine no ≅ 104 Quite good it seems.

So a longer time of measurement will give a better result, most likely.

Try Coconut oil. Δ Abs = 0

Therefore the Iodine no for Coconut oil ≅ 0.

Actual = 7. The model gave fine under measured circumstances. Now therefore have a reasonable model for estimating the iodine number of an oil based upon an absorbance differential.

Try a scenario w/ coconut oil with

$t = 15$ min $V = 100$ $Abs_0 = 1.345$ $Abs = 1.333$

Iodine no $\approx \frac{666.77}{15 \cdot 100}(0.012) = 5.3$ Spot.

This is excellent. You can see how a differential and integral methods can be applied to other problems.
As it is, you can take an average of several readings, but longer time intervals will normally be more accurate.

Now, lets look into the iodine-alcohol reaction which produces a yellow complex.

Does iodine increase solubility of a lipid or oil?

Very interesting. The yellow color reaction of providone-iodine and isopropanol is not known widely on the net. This is a surprise and it can readily be tested.

However, a youtube video shows it readily. The topic is known as Solvatochromism, ie, a substance showing a different color in various solvents.

The topic is known but it does not appear to be highly researched. Youtube has some work from Cornell.

The change in color apparently depends primarily upon the polarity of the solvent. Alcohol has a different polarity than water. Question: Which is more polar, isopropanol or water?

Isopropanol is less polar than methanol & ethanol
Water is stated to be more polar than isopropanol because Isopropanol has non polar carbon to - carbon bonds.
Also only one OH dipole in isopropanol, but two OH dipoles in water. Makes sense.

Therefore if the lipid does not dissolve in alcohol you can use yet another solvent.

These suggests that the more polar the solvent, the greater the red shift of the spectrum of iodine dissolved in that same solvent. I wonder if that holds.

There is a great lesson here. The solvent for colorimetric work does not have to be water.

However, many other solvents will damage the plastic cuvettes. Glass test tubes do not seem to be measuring properly in the VIS spectrometer; this is definitely complicating matters. Let us test the problem.

Glass tube varies below 400 and above 900 but it may be possible to use the useable range w/ rubber band or scrith

You must also not use any readings < 0.10 but you see that you can indeed use glass tubes. This will open up solvents.

We all now that even isopropanol destroyed the cuvettes overnight.

Ok, we do have an interesting result though. Even though oil is insoluble in water, with iodine added it still reacts w/ the iodine and does decolorize it. It does however leave the solution opaque which will make measurement more difficult but not impossible. We can, however, see that water is the solvent for the iodine test even with oils.

So lets go back to our methods.

Lemongrass w/ water + iodine control.

Δt	t_{total}	ΔV	V	Abs.

Before we proceed to an oil - iodine
decolorization problem we need to investigate
the stability of Betadine color in H_2O
overtime.
The color does change from orange to a
deep yellow over several min. We have,
after ~ 5 min a peak of ~ 425 nm.
& A = 1.360 (3 ml H_2O, 100 ul Betadine)
We will monitor the over time.

It has shifted to 418 nm @ A = 1.305
so there is indeed change going on w.r.t. time.
413 nm, 1.282 still change.
412, 1.231
~ 10 min 409, 1.164

So we can see that iodine is vly problematic
as an colorimetric indicator.
Can anything be done to stabilize the problem?
406 1.089

This is highly unacceptable. It can not
be used. Alcohol is also damaging the cuvette.

Ah Iodine in water turns perfectly clear if you wait long enough. Then is a significant issue.

Try adding acid & base.

Base ~~Acid~~ turns it immediately clear.

Acid! ~~Base~~ we shall see. Great news.

!Acid ~~Base~~ seems to be holding the color. Then is very promising. It looks like it is holding stable and retaining the full spectrum of color or a red orange color. Yes it is working, that's great. No disturbance in the spectrum.

You had it Completely backwards.

Acid (1 drop TM HCl in 3 mL H_2O with 100 ul Betadine added) STABILIZES THE COLOR! (not base!). This is great.

It also is very sensitive to the reaction w/ oil. Max absorbance is @ 423nm but it is a very broad absorption from 400 to 450 nm.

The solution turns opaque white in reaction to decolorizing the iodine.

With excess iodine the solution goes from opaque white to opaque yellow.

It will be easy to detect completion. Absorbance will go to an extreme value (eg 3.0) because of opaqueness. It will not go back.

You now have a method that will work. You might want to choose the colored iodine as the reference.

The test is now very sensitive. You have a problem with scaling of it.

10 ul of lemongrass oil completely negates the colored spectrum. Therefore the iodine is completely consumed by a very small amount of lemongrass oil.

Now, how to scale the property.? You cannot measure less than 10 ul oil.

Since the absorbance value of the Iodine Control is in a good range, it seem like you would have to dramatically increase the volume of Iodine of Control solution. eg

100 ml of H_2O

$\frac{100 \text{ ul Iodine}}{3 \text{ ml } H_2O} = \frac{X}{100 \text{ ml } H_2O}$ $X = 3.3$ ml Iodine

That is quite a lot of Iodine to be using.

What if you diluted the Emogram in alcohol?
eg 100 ul in 3 ml of alcohol?

$\frac{3E-3 \text{ l}}{100E-6 \text{ l}} = 30$ not enough

100 ul in 10 ml of alcohol

$\frac{10E-3}{100E-6} = 100$. Ok, this is more reasonable.

Ok, this is good.

More ~~calculations~~

a

May 02 2017

Projects:

1. Continuing w/ the iodine number titration. A complete recalibration is required after understanding how acid affects iodine color stability. Recall also the paper we found using an iodine - acid indicator solution, but it was testing for what? IT was testing for nitrites. "A simple Colorimetric method Screening of Nitrite using iodide in an acidic pH solution" Austin Journal of Analytical & Pharmaceutical Chemistry

I developed my own method Nov 2014 Shanmugan w/ acid + CuSO4

Look @ notes for Mar 19 2017, notice difficulty w/ acidified iodine & protein testing, However I believe that then we with elemental Iodine crystals, not betadine. Interesting if there is an important difference. acidified betadine seems highly stable w/ respect to color & light

From Mar 18-19 2017 Notes it certainly appears that we were investigating protein detection - Cincentration w/ acidified iodine and that we had some problems which led us to abandon the assay in favor of the development of the dye modified Biuret reagents developed.

In retrospect, we must now wonder if there is any difference between the use of iodine crystals VS Betadine.

We certainly know that iodine is highly reactive to many organics. The problems faced include light sensitivity and lack of linear response w/ concentration. We can be on the watch for these and see if these problems can be circumvented. Cobalt catalyst w/ betadine seems highly achievable w/ dilute acid. Next we can re-investigate the linear response issue.

—

Now we continue, back to the iodine number issue. First step is now to prepare a highly dilute solution of lemongrass oil. But if you dilute it in alcohol, how do you know that the alcohol is not skewing the spectrum more than the oil is?

You would need to run a difference plot between
iodine + acid + alcohol
VS iodine + acid + alcohol + lemongrass oil.
the small amount of alcohol & the alcohol lemongrass ratio is the prospective problem here

You must acidify (1 drop 1M HCl + 3 ml H_2O + 100 ul Betadine) the water BEFORE you add the betadine or you will have a skewed reference solution

Additional problem: Your cuvettes are being destroyed by either the alcohol, the iodine, or the lemongrass. You can not afford this problem. You cannot keep the solution in the cuvette except short term.

Let's now prepare the dilute oil-alcohol solution. Use 100 ul of lemongrass oil in 10 ml of alcohol.

Lemongrass Trial:

Iodine Control Abs 426	Δt	t	ΔV	Vol	Alcohol + Ref Abs 426	Alc. + Ref + Oil Abs 426
1.605	0	0	0	0	~~1.54~~	~~1.5~~
	5	5	20	20	1.57	1.546 (Visible Oil Droplets)
	5	10	20	40	1.572	1.496 Visible
	5	15	30	70		
	5	20	30	100		

The a very successful test. We learn many things from the developed protocol. Foremost, Iodine (betadine) reaction in H_2O should be measured @ ~ 500 nm, not 426 as the is where the orange - red peak is (actual value = 502 nm) We also learn that alcohol or lemongrass or providora damage the cuvettes so it must always be highly diluted eg 100 ul in 10 ml H_2O. Also gonna calculate of dilution of lemongrass by a factor of 100 was perfect.

Alcohol + Red Abs 502

Alcohol + Ref + 0.1 Abs 502

Iodine Reference 502

1.124

The data set will be substituted w/ a set of data @ 500 nm vs 426.

Before we collect the data @ 500, we can also evaluate the effect of the 100 ul addition of isopropanol & the iodine reference solution, esp. @ 500 nm.

Providence Dilute Reference Abs_{500} = 1.124

Providine Ref + Alcohol (100 ul) @ 20 min = 1.040

So there is some effect. It is not huge, but it is barely visually detectable. It can optionally be figured into the comps but it can likely be ignored also. .10/1.124 = 8.9% error. Probably the alcohol should be taken as the reference.

[redacted]

This is the basic data collection format for determining the iodine no. of an oil

Iodine Reference $Abs_{500} = 1.124$

Δt	t	ΔV	V	Iodine + alcohol Abs_{500} (ref)	Iodine + alcohol + Iodine Abs_{500} (oil)
0	0		0	~1.100	~1.100
5	5	20	20	1.089	Ø.923
5	10	20	40	1.089	Ø.560
5	15	30	70	1.213	Ø.158
5	20	30	100	1.040	Ø.043
				(x=1.106)	

You can see that the effect of the alcohol is actually fairly minimal (esp in relation to the reaction w/ the oil. Therefore we can safely ignore the result, especially if we will take a reading of oil + alcohol @ $t=0$.

For our previous work, we now investigate the relation:

$t \cdot V$		$Abs(oil)_{500}$
0.0	0	1.100
5.20	100	Ø.923
10.40	400	Ø.560
15.70	1050	Ø.158
20.100	2000	Ø.043

This is clearly an exponential relationship. It is not linear.

$-1.644E\text{-}3\,(t \cdot V)$

$Abs \cong 1.058\,e$

$r^2 \cong Ø.994$

Ok, this has now become very interesting. We have a clear exponential relationship.

Conditions are:

Reference solution: 3ml H_2O + ~~100 ul~~ 1 drop dilute HCl BEFORE adding 100 ul Betadine.

Sample solution: 100 ul Lemongrass oil dissolved in 10 ml 91% Isopropanol alcohol.

Protocol: Measure time, vol, absorbance values over time. Time · Vol is actually an integral function.

We have a reference iodine value for lemongrass of ~114.

We know that the slope of this regression will proportional to the iodine number.

$$\text{Iodine No} = K \cdot 1.058e^{-1.644E-3(t \cdot v)}$$

We may choose the end point $t \cdot v = 2000$

$$K = \frac{114}{1.058e^{-1.644E-3(t \cdot v)}}$$

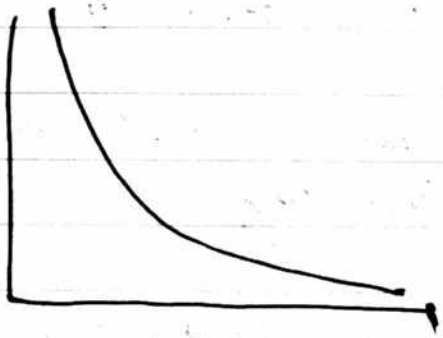
$$K \simeq 2886.55$$

Therefore, for the particular scenario of lemongrass

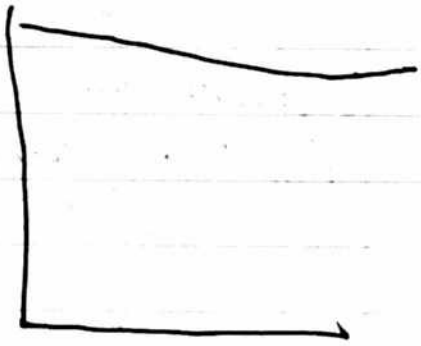
$$\text{Iodine No} \simeq 3054.0e^{-1.644E-3(t \cdot v)}$$

This is not true.

High Iodine No.



Low Iodine No



It is not the slope that is proportional to the Iodine No, it is the integral of the function that is proportional (inversely proportional) i.e. the smaller the integral the larger the integral. so our general function is:

$$Abs = a e^{-(t \cdot v)} \quad \text{or} \quad y = a e^{-x}$$

the integral of the function is:

$$y = \int a e^{-x} \qquad \int e^v dv = e^v$$

$$= a \int e^{-x} \qquad v = -x \quad dv = -1$$

$$\text{so } a \int e^{-x}(-1) = e^{-x} \cdot a \cdot -1$$

Therefore our integral of $\int ae^{-x} = -ae^{-x}$

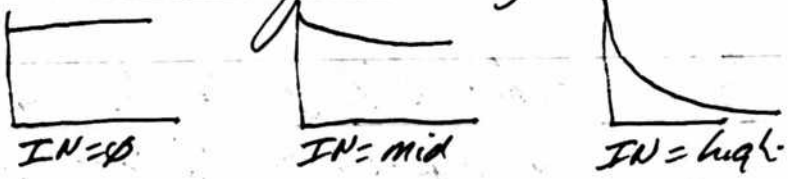
So what we really have is

Iodine No = $k(-a)e^{-x}$ but since k is an arbitrary constant (inversely proportional) we actually simply have

Iodine No = ~~ka~~ ke^{-x} or

$$\text{Iodine No} = K\int_{t_1}^{t_2} e^{-(t \cdot v)}\, dt$$

Two conditions, Iodine No = ∅ & Iodine No = 114 (eq)
Solve for two conditions in a linear form. (May not actually be linear.



The integral of our equation $1.058\int_0^{2000} e^{-1.644E-3(t \cdot v)}$ = 2112.52

If the Iodine no. was ∅, our integral is $\left(\frac{1.058}{1.100}\right) \cdot 2000 = 2200$

So	Y IN	X Integral
	0	2200
	114	619.53 ~~2112.52~~

Iodine No = 158.7 ~~1.303~~ · (Integral) + ~~2066.9~~ −.072

This is wrong, our integral should be ~625 yes, 619.53 This is our relation.

Ok, now we have it. We have a very reasonable and realistic manner of estimating the iodine number.
The only element I see @ this point is that the integral transition is not likely linear and you should probably form the exponential regression on the following data

Integral	Iodine No
Ref abs. time (eg 1.100×2000)	0
619.53	114

fit to $y = ax + b$

~~fit to $y = ae^{bx}$~~

2200 = as fit to this data & exponents

~~$\text{Iodine No} = 26981 e^{-8.824E-3 \cdot \int_{t_1}^{t_2} f(x)}$~~

where $f(x)$ is the absorbance function of the sample (a reference solution as far as I see).

No, this is far too draconian. A linear function will indeed be more realistic.

$\text{Iodine No} = ax + b$

CDB Culture Sheen Analysis:
Ref. Absorbance Prior to Sheen Addition = 1.113 (500nm)
We have taken from the top of the test tube undiluted sheen.

t·v	Δt	t	Δv	v	Abs_{500}
0	0	0	0	0	1.113
100	5	5	20	20	1.073
500	5	10	30	50	1.060
2250	5	15	100	150	1.050
4000	5	20	50	200	1.007

Our regression here is: $-1.988E\text{-}5\,(t{\cdot}v) + 1.088$ $r^2 = .80$
In this case our base integral for $\int_0^{2000}$ is $1.113/2000 = 2226$

$$\int_0^{2000} f(x) = 2136.2$$

So we have

Int	Iodine No.
2226	?
619.53	114

so Iodine No $= -.071 \cdot \text{Integral} + 158.0$

Therefore our iodine no. estimate is

$$-.071(2136.2) + 158 = \underline{\underline{6.3}}$$

This indicates a highly saturated "sheen" to the CDB layer.

Recall that this is undiluted "sheen" layer of the COB. Also understand that the sheen layer itself is likely to be highly diluted as it is mixed w/ the culture broth. Regardless of either scenario, the test results here indicate that the sheen layer is very likely that of a a highly ~~un~~ saturated oil. This would be similar to that of coconut oil, which has a iodine no. of 7.

Now the question is:

What is known about saturated lipid (oil) production by bacteria?

We have the characteristics of:

1. Gram negative
2. Saturated lipid production in culture broth
3. Unsaturated lipid existence internal to membrane
4. Oxidizes iron
5. Ferments alcohol] Facultative
6. Generate protein directly under prolonged incubation
7. Paper on bacteria theirs.
8. Filament production in sufficient media
9. Acidic environment for growth.

Engineered?

10. Phospholipide present
11. Biofilm production (polysaccharide & amine composition)
12. Coccus form
13. Degraded by Vit C & Citrate
14. Three amino acids likely identified w/ in isolated proteins.

Many, many properties & structural features known.

In reading bacterial field guide, it is mentioned that some of the films produced by bacteria, esp iron and manganese oxidizing bacteria (such as this) are METALLIC films, not oil films. This therefore causes me to use the iron detection / concentration kit which range from 0.05 - 2 ppm. On the surface, where the film is, our sample measured @ 1.75 PPM. Deeper in the solution it measures ~2.0PPM. If it is a metallic film layer the hypothesis is that the concentration should be higher than in solution - this may be a viable hypothesis. Remember ionic iron is added to the cultures to begin with so we are certain to have iron. Also, if a metallic film it should "break up". We did not see this but indeed I thought I observed this earlier.

Therefore...

The upshot is that I still am not certain if we have a metallic film (incidentally this would not be ionic, which is best suited to the test kit) or on a saturated lipid (oil) film.

What about the lipid emulsion test?

May 03 2017

The goal here is to determine a simple colorimetric test for alcohol.

Iodine is definately reacting with alcohol.
It reacts w/ lipids to turn the Iodine colorless.
With alcohol I believe it will turn the solution yellow. Recall that the Iodine is acidified w/ dilute HCl.

A visual change is already detectable w/ 91% Isopropanol, 30 ul. By 60 ul in 3 ml of control HCl - Iodine the solution has changed from dark orange to pure orange.
It is definitely sensitive to concentration.
By 250 ul it is yellow orange.
By 350 ul it is mostly yellow.
By 450 ul it is yellow.
We therefore have a good test for alcohols w/ acidified Iodine (Lugol's).

Now determine controls and test our cultures.

Our peak yellow freq is occurring @ ~ 425 nm.
The reds and oranges disappear.

These are non destructive methods that are being developed. The BC 30 is, unfortunately, destructive. Now, what characterizes the reaction is not an increase in the measured absorption @ ~425 nm but the severe reduction of frequency absorption @ ~~510~~ 500 nm.

The control - 10 day absorption @ ~~510~~ ~500 is 1.207
The absorption after significant alcohol addition is Ø.690

Therefore it is reduced almost by 1/2.
This is when the control measurement needs to take place. The BC 30 has a pellet @ 50 nm and then will suffice.

t·V	Δt_{min}	t_{min}	ΔV	Vol	Abs ~~510~~ 500
0	0	0	0	0	1.117 ~~1.54~~ 1.64
50	5	5	10	10	1.076
300	5	10	20	30	1.06
1200	5	15	~~30~~ 50	80	1.019
3600	5	20	100	180	Ø.952
9500	5	25	200	380	Ø.861
23400	5	30	400	780	Ø.629

BC 300 will require 1. adequate sample volumes
2. some flexibility of λ
3. limited regression capabilities

Now we have with ~~UV~~ VIS-NIR

	additional kit methods
proteins (total)	
lipids - iodine number	Phosphorus
polysaccarides (starch)	Oxygen
amines	Silicate
alcohols	Magnesium
nitrites	Iodine, Iodide
ammonia (w/ test kit)	Calcium
nitrates (w/ test kit)	Potassium
iron (w/ test kit)	
[albumin]	

$-2.295E\text{-}5(t \cdot v)$

Our equation is: $Abs_{500} \cong 1.070e$

$r^2 = 0.985$

This is a good formula.

We now measure the culture level C various t (10, 20, 30 min)

Culture broth

t·v	Δt	t	ΔV	V	Abs_{500}
0	0	0	0	~~LHT~~ 0	1.117
2000	10	10	200	200	1.087
4000	10	20	200	400	1.032
18000	10	30	200	600	0.951

$Abs_{500} = 1.111e^{-8.739E\text{-}6(t \cdot v)}$ $r^2 = .996$

Let us start thinking about how we use the data.
We have w/ 91% Isopropanol:

$Abs_{500} \cong 1.070\, e^{-2.295 E\text{-}5\,(t \cdot v)}$ $\qquad r^2 = .985$

We have the same critical value for absorbance of the reference HCl-Iodine solution @ 1.107.
We will not always have the limit it should be close.

In our first measurement, we have a tv of 2000.
But we also have a fixed v of 200 ml
and a fixed time of 10 min.

The theoretical absorbance of the actual reference isopropal solution is

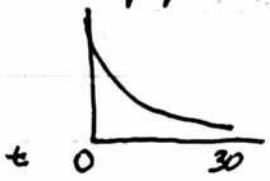
$Abs_{500} = 1.070\, e^{-2.295E\text{-}5\,(2000)} = 1.022$

but we measure 1.087 so we know that we have alcohol >0 and more than 91% Isopropanol. This is reasonable.

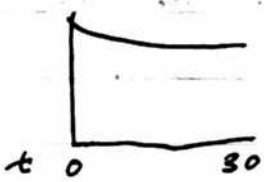
Another thing we are willing to live with are reactions that have been developed are too complex for Beer's Law. That method is relatively complex the instantaneous reactions. Your reactions and methods here w/ iodine especially involve kinetics.

Ok, now we look @ ratio of integrals for the Concentration estimate

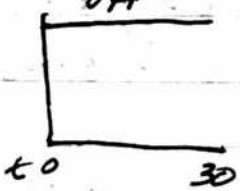
91% Isopropyl



Culture



NO OH



$$\int_0^{30} 1.070e^{-2.295E\text{-}5(Ev)}\,dt \qquad \int_0^{30} 1.111e^{-8.739E\text{-}6(Ev)}\,dt \qquad \int_0^{30} 1.117\,dx$$

32.089 33.326 33.51

$\frac{32.089}{33.51} = 95.759\%$ $\frac{33.326}{33.51} = .994509$ 1.000

$\frac{33.51}{} \approx .957594$

91% alcohol

$1 - .957594 = .042406 \Leftrightarrow$ 91% alcohol

$1 - .994509 = 5.491E\text{-}3 \Leftrightarrow x?$

$\frac{5.491E\text{-}3}{.042406} = .1295$

The means our culture solution would have an expected concentration of $.1295\,(91\%) = 11.78\%$ equivalent of Isopropanol. This is a highly significant and interesting result.

The appears to be a perfectly viable method of determining the existence of alcohol within the culture as well as determining the concentration of it.

Summary: We have
1. nitrites
2. alcohol
3. bromfion
] summary paper is worthwhile - damages & needed

a fermentation process is, itself, highly significant.
Oxidation & fermentation w/in the same organism.
facultative.

Between nitrites & alcohol, the metabolic rate (along w/ glucose content) can easily be ascertained.

You have done more w/ colorimetric data than you imagined at first.

Another major objective is that of sugars, simple sugars such as sucrose, fructose & glucose. Let's work w/ sucrose first. I believe you may have made some progress earlier?

We want

fats	protein
sugars	carbohydrate

On Mar 27 2017 we have initial signs of a successful sugar detection reagent that was discovered. Let us revisit this.

1. RIT emerald dye
2. 3 ml H_2O
3. 4 drops conc. NaOH
4. 2 drops $CuSO_4$
5. variable tartaric

??? The test does work but at a mild difference.

A shift from blue green towards blue. Moderate sensitivity stated.

Emulsion test for lipids also valuable
1. add ethanol to solution then add water.
lipids cause it to turn cloudy white.

The lipid emulsion test for lipids (lipids, alcohol + water) has failed. This is important.

This indicates that the "sheen" on the top of the culture is

1. not oil based, eg a metallic film candidate
   or
2. Very low concentration such that it is not detectable by the emulsion test.

Our contradiction comes from NIR that shows CH_3 present. We must repeat that test.

—

The sucrose test does work but it is definitely not real sensitive. Work on this."

3 ml H_2O
10 drops conc NaOH
1 drop $CuSO_4$
visible + tartaric
100 ul emerald dye

We have
Clypping.
Too strong

3ml H_2O
5drops NaOH
4 drop $CuSO_4$ 100ul $CuSO_4$
VIS + Tartaric
50 ul dye

We do have a change in absorbance @ 600 but not a shift yet. There is a major reduction in absorbance @ 600nm.
Also our solution remains way too strong.
The sugar is indeed more blue
The Control is bluegreen.

3ml H_2O
3 drops NaOH conc
40ul $CuSO_4$
VIS Tartaric
40 ul dye

600 nm is indeed bluegreen.
The sugar is reducing the
Component sharply &
should be shifting it toward
blue, ie 590.

Ok, this is looking better. It is producing a rich bluegreen. It is sensitive to a small amount of sugar. But the end product is still way too dark and it must be diluted by at least half.

What if we drop it down like more?

3 ml H_2O
1 drop conc NaOH
20 ml $CuSO_4$
4S Tartare
20 ml emerald dye.

The reaction is indeed ~~a drop~~ an increase in absorbance @ 595 nm.

Actually it increased from 0.34 to 0.39.??
It is visible by eyed.
Yes 595 is indeed blue.

So the bar worked very well. Still too strong though

We can see that the blue absorbance @ 595 nm. Either of these 2 solutions work

3 ml H_2O	3 ml H_2O
3 drops NaOH	1 drop Conc NaOH
40 ml $CuSO_4$	20 ml $CuSO_4$
40 ml dye	20 ml emerald dye
4S tartare	4S tartare

However it is still too dark & gets clipped and required serious dilution of 0.4 ml into 2 ml H_2O (Ratio of 5)

So lets try to lighten up on the NaOH.

3 ml H_2O
3 drops 1M NaOH
40 ul 0.5M $CuSO_4$
40 ul Straight emerald dye kit
vis tartaric

Ok, the solution worked very well and detected a very small amount of sugar.
It is however too dark/still and it must be diluted by a factor of 3.

You also, however get a very sharp strong solitary peak @ 595 nm so it is very easy to measure. With only a few grains of sugar (sucrose) Abs_{595} jumped from 1.413 to 2.162

UH OH! Actually it did drop. repeat and add more sugar.

Ok, there's a problem.

The test developed is useful for detection
BUT IT IS NOT USEFUL for Concentration.

Absorbance change (decrease at 595 nm) when exposed to sugar but it does not vary w/ concentration.
The severely limits the value of the test.

You may need to go to Benedicts after all, cloudy solution, not easy to work with.

Benedicts reagent:
1. sodium Carbonate
2. sodium Citrate
3. $CuSO_4$

Opaque range upon heating, this is a major disadvantage.

Is there a colorimetric test for sugar beyond Benedicts reagent?

We also know that we have a glucose meter & that work electrochemically. Does this mean that there are voltammetry methods that might work? What are the different requirements for fructose, glucose & sucrose? Iodine works w/ polysaccarides.

How would you breakdown sucrose into glucose? Acid?

May 04 2017

Today we work w/ sugars. Seeing colormetric reactions.

Sugar Chameleon reaction:

Solution 1: Very little $KMnO_4$ in 50 ml H_2O

Solution 2: 6 gm sugar, 10 gm NaOH in 70 ml of H_2O.

Scale down to 10^{20}.

Pour Solution 1 into 2.

Purple → blue → green → yellow orange → clear w/ settling of MnO_2.

$MnO_4^- + e^- \rightarrow MnO_4^{-2}$

purple + green = blue

$MnO_4^{2-} + 2H_2O + 2e^- \rightarrow MnO_2 + 4OH^-$

green — brown solid (appears yellow)

Now for hydrolysis:

Hydrolysis is the chemical breakdown of a compound due to reaction w/ water.

So hydrolysis = decomposition due to, resulting from, because of, REACTION with water.

Hydrolysis occurs in many different ways. So how do you hydrolyze a disaccharide like sucrose?

Another way of defining that hydrolysis is defined is when a molecule of water is added to a substance, and that incidentally often may cause the substance and the water molecule to break into two parts.

So two different ways of interpreting and defining hydrolysis.

Hydrolysis of sucrose:

$$C_{12}H_{22}O_{11} + H_2 \xrightarrow{H^+} C_6H_{12}O_6 + C_6H_2O_6$$

D glucose D fructose

ie, by boiling w/ a mineral acid.

May 05 2014

Two new items/instruments have arrived.
ECG entertainment 3 channel, 12 lead
Brain wave monitor

The things on list for clinic season to:
1. VIS light spectrometer - portable
2. Biochemical analyzer - bc310
3. Ultrasound 2.5 - 5 MHz laptop
4. Urine analyzer - portable strip
5. ECG 3 channel, 12 lead
6. Brain wave monitor

Great capability established here.

Today we got the ECG running and clean data recorded on 3 sessions.
Analyses of strips and waves to follow.
At this point all previous data seems to fall w/in normal bounds.

The criteria of "general wellness" is to be applied to all research conducted.
No specific diagnosis is an objective.
Health research, in general.

Page 101

My data needs to be recollected

Some initial data:

P
Q
R
S
T

QRS duration 108 ms = .110 s
Normal range is .08 to .11 sec

QRS axis is ~ +9.5°
Normal range is −30° to +100°

1 mV = 10 mm
Each minor mark is .1 mV @ 50 mm/mV.
R amplitude = +.66 mV (V1)
S amplitude = −1.09 mV (V1)

PR Interval	.162 s	Normal .16	OK
QRS Interval	.122	Normal .08	bit long
QRS axis	+9.5°	Normal +70°	low

Look into left ventricular hypertrophy
QRS duration may be too long, indicating possible left partial bundle branch block.

1.09 + .01 = 1.10 < 3.5 mV
Voltage criteria do not indicate this problem.
RV5 + RV6 would be very high

I have the leads reversed.

ECG Analyses

We should have better data now.

X	Trial #	(1)		(2)	(3)	Norm
✓ 75	HR	71		78	76	60-100
93	P Duration	74		100	104	
✓ 96	QRS Duration	84		102	102	<100
172	T Duration	174		174	168	
✓ 166	PR Intervals	164		168	166	120-200
✓ 365	QT Duration	372		372	352	<440
411	QTc Duration	413		423	397	
16	P Axis	+29°		8	11	
4°	QRS Axis	+7°		3	3	-30 to +100
26	T Axis	42°		15	22	
.01	P Amplitude	.08mV	(V5)	.01	.01	
—	Q "	None		—		
1.34	R "	1.44	(V5)	1.32	1.25	
-Ø.38	S "	-Ø.35	(V5)	-.39	-.40	
Ø.18	T "	Ø.22	(V5)	Ø.17	Ø.16	

There is a favorable report. All heart primary intervals (5) fall within normal range. QRS interval is a little on the longer side and QRS axis is a little on the low side

May 07 2017 SUGAR TEST

Very good work on setup today. I have the spectrometer device & logging software working as of today. Some additional comparisons are to be made in the future.

Would like to 1. detect sugars
2. detect free radical or oxidative stress

We are using the Fehlings test. This is a simple method. Only requires CSO_4, NaOH & tartaric acid. I have all 3.

Control solution is blue. Sugar solution is more green.
Control: max absorbance @ 650-700
Sugar + additive shift of absorbance to ~612 nm. and a decrease in from 650 to 700

650-700 appears green blue
612 shifts to blue green. There is a dynamic change and shift.
Now we check control solution #2.
Control solution II shows the same shift upon heating.

We only have 2 detectable results with adding copper to the control, & they are both slight.

1. A slight increase @ 405 & 447 nm
2. A slight increase @ 942 nm, NIR range.

~942 is Ar-OH, ~930 is C-H

Ok, we definitely have the reaction. It turned a yellow green.

Yellow & yellow green are in 400-420 range so we may indeed have picked up something.

I added more NaOH & more $CuSO_4$ which gave a nice blue color. It shifted to yellow green.

We were correct @ the increase @ 447 nm.

There are 4 changes occurring
1. Increased abs @ 447 nm. Yellow
2. Increased abs @ 591 nm w/ sufficient concentration. Blue to Blue Green
3. Increased abs @ 907 nm. CH_2
4. Increased abs. @ 942 nm CH or ArOH

Of these, increased abs @ 447 nm is the most useful & most sensitive. The initial test @ low conc. of sucrose was able to pick this up.

Now we will refine the controls and test.
Use
1. 3 ml H_2O
2. 1 drop conc NaOH - KOH
3. 2 drops $CuSO_4$
4. Visible tartaric acid

+ HEAT! This is critical to the process.
Boiling for 2 min slightly.

Now set up these controls. The key here is yellow visibility (blue absorbance) @ 447 nm.

Now we are testing 3 different Concentration levels of sugar, low, medium & high against a Control of primary solvent @ 447 nm.

Heat is applied.

Here is what happened in our tests:

1. Low Conc. of sucrose - did have a very sharp increase in ABS @ 430 - 450 With increased absorption across the board dramatically higher from 400 - 550 nm. So there is a huge change here even w/ a low Conc of sugar.

λ	Control	Low Conc
430	Ø.19	Ø.65
450	.19	Ø.58

So there is a very noticeable diff.

Now what is interesting is that little to no reaction occurred w/ the mid-level concentration. No idea why. The major change is a mild increase @ 700 nm but we do not pick it up in the yellow range. Why?

On the high solution it is only a modest change, from .185 to .288 @ 450 nm.

There are therefore, some problems here. The test was not sensitive to concentration and it was MOST PRONOUNCED @ the lowest concentration. The truly does not make sense.

Repeat the test. (a new conc. reagent was)

3 D drops Conc NaOH - KOH
3 D drops $CuSO_4$ 0.5 M
vis. test (2x)
3 ml H_2O

We do have a problem w/ the method so far. Has stayed all blue. Very slight absorbance increase @ 450 & not sensitive to concentration. You saw the reaction once very strongly w/ direct heat, no water bath.

You need to repeat the test until you can reproduce the yellow color.

1 drop conc NaOH
2 drops $CuSO_4$ 0.5M
2x tartar
3 ml H_2O

We do less success for the steps but there are still some problems.

1. The test is moderately sensitive, I assume in the order of 200 ppm or so.
2. The solution does get opaque with the color change that introduces yellow. This causes problems w/ VIS spectrometry.

3. The middle strength solution did not react well, no idea why. Low strength and high strength sugar did work well 450 nm can be used, but mid concentration did fail.

4. Interesting that 380nm might be a potential indicator as well.
5. An increase is taking place @ 650 also.
6. Broad band increase in absorption also seems to be taking place.
7. You did not use water this time as you were investigating the heat levels required. Can do any so you broke a beaker so you cannot do this again.

I am not entirely satisfied w/ the method, however. It seems variable, opaque & not entirely reliable, and relatively insensitive.

I think we will investigate the hydrolyse - glucose meter method.

It is suitable for modest detection.

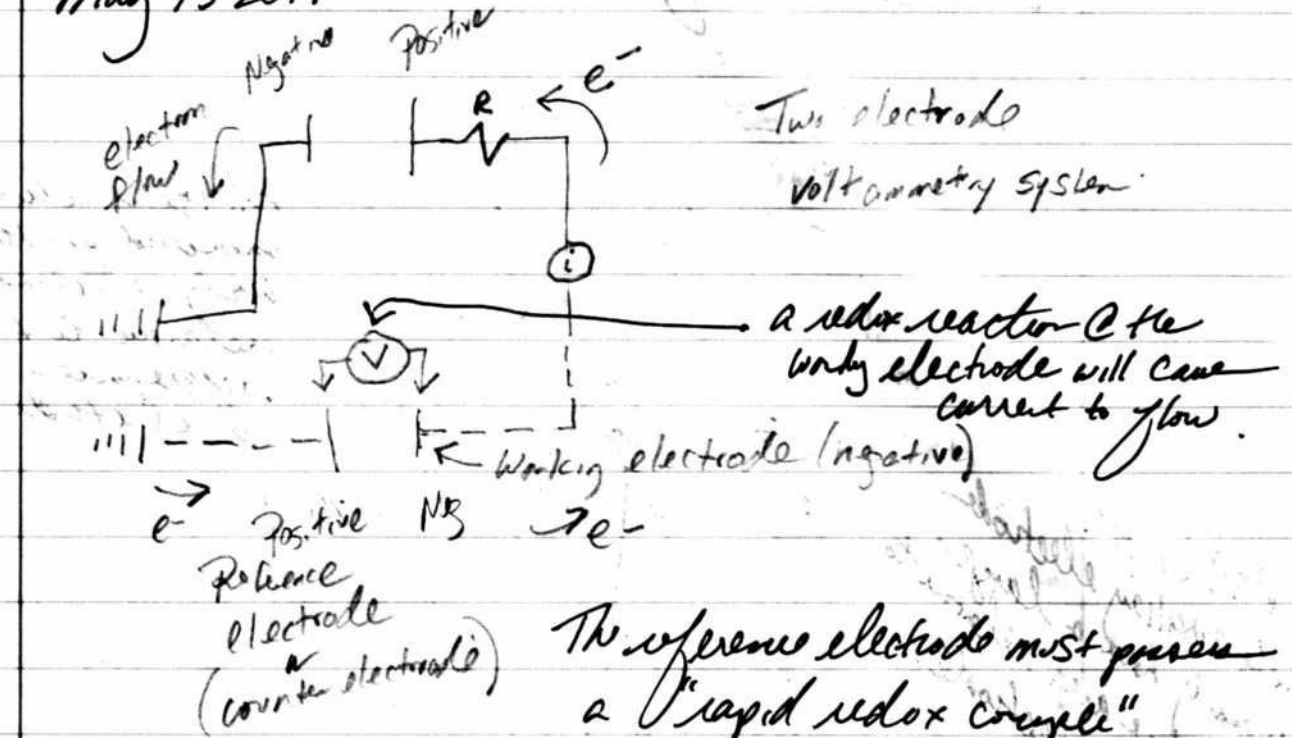
May 08 2017

I found a paper that states a yellow colorimetric reaction take place on sugars & oligosaccharides & polysaccharides with the use of Conc. sulfuric acid & phenol. I have 0.7M H_2SO_4 & 90% phenol but I cannot get the reaction to occur with either sucrose (disaccharide) or glucose (monosaccharide), even w/ heat applied. I am not sure where they are coming from except for when acid being @ a high concentration ie 95%.

I also find reference to the problem of no suitable chromophore for carbohydrates, ie sugars, being available. Would be great to discover one...

I also do have to wonder about volatility and colorimetric would be easier.

May 13 2017

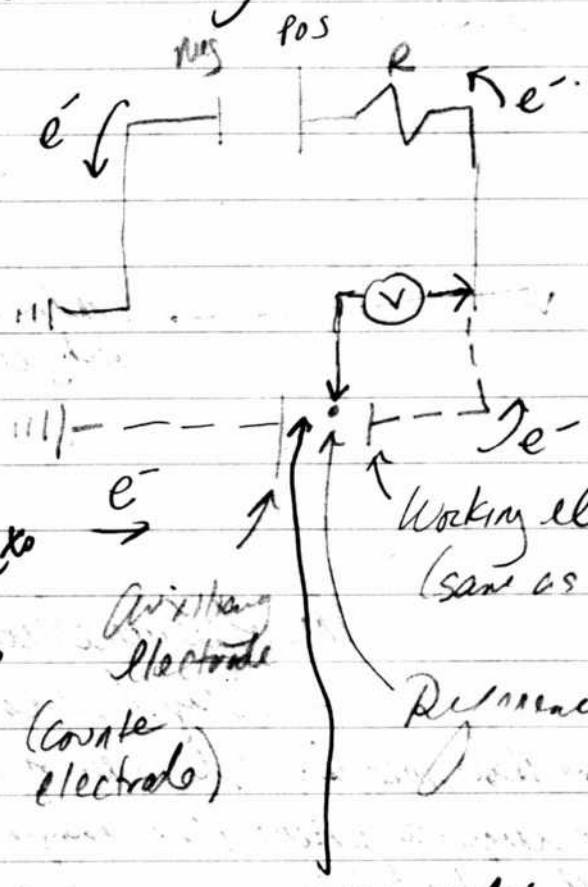


The reference electrode must possess a "rapid redox couple" (whatever that means). The area of the reference electrode is kept large to ensure that changes in current cause only small perturbations in current density.

Big problems when current is high & resistance of the solution is high since the "iR" drop" becomes a problem. Ohms $I = \frac{E}{R}$ or $E = IR$ current flow causes a voltage drop, & as you can see high current & high R causes a large E, or large voltage drop. What happens to your battery voltage when you play in [redacted] laptop?

Note that in a microelectrode system, & where very low currents are involved, that a two electrode system actually can be used w/ success. (down to picoamps) Faraday cage may be required.

Three electrode system:



Voltage is always measured at the working electrode relative to the reference electrode.

p99 Smyth
the auxiliary electrode usually has a large surface area relative to the working electrode.

current to flow to both electrodes.
But here, the auxiliary electrode provides a "sink" for current flow. The auxiliary electrode establishes the system, therefore, even with higher current flow a higher resistance.

I now understand the electrode fundamentals of voltammetry system.

May 14 2017

I love the Palmsens memory today.

I do not have my previous notes.

I recall only partly my conditioning routine:

[-3,3] E Conditn = 0

[3,-3] E Conditn = 0

I believe I also forced a condition, eg

[-3,3] or [3,-3] E Condition = +3 or E Condition = -3 but I do not see the additional condition as causing any change or producing additional data this time. The first two trials seem sufficient. Also recall that you use a differential plot, not a y=f(t) plot.

The other thing I am doing this time is making a large surface area auxiliary electrode.

[-3,3] E_0 = -3

[-3,3] E_0 = +3

[3,-3] E_0 = 3

[3,-3] E_0 = -3

} The was our Consideration set. We reduced it to 3

t_{equil} = 10 Scan rate = 0.3

E Step = .005

t_{pulse} = .008

There is no difference between

$[-3,3]\ E_0 = -3$

and $[-3,3]\ E_0 = +3$

Therefore one of these is redundant.

This may not be exactly true.

$-.62V$ .69 O_2, H_2

$-1.57V$

$-.65$

-1.56

$[-3,3]\ E_0 = \phi$ This stabilizes $C_{-.82}$

Cl^-, OH^- ?

Do not run in derivative mode!

x_1 [-3,3] $\bar{x}_0 = -3$ $t = 5$ sec!

Using 5 trials for stabilized mean

Stabilized $\bar{x}$ ~~-.89~~ -.87

x_2 [-3,3] $\bar{x}_0 = +3$, $t = 5$ sec

The stabilized $\bar{x}$ ~ the same spot, ie -Ø.83

$\bar{\bar{x}} \approx$ -Ø.85

Now

[-3,3] $\bar{x}_0 = 0$, $t = 5$ sec

The stabilized $\bar{x}$ -Ø.87

So this is very close to the mean.

Now where, if:

		Stable $\bar{x}$:	
	[3,-3] $\bar{x}_0 = Ø$ $t = 5$ sec	-.72	??
x_1	[3,-3] $\bar{x}_0 = +3$ $t = 5$ sec	+.86	
x_2	[3,-3] $\bar{x}_0 = -3$	+.83	

Notice that we have two points of symmetry

			V
Preferred	[-3,3] Eo = -3	t, 5sec	-.87
	[3,-3] Eo = +3	5sec	+.86
	[-3,3] Eo = +3	5sec	-Ø.83
	[3,-3] Eo = -3	5sec	+Ø.83
Also	[-3,3] Eo = Ø	5sec	-.87
	[3,-3] Eo = Ø	5sec	-.72?

For symmetric sets, we have a mean of

Ø.85

Notice $HO_2^- + H_2O + 2e^- \leftrightarrow 3OH^-$ @ Ø.88

This is the only one that seems plausible. Notice how close this is to the preferred set.

This is indeed an electrocatalytic redox reaction within alkaline aqueous medium

Iron PDF:
Oxygen Reduction on Graphite & Carbon

"It seems that all carbon materials have some electrocatalytic activity towards ORR in alkaline solutions"

Electrocatalytic Oxygen Reduction Reaction (ORR)
Chaojie Song and Jiujun Zhang

Our reaction is an ORR in alkaline aqueous solution involving carbon electrodes.
There is a good fit.

They list this reaction @ +0.867
This is extremely close to the mean of our preferred set @ 0.865.

We have determined w/ distilled water we can pick up an Oxygen Reduction Reaction. We must presume that the distilled water is slightly alkaline.

Now, to our 2-3 ml of distilled water we have added 10 ul of 1M HCl.

We now also assume that our preferred set is
[-3,3] Eo = -3V t=5sec
[3,-3] Eo = 3V t=5sec

You are also testing stability of the solution by no stirring to stirring stirring.

Strong stirring has made a big difference. You must mix thoroughly.

You can see the current increase w/ bubbles a magnitude of graph. Much greater current flow.

Stable
[-3,3] Eo = -3V t=5sec -0.82

3,-

Notice that current of distilled water is 1-2 mA
Notice that current of dilute HCl is ~ 17 mA.
Notice the magnitude of y' for distilled H_2O is ~0.8
" " " " " " Dilute HCl is ~ 60

One candidate reaction is
$ClO^- + H_2O + 2e^- \leftrightarrow Cl^- + 2OH^-$ is +0.81

Now we have a very interesting and active curve
from $\begin{bmatrix} 3 \\ 1 \end{bmatrix}$ $\begin{matrix} -3 \\ 6 \end{matrix}$ t=55sec

Activity @ +1.35 ? Ba Cr? Ti? } These are
+1.08 ? Br, I? N_2O_4? } intriguing.
-1.28 $ClO_2 + H^+ + e \leftrightarrow HClO_2$ +1.27

Now what we get is an observable macro min peak
(not derivative) @ -1.28
With the derivative, the sharp gradient is split
midway @ -1.26
start ≈ -1.19 (too early)
end -1.30 (too late).

Do I have Iodine contamination in the HCl? It is possible

So you have a lot of activity by adding the HCl. You have to ask if that really is necessary. You should seem sensitive enough without it?

Now you add very dilute $CuSO_4$ + H_2O with no acid added.

You should be aware of the electrode & Conditioning when in water before you move on to next sample. We have a ~~template~~.

Stable

[-3, 3] $E_0 = -3$ $t = 5$ sec ~~-0.27~~

macro peak ~~+1.53~~

Electrode must be aligned properly as you set it up.

-1.74

-1.24

[3, -3] $E_0 = +3$ $t = 5$ -2.14

Strong Peak $S_2O_8^{-2} + 2H^+ + 2e \leftrightarrow 2HSO_4^-$ +2.12

So the sulfate of $CuSO_4$ seem to definately be picked up on the [3, -3] run. There is a sharp strong peak. But I cannot say I see a Cu presence at the time.

We do have a nice smooth set of smooth curves.
Everything except SO_4 match seems to be a mono specie. What if we add more Cu?
We have now added more $CuSO_4$.

Conditioning the electrode is important before pursuing a sample.

Ok, when ya condition the electrode ya get an entirely different result.

[-3, 3] Eo = -3 t=5 -2.04
-1.76
-1.65
-.88

[3, -3] Eo = 3 t=5 +1.05
+1.35
+1.46

Now, since we forgot to condition the water, we need to select and condition a water range blank.

Water Control after conditioning.

I am not getting stable results.

		$S_2O_8 + 2H+ + 2e \leftrightarrow 2HSO_4^-$	2.12
large	-2.07	$O_3 + 2H+ + 2e^- \leftrightarrow O_2 + H_2O$	2.07
small	-1.71	$H_2O_2 + 2H+ + 2e^- \leftrightarrow 2H_2O$	1.77
small	-1.56		
large	+1.03		
large	+1.49		
large	-.78		

I see problems. I see no advantage in the new electrode. Revert to original design.

	-1.97	$S_2O_8-- + 2e \leftrightarrow 2SO_4--$	+2.00
	-.89	$HO_2- + H_2O + 2e \leftrightarrow 3OH-$	+0.88
?	+1.07	$2SO_3-- + 2H_2O + 2e \leftrightarrow S_2O_4-- + 4OH^-$	(-1.12)
	+1.62		

Ok, we seem to have a method back
1. Use the regular Ø.9mm electrode
2. Condition w/ Potentiometry in water
3. Read water w/ Normal Pulse Voltammetry as a blank
   [-3,3] E_0 = -3 t=5
   [3,-3] E_0 +3 t=5 set in ~~red~~ black

3. Now Progress to sample until repetition
   [-3,3] E_0 -3 t=5
   [3,-3] E_0 +3 t=5 set in ~~red~~ red.

Read significant red value in derivative mode

The work presents some difficulties.
I do not see copper detected.
I see some things that I cannot explain.

$Cu^{2+} + 2e^- \rightleftharpoons Cu$ @ Ø.33
Why do you not see this?

Can you reduce copper ion to copper by applying a voltage of +.33 Volts?

Testing water Control blank again.
No water is not stable.

Condition w/ acid & dry again.
3ml H_2O + 20 ul 2M HCl
Condition w/ Pot for 120sec
Current is high w/ HCl added, then is expected.

The Control electrode itself is not stable. I am not sure what goes here.

OK, we have learned some lessons

1. Little to no acid will be required. You have plenty of current.

2 The broad overlay electrode DOES seem to stabilize the system. You have 3 coincident plots on both

[-3, 3] Eo = -3 t = 5
[3, -3] Eo = 3 t = 5

for a H_2O - 10ul 1M HCl Control Solution

Our peak with HCl - H_2O as

small +1.93 ?

small −1.40 ?

large −.65 $ClO_2^- + H_2O + 2e \leftrightarrow ClO^- + 2OH^-$ +.66

small +1.22 $ClO_3^- + 3H^+ + 2e \leftrightarrow HClO_2 + 2H_2O$ +1.21

small +1.46 $HClO + H^+ + 2e \leftrightarrow H_2O + Cl^-$ +1.48

$2ClO_3^- + 12H^+ + 10e \leftrightarrow Cl_2 + 6H_2O$ +1.47

large +2.09 $O_3 + 2H^+ + 2e \leftrightarrow O_2 + H_2O$ +2.07

~~$Cl^{+3} + 3e$~~

There was a struggle but it looks like I have excellent results w/ Chlorine reaction showing up all over the place in the literature. We also had a verified result with water alone ORR reaction. It appears the wood auxiliary electrode was extremely beneficial.

We will now head toward the use of this and the construction of a unified electrode.

May 17 2017

Voltammetry Continuance.
The electrode has been improved to incorporate a higher surface area auxiliary electrode.

I do not believe that acid is required, 4 shed current labels will be sufficient.

You have reduced the stress on the electrodes w/ more flexible leads.

You are to remember to Condition the electrode prior to sample records. 0.5A applied under potentiometry for 120 secs until stable.

Water blank will be subtracted out.
The flexible lead idea came to be most better.
Reference H_2O:
[-3,3] $E_0 = -3$ to 5
Initial values are
-2.8 2.79
+1.06
[3,-3] $E_0 = +3$ to -5
+0.41, +1.86

It is possible that you have some contamination (eg soap) in the water blank, but this can still be removed.

What we do see is that we do indeed have stability with the modified electrode arrangement & flexible leads so helpful considerably.

Now lets add $FeSO_4$.
Our sample is fairly dilute.
Moving the electrodes deeper has destabilized the initial result.

What we noticed here is that setting the electrode deeper into solution stabilized the result very quickly.

Our sample may be too dilute —

We only have new peaks @
-1.78 (small) $H_2O_2 + 2H^+ + 2e \leftrightarrow 2H_2O$ $+1.77$
$+0.86$ (small) $HO_2^- + H_2O + 2e \leftrightarrow 3OH^-$
$+2.86$ (large) Ca(?)

Now we will add more sample.
We add 20ml directly to sample tube.

The envelope stabilizes very quickly here w/ deep electrodes.

Now we have a stronger signal. We have

Actual peaks @

	−1.78	(strong)	Hydr Same as before
+1.45	−1.47	(weak)	Unknown, Cl?
+0.62, 0.64	−0.63	(moderate)	Cl again?
+0.77	+0.79	(moderate)	$Fe^{3+} + e \leftrightarrow Fe^{2+}$
1.27	+1.26	(moderate) (strong)	Cl again!

Ok, this is very interesting. We have definately picked up the iron this time

We estimate 3ml of H_2O

$$\frac{3\ ml\ H_2O}{20\ ml\ Fe\ Solution} = \frac{3E\text{-}3\ l\ H_2O}{20E\text{-}6\ Fe} = 150\ \text{Dilution Factor}$$

Now, I believe we have a 0.5M Solution
therefore we detect a $\frac{0.5M}{150} = 3.33E\text{-}3M$ Solution.

Now, this is not real strong, but it is still valuable.

1M Solution = $FeSO_4 \cdot 7H_2O$ = 325.2 gm/mol

Fe is 17.2% of solution so

Fe = .172 (325.2 gm/mol) = 55.93 gms/liter

and 3.33E-3 (55.93 gms/liter) = 0.186 gms Fe/liter

$\frac{.186 \text{ gms}}{1000 \text{ gms}} = \frac{X}{1000{,}000 \text{ gms}}$ $\quad X = 186$ PPM

I suspect I could easily also detect ~~the~~ ½ of the level so I estimate that I am good down to ~ 100 ppm in ionic solution detection.

Now let's go to $CuSO_4$.

Condition electrode first.

You have learned that electrodes must be set deeply into solution.

The paint on the pencil is introducing some contamination into the work. You must unless have a clean electrode (Carpenter pencil).

With the $CuSO_4$ sample we have peaks @:

Actual		
+1.77	−1.80 (strong)	$H_2O_2 + 2H^+ + 2e \leftrightarrow 2H_2O$
	−1.40 (weak)	Cl @ 1.45?
	−1.24 (weak)	O_3, O_2, H_2O?
+0.15	−.16 (weak)	SO_4? $Cu^{++} + e^- \leftrightarrow Cu^+$
	+.89 (moderate)	HO_2, H_2O?, $Cu + I$?
	+1.24 (weak)	
	+1.39 (strong)	

There are no matches w/ the blank of H_2O.
We also have symmetry @ $\pm$ 1.39 − 1.40
$\pm$ 1.24

Actually we do have it, it seems.

$Cu^{2+} + 2e \leftrightarrow Cu^+$ +~~0.46~~ 0.15

Should this not be:
$Cu^{2+} + e^- \leftrightarrow Cu^+$???

This is therefore a difficult element to detect. Notice that it is also a weak signal.

Cu, however, was very difficult to capture. But we have done it, with possibility of SO4 detection.

Elemental detection of unknowns, however, is incredibly valuable.

Now, lets look @ sugar (sucrose)

Look @ H_2O blank first. We have

-1.94 S(trong)	Consider pencil
-1.53 W(eak)	paint contamination
-1.37 W	in this process.
-0.20 W	
+1.04 W	You will also want to
+1.16 W	clean the electrode
+1.51 strong	in the future.

With sugar, in comparison to the blank, we only have 2 additional weak peaks:

weak	-1.78	Possible ORR reaction. H_2O_2 @ 1.77
weak	+1.44	Possible Cl or I reaction.

No definitive unique profile seen w/ sucrose.

It is unsure that we have any redox reaction take place w/ success that can be observed.

Let's look @ the culture.
With the culture, we identify the following peaks separate from the H_2O blanks:

Actual		
+2.12	−2.16 (s)	HSO_4
	−1.45 (w)	Cl or I
	−1.19 (w)	Cl or I
Φ39 − Φ.30	+Φ.34 (m)	$Fe(CN)_6^{-2} + e \leftrightarrow Fe(CN)_6$
	+Φ.51 (m)	$Cu^{2+} + 2e \rightarrow Cu$, Cl, CN again
	+1.10 (w)	Cu, S_2O_3, $Fe(OH_3)$, HO_2
	+1.39 (s)	Cu, I, SO_3
	?	

AC Voltammetry.
I am now attempting AC voltammetry.
Very interesting results w/ sharp peaks that reproduce.
H_2O sample settings:

[-3,3] $E_0 = E - 3$, $t = 5$

$E_{step} = 0.15$	Sharp peaks @
$E_{ac} = .05$	-2.85 (s) Ca?
Scan rate = 0.1	-.90 (s) HO_2^- (ORR)
Freq = 800 Hz	-.60 (s) Cl?
	+1.34 (w)

Also an interesting curve upward @ +2.21...

Now let's add Fe SO4.
Fe should involve 2.07, 1.90, 0.77, 0.36
-.04, -0.44, -0.56

I am getting some very reproducible curves but
I do not see how to interpret them.
Now the curve is smoothly out and lacks
some detail.

I may be picking up some detail now.
-1.90

I am picking up a very interesting course.

inflection points, min, max, etc?

-1.88	1.90
-.24	-.28
-.54	+.31
+1.00	+.59
	+1.86

Also notice the inflection points @ ~2.07

If you look @ the key picture you get

-1.90		1.90
-.27	Symmetry here vs	.36
+.59		.56
+1.87		1.90

These are a powerful indicator for Fe

AC Voltammetry is very intriguing. It is slow to pick up the detail you need.

I think continuing it with differential NP voltammetry would provide your greatest chance of success.
Your curves are very smooth & reproducible.
A scan rate of 0.03 to 0.05 V/sec seems acceptable. Stability does take a while. I have about 12 curves inverted.

Again:

-1.95	1.90	Two points
-.54	.56	of Symmetry here
+.19		@ .56 & 1.90
+.60	.56	
+1.31		I also have a
+1.96	1.90	point @ 0.00

I have now picked up. Thanks.
Sharp peaks likely indicate an overload of some. You are after a smooth curve to the degree possible. Overloads are not good.

Remember, there is only the upslope so far. You still need [3, -3]

Ok, I have worked the [3, -3] series. AC voltammetry combined w/ diff NP voltammetry look to be a very powerful combination.
You are definitely picking up Fe in soln.

Discontinuities must be disregarded and avoided w/ AC voltammetry. You can narrow the range accordingly to isolate these sections.

Actually the discontinuities might be really important but the graph process does not always want to show them.

Tightening up the range can be very useful e.g. with Cu [-2, 2] I picked up a few points right away. Smooth curves are indeed best.

May 10 2017

New electrode w/ Carpenters pencil for auxiliary electrode has been developed.
Running potentiometry conditioning routine
Our test involves a $FeSO_4$ & a $CuSO_4$ mix

Water Control gave the following:
-2.58 V(w)
0.33 V(w)
0.47 V(w)
+1.63 (VW)

With $CuSO_4$ & $FeSO_4$ mix we have: w/diff NP
-1.76 (S) H_2O_2 1.77 Notice Symmetry @ 1.50
-1.56 (W) No good match?
-1.50 (W) No good match? MnO_4 1.51
-1.02 (S) (M) No good match?
+1.31 (S) No good match?
+1.51 (S) No good match? MnO_4 1.51

What is happening here?

We have a problem here. What about we just pick up?

Now to AC voltammetry
[We will ~~reduce~~ to [-2,2]
[-3,3] parallels [-2,2] so there is favorable.
We get

- 2.40 Mg 2.37? +2.36

-2.16 SO_4 2.12 +2.50 ?

-1.69 Mn 1.69

-1.49 Mn 1.51

-.06 Fe -.04

+0.43 Fe -.44

-.79 Fe .71

-.61 Mn 0.60

Notice Fe has been picked up 3 times.
No Copper?
Mn Came in strong w/ diff NP also. Why?

1. A real curiosity here. We were able to pick up Fe w/ AC voltammetry, 3 signals. But not Fe w/ diff NP. Actually, it a diff AC Voltammetry key used.

2. Second, we pick up Mn on both diff NP & AC Volt but we did not add Mn. Is there a source of Mn in the pencil? Either the lead or the paint? The can be tested using all three leads as cylindrical.

3. Very interesting that Cu was not identified w/ either method. This raises another key question as to why not.

You do not want overloads, this means too much current. Sample requires dilution in that case

May 19 2017

Today we work on the manganese my study.
Switch the electrode to a unique set.

If Mn was on the electrode at the point of the pencil, why does it not show up in the sample blank or well? The only explanation for its subsequent appearance would be an interaction between Mn and (Fe or Cu).

—

You can use your reg plated electrode (broad auxiliary) also as a comparison test you did have some overload so we could reduce the Cu - Fe concentration.

First step: Condition electrodes.
First off, let's compare H_2O control between Carpenter pencil & reg pencil.

Our conc. level for sample will be 2ml Cu & 20ul Fe in 6 ml H_2O to try and reduce current level.

What we see first is that the control for H_2O w/ the Carpenter pencil does differ significantly from the regular pencil H_2O control.

Regular pencil:

-1.97 (s)	Notice our symmetry here
-1.62 (w)	@ ± 1.51
-1.50 (w)	also we have +1.33 (vs +1.31)
-0.53 (vw) (m)	and there is congruency w/
0.93 (m)	pencil control w/ Carpenter
1.33 (w)	pencil sample with Cu & Fe
+1.53 (s)	added! So there are

indeed some real questions here.

Now add sample.

Now w/ Cu + Fe added (20 ul / 6 mL) notice the current level is much higher (5 uA).

Notice we also had 1.62 w/ Carpenter pencil control w/ H_2O so we have a great deal of overlap taking place that is not allowing any discernment in between control & samples.

Our 2hrs/6 ml H_2O of $CuSO_4$ + $FeSO_4$ now gives us the following results:

	−1.49 (s)	Mn? (1.51)
	~~−~~	Cl ions around 1.47 − 1.48
	−1.32 (w)	No good match
	−1.27 (w)	O_2 (1.24) Mn (1.23) Cl (1.27)
	−.88 (m)	HO_2^- (.88) Cu + I (.86)
SO_4 (1.12)	+1.16 (s)	Mn (1.15), Cl (1.15) Mn (1.19)
	+1.43 (s)	No good match (I_2? − 1.44)

The general magnitude of the ~~the~~ ~~pe~~ produces much higher current (~15 mA vs ~2mA w/ control) and the makes sense.

There is uncertainty. No good pickup of sample constituents here. Now lets go to the AC volt.

You are definitely measuring something here but what exactly are you measuring? Why the appearance of Mn & Cl ~~as~~ candidates?

With ACV, there is always a sharp dropoff around +2V, I wonder why there is.

An average of +ACV diff curve yields:

-2.46 ?

-2.22 H_2 (2.25)

-1.52 Mn (1.51) Cl (1.48) Mn (1.56)

~~-1.94~~ -0.20 SO_4 (.17) Cu (.15) SO_4 (.20)

~~+2.66~~ +.28 ?

+.64(?) Cl (.66) I (.62)

+1.00 I (.99)

+1.20 Mn (1.19) SO_4 (1.12)

+1.90 Fe (1.90)

Our candidates are therefore
Mn, Cl, (SO4) (Cu), I, & (Fe)

We are having much better luck we are seeing w/ diff AC voltammetry than with DiffPP.

We have a reasonable candidate list w/ 3 out of 6 candidates accurate and 2 out of 3 metals identified.

You would then need to develop secondary verification tests for the candidates, voltammetry would not be sufficient in this case.

I do not know when the strong Mn signal is coming from across the board. Does pencil lead contain Mn in any way?

Our dilution ratio is

~~20E~~ $\frac{6E-3\ H_2O}{20E-6\ Cu\ or\ Fe\ solution} = \frac{300}{}$

1M $FeSO_4$ = 55.93 gms/liter
but we have a 0.5M solution so 27.965 gms/liter
but our dilution ratio is 300 3.107
so we have .093 gms/liter

$$\frac{.093 \text{ gms}}{\text{liter}} = \frac{93}{1E6 \text{ gms}} \cong 100 \text{ PPM}$$

so we appear to be detecting @ ~ 100 PPM level

I would like to know what is happening w/ diff NP.
Our candidate list there is:
Mn, Cl, Co, I
so we miss SO_4 & Fe
It is a start but AC voltammetry diff seem best.

Now we need to use our regular electrodes.

Averaging our curves in ACV did seem beneficial.

Also the regular pencil electrodes seem to perform somewhat better than the Computer pencil.

Notice however, that Mn showed up in the control sample. We must run a trial w/ the small pencil leads.

I have learned that my $FeSO_4$ solution has been oxidized and is no longer in the Fe^{+2} form. No wonder she has complicated detection. I assume Fe^{+2} will be much easier to detect than Fe^{+3}.

We are now using the uniform D.9 mm electrodes.
Remember to set electrode deep into solution!

We stabilized VERY QUICKLY w/ the H_2O control on the upswing! That is great.
Also stabilized quickly on the downswing. Good. The electrodes were conditioned. That also stabilized quickly & smoothly.

Now we go to sample. 40 ul Cu & Fe in ~~6~~ 6 ml. H_2O. This is ~ 200 ppm.

The deionized and current are very clear. Now let's look @ results.

Diff NP Results w/ Cu + Fe (Oxidizer)

Fe 1.9	1.82 (s)	Interesting results here.
Cl 1.48 Mn 1.51	−1.49 (w)	Very clean behavior under
I2 1.44	−1.38 (w)	all circumstances
Cl .66		
O2 .69	−.64 (m)	
I2 .62		
SO4 .93	+.96 (m)	Symmetry again w/ 1.49 & 1.50.
?	+1.33 (w)	Ø.64 repeats also.
?	+1.36 (w)	1.33 repeats
Cl 1.48 Mn 1.51	+1.50 (s)	.93 repeats vs .96

Now ACV: We also see more stable behavior here w/ no sharp drops @ ~2V.
Adding on ACV to NP did not show the graphs properly. You must apparently keep the methods separate.
ACV also stabilizes very quickly.

−2.63	Mg (?) 2.68	Use next page for average of results
−1.95	Fe +3 1.90	
−.68	O2 (.69) Cl (.66)	
−Ø.12	Cu Cl (.12)	Cu (−.08)
+.46	SO2? (.45)	Fe (.44)
+1.61	Cl (1.61)	Mn (1.56) Al (1.66)

Averaging 3 good curves does help.

Refined and averaged list for [-3, 3]

-2.61 (m)	?	
-1.97 (s)	Fe (1.90)	
-1.23 (w)	O_3, O_2 (1.22-1.24)	Mn (1.23), H_2O, OH
-.71 (m)	O_2, H_2O_2 (.69)	
-.25 (m)	SO_4 (.20)	
+.39 (m)	SO_3 (.40)?	
+.73 (m)	O_2 (.40)	
+1.61 (w)	Cl (1.61)	Mn (1.56)
+1.86 (w)	Fe (1.9)	
+2.15 (w)	SO_4 (2.12)	Cl (2.10)

[3, -3]

-1.86	Fe (1.90)	(1.86+1.06+1.97)/3 = 1.80
-.99	I, N, Cl?	SO_4 (.93)
Fe (.56) -.56	Mn (.55)	Cu, Cl (.54) Cu (.52)
+.006	Fe (.04)	
+.98	I, N, Cl,	SO_4 (.93?)
+2.57		

Candidates are

Fe (n=4) SO4 (n=2) Mn (n=3) Cu (n=2)

We now approach the sugar problem (sucrose)

First: Condition each sample

Next: Diff NP H_2O sample control

We look result to here.

Notice however that they differ from previous H_2O control

NP Diff:

H_2O Control (Current)	H_2O Control (Previous)
-2.37 (s)	-2.58 (w)
-1.01 (w)	.33 (w)
0.61 (m)	.41 (w)
1.40 (s)	1.63 (w)
1.42 (s)	

Now lets look at CV diff: H_2O Control

You have now run a diff CV plot of sucrose. You have 3 runs from $[-3,3]$ and $[3,-3]$ averaged & compared to H_2O control.

The main observation is that H_2O control has a min @ -1.38V.

With sucrose, the min shifts to -1.66V so a discernable shift is observed.

Inside the [-3,3] dip AC plot
there is also a shift, even though
the curve has overloads to clay peaks.
Nevertheless, there is a discrete shift from

	-.498		-.902
to	-.804	and to	-1.194
Δ =	.306		.292

and our shift of decay [3,-3] is
-1.38
to -1.66
Δ= 0.280 on the smooth curve

And we see between all of them we have
an avg shift of -0.295 V
and this may indeed be a characteristic
of a sucrose solution that is repeatable.

Ya now, for the first time, would
like to investigate electrical impedance
spectroscopy.

We are also using the diff AC Voltammetry a much discerning than Diff Normal Pulse. It seems to offer the diff in the Advantages.

It looks like very good success w/ Electrical Impedance Spectroscopy of the sega solution vs the water control. There is a very discernible difference between the two solutions @ -1.5V, the point of significant shift (approx location) of the diff AC Voltammetry curve.

May 20 2017

Continuing w/ Electrical Impedance Spectroscopy (EIS)
We need the equivalent circuit software.
We start today by looking @ the test sensor and the settings. We get good & expected results w/ the test sensor.

Next we run a potential scan w/ the test sensor for a range of $[-2, 2]$, $\Delta = 1$ with a freq. range of 1 to 10kHz.

We can see that EIS is modeling all reactance as Capacitive reactance. It does not seem to be any inductive reactance in the process or the model or the mathematical result.

When our phase angle is very high, you know that capacitive reactance is playing a large role in the circuit and that resistance is playing a very small role.

When the phase angle is low, you know that resistance of the circuit is playing a stronger role and that capacitive reactance is playing a much smaller role.

A Bode plot will show you the relative amounts of these two factors, resistance and capacitive reactance, as a function of frequency.

The tells you how the circuit is reacting to a signal (ie, frequency).

You have learned a lot here, and it all relates to your radio study that you have taken or the last year.

Next you need to learn how voltage applied relates to the circuit behavior, and how they relate to current flow when $E_{DC} = \emptyset$ or $E_{DC} \neq \emptyset$.

Notice the difference between E_{DC} & E_{AC}!!! you generally will want a small value for E_{AC}.

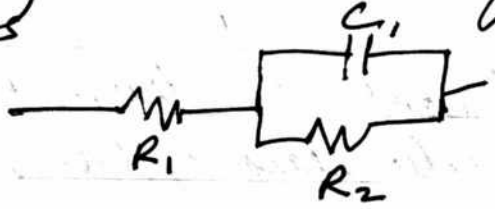
Now we work towards sugar (sucrose) again.

f Hz		Z_a	-Phase Angle
9709	H_2O	5.475E4	26.38°
9709	High Sucrose Conc	1.311E4	9.65°
9709 Hz	Low Sucrose Conc	8.341E3	5.05°

There is also a shift of the peak phase angle.

H_2O	~ 32.5 KHz
High Sucrose	~ 50 K Hz
Low Sucrose	> 50K Hz

We now have fitted simulation models developed for the circuitry of our H_2O & Sucrose EIS work. The program used is EIS Spectrum Analyzer, as referred to by Palmsens. Only one open source freeware program located, from here it is $ 2000 a pop, such as the Commercial program ZView. We are lucky here. Our model form, per Palmsens is



1° 6639
50° 11004

	R_1	Err%	R_2	Err%	C	Err%
H_2O Control	4146.5	~~50%~~ 6.55%	5161	.58%	2.36E-10	2.6%
Low Sucrose	2016.9	0.72%	6639.2	.24%	4.81E-10	12.7%
Hgh Sucrose	2752.5	1.24%	11004	.30%	4.85E-10	2.73%

Basis for Concentration Studies

What do we observe? R_2 looks like our best indicator for concentration studies. R_1 is the ohmic resistance by the electrolytic solution. R_2 is the resistance due to the electron transfer of the faradic reaction. C_1 is the capacitance by the double layer of the working electrode.

May 23 2017

I am back @ the lab!

Wallace Idaho

The lab may end up moving this year (Moab UTAH?) but for now it remains

Full Steam Ahead! for the summer of 2017

We acquired some new instrumentation over the last winter season, even while on the road:

1. A Urine Analyzer

2. An ultrasound machine

3. A Biochemical Analyzer (ie, a production based colorimeter)

4. An electrocardiograph (ECG instrument)

And today, the first day back, my savings established about a year ago, I have purchased a

5. UV-VIS-NIR spectrometer 190-1100 nm

This will be the first time the lab has full UV capability & also a long overdue Chemical instrumentation. Have made all of the possible @ approximately 1/3 to 1/4 the cost of US instruments. And they are indeed capable.

6. A brain wave measurement instrument. Incredible capability w/ the right software (found it for free) @ $100.

—

It is definitely time to regroup for the summer.

With the stalling of re[redacted] process, CI must now move forward with the Legacy Project and advancing the scientific front & distribution of information.

A Primary Objective: CI hope to acquire DNA test results. This has been possible for several years (Given sufficient funding & protection of IP) but since the process has been blocked, the work will be attempted independently & within CI.

There are, of course, more of other projects but these will be paramount.

Steps

1. Attempt extraction of Jamelian DNA sample, eg tomato, etc

2. Progress to mice again (it has been done twice before over the years) extract the CDB DNA.

3. UV (first time capability) will be used to determine DNA purity.

4. Initial sequencing of the DNA:

Projects (1)

There are innumerous projects, in addition, on tap.

1. Release of ICMP test results
2. Ear wax samples
3. Received samples from citizens
4. Electrolyte colorimetric tests
5. WP paper revised, lit. on lead, prostate etc
6. Official record interviews
7. Compile & summarize IR data
8. Culture protein (aerobic anaerobic) analysis — Important Topic
9. LC work would be good
10. EIS Electrical Impedance Spectroscopy would be good. AC voltammetry also, NPV
11. Develop GC further
12. Laboratory notebooks scanned & ~~returned~~ — In progress
[redacted]
~~14. What is polymer formation as an w/ pipette?~~ — Observed
15. GC separation of diethyl ether
16. Headspace analysis further developed — In Process
17. Truck & supplies unloaded
18. HEPA filter air analysis
19. DNA / Lab projects?
20. Re water pollution set transmittance Colorimeter ~~tests~~.
21. Oxidate test

How about tomato and banana first?
Then Potato.

I have acquired

1. onion
2. tomato
3. banana
4. potato

I have also acquired my note of Nov 03 2014 which describes and declare the method of CDB DNA Production. (V016)

General method is (eg banana)
1. 100 ml sample
2. 4ml OX clean detergent
3. 3 gms salt
4. Two full scoop of enzymes (microscoops)
5. Pulse on blender to break up. keep to a minimum
6. ~~Blend @ low speed for 30 sec~~, Strain, Cool down
7. Adjust temp proportionally for density of sample.

Pulse to Coarse Puree add minor water as required

Cold alcohol method

Nov 03 2014

Our method of DNA extraction seems simple, straightforward & reliable.

1. Measure 100 ml of sample/water combined. Adjust properly.
2. 4 ml Oxi Clean detergent (4 gms)
3. 3 gms salt
4. Two full scoops of enzymes
5. Pulse the sample to break it up in the blender. Keep this to a minimum.
6. Blend @ low speed for 30 sec

In other samples adjust blend time by ratio:

$$\frac{\text{Volume of sample} + 30\text{cc}}{100\text{ml}} \times \frac{\text{Density of Sample}}{\text{Density of Banana}}$$

Do not overblend, it breaks up the DNA into smaller filaments & eventually particulate debris.

Question:

Can we try DNA on the COB today? ?

I think we have enough to try.

Maya success today!
COB DNA Extraction.
Verified
Nov 03 2014

Method:

1. 50 ml COB (approximately a 3-4 week old culture)
2. ~~Dawn~~ 2 ml Oxi clean detergent (2 gms)
3. 1.5 gms salt
4. 1 full microscop general enzyme.
5. Blend on low speed for 2 minutes
6. Strain
7. Cold alcohol
   Let it set in a half hour
   Maya success!!!

2nd batch not as successful as the 1st batch but still ok and usable
Suspect not enough enzyme?
Maybe blend! Less - 30 sec
It is building up ok eventually.

Try:

1. 50 ml COB
2. 3 ml soap
3. ~~1.5 gms~~ 2 gms salt
4. 2 Scoops enzyme
5. Blend ~~30 sec~~ ~~45~~ sec on low ~~30~~ 45
   Next time Blend 1m 15 sec

I am not sure that straining is actually necessary. A mga Complex is forming w/ the raw filtrate.

You have left to let Consumption take place. There is essentially nothing left except for what is floating.

It might be a DNA Complex VS pure DNA.

It seems to me that the og anion has a very high DNA Content.

Whatever you pour off eventually appears to be transforming itself to a DNA Complex.

Even the 30 second first run is producing volcanoes of a nice light color.

Ok, I do have onion DNA being produced. It is not high volume but it is visible upon defrosting. You might use onion to improve your technique. There is a question on the amount of blending. I used 30 secs. Suggest a trial w/ 15 secs & 60 SECS

Letting the samples set for a while. Maybe it was microscope & salt also. No, it says 3 gms. That is substantial.

The trial ya only pulsed the onion until it was broken up. No sol'd blending for 30 secs, it pulsing kept to a minimum. The production of DNA was much more successful and DNA was visible immediately by eye. You also had remainder in a beaker and it also produced a highly visible sample.

Method was to place 3 ml of strained solution in a test tube and add 2 ml of cold cold alcohol.

--

You did NOT cool down the stained solution pot prior to the addition of alcohol this time. You added very cold alcohol to the stained solution immediately.

You probably have 5-10 times as much product w/ the second trial as the first.
COB may likely require longer blending however, I am sure that ya will need some heat.

May 24 2017 - DNA Extraction

Tomato today. I have saved onion DNA from yesterday.
Pulsing to puree (coarse)
Will let salt, enzyme, soap puree (coarse) set for 10 minutes today prior to straining.

—

The tomato DNA extraction today was highly successful. It may have been helpful to let the coarse puree set for 10 minutes before adding the alcohol. Also, as I recall, tomato is one of the easier species to work with.

Also, tomato DNA readily floated to the surface in the test tube and was very easy to collect. The bleach method was also highly successful and would have been sufficient by itself.

The tomato DNA does retain a pinkish color from the tomato. I recall the onion and earlier samples as well.

I have plenty of material to work with here to investigate the 260/280 ratio.

*

Carrot:
More pulsing required, slight water addition.
Will let set for 15 min before stationary.

[redacted] Application
rejected on grounds of
"the peer literature claims that the issue
is "delusional".
[redacted] international [redacted] filing
[redacted]

The carrot DNA is ostensibly much harder to collect, as I recall from before.

But it appears that in the end, it has been done very successfully.

The 3rd test tube sample failed. One of the weekend beaker samples (~20 ml) succeeded only moderately.

But one of the two beaker weekend samples (~20 ml) appears to have done quite well. The DNA appears to be of a much finer texture (what is the size compared to onion & tomato for example?) and it too absorbs the color, in this case, of the carrot.

I have a very good sample to work with. The blanks for 260 / 280 will end up being dilute alcohol.

Next is potato and last will be banana.
But for now, let's start looking @ 260/280
(actually 254/280) on the carrot sample
dissolved in water, kept to a minimum.

The tomato DNA sample does dissolve
nicely in water. Approx 1½ ml of solution
was used (ie, alcohol and DNA) and diluted
to approx 3.5 - 4 ml w/ H_2O.

We will construct the blank as
1 ml ethanol
2.5 - 3 ml H_2O
for the UV 260/280
absorbance test.
There could be some detergent in the sample
to cause a problem. You may have to rerun several
generations on alcohol.

Blank to Water = Ø
H_2O + Alcohol (3 to 1) 254 = 79.Ø @ Ø.1
Tomato DNA @ 254 = 102 @ Ø.5

Blank to H_2O = Ø
H_2O + Alcohol (3 to 1) 280 = 39 @ Ø.1
Tomato DNA @ 280 = 68 @ Ø.5

Pure H_2O reserve perfectly.

Lets compute ratio. Understand that 260 would be a little higher than 254.

254:

Blank 79 @ Ø.1

Tomato$_{254}$ 102 @ Ø.5 = 510 @ Ø.1

Δ = 510 − 79 = 431

200:

Blank: 39 @ Ø.1

Tomato$_{200}$ = 60 @ 0.5 = 340 @ 0.1

Δ = 340 − 39 = 301

$$\text{Ratio} = \frac{431}{301} = \underline{\underline{1.43}}$$

We desire 1.8 for pure DNA.

We understand 260 would be a little higher, possible 450-460

$$\frac{455}{301} = 1.51$$

This is our best estimate of our ratio @ this time. We may also have some interference w/ soap.

If the ratio is actually correct, it would indicate that we have about 80% protein and ~ 20% DNA in our sample. This is nevertheless a great accomplishment and can be used in various ways.

The question of soap + enzyme interference does exist and. This means that the DNA sample will be rinsed repeatedly in alcohol to eliminate the question. It does seem unexpected that my sample will contain protein of this magnitude but we will test this possibility.

Another question: Assume you do have both protein and DNA in the sample, how do you separate either the protein or the DNA?

Ammonium sulfate?

May 25 2016 — Potato Run; DNA

Potato appears to have worked also, but to appear of equal difficulty to that of Carrot.

You are letting the coarse puree sit for a good 15–20 minutes before straining and adding alcohol you have also used a Buchner–vacuum pump funnel system for straining the time as it is much quicker.

It does however, show how much detergent do in solution as you had considerable suds form in the vacuum process.

The DNA production in the 3 ml test tube is marginal and difficult to identify a separate clue to lack of volume production.

The residual header of ~20 ml strained material is not as the success story. The DNA layer take some time to develop in the colder alcohol – eg ~15 mins or so.

The DNA layer, however, as opposed to the carrot & tomato samples is a nice white color, so the looks good. It has a soft appearance with only a few but identifying and distinctive filament examples that protrude ~~into~~ w/ small bubbles from the primary layer of DNA @ the alcohol - H_2O interface.

UV spectroscopy will be very valuable here, that equipment is also long overdue.

The 3 ml test tube production is very limited but it too is workable.

For difficult materials, the beaker method (larger volume) does appear superior due to volume production.

The amount of alcohol added is ~ equal to the sample volume. Cold & slowly & carefully added along the inside edge of the container.

The Banana DNA Trial:

With respect to the banana DNA, the puree here was even a little too thick & was hard to pull through the vacuum. That sample retrieved, however, was highly productive.

The sample material was deluted w/ approx 50 ml of H_2O and the process repeated. The second vacuum run has also been highly productive.

Both sample use the blender method (~20 ml) and each sample is similarly productive.

The DNA in banana form a very substantial Cohesive layer and is easily extracted w/ a more coarse pipette. The sample is clean and abundant.

Banana is definitely the easiest sample to work with.

I now have 5 different reference samples to work w/ before attempting the CDB trial. All trials were successful with varying levels of production and possible purity variations. UV spectroscopy will be used to assess that situation.

The five samples are (DNA)

1. Onion — easy
2. Tomato — moderately easy
3. Carrot — apparently the most difficult
4. Potato — moderately difficult
5. Banana — easiest

Pulsing is only to the point of a coarse puree. Sufficient water for the blended samples to pour is desired.

And the question is, are we now ready for the moment of truth (again) with the real CDB DNA?

We do get to take a break, regardless ...

CDB Trial:

1st run:
Scan for 1 minute
~ 30 ml
1.8 gms salt
~ 3-4 ml oxiclean detergent
slight enzymes added
will set for 15 min.
no vacuum used, not enough mass

This project will undoubtedly be difficult. There are some small bubbles & activity in 1st tube but not dramatic.

It is difficult to say whether we have any success here or not. There are no visible strands or strong bubbling activity as I recall from earlier times. There is, however, a slightly colored layer that is forming @ the interface but it is unclear whether or not it will contain any DNA.

As the test tube stands it does appear that the culture stably is separating by a density gradient, not DNA based.

I find no direct evidence visually of this even after so far a 10 second run & then a 3 min run.

Trial 2: (2)

3 min blend low
Easy.
2 gms Salt
30 ml CDB Culture
set for 15 min

At the turning cream colored under these circumstances.

The flakes on top of the long term culture (re film) definitely appear to be metal flake production as described in the bracketed field guide.

I see no need to filter the blend.

I verify that the source culture does contain the CDB @ a manual level. Also I find a dominant filament immediately after the alcohol extraction. It is the classic CDB filament w/ internal CDB, it is NOT DNA.

Microscopic examination of alcohol extract shows no sign of anything being broken down.

Projects (2)

Additional Projects:

22. Analyze film on top of COB cuvette (Partially - looks to be metallic film)
23. Analyze detergent - alcohol - H_2O blank w/ UV
24. Look @ variation in IR between different DNA samples.
25. Brainwave project.

I suggest 10 min of blendy next.
Likely more enzyme as well.

Trial 3:

~15 ml CDB
~10 ml H_2O
1 gm salt
More enzyme
More detergent

Liquify on blender for 10 min, Blender is actually on low setting

The data not look promising. One curious, subject I saw before on a smaller scale, is the production of a small amount of floating material (brown) from a rather large initial sample (~25 ml) Microscopic examination required. No direct filaments observed but there is a relatively large diffuse layer above the interface in the blender samples.

—

I am not sure what we have in the diffuse alcohol layer. I have looked @ it under the microscope @ high power. It is definitely not the original culture, most of the material has definitely been transformed. It also has numerous tan patches w/ a purple color which is a

definite reaction has taken place. It does look like organic mass, it is not in the majority crystalline. I have let it dry with a drop on the microscope slide.

Can I verify its composition & compare it composition to known DNA such as banana? I would say that you have definitely broken something down.

Even the test tubes had the substantial layer - it is easily visible.

We may have something here to be looking @. UV spectroscopy would be helpful. I'm betting that even in the test tubes the top of the alcohol layer is slightly more darker.

The entire sample (culture) is not transformed but a large portion is, approximately 70%. Remaining 30% is in COB form.

It should not be too difficult to produce IR ATR spectrum with alcohol has been used.

UV to come later.

What about Index of Refraction w.r.t. DNA?

I do have an excellent spectrum of banana DNA on IR using the KCl crystal. With CONH I have the molecule w/ amines, amides, glycoside linkage (O-C-N) and phosphate ester. It appears to be a perfectly representative example w/ good resolution on the IR plot. The alcohol on the crystal needed to evaporate completely for the spectrum to settle down.

May 26 2017

I do have a problem on my hands. How to get the DNA from the COB? The bacterial form seems to present special difficulties. You can try a run with

1. Alkaline solution in addition to what we have tried. It does not look like a 30 sec label is going to be able to do anything?

2. Since ya have proven equivalency of the wire filament (grape etc) and the Vit C extracted filaments + the culture process, it may be most easiest to work w/ the wire extract filament network.

3. Ya probably need to get more advanced culture in place as soon as is possible.

There is no visible DNA on any of the samples under there for.

You can repeat the 260/280 ratio w/ the banana DNA since you know that it is highly pure. You can rinse a few times and bleach w/ alcohol.

We are starting cultures again. We need materials for DNA extraction w/ special emphasis upon filament development.

#1-8

8 cultures:
~150 ml H_2O
1/4 tsp $FeSO_4$
1/2+ tsp Brown Sugar
1 drop CDB
1/4 tsp liquified peeled potato

#9-10

2 cultures:
~150 ml H_2O
1/4 tsp $FeSO_4$
1/2+ tsp Brown Sugar
oral filament seed *
1/4 tsp liquified peeled potato

I now have 10 cultures underway; color is good. 8 are CDB based and 2 are filament based.

We also have one portion filament sample (oral) to work with but highly risky to attempt DNA work w/ it.

Since we can work w/ 15 ml now instead of 30, let us continue our trials w/ the CDB form.

You have a good system for normal DNA production but shielded a CDB product after our challenge. Would you rather try & obtain DNA from a seed or a fruit. Or seed is definitely more difficult, and you see w/ carrot also & roots.

Lets try a NaOH trial w/ one minute blend @ low speed.

	Trial 1	Trial 2
Use	10 ml CDB	
	1 gm salt	
	1 ml soap	
	1 scoop zymase	large scoop
	5 ml H_2O	
	2 drops conc. NaOH-KOH	3 drops
	1 min blend low	5 min
	Sit for 15 min.	

Less Disruptive

The reaction set appears to be definitely more disruptive than yesterday's trial. The surface of the culture (alcohol-culture) interface is certainly more textured. Also there is some bubbling that is persisting for a longer time. These are all good signs.

I do not see filaments emerging from the interface but there are particles in solution and a broad based diffuse layer above the interface. We will subject to infrared.

—

We have some very interesting results of the bleach sample (~20 mL) of the interface layer. We definitely have some overlay of the Banana DNA spectrum. Our main loss seems to be that of the aromatic glycoside linkage O-C-N. (this is amide) The indicate that a base or certain bases are not present. This was @ 1744 in Banana DNA.

But if you look closely, you do see a "bump" @ ~1730. This is indeed ester linkage.

Our second trial was less disruptive.
So now we try:
Trial #3
10 ml CDB
10 ml H_2O
1 gm salt
1 ml soap
1 small scoop enzyme
2 drops NaOH & KOH
15 sec blend!

Sit for 15 min

Trial #3 does have some bubbles forming in the beaker. The does seem to be a necessary requirement.

There are as an small particles in the alcohol layer. There is as also a diffuse layer being formed @ the interface.
Visible disruption of the interface is taking place. Some materials do seem to be floaty more than others.

Yo have a curious very small sample here which is deserving of a UV spectrum. There was some material which was floating @ the interface more than other material. I have successfully segregated it. It is about 1 ml of solution so it must be protected until UV arrives.

—

We have 3 samples to look @ w/ Cary UV & IR:

1. Tantalizing slightly levoyant material in 1 ml
2. Darker lower interface layer, least desirable
3. Diffuse layer

This is our most important product to look @ these for.

It looks like I have done it and succeeded in extracting DNA directly from the CDB.

Many signs point to success. The activity level of the trials can be used to indicate success. Trial #3 looked to be active, Trial #2 was a failure, and Trial #1 also appear, in retrospect to be successful as well.

Trial #3 will be adopted as the reference @ this time.

10 ml CDB dense coccus culture from
10 ml H_2O
1 gm salt
1 ml soap
1 small enzyme scoop (1 microscoop)
2 drops conc (~9.0 M) NaOH - KOH
15 sec blend only @ lowest speed

Let set for 15 min
Pour into beaker
Add cold alcohol & monitor activity carefully.

3 different layers a materials will form

1. MM size buoyant fragments along edge (1 or 2 thick time)
2. Coarse layer immediately at the interface
3. A diffuse layer above the interface.

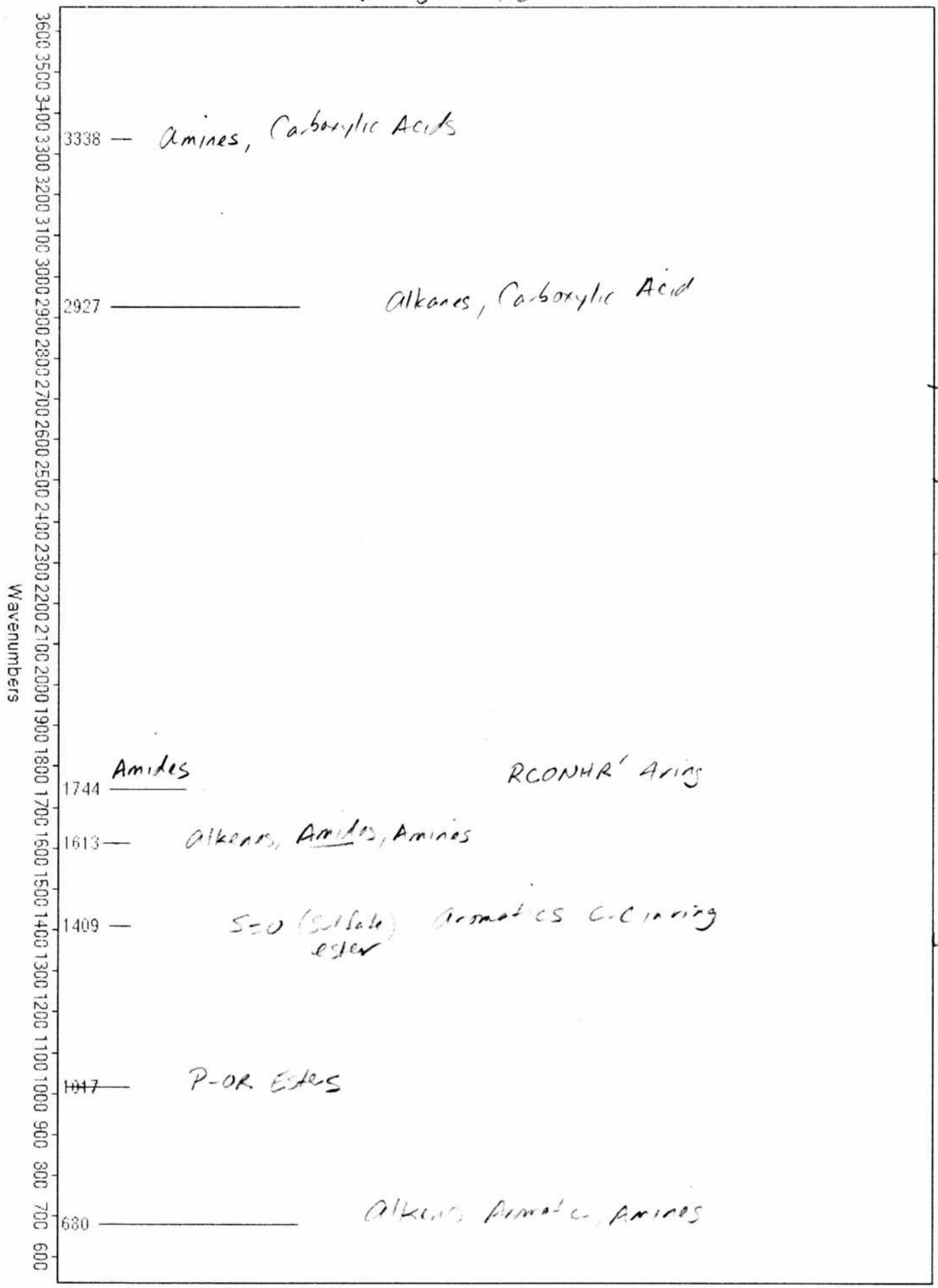
We are saving #1 for the UV as we only have 1 ml of solution. But #3 is the ticket for now.

Confirmation of DNA result has been made via IR. We have a match on 5 major peaks w/ the reference banana DNA

Banana DNA	COB Trial #3 - Diffuse
~~3306~~ 3338 cm^{-1}	3306 cm^{-1}
2927	2918
1613	1593
1409	1404
1017	~1100

We do not pick up the amide peak (CONH) @ 1744 yet.

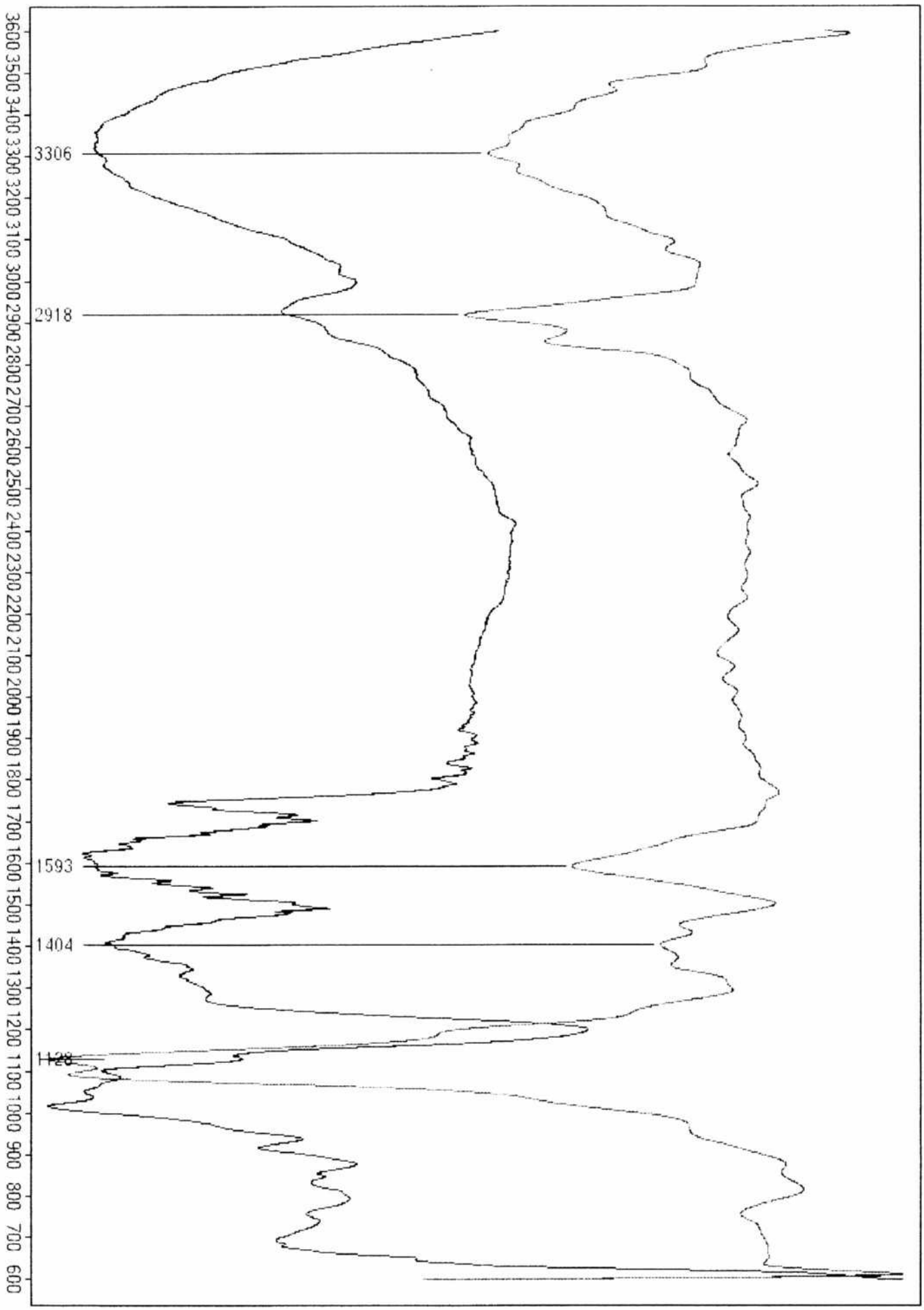
Our sample is undoubtedly not as pure as the banana as it has color to it. But it is pure enough to prove the case.



DNA Banana May 26 2017

Functional groups of DNA are:
Amine (NH_2), amide ($CONH$), hydroxyl (OH), glycosidic linkage ($O-C-N$), & phosphodiester ($R-O-PO_2-$)

Pase 188A



DNA CDB NaOH Trial 3 15 Sec Blend Against Banana DNA May 26 2017 - 03.JPEG

Page 188 B

What are the functional groups of DNA?

Organic Chemistry Functional Groups Quick Introduction of Structures

1 Answer

Ernest Z.
Jul 11, 2016

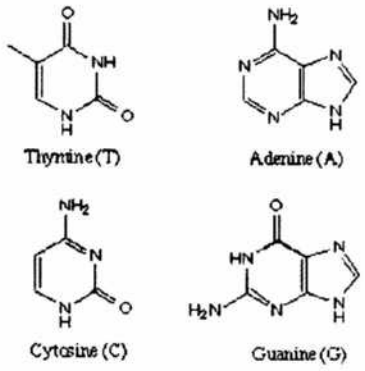
Share

5

Answer:
The functional groups are amine, amide, hydroxyl, glycoside linkage, and phosphodiester.

Explanation:

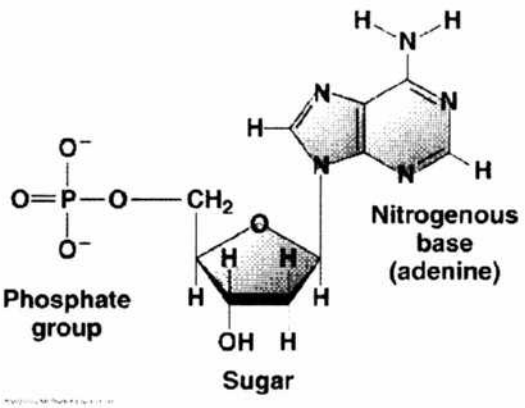
First, let's look at the bases in DNA.



www.education.mrsec.wisc.edu

Adenine, guanine, and cytosine have amine (-NH_2) groups, while thymine, cytosine, and guanine have amide (-CONH-) groups.

The bases are joined in DNA to form nucleotides with the general structure



Nucleotide

(from cahsbiology.weebly.com)

We see a glycoside linkage (O-C-N-) between the sugar and the base, and a hydroxyl group (-OH) and a phosphate ester ($R\text{-}O\text{-}PO_3^{2-}$) on the sugar.

Just asked! See more

What is the arc length of the polar curve ... Answer
5 minutes ago

2.00 grams of a straight-chained hydrocarbon ... Answer
11 minutes ago

How do you divide 2: 4 ... Answer
20 minutes ago

What event led to Ayatollah Khomeini's ... Answer
20 minutes ago

Related questions

How are chlorides formed?

How do ketones get in urine?

What are the the functional groups for carbohydrates, lipids and proteins?

How do you count pi electrons in aromatic compounds?

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See more

Trending questions

What is the formula for finding volume if you know the density and mass? Che

What are some examples of concave mirrors? Phy

Why is it important to study Earth Science? ...

How do you show that ... is ...? Tri

Why is imidazole (C3H4N2) aromatic? Org

Why and how does the skeletal system interact with the digestive system? Ana

What is the difference between a trapezoid and a rhombus? Geo

What is an energy pyramid? Env

What observation provides the best evidence that Earth revolves around the Sun? Ast

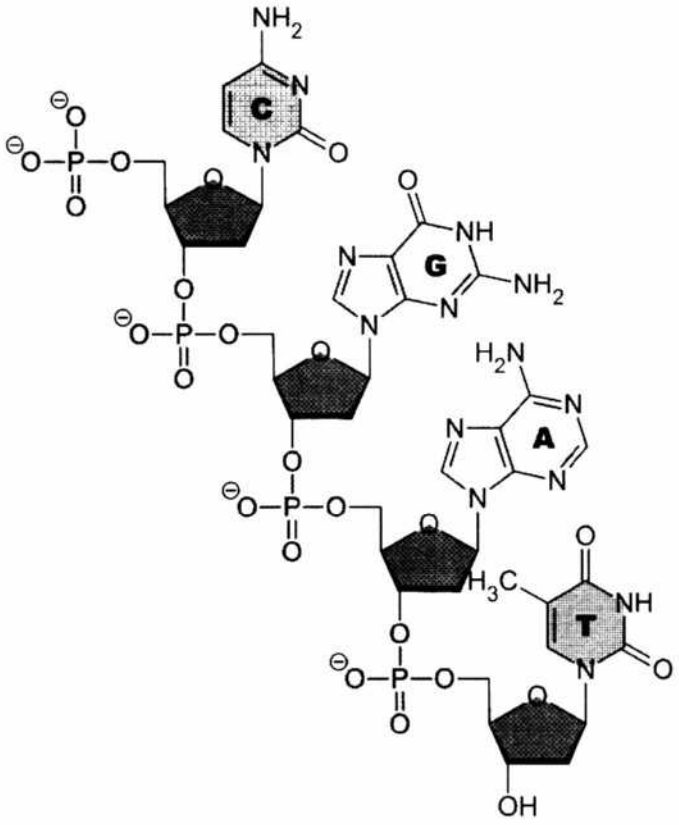
What is the source of energy for nearly all life on Earth? Bio

What is 2 times the square root of 5? Alg

How do you find the derivative of Cal

Page 188C

Finally, we look at a DNA strand.



DNA Strand

(from en.wikipedia.org)

We see that the nucleotides are linked by phosphate diester groups from one sugar molecule to the next one in the chain.

Was this helpful? Let the contributor know! Yes

Show activity

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Add an answer

Write your answer here...

Which planets is least like earth in size and composition? Ast

What was the purpose of Mao's Little Red Book? Wor

What do motivation and emotion have in common? Psy

What is the difference between a metaphor, personification and a simile? Eng

How do you write the fraction $\frac{2}{4}$ in simplest form? Pre

Why did the British raise taxes in the American colonies? How did colonists react? U.S

What's your opinions on the Socratic app? Soc

What is the IQR (Inter Quartile Range) for the data set? 11 5 19 14 Sta

Can someone solve $\cos 2x = \sin x$? Tri

Impact of this question

849 views around the world

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May 21 2017

The 10 cultures are incubated and active. Bubbling is taking place w/ in the cultures so this is a good sign.

The CI Legacy Project + Wiki CI have been announced today. Distribution of the information on a global basis is the priority.

We had quite good fortune yesterday w/ a level of success on the CDB DNA extraction. It would be a good idea to compare the variance w/in the DNA samples, eg tomato, onion, etc.

There is a question why the amide link is not showing up yet. How do the other samples compare to this?

—

I would like to start today w/ examining the black formation (polymer?) on the pipettes that have been sitting across the winter. Microscope work.

There an extremely dense and fine filament network that has developed on the base of the pipette that were left in solution prior to departure in Oct 2016.

The solution most likely had detergents in it. It may a may not have had hydrocarbons in it.

What detergents have the black form vs white. They are also very fine. They appear to be of classic form. Can ya reinvent them as a culture?

150 ml H_2O

1 Detergent? ~~(10 squirts)~~ 1 ml pure
2 Detergent + Brown Sugar (1/4 tsp)
3 Detergent + Brown Sugar + Potato? (1/4 tsp each)
4 Detergent + Br Sugar + Potato + Iron? + H_2O_2
1/4 1/4 1/8 6 drops

Potato is peeled & liquified.

Let see how we can do with it.

Four culture are in place under the conditions cited. A total of 12 cultures now operative.

We will save residual black filament network and preserve in H_2O.

We now have two well developed filament network seed samples in temp storage (in H_2O) for future projects. IR analysis is an obvious first.

1. Oral sample - was hard & rinsed
2. Pipette long term growth - anticipate detergent role in growth.

Let's get another container unloaded from the truck. Done & unloaded about 6 more to go to bring the lab back to full inventory.

I have unloaded the culture test tube sets(2) that have not been looked @ since Oct 2016.

These cultures are important in that they demonstrate signs of altered protein production. The strongly suggests a genetically engineered life form. I also recall alcohol production. As I recall, these were standard cultures w/ 2 exceptions:

1. A balloon is capped onto the top of the test tube as the original intent was gas analysis via GC. The goal has been achieved to some degree and has been posted.

The discovery of a protein produced was accidental and potentially is very important.

2. The second change was incubation @ a moderate temperature, ~ 85°F. The requires ~ 3 weeks of time upon which a color separation takes place.

We have strong Color formation w/in our two sets.

Set 1 has about 11 tubes. The color of each tube is identical and of light green - olive color

Set 2 has 11 tubes also but they are of a golden brown color.

The color is bright & bold in both cases and transparent. There is a small solid residual @ the bottom of each tube. The color of the solution is not uniform, there is no layering now. Recall that the original color after discovery via incubation was red. There is no odor.

The entire process will need to be repeated. Analysis of the two colored results could be both tentative and difficult.
Fe^{+2} & Fe^{+3} are candidates.

I have no idea why the color difference exists between the two sets.
Water in the sample presents difficulties - Another UV candidate.

OK, already a great important discovery.

The light green solution from does indeed contain Fe^{+2} in high concentration.
There is fascinating since since it tells us that reduction of Fe^{+3} has taken place.
The is not real clear to do so how did the happen? Is it the result of an anerobic process since we know that all tubes were capped for about 8-9 months

Test Method: 0.3% 1,10 Phenanthroline

So even there is a difference in color between the two sets they both have a shade of green-olive within.
What we see now is that the second set of tubes also tests positive for ferr.

So anerobic condition cultures appear to produce:

1. A protein (directly?) genetically engineered
2. An alcohol (A fermentation process)
3. Fe^{+2} in solution given adequate time (~ 9 months?)

The set 1 solution (light green) did not test positive for Fe^{+3}.

You need to check the viability of the Fe^{+3} reagent but the days given enough time and under anaerobic conditions all of the iron in solution is reduced to Fe^{+2} form.

This is undoubtedly significant biochemistry with w/in the body. Imagine the ability to convert back & forth between Fe^{+2} & Fe^{+3} and the energy transport system that accompanies that ability.

Important Notes on the topic of the "red protein logs" are available with a

Vol XVII Sep-Oct 2016 notes, also see Oct 2016 01.
Vol XVI See Sep 15 2016

My notes on the constituents of the cultures & tensions
One tube in the light green set is labeled:
H_2O + Salt + Fe ($FeSO_4$) + Sugar
The clear set is labeled
Fe + Sugar alone

therefore, we may have removed most important information.

No mention of H_2O_2 is made. It appears the culture medium was kept to an absolute minimum.

It also appears that the addition of salt allowed for a higher long term production of Fe^{+2}.

Also when you dilute the solution, it behaves like waxy alcohol and water. Recall that a Bradford test is what tested positive for protein.

You need to set the set of cultures aseptically again.

—

Within 4 hrs, it is possible that filament growth is progressing rapidly within Culture #4 of the black filament network that originated from the 9 month pipettes extraction.

Probably the most crucial questions before us now are:

1. What is the nature of the DNA?

2. Is the microorganism genetically engineered or not?

1. In the near future, we will attempt to validate the DNA production and develop a sizable sample amount. Test of variance (IR) between specie will be completed along w/ UV analysis (variance possible there also)

2. We will reestablish the anaerobic culture process in an attempt to substantiate the protein production and alcohol. Any and all of the anerobic properties.

Test tube

Ok, 2 cultures have been set up for ~~aerobic~~ anaerobic & gas trials / protein / alcohol

11 with	3 week period expected for layering.	11 with
Salt		$FeSO_4$
$FeSO_4$		Sucrose
Sucrose		H_2O (~12ml)
H_2O (~12ml)		No Salt

Question: What is in the Fe+2 solution besides Fe+2

1. Is there protein?
2. Is there alcohol?

Well it does indeed look like protein is there. It seems to pass the Bradford test quite easily w/ a nice rich blue color.

It also really looks like alcohol is mixing w/ water when you dilute the Fe+2 solution.

You should really be able to put your Colorometric tests to work here I believe you developed tests for both protein and alcohol.

You have lots of ways to go about then Boiling point & distillation might be one of the easiest & most definite.

We shall go after boiling point & index of refraction of first distillate. We may [illegible] attempt density to some degree. Then [illegible] then UV.

~20 ml sample

BP: 98.1 + 2° Elev. Correction = 100.1°C

Boiling is slowing down after ~9 ml. It is water. Not alcohol! There may be more than ~~one solvent~~.

Index of Refraction

1ST DISTILLATE ONLY

140°C

Approx ~~240°F~~ in gravel bubbles are starting. Boiling has Commenced. Now to go up tube.

However, please notice that butanol, an alcohol, has a BP above that of water so follow the distillation process closely ~~strong~~. Distillation apparatus is working beautifully for micro distillate to ~20 ml. Notice the solution is now ~~slightly~~ more yellowish, indicating oxidation of the iron is taking place.

Page 200

We can see the solution solidify on the bottom of the flask.

The is likely a metallo-protein, therefore, that is soluble in water.

The only solvent that appears to be water. Check index of refraction.

It looks like full oxidation of the iron has taken place. The residual is now strongly rust colored.

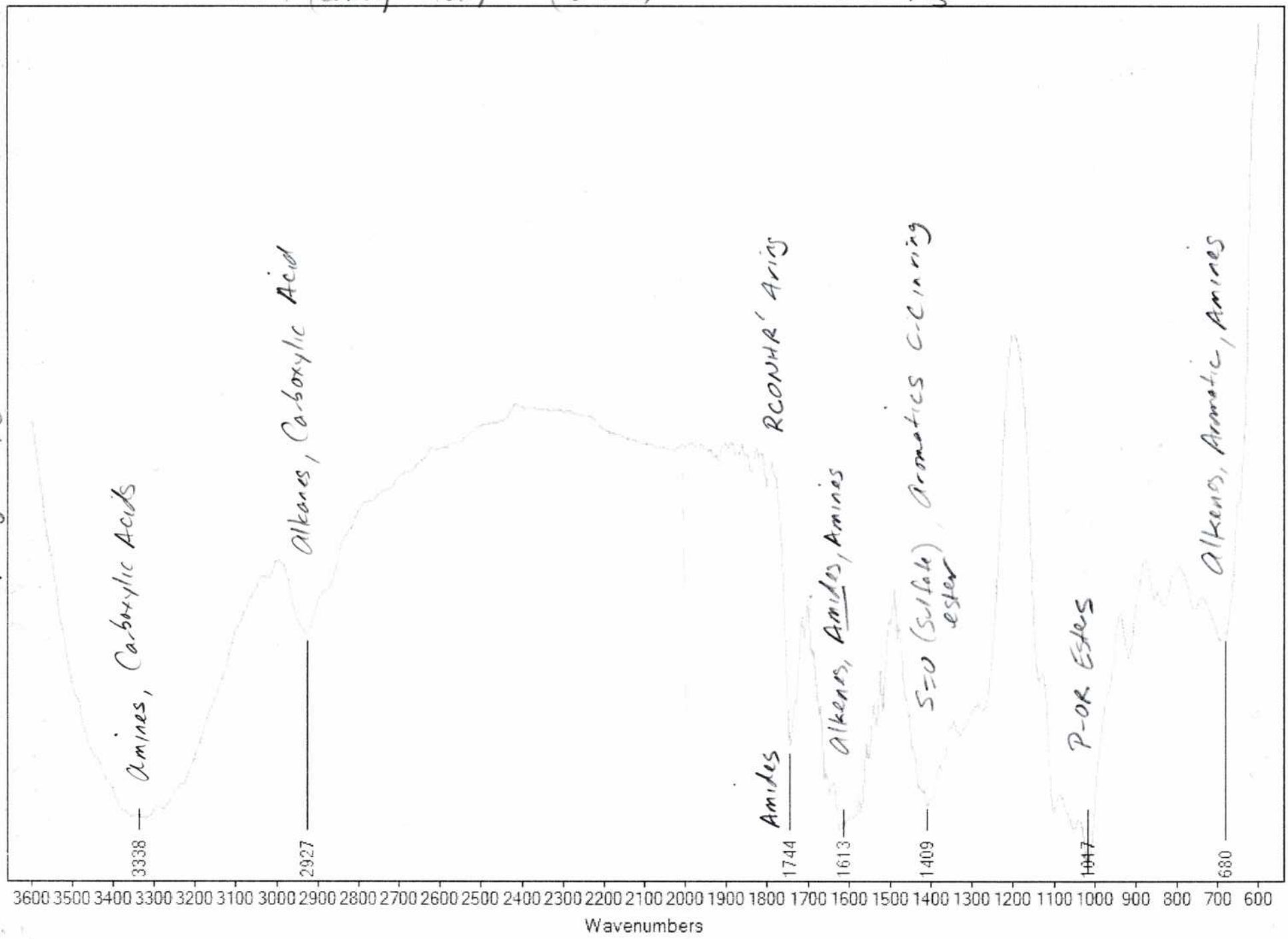
It worked well to increase the heat of distillation w/ a offset torch & look letting into the gravel surrounding the flask.

Index of refraction is indeed zero w/ reference to blank of water. It is/ was indeed water.

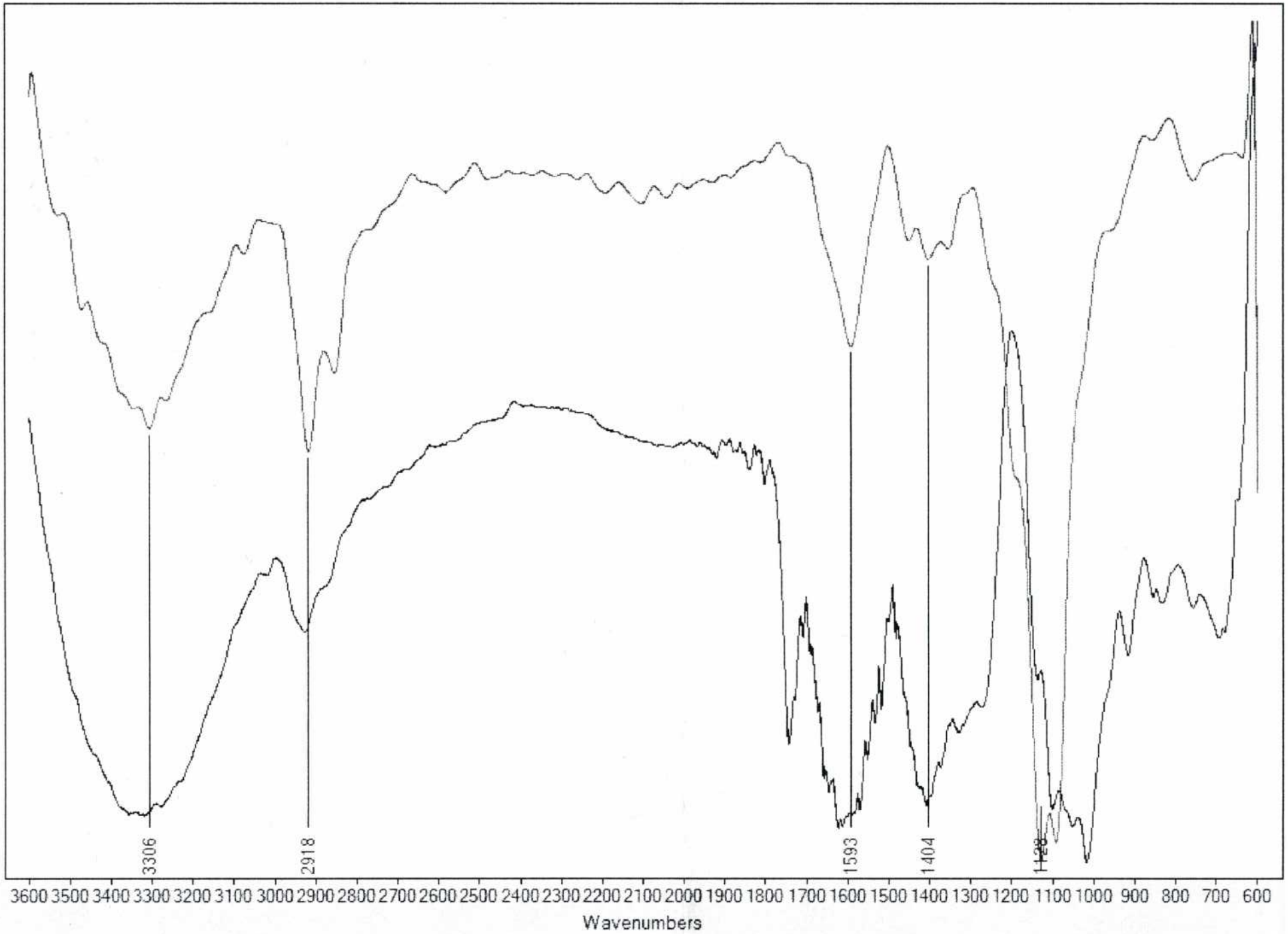
DNA Banana May 26 2017

Functional groups of DNA are:
amine, amide, hydroxyl, glycoside linkage, & phosphodiester
(NH_2) ($CONH$) (OH) ($O-C-N$) $R-O-PO_3^{2-}$

Base 188



DNA CDB NaOH Trial 3 15 Sec Blend Against Banana DNA May 26 2017 - 03.JPEG



Page 188A

Page 188 B

What are the functional groups of DNA?

Organic Chemistry Functional Groups Quick Introduction of Structures

1 Answer

Ernest Z.
Jul 11, 2016

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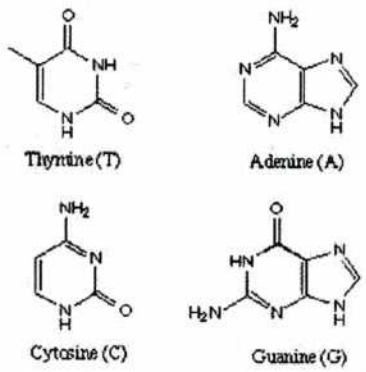
5

Answer:
The functional groups are amine, amide, hydroxyl, glycoside linkage, and phosphodiester.

Explanation:

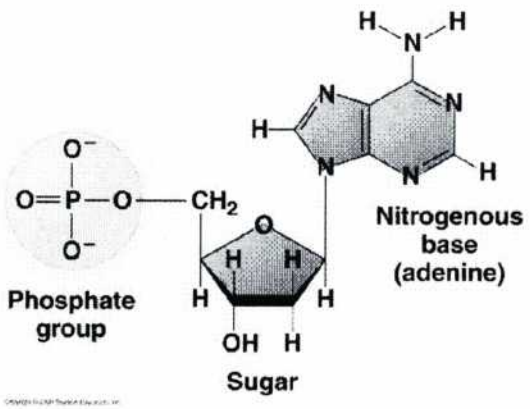
First, let's look at the bases in DNA.



www.education.mrsec.wisc.edu

Adenine, guanine, and cytosine have amine (-NH_2) groups, while thymine, cytosine, and guanine have amide (-CONH-) groups.

The bases are joined in DNA to form nucleotides with the general structure



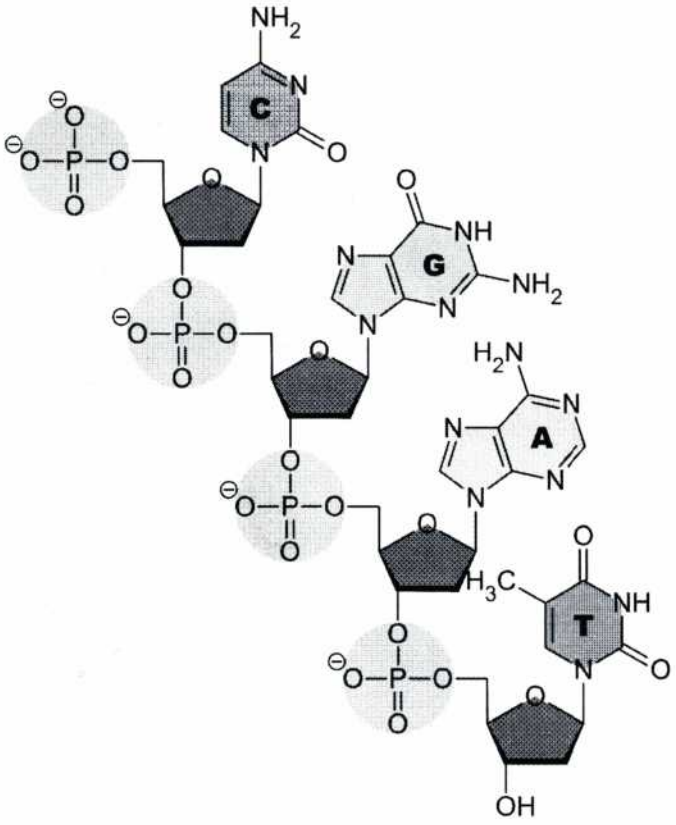
Nucleotide

(from cahsbiology.weebly.com)

We see a glycoside linkage (O-C-N-) between the sugar and the base, and a hydroxyl group (-OH) and a phosphate ester ($R\text{-}O\text{-}PO_3^{2-}$) on the sugar.

Page 188C

Finally, we look at a DNA strand.



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Can someone solve cos2x = sinx? Tri

Impact of this question

849 views around the world

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The brown (oxidized) protein is very sensitive to pH

Acidic - yellow (as in the Fe^{+3} solutions)

Alkaline - dark green

(Like what happens w/ wire in a lake)
We have to assume the protein has been denatured in some way.

IR can be used to verify amine existence.
Bradford shade of blue can also be analyzed.
Additional protein tests can be made colorimetrically and concentration determined.
One thing we do know, there is no alcohol in these forms after 9 months of storage.

Red wire, when made alkaline, does turn green when acidified, but turns back to the reddish color.

We definitely have a metalloprotein

May 20 2017

The paper is to be entitled:

"Bacterial Protein Production: Implications & Consequences"

We have definitely verified protein production w/ the anaerobic culture approach.

The big problem and question is how we divert protein production caused?

I see two methods so far, and neither one of them bodes well for the public

1. Genetically engineered bacteria are used to produce proteins, eg, insulin

2. "Bacteria Protein Secretion" is a known mechanism, but it hardly appears commonplace. In addition, it is usually associated with pathology.

Bacterial protein secretions are some of the most important toxins known. Examples include diptheria, cholera and tetanus.

—

It is an interesting question how I purified the protein from lysed plant cell from w/in the "bed layer". That was indeed quite an accomplishment. You can review those notes to try and recover the process. I also recall some strong disagreements in the IR analysis that led to their specific amino acids. We know now that we have a water base and a ^{H_2O} soluble protein @ hand w/ Fe^{+2} ions in solution. It may or may not be a metalloprotein.

Can we work w/ IR given that we know we have water in the solution? Can we salt out the water? Ammonium sulfate? We know that acid & base change color but they do not precipitate.

—

I have attempted "salting out" w/ ammonium sulfate and it appears to be successful from all that can be seen. I definitely appear to have a precipitate.

I do think we have a precipitate.

NaCl dissolves ~ 36 gms / 100 ml @ ~20°C
Amm Sulfate $(NH_4)_2SO_4$ dissolves ~ 75 gms / 100 ml @ 20°C !!!

This is quite high and you did not add that much and you have a significant precipitate that has now formed after setting for some time. It is hard to imagine that this is the salt.

Next, how soluble is amm. sulfate in ethanol?
It is insoluble in ethanol.

—

Yes, the salting out was a complete success. You have a precipitate and it is separable in proportion. I can see now that dilution of the Fetz solution caused a reaction that, although transparent, is visible to the eye. This is what you thought was alcohol that is not.

You made a mistake adding water back into the sample so that has redissolved the protein (but it is only you observed the "transparent reaction" that is taking place when you mix the precipitated protein and water.

You must transfer it into ethanol.
Salting out for a second time has produced what seems to be a very high yield.

The procedure to purify the protein is as follows.

1. Given red layer or mature tet+2 layer: (It is in H_2O)
2. Add ammonium sulfate gradually until precipitation starts to become visible
3. Drain off water after precipitate has fully formed (completely in both case)
4. Add ethanol to store and to allow it analysis.

pH of protein solution is phenomenally low, ie ~2.0.

A strong IR plot of the purified protein has been acquired. It is all as it should be. Strong signs of acid & aromatics, & amines. Amino acid candidate can almost certainly be identified.

Guess what I have sitting on the desk for more than two years now? three Edvotek lab exercises on the determination of properties of proteins such as molecular weight.

A great deal of achievement has taken place here since May 23. The stage appears to have been set.

1. Flawless DNA extract of 10 or 5 reference species
2. Skillful hominy stolen by specific DNA extraction process required for the new CDB.
3. Serendipity and skill and experience to isolate and purify a protein in a way that has never been seen here before.
4. CI Legacy Project & Wiki CI have been started

[redacted]

5. Reestablishment of the culture environment. Three different sterilizations are in place.

6. UV spectrometer is on its way; that will be very useful.

You have numerous ways now in which the protein can be verified:

1. Bradford Test
2. IR Analysis
3. UV analysis
4. Ninhydrin analysis
5. Carnicom colorimetric method developed that assists with modified Biuret reagent (Concentration determinable)
6. Edvotek lab experiments applied?

May 30 2017

There is some activity taking place w/ our "Black Culture" #s 3 & 4.

We also see that in Black Culture #1 that the filaments (and possible growth extensions) are indeed white again. The black color may have been a result of oxidation as the pipette material was gradually dehydrating.

We will inspect black culture 3 & 4 under the microscope. From previous microscopic examination we are already seeing signs of filament growth within surrounding potato cells.

Also today I would like to examine effects of Vit C, bleach and urine.

We definitely have filament production taking place in Black Culture #4 @ bottom of culture jar. Inspection @ 500X shows full filament production occurring

What appears to have happened here is that you may now have a method of developing two different cellular formations, one being the coccus form and the other the filament form. Filament form requirements may be considerably different from the coccus form.

Coccus form seems to be carbon & iron based.

Filament form thus far may be dairy based w/ polysaccharides (starch) and enzyme based surfactants.

This opens up many curious leads and possibilities here.

In "Black Culture #3) (Potato, detergent (enzyme) brown sugar) but no iron, the filament growth is extremely high interspersed w/ potato cells.

You can see the filament production by eye.
You can see the COB @ 5000X
You can see yeast activity on the microscope slide @ 5000X. You can see contaminant bacteria in the culture.

Question:
1. What kind of enzyme is in Oxyclean?

2. What is the pH of "Black Culture #3"?
6.0

CDB and filaments are literally swarming in growth in Black Culture #3.

1. Filament seed
2. Brown sugar
3. Potato liquified
4 Enzyme detergent.

Since the pH of Black Culture #3 is ~6.0 what we have now is an entirely different medium of culture growth that is dramatically more productive than the non-sugar medium. We also have an entirely different method of purifying (and extracting a protein). We have also accomplished DNA extraction which we will eventually attempt to extract from a filament culture.

Maya Events as we Place Here

These are maya discoveries and processes that have taken place. The protein and different culture have been discovered BECAUSE of the 6 month heater in the lab which nature has quietly taken its course ie

1. Production of filament growth @ base of pipette by sucking in detergent solution for 6 months, & almost entirely degraded. You have now seen this take place twice so you know the enzyme detergent is a factor.

2. The change in the anaerobic cultures over an 6 month period to produce a green color that warranted investigation. From this we determine
   1. High concentration of Fe^{+2} in solution (indicating a reductive reaction over time)
   2. Distillation to prove the solvent is almost entirely, if not entirely water
   3. By salting out w/ ammonium sulfate the extraction of a pure protein, subsequently analyzed by IR and a pH of 2! Also it is water soluble

We now want to see if we can produce a filament rich culture from only a seed of the coccum form.

Call it Filament Trial # 1 or "FIL 1"

150 ml H_2O
1/4 tsp Brown Sugar
1/4 tsp liquid plato
1 ml enzym detergent
1 drop Coccum from CDB
Incubation @ 85 -90°F.

The enzyme on OX clear may not be easy to identify.

One candidate is protease.

Tide has protease, amylase and mannanase. Pectinase is another candidate.

Another source lists candidates as:
1. Amylase (starch)
2. Lipase (lipid)
3. Protease (protein)
4 Cellulase

Since the potato certainly is a major factor here, we can infer that the most likely enzyme at work is amylase.

So we try it independently to test.

Guess what amylase is? SALIVA!!!

Test it,

150 ml H_2O
1/4 tsp brown sugar
1/4 tsp liquid potato
Significant saliva sample (no detergent)
Incubation 85-90°C
1 drop COCCUS COB

I am going to look @ ORP a lot:

	Wallace ~~distilled~~ tap water	+358
	Distilled water	+275
Distilled	water w/ 1 drop bleach	+635
	Urine	-40
Distilled	~~Tap~~ water w/ acid (HCl)	+315
	Tap water w/ base (KOH, NaOH)	-66
Distilled	~~Tap~~ water w/ vit C	+175
	Black Mg Culture	+225
	Long Term CDB Coccus Culture	-20
	Green Fe+2 Long Term Culture	+290
	Sucrose in Distilled Water	+135

ORP of tap water increases upon stirring, decreases & stabilizes upon rest.

—

ORP is actually a very interesting measurement and it does have potential, open intended

I see some immediate applications:

1. Process Control! It will be very good for chromatography as it is very sensitive to change.

It is basically a readout of the total redox potential of a solution, and that is a very powerful measurement. Most everything should change.

2. Oxygen rich water (positive ORP)

3. Oxygen starved water (negative ORP)

All in solution can give a strongly negative ORP so don't overcomplicate the issue. There is a correlation w/ pH but it's not arrow. It is linear & symmetric.

4. Also a very good indicator of chlorine and even Chlorine Concentration if you know what it is present as a single species.

You can see that it picked up a change with sucrose very easily.

Can interesting LC problem to reintroduce the methods. Can you split sucrose into fructose + glucose?

I think what you can set up Chromatography (LC) w/ ORP fairly easily. The idea is to flow to 50 mL beakers instead of G bar start tube w/ the fraction collector.
Is there an adjustable fraction collector w/ time and cabling capacity?

An ORP meter that gave an alert both up and down would be great!

This looks very doable. 15 min segments.

I have my doubts that the fraction collector that is required is any more. It is, however, expensive to replace. 50 mL beakers could be just fine.

W/ Chromatography (LC) you have 4 methods you can apply fairly easily:

1. ORP
2. pH
3. Index of Refraction
4. UV Absorbance

It would be good to have a spreadsheet. I have one now. I have increased the pump pressure for the column by making an X connector. The pressure is also adjustable w/ the pump. I have a much more efficient column now & sample w/ no fraction collector involved except SD and beaker w/ ORP probe immersed along w/ a variable pump @ high pressure.

Add other meters for simultaneous input of EC & pH.

May 31 2017

I would say we have a very good simple & efficient chromatography (LC) system in place now.

You are still working in fractions but globally C D ml per segment. You can drop to 5-10 ml if circumstances warrant. You have fractions identified & they can be segregated upon need.

Ya have a good monitoring system in place.

It consists of

1. ORP
2. pH
3. Conductivity

} are primary flow & process indicators.

4. Index of Refraction is next upon need.
5. UV flow through C 254 nm is available
6. Full UV - Vis - NIR spectrum should be available if all goes well by the end of the night.

food dyes are indeed acidic according to net article. The fact w/ chromatography, they react w/ alkalai. It still seem a trial to get these dyes to run through the column, however.

"The critical part of chromatography is finding the solvent mixture that gives the best separation.
We only given for food dyes the solvent is a combination of
5% NaCl
H_2O
Vinegar
NH_3

These were on my list except for salt but it would have take me several days to work on them.

You start out separately & then mix.
But how could we predict?

Incidently about salt.
You can drain the column w/ a syphon pump and you can rinse the column w/ small amounts of solvent.

I love the new UV-VIS-NIR instrument up and running and controlled by the PC. It is going to be good.

1. It will require that UV, VIS & NIR problems and examinations all be handled separately and in detail. There will be no mixing.

2. Cuvettes, never test tubes, will be required in all cases. It can not handle test tubes for VIS or NIR. I do not know why that it seems to be the case.

3. It is a good feature to be able to turn off either or both UV & VIS lamps. The will extend life considerably. We need a spare VIS lamp as soon as possible.

4. The instrument is very sensitive when used correctly and gain adjustable as to resolution.

5. The graphic capabilities of the software are not extensive, but the software does the basic job any graphics (eg annotations) will be handled in photo impact.

6. It needs a hardware key (dongle) which I do not like. But it is the case so don't ever lose it!

—

A very nice feature of the software, however, is that it allows for overlay of spectra during a run. The no of overlays must be determined ahead of time, however. But that is still very useful, it allows you to compare runs in real time to look for differences.

Can we control the gain? I don't see it. Only by dilution I presume.

—

You can have as many overlays as you want. That is a very helpful feature.

Gain least scans look to be keeping the Abs ≤ 1 or so. High gain/low are creating distortion in the data. The enrichments are fairly sensitive.

The instrument is highly sensitive. Apparently I can not adjust the gain therefore dilution will be the mechanism for stat of clipping occurs. The instrument will be very valuable.

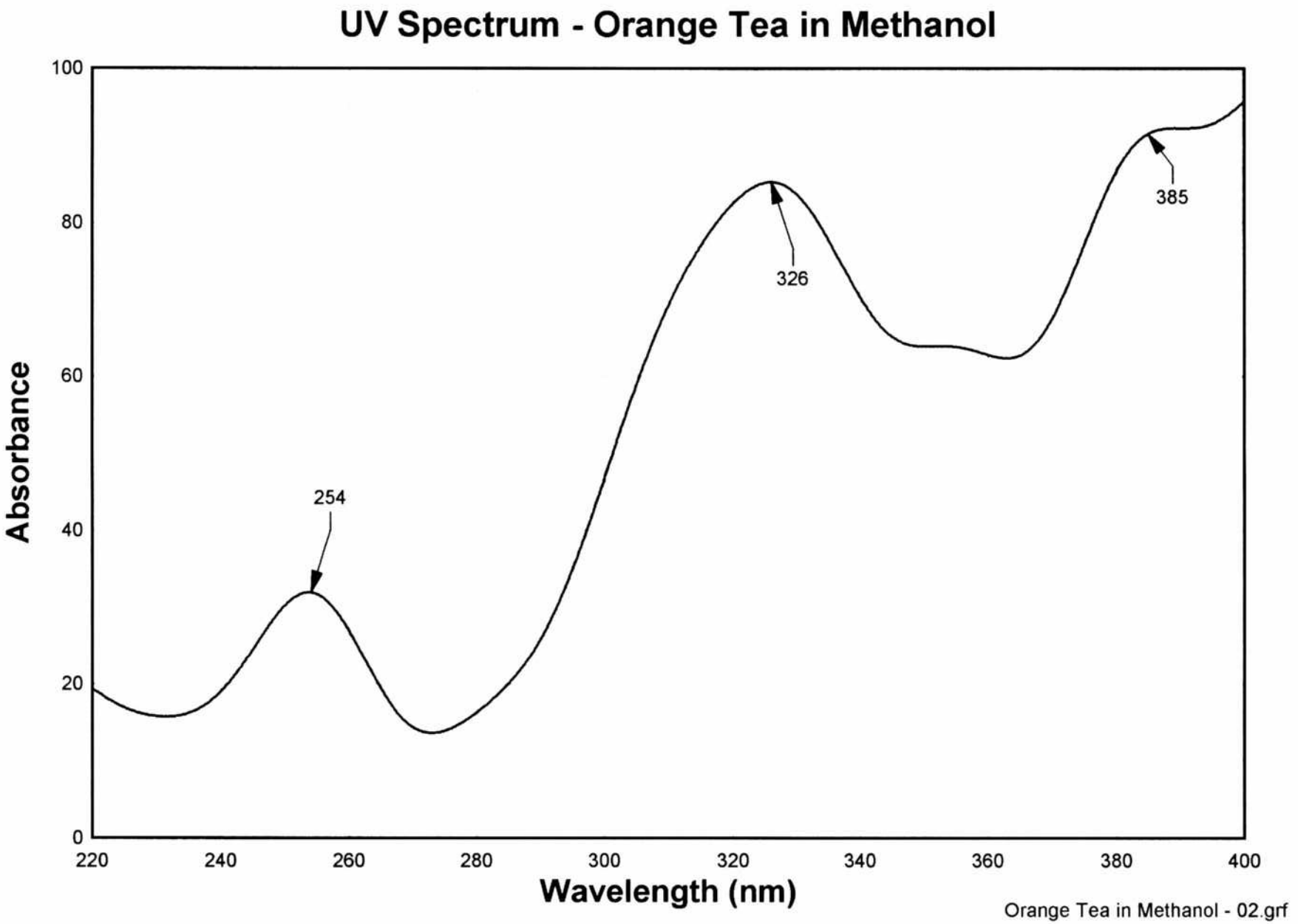
" FIL 1 " is a highly successful culture. Major filament production in short time based upon a single COB coccus from drop. This is rather monumental to have such good control over the filament growth.

Let's look @ green FITC - protein solute w/ VIS and UV & maybe NIR

I was concerned that the UV segment of the ~~instrument~~ was not working. Test w/ Fe+2-Phelan Came out very weak but there was a signal
Also sucrose with a weak solution showed no absorbance.

But sucrose at VERY HIGH CONCENTRATION, as in plot on left, does show significant absorbance near 220. So it may be fine. It is however, a very noisy plot.

UV must be done @ very high resolution.
ie 0.2 from 220–400
Remember to blank w/ the proper solvent, eg methanol if required.


UV Spectrum - Orange Tea in Methanol
Absorbance
100
80
60
40
20
0
220
240
260
280
300
320
340
360
380
400
Wavelength (nm)
254
326
385
Orange Tea in Methanol - 02.grf

June 01 2017

Voila! I have solved and rectified a major concern I had w/ the UV results. There was no adequate signal, as if there was a scaling problem of some kind.

It ends up that the cuvette tray WAS NOT ALIGNED PROPERLY w/ the light source. It needs to be engaged or clicked in. Nothing in the manual on this.

But now you have it! Major results are now starting to come in!

The lack of sensitivity and gain issues have been solved!
Another source we are seeing, however, is a discontinuity @ 290.7. No idea why or this. The instrument remains very sensitive in UV!

Ok, we have now confirmed the extraction of Musa banana & COB DNA w/ observed peak @ 260 nm for both. *

Now we compare the three CD3 layers

1. Diffuse layer

2. Interface layer

These two have equivalent spectra. There is no significant difference between the two.

With a high concentration of DNA introduced we get a much different peak. However we notice introduced a discrepancy @ the UV-VIS light switch over.
We will run the scan from ~~223~~ 220-330 nm to avoid this problem.

Now I have the Fetz-protein solution UV scanned.

Mega Achievements

One of the great things about UV, along with the UColby database, is that it gives you a quick indicator on the type(s) of components or functional groups that might be present. This is a good lead into IR & physical property work. It is, of course, much simpler than IR with 1-3 peaks total usually involved. Another huge advantage is the ability to use polar solvents. We now, within 2 weeks have:

1. An entirely different and radically more productive (w/ filament mode) culture medium.

2. We have extracted DNA from the CDB

3. We have proven (now via UV) that we have extracted the DNA from the CDB

4. We have demonstrated the role of sugar (brown appears for sugars), starch and amylase (or other enzymes) in the filament growth process.

5. We have isolated a pure protein from the CDB via salting out, apparently produced via an anaerobic process. water-soluble

These are indeed mega achievements in a very short time window.

6. You also now have a much improved LC column selection along w/ convenient MS

7. You have the UV instrument fully running now.

If you want sugar identification IR is certainly a way to go. However!

Sucrose, Fructose & Glucose — Have essentially the same spectra. There is no meaningful difference!

At least @ the lower concentrations chosen!

Colby does not pick these up via the two small peaks @ 265nm & 274nm
Sucrose is not in the database, or fructose

Jan
So be careful w/ Colby, it is not complete.
It doesn't look ideal easy to come up w/ spectra for just anything. ~2500 Compounds but no sucrose, fructose, glucose.

What if we drove the instrument to 190 nm just for kicks?

It just drops immediately @ 220nm.
It just cannot be used below that point.

UV is great in that 1 drop of acetone can be placed in 3 mL of H_2O and the instrument is easily reductive to the dilution. We also learn from this the effect of solvents upon the spectrum; they indeed do affect it.

Blue shift of n to p^* with increasing polarity of solvent (e.g. water diluted w/ acetone changed from reference 270 nm to 263 nm)

Red shift of p to p^* with increasing polarity.

Blue shifts are of much greater magnitude than red shifts.

Jun 02 2017

Our test tube cultures are now already starting to form the red layer. This is a perfect case for UV analysis.

Our iron based cultures mostly stall out @ the COCCUS stage. Limited filament production occurs. We can + should now consolidate all 8 of those cultures into one.

The "FIL 1" culture is by far our most dramatic production to the recent series. FIL 2 does not seem to be productive so this says there are other factors involved such as detergent vs no detergent and/or amylase vs other enzymes that may be within the detergent. We will need to identify the chemical constituents that cause the success to the degree possible.

Our spectroscopy course from Univ of Manchester is most certainly beneficial.

The red layer can be monitored to see how and if it progresses toward the green Fe+2 layer.

—

Was it not the Fe+2 green solution that had the pH of 2.0? We also have the UV spectrum of this - it shows broad and strong absorbance in the lower end of the UV spectrum. We now understand the significance π to π^* bonds. Why no protein max absorbance @ ~280nm? Review this.

—

We will start extracting DNA again soon to build the sample supply w/ contact to sequencing labs.

—

We need to develop the Fil series of culture, consolidate the iron coccus series and begin analyzing the anaerobe red layer. We also once again have some gas production in the GC. We know that CO_2 is high.

—

The ~~Cocci~~ Coccus series 1-B from May 26 2017 have been consolidated into 2 cultures for long term coccus development.

Now lets start developing the "FIL" cultures for tests. From May 30 2017 work.

Next trial is:
The base reference culture is now:

150 ml H_2O
1/4 tsp Brown Sugar
1/4 tsp liquid potato
1 ml enzyme detergent
1 drop Coccus CDB
Incubate @ 85-90°F

We need to vary the enzyme and vary the detergent.

FIL 3 will be

150 ml H_2O
1/4 tsp Brown Sugar
1/4 tsp liquid potato
1 multienzyme tablet
No Soap
Incubation
1 drop CBD

FIL 4

150 ml H_2O
1/4 Tsp Brown Sugar
Liquid Potato
Very Soap + Enzyme
Both Soap & Enzyme
Incubation
1 drop CBB

FIL 5

150 ml H_2O
1/4 Tsp Brown Sugar
Liq Potato
Ivory Soap
Nr Enzyme
Incubation
1 drop COB

The culture will be built up until the pattern can be separated out.

Now let's start looking @ the red layer w/ UV. The red layer is too strong. It must be diluted. 2 drops in cuvette now. Looks a bit weak. Now 5 drops total Now 10 drops.

The original culture set of 22 tubes (11+11) was started only on May 27 - 6 days ago and you already do have the visible layer of color forming, albeit weak. It will turn red eventually.

Jun 03 2017

Today:

1. Continue w/ Univ Manchester Molecular Spectroscopy course
2. "FIL" 3 cultures appears to be very active. Additional FIL cultures may also be active but they are too turbid to examine right now. This indicates that the enzymes are likely playing a crucial role here.
3. We will continue UV analyses w/ the red layer cultures.
   1. How does a sugar compare to the red layer (sucrose, fructose, glucose)?
   2. How does the red layer compare to the base layer?
   3. How does the red layer, base layer & sugar spectrums compare in all combinations?
   4. How do you interpret the spectrums?

4. Start taking a look @ [redacted] book.

5. At a time to start probing the DNA

6. Protein assessment from the Echelon labs after sufficient production.

7. Analyses of room air by HEPA by both LC & UV would be a useful project.
All work would be augmented by both
   1. IR
   2. Electrochemistry
   3. Physical Property Analysis

8. Computational Chemistry software reactivated.

9. Revisit old-school methods of molecular weight determination.
Freezing point, boiling point?

Jun 05 2017

1. [redacted]
2. Check UV of methanol
3. Need pH of wd layer & bac layer + repeat on Fe+2 + Fe+3 bnf cultures (2.3) (2.6)
4. [redacted]
5. Extended microscopic examination of FTL culture series
6. DNA extraction top, what is UV absorbance < 260? Got it, methanol.

Soaking 7. Room air filter analyses - LC?
Methanol UV a priori, exp debate.

8. Info wd close today!

Yes the attenuation of the UV DNA spectrum < 260 nm appears to be directly or largely attributable to the presence of methanol (denatured alcohol) with the sample analyzed. The disturbance @ ~270 is visible as well as the sharpness < 234 nm.

Good work. Turn off lamps.

Determing molar mass by freezing point depression is not too difficult.

Anything that dissolves in water that can be weighed accurately should suffice. Adding salt to water will allow for a below zero freezing temperature. -10°C seems achievable. This is a good method.

Now, it should not need to be distilled, any solvent that is pure should be able to be used, as one that you know the freezing point of.

The 8 day old old layer has a pH of ~~2.72~~ ~2.3 This is remarkable, same result as 9 month sample in only 8 days.

We theorize how a highly acidic protein w/ an aromatic (tryptophan) and almost certainly glutamic acid involved. It also tests highly positive for $FeCl_2$ in solution.

(We understand the culture medium by definition has $FeCl_2$ also)

We understand the culture medium has Fe^{+2} in it, however we might be able to distinguish a concentration difference between the base layer and the red layer.

The pH of the base layer is 2.6 so it is not quite as acidic which is what is to be expected. It is, however in the process of changing. The culture of the medium is best between $C \sim 4.5$.

Now lets see if we can distinguish a concentration of Fe^{+2}.

There is no visible difference in concentration of Fe^{+2} between the red layer and the base layer. The defining differences at this point are the color, the pH and the UV spectrum.

It is time for microscopic examination.

1. "Fil 1"

We have a very interesting transformation that has taken place here. We no longer have an abundance, if, none of long filament structures w/in the cultures. We do have a massive culture growth taking place only visible @ 500X, however.

We have postulated two of:

1. Filament structures have degraded, deteriorated & broken into smaller sections
2. CDC Cocci form are developing on a massive scale and now assembly into short filament fragments which presumably would develop further over time.
3. The development of a concurrent second bacterial species (rod shaped) which is now in competition w/ the CDB (also visible on a massive scale)

Terms will be needed to sort these out.

Fil 1: 15ml H_2O 1 drop Cocci CDB
Brain Sugar Incubation 85-90°F
Liquid Potato
Enzyme Detergent

Next we are looking @ "Black 3"

We have the same general result as with FIL 2, except that there seem to more frequently be the existence of longer (but not long) filament growth sections. Since they are of uneven length and too long in many case, they do not qualify as a completely bacillus species.

I have to wonder if the temperature might be a little too high & whether it has caused a deterioration in the filament form. We will decrease temp towards 80° & maintain ~90°F.

Remember that "Black 3" is what started it all.

Now for FIL 2

FIL 2 is saliva based. On the top of the surface was a small amount of floaty material not representative of the solids that are settled on the bottom of the culture.

The material is ambiguous and may simply be salivary solids that remain intact. There is no obvious CDB of filament production upon examination of the culture.

Now for the solids @ the bottom of the culture.

By all appearances, macro & micro, the does not appear to be a productive culture. The does not speak well for amylase as being a primary enzyme of influence.

The culture will be disregarded.

Now FIL 3 - will be discarded.

FIL 3 seems to be only moderately productive.
I allow no leeway to it @ this time.

FIL 3 has enzyme but no soap.
Indication remains that soap and/or enzyme could remain important.

Now FIL 4 (Enzyme table + Ivory soap)

It can be seen that FIL 4
macroscopically is not productive.
It will be discarded.

FIL 5 - Has Ivory soap, no enzyme. (Save this)

Very interesting line. FIL 5 actually is showing the most advanced growth of the recent FIL slides (3-5). Filaments clearly visible, many already developing cola. This indicates that soap is more critical in the process than enzyme were. Soap concentration will be increased.

Cultures Status & Set Up June 05 2017

What remains in FIL 1 & FIL 5

FIL 1
150 ml H_2O
1/4 tsp brown sugar
1/4 tsp liq potato
1 drop Coccus COB
1 ml enzyme detergent

FIL 5
150 ml H_2O
1/4 tsp brown sugar
1/4 tsp liq potato
1 drop Coccus COB
1 ml solid iron soap

Now we work on these variations.
Increase soap supply, add more enzymes, and add blood.

FIL 6
150 ml H_2O
1/4 tsp. brown sugar
1/4 tsp fresh liq potato
1 drop Coccus COB
4 ml Oxiclean
pinch enzyme

FIL 7
150 ml H_2O
1/4 tsp brown sugar
1/4 tsp fresh liq pot
1 drop COB
4 ml Oxiclean ~~+ blood~~
pinch enzyme

FIL 8
150 ml H_2O
1/4 tsp brown sugar
1/4 tsp fresh liq potato
1 drop COB
1 ml Navy + blood
pinch enzyme

I have added a bit of citric acid to the leftover potato to see if that keeps it from oxidizing so heavily & quickly.

We also have Black Cultures 1-4 Oplative

Doing very well on the bet today.

The air filter (~ 9 mos old) is now soaking in denatured alcohol. We will let it sit for a day a two.

I am starting w/ UV & IR plots of the air filter methanol extract.

UV: I added some notes to a file.

1. Reference solution = dilute methanol (5 drops in cuvette)
2. Absorbance of sample is negative (!) < 300nm. This indicates a relative lack of absorbance in comparison to the dilute methanol. The methanol sample extract is @ the same dilution ie 5 drops in the cuvette of water. The sample in pure methanol is absorbing way too strongly and needs to be diluted further.

The better thing to do here is to dilute w/ more methanol, not water!!!

We will do this and also record a VIS spectrum probably undiluted yeast.

Now, with the IR plot, something very interesting is going on. The airgitter mat closely matches a spectra of the hair on my forearm! The next best match, almost @ the same level as with an ATR spectra of another HEPA filter last fall. So the particles highly concordant.

Question: How can it closely match the hair on a forearm? I dont practice that much hair in the room. It could indicate another protein heavy dissolved. Remember also that hair on my forearm IS NOT GOING TO DISSOLVE in methanol. FAT Chance. So it looks like we dysenteries need to be looking @ protein candidates!
A Bradford test would already be helpful here.

The Bradford test come out NEGATIVE, @ least @ the concentration level tested. Then is interesting, may be not a protein.

Big Question: How did I process my hair to get an ATR IR plot last fall?

The forearm hair analysis was actually done on Aug 05 2015 (!) so we can review our notes then to see if any methods are described.
Hard to imagine having any success w/ forearm hair placed directly on ATR & matching a HEPA air filter so closely.

—

Ninhydrin test is underway.

—

The new cultures, esp FIL 6 seem especially active.

—

HEPA Fit Filk abs. peaks seem to be @ 242 & 292 relative to methanol blank. Of interest we have a discovery @ 292 as well.

—

The ninhydrin that will need to be run w/ the spectrometer.

Now for VIS spectrum on HEPA filter
(also NIR)

Reference will stay methanol but will
use undiluted solution.

—

We have a much better UV plot now.
Best results are with full strength extract
from 220 - 1100 nm.

Our most intensity results are UV peaks @

243 nm (moderate)
290 nm (weak) slope break only
316 nm (very strong)

w/ gradual increasing absorbance from 600 to 400
w/ 400 peak elevated w/in baseline range.
The means absorption of violet + appearance
is yellow green.

~~Let's~~ pick up

Jun 06 2017

1. La FIL series of cultures appears to be highly active & productive. Addition of blood seems especially interesting also. Oxidation of iron appears evident. Microscopic evaluation required.

2. The HCPA filter is providing an abundance of material to investigate. Might have significant lead on methanol extract w/ use of Colby database & GAMESS computation on proposed structure (benzaldehyde example) and simulated IR spectra & simulated UV spectra vs recorded spectra. Many tools now to investigate unknowns due in place. We have a solid that settles (black) and a methanol extract. Plenty of sample material.

3. Determining molecular mass would be a major benefit. Develop these methods further such as freezing point depression & boiling point elevation.

4. UV Course is in full swing. Now on IR.
Today is the day to move the forward.

5. [redacted] - you must begin review process.

6. DNA production it is now final.

7. Glorious simulations & Computations are becoming increasingly valuable along w/ Colby database, yor telespectroscopy channel, & property prediction from one source. (molinstincts.com). Lots of good software now in place. Avogadro is more useful than was known; it accepts GAMESS. Scaling of IR plots is a critical factor

8. Chemission is our strong UV lead.

Lets start w/ microscopic examination of some a very active culture.
FIL B - Blood Addition
We do indeed have major filament production taking place within 72 hrs of culture inception. This is a remarkably advanced culture.

FIL B Constituents

150 ml H_2O
1/4 tsp brown sugar
1/4 tsp fresh liquified potato (peeled)
1 ml Ivory soap + HUMAN BLOOD
Pinch of broad spectrum enzyme
1 drop COB Coccus form.
Incubation ~ 80-86°F
We certainly have a jackpot here. Oxidation of the iron in the blood is certainly taking place by the color change from red to brown.

COB and the circular (apparent convex) cell formation is also visible. The process probably took weeks to accomplish under unknown or unidentified conditions.

The is obviously a highly favorable culture medium. Droppy temperature some clots appear to be favorable also.

Potato cells are of course visible also but these are characteristic and easily identified.

One question that will arise is how to isolate the filament network from the complex culture medium. This is one major advantage of the coccus restricted culture medium which is pure.

The blue color in advance culture from these parts is also visible here.

The use of blood dictates a source. This suggests the use of a small amount of meat may also be useful. This could be liquefied hamburger like the potato.

We know that ether is the most advanced & controlled culture to date.

FIL 6 + 7 are sufficient to produce the filament stage by growth but it is not as advanced as FIL 8.

FIL 8 stands as a model for advanced and rapid growth in a controlled culture medium.

Questions include:

1. Enzyme or no enzyme?
2. Blood:
   a) human only or mammal in general
   b) Liquified meat substitutes

Liquification of the nutrients is a defensive benefit to permit rapid absorption.

Item No. 1 is complete for the day w/ amazing results now before us.

All other topics are in high demand.

It might be that a sufficient filament network could now be isolated onto a solid medium such as agar.

Your work on the coccus based is indeed incredibly important as you wanted to inhibit the growth @ an early stage & to a large degree you have succeeded in this strategy.

Trying to thwart more advanced forms of growth are much more difficult since it has already found the nutrients that it requires.

To soap a polymer:

Traditional soap is not. It is an alkali metal salt of an aliphatic acid with usually 14-18 Carbon atoms.

Some synthetic detergents are, however, polymers

We know now that the CDB can use soap to its advantage to make a polymer.

Navy soap: (Classic) Sodium tallowate, sodium cocoate or sodium palm kernelate, water, sodium chloride, magnesium sulfate & fragrance.

Meat, enzyme & SOS will be next test round.

—

Test our solution for volatility from the air filter. I believe it will and will therefore be suitable for GC?

Is amero benzaldehyde volatile?
No, it hardly is the least and we see that from the residue on the KCl IR plate. LLGC is a subtle technique here.

—

LC being applied to the HEPA air filter extract. There is something unexpected there for us. Adding water to the solvent in the column we had a very rapid elution of an emulsion take place. You do not produce an emulsion when you mix the extract with water therefore the column does seem to have played a role in the emulsion formation. You also have ORP, conductivity & pH info along w/ the drop rate to indicate change. A change of some sort definitely took place in the column.

Now the interesting result is that Bradford does indicate that a protein of widely concentrations may indeed be present in the emulsion from. The would not be expected from an air sample.

Secondly, when you look @ the Bradford test tubes closely you can see that Coomassie dye has reacted to some degree w/ at least a partially insoluble protein. You have seen this before. The Bradford test is not complete, but Coomassie will bind most w/ the protein. A Bradford wavelength analysis will be helpful here.

I have to wonder if salting out might be applied here. Let's see what info NC says in the extract, we have the plot from yesterday. We also know we are almost certain to have an OD on a UV ~~but some~~ ~~then~~ a look from the UV analysis.

The ninhydrin test came out negative here so protein is suspected more than amino

Column separation appeared clean & straightforward except I have no explanation for the formation of an emulsion.

Extract + Water solvent in Column → led to immediate elution of an emulsion.

The implies a separation which also implies something else may still be there.
How do you know if something else is left on the column? If you can't see it?

1. I would say drop rate would be one of the main indicators
2. If you flush w/ acid & base first is the required.

3. If column is stable and drop rate is even and controllable does that indicate a clean column?

Let's try the run again w/ clean extract and see if we get the same result.

We deliberately did reproduce the results and collected additional material. We anticipate that we have separated off an alcohol and we must wonder whether some level of protein exists within it.

Now we all that we have some color retained in the column. Now it stands to reason that the material is of a less polar nature.

But how would acid or base work here?
HCl & NaOH are both polar so how would they affect the situation. I have no idea.
A Base seems to react more strongly.

—

Acid & Base both dissolve the solid materials that settle from the fuller.

The colored material that remained on the Columbia cosmic NIR absorbance.
@ 907 nm CH2
@ 1066 nm RNH2

It is very weak but it is there.

Bradford test is negative for protein.

—

The methanol extract from the HEPA room air filter is positively turning the Bradford a shade of blue. This tells us that we have a protein in the air. It does not exist in the solids from the air filter, it exists w/in the methanol extract. These results profound. It is anticipated that protein will have features or identity in common w/ COB produced proteins.

—

I just ran a very important visible spectrum control w/ the Bradford test & applied it to milk. When you have a positive protein sample, ie milk

it will shift the control Bradford solution (just acidified water) with a max absorbance of ~ 644 nm to the left (ie, a blue shift) to ~ ~~620~~ 602 nm.

Your methanol extract is not doing this. It is increasing the magnitude of absorbance considerably @ 644 nm and it does visually appear more blue than the control but it did not actually shift the frequency towards 602. The chromatography calls into question any determination that you have a protein in solution in methanol from the air filter. Obviously more work needs to be done. Concentration is a variable factor.

While we are @ it, let us test our anaerobic culture conclusion.

I can tell what is happening. You are putting way too much acid in the Bradford test. You only need 1 drop of 1M HCl. You were distorting your results terribly.

Run all tests again.

The protein test you developed ~~the~~ would be far superior and you can use that method to verify the results.

I take the most recent statement back. You absolutely have a protein within the methanol extract from the HEPA air filter. It actually seems highly pronounced.

Bradford Caution a shift (can be minor and I list it usually detectable by eye) to the blue side of the spectrum. It is not just a limit when the magnitude of the shift, ie the integral of the curve.

Bradford control had a max λ of 633 nm. A known protein (COB ~~a~~ albumin) has a max of 624 nm. This is the shift that verifies the protein.

Our methanol has a shift to 629 nm. & the magnitude of the shift is also dramatic. We definitely have protein in the methanol extract.

You can also see when weak proteins are left for some time they will also absorb the Coomassie blue.

Your tests that you developed are for a more sensitive, you may need to use them.

In the LC column I suspect we have a separation of the protein taxy place inside an alcohol and an amine structure.

I would like to test the pH of the methanol extract by diluting w/ an H_2O.

The pH of the methanol extract is ~ 7.4. It is not acidic in any way.

Ammonium sulfate is not soluble in methanol so you can not "salt out" with it. NaCl might dissolve it appears.

OK I have unequivocally proven the existence of the protein in the HEPA air filter.

Bradford Control max λ = 633 nm

Purified Air Filter Protein Sample max λ = 619 nm

The is a significant blue shift.

The method is a bit involved; here it is.

1. Take methanol extract and dilute it with about 2 parts water.

2. Now salt out carefully & steadily w/ ammonium sulfate. You will get visible slight but visible results. Total volume ~ 20 ml.

3. Now centrifuge the results.
4. Decant off the liquid to leave small amount of residual solids (protein) @ the bottom of the test tube(s).

5. Add 1 drop 1M HCl to each tube & approx 3 ml of H_2O to fully dissolve the solids.

The protein form would be much more concentrated than that which exists in the methanol alone.

6. Combine any Concentrated solutions, OK to add one more drop of 1M HCl (you learned to not add too much Conc HCl, it only needs to be acidic). We have learned the methanol extract is near a neutral pH when mixed w/ water.

7. Now conduct the Bradford test and record the spectrum. The concentrated form will produce the deeper blue color. Results have been strongly verified.

Atmospheric (Filter) Protein Isolation:
I did some great work today. A very difficult and involved endeavor.

I extracted and isolated and verified a protein that exists now in the general atmosphere.

1. The first requirement was a HEPA filter that has been running non-stop and continuously for the past 9 months in a commercial building within a rural section of N. Idaho.

2. Second phase was an extraction of materials within that filter into a solvent of methanol (denatured alcohol).

3. Third phase is filtering the solvent & further separating by either gravity or centrifuge into liquid & solid portions.

4. Fourth phase involves diluting the methanol (1 part) with ~ 2 parts water.

5. 5th phase is salting out ~ 20 ml of solution with ammonium sulfate

6. 6th phase is centrifugation

7. 7th phase is drawing off solvent and placing dissolving residual solids (more concentrated proteinaceous proteins) in dilute HCl

8. 8th Phase is conducting a Bradford test on the more concentrated protein foam.

9. 9th phase is recording the visible spectrum to verify the blue shift of the Bradford test.

And by the way, the protein originates from the CDB filaments collected in the HEPA filter, which can be viewed easily w/ modest microscopy equipment.

Your next main project is (plural)

1. Become more fluent in determining molecular mass. (My 7 classes - you like)

2. Build up to DNA sample & start talking to labs.

[redacted]

4. IR Course now!

5. Citrus samples

Molecular mass of sugar is a good project example.
We need a method for molar mass of an unknown substance.

Pasc 267

Search term: Determine molar mass of an unknown substance by freezing point depression

The Method
for an Unknown ~~known~~ for water (1.86) a boiling point elevator

$\Delta T_f = K_f \cdot m$ $\quad m = \frac{mol}{kg}$ (molality)

$m = \frac{\Delta T_f}{K_f}$

$m = \frac{5.10^\circ C}{1.86^\circ C/m} = 2.74\ m$
(amt of water)

$\frac{2.74\ mol}{kg} (.07500\ kg) = 0.206\ mol$
(no. of mols)

$\frac{26.4\ gms}{.206\ mol} = \frac{128\ gms}{mol}$ Ya tube Channel
The Science Classroom

Given: 1. 26.4 gms of unknown Compound
2. Dissolved in 75 gms of water
3. Freezing point depression is $-5.10^\circ C$
~~This is all you need to know~~
4. $K_f = 1.86^\circ C/molal$

This is all that is required. Very cool method.

June 08 2017

FIL 8 culture, which is composed of
150 ml H_2O
1/4 tsp brown sugar
1/4 spat liquified potato (peeled)
1 ml Ivory soap
Piece of bread speckled enzyme
Human blood (~ 1/2 ml)
1 drop Coccus COB
Incubation 85-90° since Jan 05

has been our very active culture.

Change over last 48 hrs:
1. Less blue color formation
2. Decrease in filament existence
but still present
* 3. An increase in the spherical cell form that is another sign of more advanced growth
4. Abund and coccus & short filament & rod like assemblages.

The solution was originally red from the addition of goat blood. Within 24 hrs the culture had turned brownish, from all signs because of oxidation of the blood. The solution has subsequently become more clear.

I have added 1/8 tsp (~0.6 ml) of organic blood meal.
The culture will be monitored.

---

additional observations:

FIL 1 has a discrete layering that is taking place. There is two color, however. The top layer (~1/3 of volume is clear) & lower 2/3 is more opaque. Picking up the jar has mixed the layers so I will need to be more cautious next time. A good candidate for UV examination.

FIL 6 & 7 are identical and of good clear colony development (no blood added)
I will observe FIL 6, add blood meal & monitor for changes.

Block #2 has a singular but substantial filament structure forming slowly w/in a largely transparent solution. They appear to be similar to long term development of a polymer with the soap solution for long term growth.

—

The test tube cultures (both sets of 11 each) are all forming the net basically slowly and steadily. They are under observation w/ UV.

—

Liquid Chromatography (LC) is under way w/ the HEPA 9 month room filter test separations are efficiently being made & the process is becoming repeatable w/ a good spreadsheet - monitoring system (ORP, pH, and Conductivity) in place. Methods development are proceeding well here.

—

FIL 5 does not appear especially on table @ the time relative to select cultures & FIL 6 & 7.

Black 1 & Black 4 also do not seem noteworthy @ the time. Black 3 is clear & uniform & with the base for heightened interest is detergent & brown sugar results.

—

Let us observe FIL 6 (identical to FIL 7)
150 ml H_2O
1/4 tsp (1.1 ml) brown sugar
1/4 tsp fresh l.g. potato
4 ml Oxi clean detergent
pinch broad spectrum enzyme
1 drop Coccus CDB.

Does have significant uniform growth. A Clean Culture.

It does seem beneficial to set incubation temperature dropped, now about 87° F.

The cultures appear, without doubt, to have developed the most involved & sophisticated filament network. It is also a rather uniform culture w/ remaining & surrounding potato cells (huge & characteristic and distinctive) visible. One portion of slide has a remarkable growth based w/ clear cellular division taking place & what most certainly appears to be a cell nucleus. The culture will not be changed nor blood meal added @ this time. The means FIL 6 & FIL 7 will be monitored closely. A more advanced growth form than has ever been seen before.

Today, therefore, we have

✓ 1. Inspection and monitoring of cultures.
FIL 6 & FIL 7 now also have heightened interest. FIL 8 blood culture is a bit more complex right now.

[redacted]

3. NMR Study

4. LC work continues, slow & steady.
A good system that is developing here.

5. DNA sample buildup

6. Cotinine samples

7. Molecular mass determination:
Develop the skill!

A spare bulb for the VIS section of the UV spectrometer has been ordered. A bit pricier than I expected @ $85 but necessary. — Dilute (HCl did clean the cuvettes well)

Let us analyze LC elution from last night's run. We should probably use water as the reference since it cleans the solution. 220-400 nm.

Beaker	Plot	Description
	0	Water Reference
	1	Methanol (Den. alcohol)
1	2	1-1 JUN 08 2017 0130
2	3	1-2 @ 0145 5 drops in H_2O cuvette
		This is the opaque separation
3	4	1-3 @ 0145 Full Strength
4	5	1-4 @ 0200 Full Strength
5	6	1-5 @ 0200 Full Strength
		Plot 5 & 6 are identical w/ reduced absorbance w/ $A_{max} \approx 0.67$ @ ~220.
6	7	1-6 @ 0215 Full Strength
		Plots 5, 6 & 7 are identical and represent stable output from the column.

Now let's analyze the results.

Beaker #
4
5
6

Plots 5 (1-4) are equivalent and of
6 (1-5) relatively low abundance.
7 (1-6) They represent stable output
from the column exactly as proposed within
the spreadsheet notes.
Conclusion: the beaker output from beakers
4, 5 & 6 can be discarded.

In addition, Plot #1 (no beaker) is the
reference of methanol. It is quite unique on
its own right and it shows no strong a
direct relationship w/ any of the primary clusters
ie #4 (1-4), 1-5, 1-6. So we know that
methanol is not coming out of the column.

The remaining 3 plots (1-1, 1-2, & 1-3) are
highly similar. We call however that beaker #2 (Plot 3)
(1-2) is highly diluted w/ only 5 drops per
cuvette while 1-1 & 1-3 are full strength.

Plot 3 is already higher abundance than
the others so the beaker #2 is clearly our
separation of interest (1-2).

1-2 as they are the only elution that needs to be saved and it should be the most pure of anything that needs to be saved & analyzed. Everything else from previous runs can be discarded as can the last controlled sample.

—

It can be seen that UV is incredibly value to distinguish & characterize colorless organic solutions. At its most fundamental level it acts as an important separation tool in its own right.

I would like to compare the prior opaque solution saved on 06-06-17 to our current observed sample of 1-2 on 06-08 @ 0145. UV

10 drops used in cuvette. Expected to be more dilute & subject to more cross bleedover. This is Plot #8, (Scan #8). (Keep at it team builder on) We see that it is not the same and all signs are that it is more contaminated or bled into. Discard.

Now we are after the undesired color in the column. Acid has pulled it out.

Beaker	Run	Time	
4	01-04	1600	} Save
5	01-05	1600	

are the same.

6 01-06 1600 is also similar but not same

—

Now out of curiosity check previous days runs in comparison.

Yes is certainly similar but not exactly the same. Will discard previous runs

—

4

We have now three different compounds separated now from the HEPA filter.

1. A protein
2. An opaque apparent aromatic
3. A light green solution
4. A solid material by gravity.

HLB appears to be very active again w/ added blood meal. Will continue to monitor.

—

Check HEPA methanol extract for Fe^{+2}. The test is negative. No free Fe^{+2} ions in the HEPA methanol extract.

—

The is telling us that the IR plot we have of the methanol extract is not of a single compound. We need to work on the components individually with

1. physical properties
2. Crystallization
3. Index of refraction
4. molecular weight if possible
5. UV & IR analysis.

Methanol Brix is 8.4 ⇒ IOR = 1.344
(Denatured alcohol) Actual is 1.329
We know that den. alcohol is not pure methanol, however!

Our sample also reads Brix = 8.4 ⇒ IOR = 1.3444
but we know from LC & Bradford work that it badly is just denatured alcohol!

Therefore Index of Refraction is only one tool of many to be used.

In 01-02 on 06/08/17 @ 0145
We read Brix of 9.6 ⇒ IOR = 1.346

Let's try to get a density estimate also.

But before that, how close did to get IOR? You know that it worked on a weighted average.

The only UV peak we have w/ 01-02 @ 0145 is 222 nm.
So we have IOR of 1.346 (presume highly diluted) and λ_{max} of 222 nm.

UV peaks along w/ functional groups can go a long way.

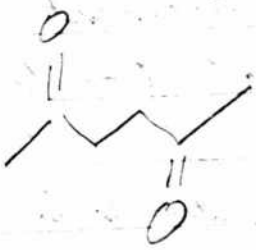
IR gives us alkane & Carbonyl group. Also it appears ketone is likely.

IR peaks @ 2954 Alkanes
2858
1720 Carbonyl, Ketone

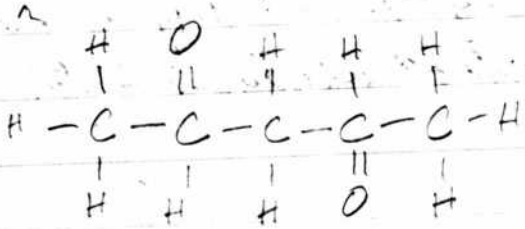
a ketone is 

IR plot might also fit. We are on the right track.

Our last candidate is Acetonylacetone



from Colby Combining UV & IR functional groups

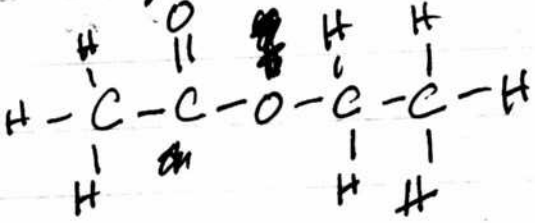


It is an aliphatic diketone. It is a toxic metabolite of hexane

Another candidate is: Cyclooctenone
This is a ring structure.
IR plot does fit.

THIS IS A KETONE ALSO

The IR spectrum says that it is an ester. You produced an excellent plot by dehydrating the sample.



UV Peak @ ~220nm

HEPA Methanol Extract First Elute Opaque Jun 09 2017 - 01.JPEG

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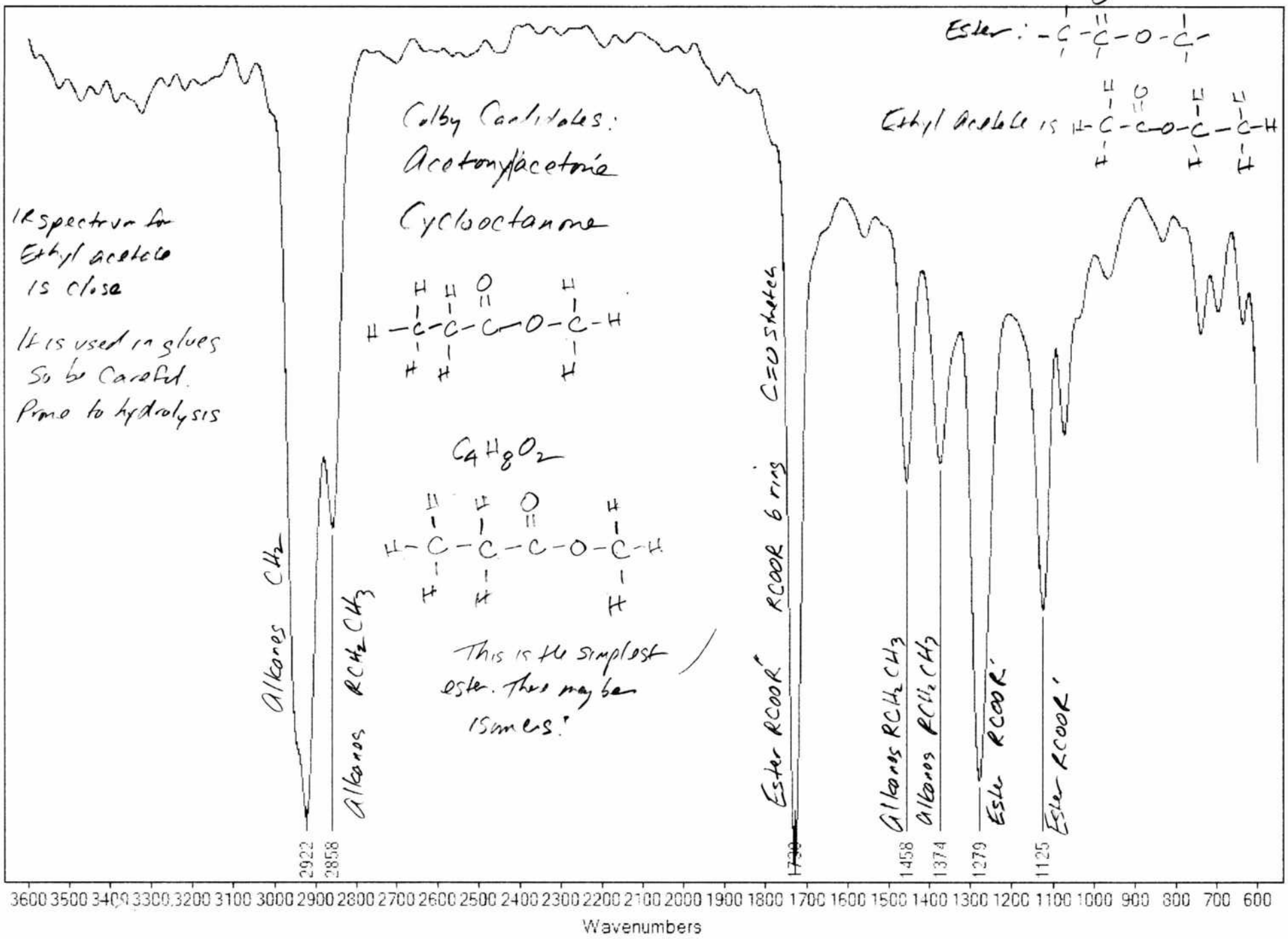


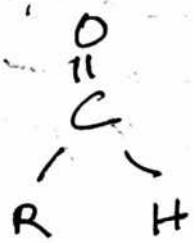
Jun 09 2017

1. Start w/ the oxidate test today.
Measurement estimated @ 3.0 to 3.5
on a scale from 0 to 5.25

This appears to be a significant improvement over previous tests which always maxed out @ 5+.

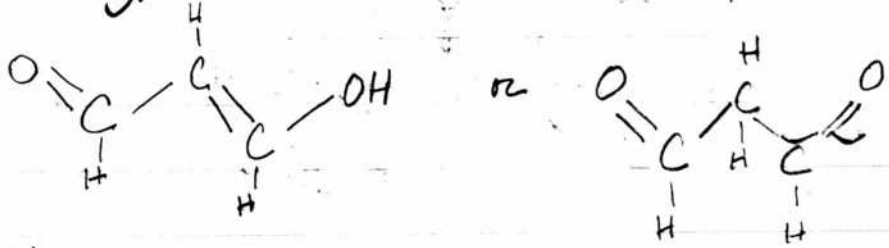
Apparently the oxidate test may be measuring aldehyde levels in urine.

"Oxidate is a colorimetric reagent that reacts in the presence of Malondialdehyde".

Aldehydes are $R-\overset{O}{\overset{\|}{C}}-H$ actually 

aldehydes are most concentrated in the urine.

What type do in urine? Malondialdehyde!



$C_3H_4O_2$

UV absorption of malondialdehyde is pH dependent.
Apparently 267 nm can be used as a
point of reference for concentration.

You can calculate a current UV reading @ 267 nm
to an oxidate test value of 3.5.
This should be a decent starting point.

Since the blood culture is so successful we are going to increase production and create a new series ("Blood")

Blood 1-4
150 ml H_2O
1/2 tsp brown sugar
1/4 tsp liq. potato
1 ml Ivory Soap
1/4 tsp blood meal
pinch enzyme
1 drop coccus
Incubate 80°F

Blood 5-8
150 ml H_2O
1/2 tsp brown sugar
1/4 tsp liq. potato
4 ml Oxiclean detergent
1/4 tsp blood meal
pinch enzyme
1 drop coccus
Incubate 80°F

Blood culture series now in place.

Now for UV analysis of urine @ 267nm for malondialdehyde.
Urine must be diluted.

Use 2.5 ml of water w/ small calibrated syringe and 500ul of urine w/ micropipette as the sample.

Measured ~3.0-3.5 Jun 09 2017

DIRECTIONS: Lightly tap the ampoule on the countertop to be sure all the liquid is at the bottom. Break the top off the ampoule using the plastic safety top. Discard the top. Draw up 1 ml of sample with the dropper and squeeze from dropper into ampoule. Evaluate reaction when color stabilizes (do not wait more than 5 minutes to evaluate). The included color evaluation chart may be used as a qualitative, semiquantitative index.

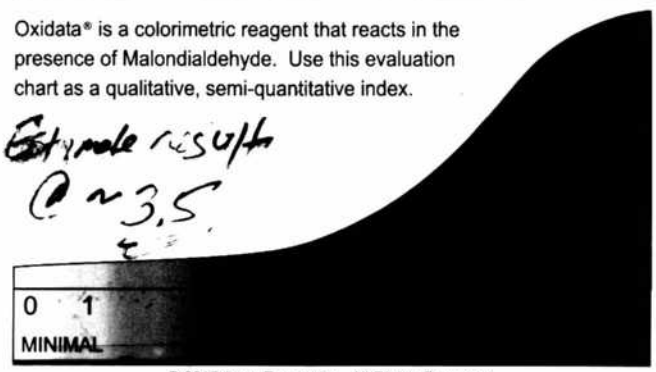
= Abs 267nm of 0.9763

WARNING: Not for injection or ingestion. Do not inhale. Contents of vial are irritating, and the intense dye will cause stains. Add test sample to the ampoule. **DO NOT ADD AMPOULE CONTENT TO TEST SAMPLE. KEEP OUT OF REACH OF CHILDREN.** In case of accidental ingestion, or if the contents of the vial get in your eyes, **CALL A HEALTHCARE PROFESSIONAL IMMEDIATELY.** This product is not intended to diagnose, treat, cure, or prevent any disease.

OXIDATA® EVALUATION COLOR CHART

Oxidata® is a colorimetric reagent that reacts in the presence of Malondialdehyde. Use this evaluation chart as a qualitative, semi-quantitative index.

Ampoule result @ ~3.5



Our dilution ratio is therefore $\frac{2.5\,ml}{0.5\,ml} = \underline{5}$

5 parts water, 1 part urine.

Absorbance @ 267 nm = 2.5204
Still a little high but it seems usable.

It would be better to run another case of
2.5 ml H_2O, 200 ul urine.
Then we likely done.

Absorbance @ 267 nm = 1.7100
Then a better limit we see now that
you should use 2.5 ml H_2O, 100 ul urine.
Again:

2.5 ml H_2O (careful measurement), 100 ul urine:
Abs_{267} = 0.9763 good. ≈ 3.5 Oxidate Test

Notice there are two peaks in urine:
241 nm
& 290 nm

Trust us there is a local minimum of absorbance @ ~ 267 but measurement will still be taken there.

Urine appears to give a very distinctive curve w/ well defined peaks.

Oxidata test is much improved relative to the past. Means in past have always been 5+.

—

On source on days that 230nm + 290nm (urea) (uric acid) are appropriate wavelengths for determination of urea and uric acid. These correspond fairly well to my peaks also.

You should have a reasonable test procedure in place for malondialdehyde detection and graphical calibration. You have already calibrated per the oxidata test. Notice what appear to be a non-linear concentration curve, however.

—

UV spectrometer rents out for $125 per hour in New Zealand. I had already paid for the instrument after one week.

Apparently the activation would be improved by having the oxidation level (eg Abs 260) even lower than it is currently. But I estimate in the past it must have been much higher.

We should now have an alternative to the oxidate test in place.

I found a second source that corroborates max absorbance near 266 - 267 nm. Good.

On the molecular mass determination problem, one of the main difficulties will be that you need a pure substance in order to work with it. That is frequently not the case, in fact, usually the case. But it will BE A REQUIREMENT. So you must strive for that.

A first example of this will be your proposed ethyl acetate. Do you have enough to distill and separate for example?

It would be good to see if we can specify the ester as other would be required before you can try to determine molecular weight.

BP is: ~~77.7°C - 78.3°C 79°C~~ (1) 78°C

Ethyl acetate is 77°C - close
Second distillation @ 85°C

We have purified elute #1 (opaque) by distillation into two fractions

Fraction #1 BP ≅ 78°C ~6 ml
Fraction #2 BP ≅ 85°C ~3 ml

UV analysis and index of refraction always a good start.

We have 3 pieces of information:
1. Boiling Point
2. Index of Refraction
3. UV Peaks - spectrum

76°C Elute.
Great spectrum. Peaks @
233 nm
271 nm (small)
283 nm
IOR: Brix 13.2 = IOR 1.357

Even w/out IR WI should be able to do something here.
Nothing in Colby is showing up here?

85°C ~~Elute~~ This looks like it can be disregarded.
IR Plot: It shows a protein prospect!
Bradford Test: A Positive result for Protein!

633nm is the Bradford Protein Control.
A protein will cause a blue shift.

We have 633 nm. This means no shift.
Work on this again. It is not ~~blue~~ green it is blue.

Repeat this test.

You can see the shift (blue) with your eye so you know there's a shift. Now to the spectrometer.

Bradford Control: 15 ~633

The sample has a peak @ 633 so there is no shift @ that point.

However, this is not the end of the story. You can see by eye that it has shifted strongly from blue green to blue.

So how can this be?
This is how it can be.
What you see w/ the sample is a dramatic increase in absorbance, relative to the control from 400 to 520, and it is more pronounced closer to 400.

The means the solution is absorbing dramatically more in the ~~yellow~~ region. blue region.
The means that portion is appearing yellow.

OK, I have it, and I have ~~it~~ strong Bradford Control ~~633~~ ~640nm

Upon
1. Separation by LC (opaque eluate)
2. Distillation (Boiling Point @ 78°C, 10R = 1,351
3. UV Peaks @ 233, 271 (weak) & 203 nm

4 And no match of any kind w/ Colby database
IR plot is run in ATR mode under a glass slide
(this is now a volatile material w/ BP = 78°C)
The IR plot clearly shows strong evidence of protein
w/ amines & amide.
The concentration however, is not sufficient for the
Bradford test.

#5. You must therefore dilute w/ H_2O by a factor of 1 part each and patiently salt out with ammonium sulfate.

6. Upon completion you must centrifuge to settle materials, draw off majority of liquid, then redilute w/ water and perform again the Bradford test under acidic conditions.

See Next Page

HEPA Protein Isolation

Upon elution, a very deep strong blue solution is obtained inside the Bradford test and

I GET A SHIFT from ~640 nm to ~600 nm.

Very definite protein here.

—

for summary, the methods were quite involved +

1. HEPA filter in methanol
2. LC – two eluates, 1st one opaque
3. Distill Elute 1 (opaque) to clear solution w/ BP of 78°C. and IOR of 1.357 No Colby match, separate from Ethyl acetate finding.
4. Run UV, IR ATR on glass slide.
   UV peaks @ 233, 271 (weak) & 203
5. IR shows strong amine & amide presence
6. Dilute w/ H_2O 1 to 1
7. Salt out potently w/ ammonium sulfate
8. Draw off liquid
9. Redissolve solids in H_2O
10. Acidify & perform Bradford Test
    strong blue color test
11. Record blue shift in VIS spectrum.

Jun 10 2017 Lab Status Report - Summary

There are many events & happenings & findings that have take place in the lab upon return this season, all within the space of a month. Many lab procedures have been improved & developed as well.

1. DNA extraction from COB has been accomplished w/ reliability & reproducibility.

2. Radical improvement in culture mediums has been achieved, with ability to manage growth between the coccus and filament stage. The most advanced culture forms of growth ever are now available upon the space of a day.

3. Protein extraction from the anaerobic culture is now reliably in place.

4. Protein identification within HEPA air filter samples has been accomplished.

Similarities in protein primary sequence is an intriguing subject of research if conditions permit.

5. Liquid chromatography methods have been improved considerably with tangible results applied to the HEPA air filter sample/extract. Ethyl acetate protein extraction & subsequent purification through distillation all together and are working in tension.

6. The use of relationships between physical property determination, UV spectrum, IR spectrum and the Colby database has provided tremendous improvement in spectra elucidation. Major progression here now w/ reduced dependence on IR for the complete picture almost always inadequate w/ IR) - but also crucial to shift the focus properly.

7. Great work has been started with computational chemistry tools, especially GAMESS software for property prediction, IR analysis, etc.

8. A helpful online course in Molecular Spectroscopy is active via Univ. of Manchester. Good exposure & reinforcement of UV, VIS & NMR.

9. Interesting project for a protein that acts as a VOC - volatile organic compound with a boiling point of 78°?! - Coming from the steps of

1. HEPA (9 months) filter extraction into methanol (recall that the protein is available immediately here via salting out, centrifugation (remove water) & alcohol combination (under dilution)

repeat dilution in water, & application of Bradford test.

2. LC methods applied, producing 2 separate compounds

3. Distillation of opaque compound (presumed to contain ethyl acetate - potential adhesive from HEPA filter) to provide solution w/ BP of 78°C

4. Dilution of distillate w/ H_2O, salting out, centrifugation, dilution of solids, Bradford test proves protein again.

10. During the winter several colorimetric methods were developed for sensitive protein detection & concentration determination. These can be applied as the case & need arises.

11. The value of purified substances can be appreciated in many ways & they can be developed. The requires extensive & complex work. However such compounds are then
    1. simpler to analyze & identify, even if only w/ related compounds.
    2. Essential for any hope of molecular mass determination.
    3. Colby database, UV & IR work are much more efficient by this type of sample development.

12. Good coursework is now available, esp Davis w/ latest courses - 3 full & high quality courses now available along with the pleasures of the Duke Univ. course.

13. There are so many projects active, of increasing complexity, sophistication and consequence that it becomes increasingly difficult to:
    1. Choose from established priorities
    2. The ultimate manpower limitation, i.e., one person for all
    3. manage the reality of time
    4. Write papers on a Continuous basis.
    The work is far too productive now to keep up with that task, probably on an order of @ least 10 to 1. The notebooks will need to suffice for almost all now.

14. UV acquisition is a great benefit:
    1. Uniqueness of Colorless species
    2. Fundamental structure elucidation or structural similarity is possible to some degree, esp w/ physical property supplementation, ie primary functional groups + use of the Cats Colby database.

15. Oxidative Stress measurement developed w/ UV & Malondialdehyde absorbance @ 267nm. An alternative to the OXIdata test.

Continuing.

Today there are some calls to be made.
Projects of high priority are:

[redacted]

2. Molecular mass determination
   - method development
3. Data production
4. Culture monitoring - increase volume
5. Follow through and organization of the complex HEPA air filter project.
6. ICMP data release
7. Citizen samples
8. Molecular spectroscopy course -
   keep ahead of schedule - NMR now.
9. LC Colored resin

Sucrose Molecular Mass Determination.
Boiling Point Method - Calibration First:
100.00 gms boils @ 98.0 Distilled

Realize that mass of water is changing as it boils.

Ext t°C	100.00 ml	min sec	temp	Notes:
	t	0	60°C	Bubbles start on probe
175°		3 30	71°C	More bubbles on Probe
179		5 15	77°	1st small bubbles rising
182°		9 30	88°	Extensive bubbles on probe
184°		10 15	90°	Bubbles on probe start to rise
187		12 00	94°	Small Continuous bubbles
187		12 30	95°	Bubbles increasing
189		13 00	96°	Steady bubbles
189		13 30	97°	"
189		14 00	97.6	Steadying temp
189		14 30	97.8	"
189		15 00	97.9	"
189		15 15	98.0	"
192		15 45	98.1	"
192		16 00	98.0	Max. reached

Now weigh 3.07 gms of sugar into 100.00 ml H_2O

Use 100 gms of H_2O:

Ext t°C	t^m & sec	temp °C	Notes
192°C	4^m 30^{sec}	53°	First bubbles on probe
189°C	13^m 00	84°	Bubbles rising
189°C	13^m 30^s	86°	Extensive bubbles on probe
187°C	16^m 00^s	92.8	Bubbles on probe rise
187°C	18^m 48s	98°C	Probe "boiling"
187°C	22^m 00s	98.3°C	Max temp beg reached Boils on surface rather than bottom.

This is my answer. 98.3° $\Delta T = 0.3°C$

$\Delta T = K_b \cdot$ ~~mol~~ "m" $\quad m = \frac{\text{mols}}{\text{kg}} \quad K_b = 0.513\ °C/m$

So $m = \frac{\Delta T}{K_b} = \frac{0.3°C}{0.513\ °C/mol}$

$= 0.585$ ~~mol's~~ molality $\quad = \frac{1.709\ \text{g}}{\text{mole}}$ ~~= 1709 gms~~ mole?

so 3.07 ~~gms~~ kg

3.07 gms = $3.07E{-}3$ kg

Page
300

Van't Hoff Factor (?) Look up.

But we used only 100 gms of solvent, not a kg. Maybe 1410.9 gms / mole

Molar mass of sucrose is 342.3 gms/mol

$\Delta T = K_b \cdot \frac{\text{moles solute}}{\text{kg solvent}}$

Moles of solute = .585 moles/kg × .1 kg = .0585 moles of solute

Molar mass = $\frac{3.07 \text{ gms}}{.0585 \text{ moles}} = \frac{x}{1}$ $x = 52.5$ gms/mole?

$\Delta T = K_b \cdot \frac{\text{moles}}{\text{kg}}$ $\left[m = \frac{\text{moles}}{\text{kg}} \right]$ $\Delta T = K_b \cdot m$ $= \frac{.3 \ (.1)}{.513}$

$= \frac{.058 \text{ moles}}{3.07 \text{ gms}} = \frac{1}{x}$ $x = 52.9$ gms/mol

Our ΔT would need to be $\approx .05°C$ not .3!!

off by a factor of 6.5?

Max abs. of malondialdehyde @ 266-267 nm has been corroborated by a second source.

Measured ~3.0-3.5 Jun 09 2017

DIRECTIONS: Lightly tap the ampoule on the countertop to be sure all the liquid is at the bottom. Break the top off the ampoule using the plastic safety top. Discard the top. Draw up 1 ml of sample with the dropper and squeeze from dropper into ampoule. Evaluate reaction when color stabilizes (do not wait more than 5 minutes to evaluate). The included color evaluation chart may be used as a qualitative, semiquantitative index.

= Abs 267nm of 0.9763

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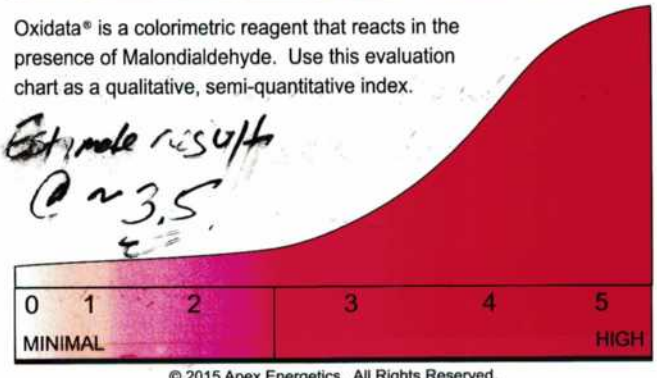
Estimate result @ ~3.5



This did not work @ all. We theoretically would have to have used a ΔT of ~ 0.05 deg C which is beyond thermometer accuracy and also did not fit the data even closely. We measured 0.3°C ??

Your sugar solution would have to be far more concentrated.

Lab example was NaCl he gets $\Delta T = +4^{\circ}C$
He gives has 100 ml of H_2O mass of salt = 11.57gms
and has
He also has $K_f = 0.513^{\circ}C/m$
This means

$$\Delta T = K_f \cdot \frac{\text{moles}}{\text{Kg}} \quad \text{or} \quad \text{moles} = \frac{\Delta T \cdot \text{Kg}}{K_f}$$

$$= 4^{\circ}C \cdot \frac{0.1\,\text{Kg}}{.513\ ^{\circ}C/m} = \frac{0.780\,\text{mole}}{11.57\,\text{gms}} = \frac{1\,\text{mole}}{X\,\text{gms}}$$

$X = 14.83$ gms/mol actual = 58.44
Error factor ≈ 4.0?

He did not give lab computations but the same problem is arising again. (33)

Written example:

Ethanol solvent 19.63 gms = 19.63E-3 kg

$K_b = 1.22 \frac{°C \cdot kg}{mol}$

$\Delta = 2°C$

$\Delta T = K_b \cdot \frac{moles}{kg} \Rightarrow moles = \frac{\Delta T \cdot kg}{K_b} = \frac{2 \cdot 19.63E\text{-}3\ kg}{1.22 \frac{°C \cdot kg}{mol}}$

$= .0321 \frac{moles}{3.2361\ gms} = \frac{1\ mol}{x} \quad x = \frac{100.6\ gms}{mol}$

Actual answer given is 100.6 gms/mol

This means your computations are correct.

The question now is the experimental data.

The method then is simple. The experimental data apparently is not a subject to great error. Why?

Notice he is dissolving $\frac{3.2\ gms\ solute}{19.6\ gms\ solvent} = 16\%$ solution.

I have a 3% solution.

You would have needed a whole lot more sugar g 34 gram grams

That is a lot.
Lets try 15 grams

We have 17.82 gms

Use ~~102.6~~ 2 gms H_2O
101.45

Maybe the boiling point method is not as sensitive as the freezing point depression method?
BP ≅ 98.43 $\Delta T = .43°C$

$$m = \frac{\Delta T \cdot k_b}{k_b} = \frac{.43°C\,(.10145\,kg)}{.513\,\frac{°C \cdot kg}{mol}}$$

$$= \frac{.085\,mol}{17.82\,gms} = \frac{1\,mol}{X\,gms}\ X = 209.6\,\frac{gms}{mol}$$

VS 342.3 $\frac{gms}{mol}$ actual

So ether method is just not sensitive enough. Apparently freezing point depression is more accurate method.

The method is only theoretical as far, not practical. Large sample size required, large error in measurement, not a sensitive method.

You tube lab demo again
Also look up Van Hoff factor

He uses 5 gms of sucrose. (4.992)
He is using a small test tube, only 10ml of solution so it is again a very highly concentrated solution. and a salt that w/ 25 gm salt.
The K_f is approximately 6 times greater than K_b.
Thus increase accuracy by factor of 6.

Water 28.90 gms
5.24 gms sugar

Temp Log

Ext	time m	s	temp	Notes
-11.4°C	1	00	-2°	
-10.5	8	30	-8.8	Min reached
-10.4	10	30	-8.9	Peaking - Stabilizing
-10.3	12	15	-9.0	" "
-10.3	12	45	-9.1	" "
-10.3	13	30	-9.2	" "
-10.3	14	15	-9.1	Reversing
-10.0	17	45	-9.2	Returning
	When you stirred the thermometer everything changed			
-8	20	35	-1.2°C	
	21	00	-1.3	Still dropping however.
-7.9	22	00	-1.5	
-7.4	23	45	-2.0	
-7.0	26	00	-2.4	
-6.5	28	00	-3.0	
-6.1	31	00	-3.5	

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Water $K_f = -1.86$ C° kg/mol
$K_b = 0.512$

$m = \frac{\Delta T \cdot kg}{K_f} = \frac{-1.2^\circ (.02890\ kg)}{-1.86} = .0186$ moles

$\frac{.0186\ moles}{5.249\ gms} = \frac{1}{X}$ $X = \frac{282}{}$ vs 320 342

Not bad

So we have gone overboard now
At a depended freezer. You needed to the
story the solution occasionally.
So you need to catch the beginning of
the freeze cycle & look @ it. Not the
end of the cycle as afterwards.

The actual depression point should be ~ 0.9°.
This means that it got for too cold & your
1.2° up on stirring was closer. Still the method
seem subject easily to error.

Shaking & stirring does stabb to stabilize.
Ok, I get −1.0 on a slurry.

Ok, what you need to do apparently is produce a slurry.

O/c I see how the work. You must just produce or melt the ice crystals. It is an extremely sensitive procedure. It requires continuous mixing @ the point of crystal formation.

You did get $\Delta T = -0.9^\circ C$ over and over w/ careful calibration.

So now we have

$$m = \frac{0.9^\circ}{-1.86\ ^\circ C \cdot kg/mol}(.02890)\,kg = .0140\ moles$$

$$\frac{.0140\ moles}{5.24\ gms} = \frac{1}{x} \qquad x = \frac{374\ gms}{mol}$$

VS 342 error = 8.5%

You would not be able to do any better than that.

You need some differential error analysis here

$$MW = \frac{\text{wgt of unknown Compound/solvent}}{\Delta T \cdot \text{sample mass}} \cdot kf$$

or

$$MW = M_{solvent} \cdot \frac{kf}{\Delta T} \cdot mass_s^{-1}$$

$$\Delta MW = \frac{kf \cdot mass_s^{-1}}{\Delta T} \Delta M_{solvent}$$

$$\Delta MW = -1 \cdot \frac{M_{solvent} \cdot kf}{\Delta T \cdot mass^2} \Delta mass_s$$

Therefore the error in MW is a function of the mass of the sample^2 (squared). This means you want the mass of the sample as large as possible relative to the solvent.

Try again w/ 10 gms of sugar!

Jun 12 2017 Molecular mass of sugar (cont)

60 w/cork adherence
36.60 gm H_2O
10.95 gms Sucrose
$\Delta T = -1.55°$

$MW = \frac{(n)(36.60E-3\ kg\ solvent)(-1.86°C \cdot kg/mol)}{(-1.55°C)(10.95E-3\ kg)}$

$n = \frac{1.55°C\ (36.60E-3\ kg)}{1.86} = .0305\ moles$

$\frac{.0305\ moles}{10.95\ gms/sucrose} = \frac{1}{x} \quad x = 359.02\ \frac{gms}{mole}$

vs actual 342 excellent work

$E_{MW} = 5.0\%$

Okay, you finally have the methods down & in place to determine molecular mass. There definitely are some tricks to the procedure.

1. You want to achieve the highest concentration you can by choice of both solute & solvent.

2. Total solvent volume needs to be ~ 35 mL to measure and contain large test tube w/ a large cannery jar.

3. Ice should be removed of water w/ plenty of salt.

4. You can work either direction, melting point or freezing point. You need only the first appearance of ice crystals in solution, both melting & freezing directions.

5. Continuous stirring w/ glass rod & close measurement monitoring in real time w/ an accurate thermometer. Read to ±.05°C if possible.

6. Study and use the rule of 13 (gas tube - spectroscopy) to make first estimate of molecular structure.

7. The additional, and most important condition is that the solute must be a non volatile and it must dissolve completely w/in the solvent.

8. The end formula is:

$$MW = \frac{\Delta T_{(°C)} \cdot \text{mass of solvent in kg} \cdot K_f \left(\frac{°C \cdot kg}{mol}\right)}{\text{mass of unknown in GRAMS}}$$

the downgrade Van Hoff factor, which must be studied and accounted for.

Rearranging this:

$$MW_{(gms)} = \frac{\text{mass of unknown in grams} \cdot K_f}{\Delta T \cdot \text{mass of solvent in kg}}$$

Example w/ Sucrose:

$$\frac{10.95 \text{ gms} \cdot 1.86 \frac{°C \cdot kg}{mol}}{1.55°C \cdot 36.60E-3 kg}$$

= 359 gms/mole OK vs 342 actual

5% error case

Jun 12 2011 - (Cont)

Today - General Topic List

1. A Call is in place - always a significant interruption in the schedule

2. Molecular mass determination has worked for the first time. A requirement (often difficult) will be that the material is

Conditions:
1. pure
2. dissolves completely in something
3. is not volatile
4. You know the Van Hoff - factor
5. adequate sample material is available.

These are actually fairly strict requirements but we will need to meet them

A volatile liquid is handled through an entirely different process, eg oils also. This would be good to work on. Getting adequate protein material is an entirely different matter.

Edvotek Protein Labs?

3. We must study NMR today - there is extremely new material.
   Maybe we can accumulate via GAMESS?
   Reading software could also be helpful.

4. LC run on colored materials of ethanol helpful. Maybe repeat on opaque elute?

[redacted] book - must term critical

6. DNA production
7. ICMP data release
8. Citizen samples

Molecular weight of powdered milk? 150 kDa

Caseins: 25-35 kDa $\cong$ 4.98 E-20 gms
separated by acid

1 gram = 6.022 E20 kDa

a kDa is a "kilounified atomic mass unit"

A dalton is equal to 1 gm/mol. This is the reference

There something that is 30 kDA is equal to 30K gms/mole

So a protein of this type:
1 gm of this protein = 33.33 E-6 moles
1 mole of this protein = 30,000 gms.

It is not that a protein is very heavy, it is that it takes a lot of protein to make 1 mole.

Eg for Carbon, one mol weighs 12 grams.
But one mole of this particular protein weighs 30,000 gms!

So obviously it takes a lot of protein to make one mole.
It would be equiv like imaging 30 liters of water to make just one mole of that protein! Obviously the scale system is different.

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Molecular mass? Can anything dissolve charcoal, for example?

Definitely not an easy task, so that is a typical example of a problem not approachable by that means.

Since we know what seem to be a volatile form of distillate protein (BP = 78°C) we should be able to estimate the molecular weight based upon the volatility method of

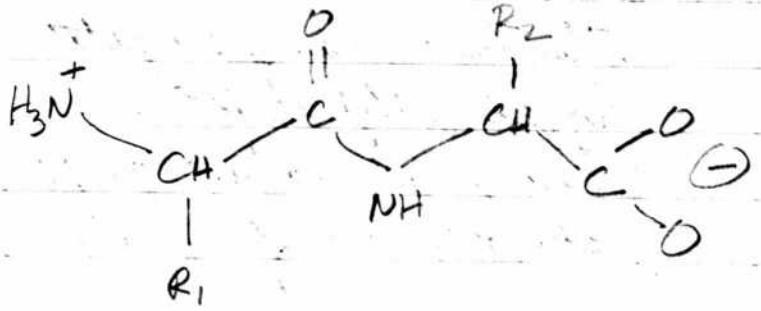
$\frac{m}{V_{STP}}$ = $\frac{\text{molar mass}}{\text{molar volume}}$

m = mass in grams of a sample gas volume
V_{STP} = volume of the gas @ STP (22.4 liters)
molar mass of the gas (mass of 1 mole of the gas

This could be a very interesting project.

Notice that proteins have the following functional groups:

CH
NH
C=O
R groups



A peptide bond!

Notice in our LC columns of opaque # that we do have a precipitate ~~that has settled~~ out. A clear solution remains. We have to wonder if the precipitate is actually ethyl acetate in a relatively pure form and that the clear solution is an protein of extract w/ BP 70°C.?? We have a gravitational separation.

We have some good LC protein tests in place.

Proteins Continued from HEPA File

LC Protein Tests: & LC

1-1, 1-2 Combined 06-08, 06-09
The tube has precipitate settled.
Suggest ethyl acetate.
Clear solution above, ~ 10 ml light
Tests negative for protein, blue green

1-04, 1-05 on 0608 Combined
Slight greenish tint to sample

Control Bradford peaked @ 636 nm
01-04, 01-05 Combined measured @
622 nm. This is blue shift that
confirms protein content.

Next is 01-05 on 06/13 @ 0015
Also a slight greenish tinge (very weak)
Measure @ 636 nm
Low protein content indicated but
detectable @ trace bradford levels.

01-08 on 06/13 @ 0045
Has the darkest greenish tint.
Measure @ 619 nm
Positive Protein Content.

What we first theorize is that either acid or base can induce the general elute to leave the column. Base may be more effective.

Protein Content is directly tied in with the intensity of the general test liquid eluted from the column.

The theory that we have measurable protein content with the HCPA filter. The most convenient way to extract the protein in concentrated form would be to salt it out, 1-B on 06/13/17 of to your preferred sample to work with.

Compare the protein against the CDB anaerobe cultures.

Jun 13 2007

1. Spectroscopy course - NMR
[redacted] book
3. Continued molecular mass determination
   1. Volatile liquids
   2. Echotech labs
   3. Practical cases?
4. Keep on top of GAMESS
5. UV software purchase decision?
   (mostly theoretical vs Colby?)
6. DNA production
7. ICMP data release
8. Citizen samples
9. Culture monitoring & resets

10. The question of comparison of proteins -
    Three different proteins may now be in place
    1. Original CO3 breakdown - non H_2O soluble
    2. Anaerobic CO3 protein production?
    3. HEPA Air Filter Analysis - separated by LC

11. Is the salted precipitate ethyl acetate?

Questions which arise:

1. How does the LC HEPA filter isolated from the compare w/ the anaerobic system?

UV analyses is the easiest place to start here.

You have reorganized the LC workstation.
Very good. 254/280 UV Gilson instrument no longer has value & the space has been cleared.
We now have a dedicated UV - LC workstation
We can measure easily almost simultaneously:

1. UV - VIS - NIR spectrum
2. Index of Refraction
3. ORP
4. pH
5. Electrical Conductivity

] with pressurized LC column adjacent to all.

Let's compare UV spectrum.

VIS + UV

We UV scan

(1) 01-04,0105 on 06-08 @ 1600 λ_{max} = 622nm
Confirmed w/ Bradford

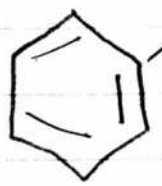
(2) 01-08 Con 06-13 @ 0045 λ_{max} = 619
Confirmed w/ Bradford strong signal.

We have similar UV scan for each sample although Scan #2 is much stronger. Our peaks are @ ~ 280 & ~230nm

We now understand from the UV scans that we have positively isolated and identified a protein from analysis and separation of materials collected in the long term HKPA filter.

We also know that the protein contains the aromatic amino acid tryptophan.

Colby database comes up with Aniline



Not bad! Described as the "prototypical aromatic amine"

Note
//

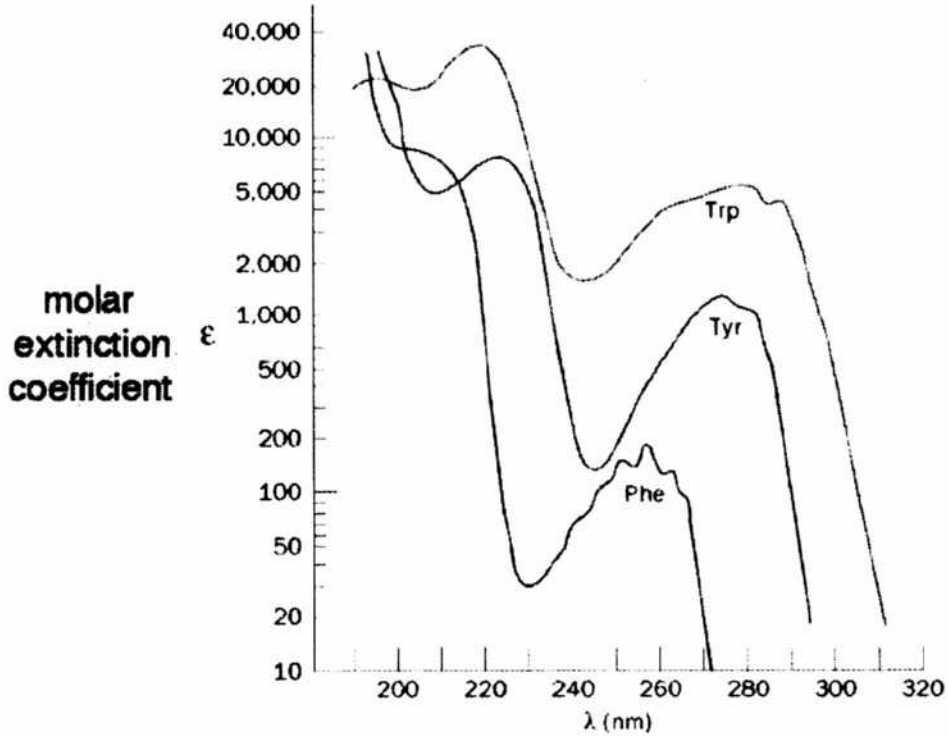
Now we compare these scans to the anaerobic cultures. First they will learn if it must be significantly diluted.

If it is even more concentrated than I expected 1-2 drops in the cuvette will be sufficient.

(3) Yes, we do have the same spectrum as Scan (1). It appears to be shifted slightly to the right. This means that the protein being produced is highly concentrated & we need only one drop - 2 drops in the cuvette. IR analysis of the well is helpful. Molecular weight determination could also be very interesting.

Perform the Bradford test.
Bradford test of the anaerobic protein & secreted protein is highly positive w/ λ_{max} @ 622nm.

We also know that both proteins are highly acidic w/ secreted protein measuring @ ~ pH 2.0. This strongly indicates that glutamic acid is involved along w/ tryptophan. Also a water soluble protein.



UV absorbance spectra of the three aromatic amino acids, phenylalanine, tryptophan, and tyrosine

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Now we have a question about the rainwater sample.

Ok, the amazing finding is that we have the same general UV spectrum showing up in the Concentrated rainwater sample.
Concentration factor on rain sample is 14.55

Let's see if we have enough material to conduct a Bradford test.

Yes, we have just enough material (~ 3ml) left in the particular rainwater sample to conduct the Bradford test.

The Bradford test is highly positive w/ the rainwater sample (Concentrated 14.55) w/ $\lambda_{max} = 619$ nm.

This work proves, to the level of UV analysis and the Bradford test that the

1. CDB secreted protein (Culture)
2. The HEPA LC isolated protein
3. And a rainwater Concentrate protein

] ARE ALL THE SAME PROTEIN.

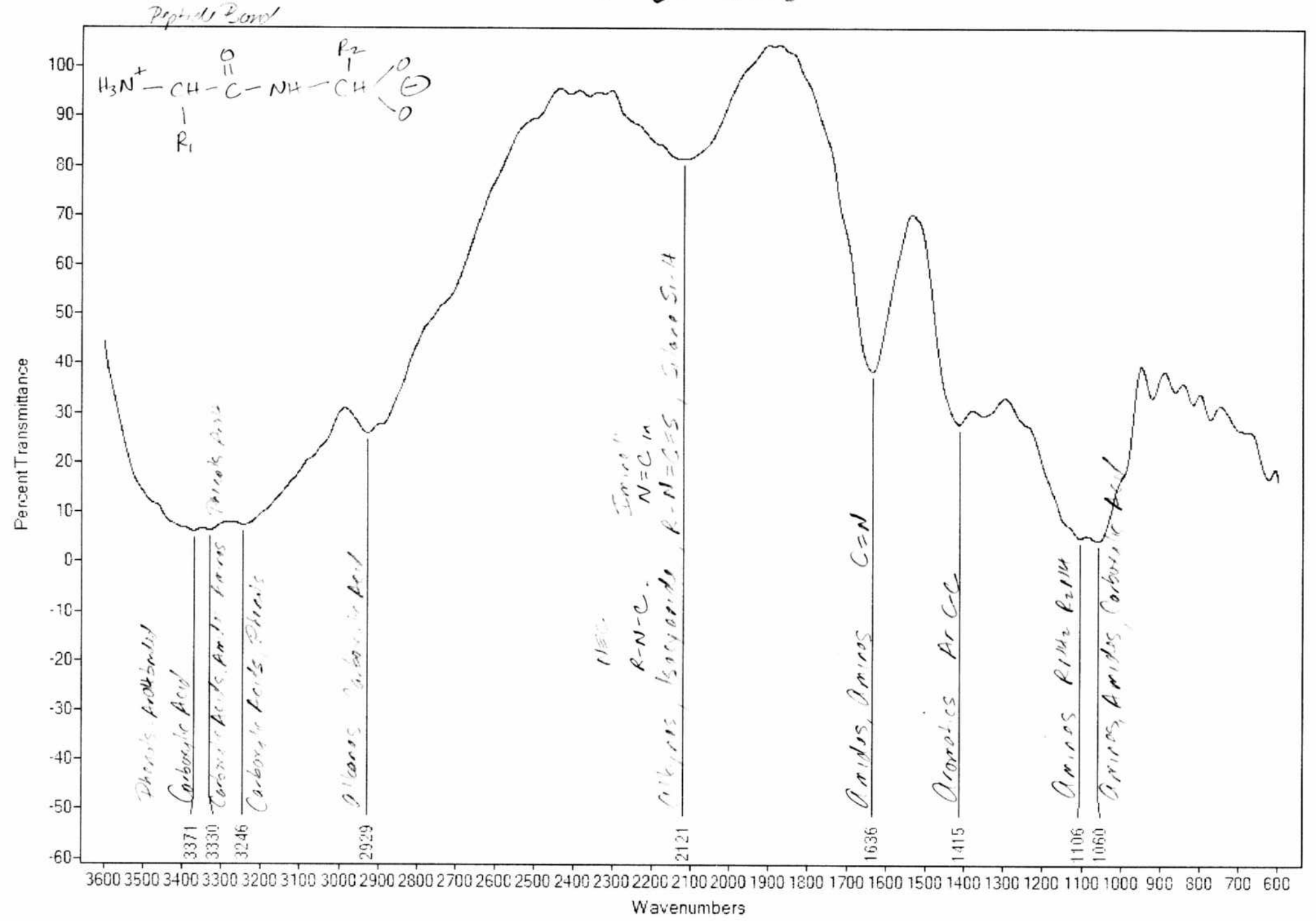
This is a highly significant finding.

Another way that you could run LC is to collect in one large container that will accomodate all electrodes and sensors, and then draw off fluids @ intervals desired. The bar potential also.
The transfer device is an bugged quartz.

A basting syringe looks like it will work quite well. Now I am not moving the containers + electrodes only 15 minutes — Also less salyes to overflow.
Careful on IR ATR, pH of protein is low enough to affect crystal.
We better use KCl crystal

Decent IR plot obtained

Notice potential Imines or Isocyanides.


Peptide Bond
Percent Transmittance
Wavenumbers
3371
3330
3246
2929
2121
1636
1415
1106
1060

Next topic is to scan w/ IR the LC isolated protein. We know that we have 3 proteins but there is nothing to say they are the same. We have

1. Original CDB protein (precipitate from)
2. Secreted Protein
3. LC separated protein - HEPA filter
4. Denaturate protein.

We can already see that the secreted protein and the LC isolated protein from the HEPA filter are not exactly the same, even though they both have

1. similar UV spectra
2. both water soluble
3. both very low pH.

June 14 2017

Molecular Mass of Salt:

Mass of Water 39.13 ml - gms

NaCl Mass 4.17 gms

Subtract two drops from water = .12 gms

⇒ Mass of water = 39.01 gms

Mass of NaCl = 4.17 gms

Freezing point: −5.45°C (1) −5.3° (2)

Hold thermometer up high to make room for stirring rod.

$MW = \frac{\text{mass of unknown in gms} \cdot K_f \cdot \text{Van't Hoff factor!}}{\Delta T \cdot \text{mass solvent in kg}}$ (gms)

$= \frac{4.17 \text{ gms} \left(1.86 \frac{°C \cdot kg}{mol}\right) \cdot VHF!}{(1.1°C + 5.45)\ 5.45°C\ (39.01E{-}3\ kg)}$

VHF! $30.36(2) = 60.7$

~~375~~ gms

$= \frac{36.5 \text{ gms}}{mol}$

Actual is 58.44 Error = ~~37~~% 4% excellent ~~Abt right~~

Just MW is so low it is easy to introduce error. However error does not seem like it should be this high. Your mass would have to be ~6 gms. No way with this error.

June 14 2017 (cont)

Today we:

Getting there! It is work..

1. Keep up w/ adv molecular spectroscopy course. Increasingly demanding as anticipated.

Not bad! ✓ 2. Working on molecular mass of NaCl.

[redacted] work

✓ 4. Compare IR spectra of CDB elevated proteins against LC protein isolation.

Then try to locate in saliva protein and original CDB separation protein.

5. Ethyl acetate LC examination.
6. LC produce additional elute volume ethyl acetate & protein and?

7. Clean HEPA filter analysis, esp for ethyl acetate?

8. Monitor cultures
9. Produce DNA
10. Cytogen sample
11. ICM release

There is no way that the molecular mass of salt should have that much error in it. Lets try again. We do need a cap for a large test tube that does not leak.

Notice you do not need very much salt to produce a significant freezing point depression.

Again:

Mass of H_2O ~~35.24 gms~~

Salt gms NaCl 3.28 gms salt

2 drops lost again $\Rightarrow$ 35.12 gms H_2O

Freezing point: $-4.3°C$

Calibrate thermometer. Calibrates @ $+1.1°C$!!!

IT IS WHAT IT IS! ~~But it is the wrong direction.~~

Without thermometer calibration

$MW = \frac{3.28 \text{ gms}\ (1.86)}{4.3°C\ (35.12E\text{-}3 \text{ kg})} = 40.40 \text{ gms/mol}$

Need Van't Hoff factor

Better, but still low and remarkably close to previous run. I do not know why it came out lower, method seem very sound.

We now have a comparison between the CDB secreted protein and the LC HEPA air filter separated protein. We can see that they share the same fundamental characteristics.

The alkane and alkyne properties are also of much interest. We should be able to test for alkynes.

Recall also the high silica tendency in the rainwater analyses - this was surprising @ the time and still is.

The next goal would be a rainwater protein but this is difficult to concentrate sufficiently and also remove the water. Salting out is a possibility here?

IR of LC HEPA separated protein + comparison to CDB Secreted Protein - Also see Jun 13 2017 IR Plot

HEPA Air Filter Protein Jun 13 2017 - 01.JPEG

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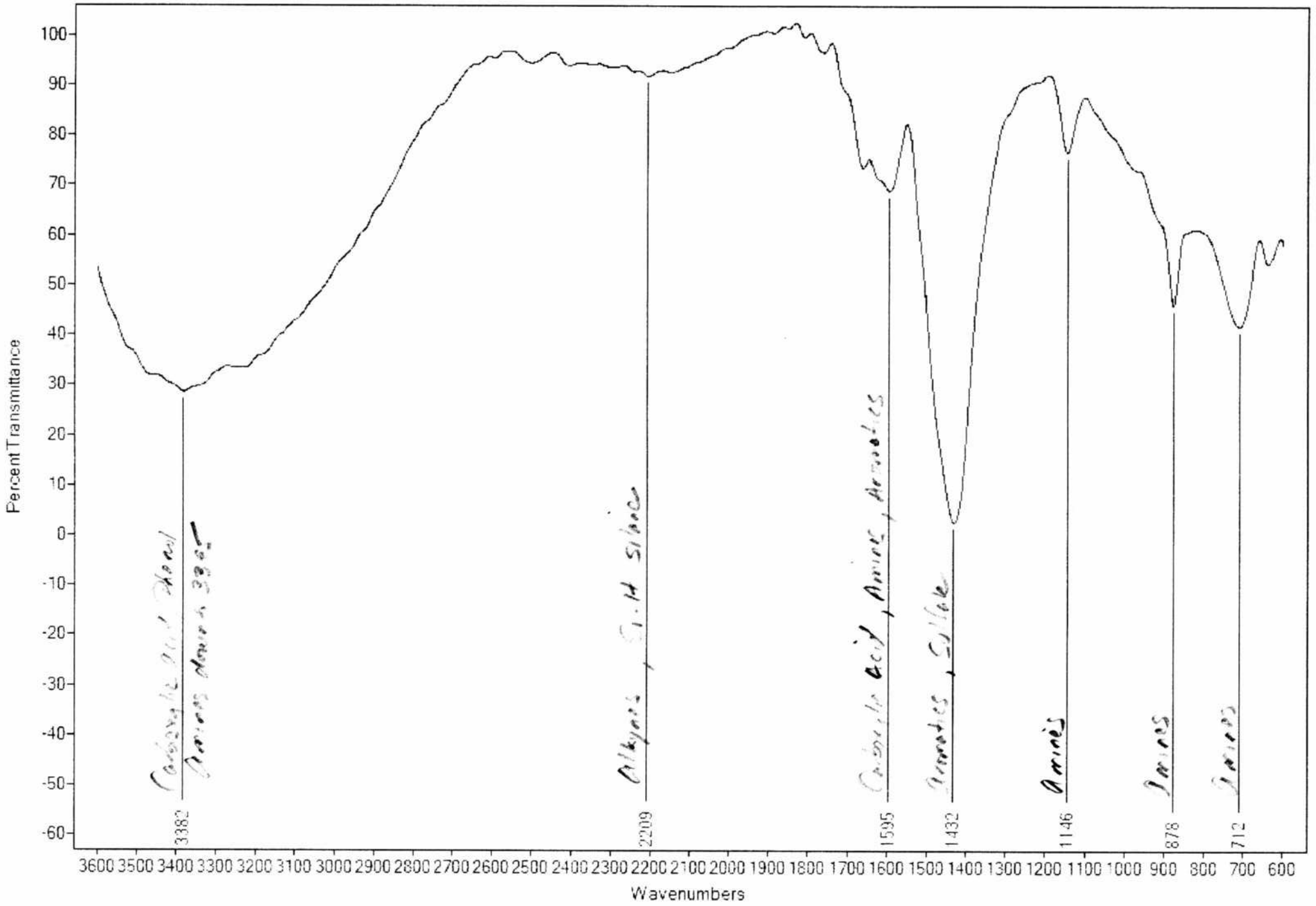
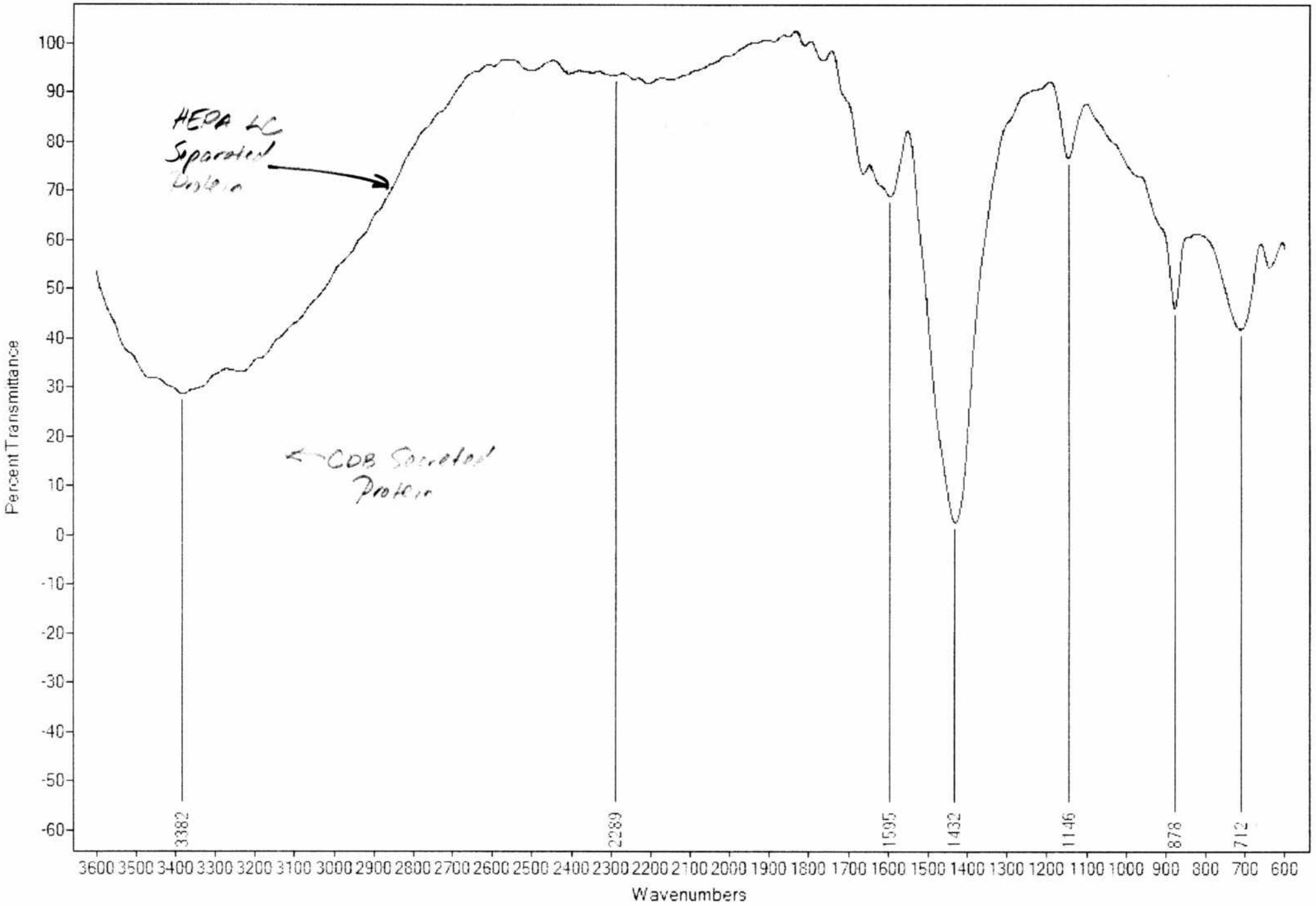



HEPA LC Separated Protein
CDB Secreted Protein
Percent Transmittance
100
90
80
70
60
50
40
30
20
10
0
-10
-20
-30
-40
-50
-60
3382
2289
1595
1432
1146
878
712
3600 3500 3400 3300 3200 3100 3000 2900 2800 2700 2600 2500 2400 2300 2200 2100 2000 1900 1800 1700 1600 1500 1400 1300 1200 1100 1000 900 800 700 600
Wavenumbers

Method Development —— Molecular Weight Determination

It is interesting when the salt water crystallized immediately upon stirring @ a certain ~~point~~ temperature of tube registered ~ −10°C and it was still liquid. The solution was stirred, the entire volume immediately crystallized and the resulting temperature equalized @ ~ −5.5°C. The actual melting point was determined @ −4.3°C.

What is really odd is that the thermometer Calibrates @ +1.1°C w/ freezing distilled water. Therefore when it says +1.1°C it is actually zero. The says the thermometer is really too high. However this is in the opposite direction of our error.

You would think that this would mean our actual measured temperature is −4.3°C − 1.1°C = −5.4°C However, this only make our solution worse. Incidentally what is the Van Hoff factor

Answer. −5.4°C

$$MW \stackrel{?}{=} \frac{3.28\,(1.86)}{5.4^\circ\,(35.12E{-}3)} = 32.2 \text{ gms/mol}$$

Van 't' Hoff factor is 2.!!!!

Therefore our answer must be $2(32.2) = 64.4$ grams/mol!
Then we better.

Thermometer may have actually only been ± 0.8
So $-4.3 - 0.8 = -5.1°C$

$\frac{3.28 (1.86)}{5.1° (35.12E-3)}$ = No this would only make it worse.

$\text{Error} = \frac{64.4 - 58.44}{58.44}$ = 10% error. Then we must more better.

Then tells us that
1. We must calibrate our thermometer w/ freezing solvent.
2. The Van't Hoff factor is critical to know.

Non ionic Compounds: Van Hoff factor = 1
for Ionic solutions it is ≥ 1 (as an integer)
$NaCl = Na^{+1} + Cl^{-1}$ Van Hoff factor = 2
$MgCl_2 = Mg^{+2} Cl^{-1}$ Van Factor = 3
$Ca_3(PO_4)_2$ Van Hoff factor is 5.

On your molecular mass of NaCl determination w/ consideration of calibration of thermometer and the Van't Hoff factor you had two answers:

60.7 gms/mol
64.4 gms/mol

$\bar{X}$ = 62.5 gms/mol
vs actual
58.4 gms/mol

Error is 7% this is quite respectable esp. for such a low molecular wt compound.
You have now completed sucrose & NaCl successfully.

The catch 22 is, if it truly is an unknown YOU DO NOT KNOW WHAT THE VAN'T HOFF FACTOR IS!

You might determine if it is ionic or not w/ electrical conductivity but if it is ionic you still do not know that ratio of composition. Your result could easily be off by an integer value, therefore.

However the Vant Hoff factor is not quite so simple.

1. The VHF does not have to be an integer, it is a measure of dissociation
2. VHF for a non ionizing molecule is 1
3. VHF for an ionic compound is the number of ions that form when the compound dissociates
4. VHF for materials in non polar solvents is usually 1 (because they are typically non ionizing).
5. VHF for an ionizing molecule, an acid, is the number of ions that form.

There are therefore some very serious limitations to determining the molecular mass of a substance this way:

1. You must have enough material to work with
2. You must know what it dissolves in
3. You must know if it is ionizing or not
4. If it is ionizing then you are going to need to know (or guess as a multiple) the Van't Hoff factor
5. The material must be pure and a the concentration in solution must be known and it must be pure in solution -

this can become problematic fairly easily.

I am quite curious, what if you only had to make smaller sample, like 5 ml solvent and 0.2 gms of salt?

Mass of H_2O 6.78 gms
NaCl 0.18 gms
Freezing Point (no calibration) = ~~+1.0°C~~ -0.9°C
We cannot alter the tube or lose any water.
Dissolving should take place through convection or mild agitation

$$MW = \frac{0.18\,(1.86)(2)}{-0.9^{\circ}C\,(6.78E\text{-}3\,kg)} = 98.8\ gms/mol$$

w/out Calibration of thermometer

$$MW = \frac{0.18\,(1.86)(2)}{2.0^{\circ}C\,(6.78E\text{-}3\,kg)} = \frac{49.4}{\text{~~47.0~~}}\ \frac{gms}{mol}$$

not bad. Actual Calibration of thermometer $+0.8^{\circ}C$
Therefore

$$MW = \frac{0.18gms\,(-1.86)(2)}{(-0.9^{\circ} - 0.8^{\circ})\,(6.78E\text{-}3kg)} \overset{VHF}{=} 58.1\ \frac{gms}{mole}$$

vs 58.4 gms/mole actual

Ok we have succeeded in scaling down the operation considerably to a more practical level.

1. Point of freezing/melting is @ point of last visible crystal existence

2. Be gentle with agitation, it is mostly a matter of good observation & consistent procedure.

3. Calibration of thermometer must be included in the process. (Current is +0.8°C)

4. Van't Hoff factor will be required

5. Success now w/ 0.18 gms unknown compound w/in 1 ml of solvent. Then re-great.

5b. Keep your scale very clean.

6. Do not lose any glass, do not invert tube. Use mild convection & agitation to dissolve a water bath.

6b. You need even crystal distribution for good results.

7. Every thing must be kept very clean w/ no contamination.

8. 10 ml test tubes can work fine if you are careful.

You might be able to try things like vinegar and glycerin next. Alcohol have a freezing point of -100°C or so. so these could be difficult.
Volatile liquids probably must be studied separately but would be good to try.

Since you are not using a boiling point set seems like you could use a soluble semi-volatile liquid? or a non volatile liquid.

Seems like glycerine is a good example to try.

H_2O: 8.06 gms
Glycerine: 9.08 gms - 8.06 gms = 1.02 gms glycerine
Total

Freezing Point -1.8 to -1.7 Call it -1.75
Clever use of the scale & tare allows for no pouring or transfer. Direct addition of solute to tare weights

$$MW = \frac{(1.02 \text{ grams})(1.86)(\text{VHF})}{(-1.75 + -.8)^{\circ}C \ (8.06E-3 \text{ kg})} = 92.3 \text{ gms/mol}$$

Actual: 92.1 gms / mol FANTASTIC!

Since glycerin has a freezing point above water, water may not be the best solvent to use. It was a mess at the bottom of the cold tube. I have tried to dissolve better.

It has frozen, however just fine.

It has worked, fantastically well.
I have now determined the MW of an unknown non volatile non ionic liquid.
Measured: 92.3 grams/mol Actual 92.1 grams/mol
Error = 0.2% !!!
So now we have learned that we can apply the method to certain liquids.

Lets try the protein - COB received for kicks.
We do not know how much water or anything else, but it still has a molecular weight.

COB secreted protein trial:

H_2O: 1.58 gms
COB Secretin 1.82 gms

You can just put the tube into the freezer and then let it melt.

MP 0.45°C

Then I do not even need a salt bath. Just a timer as I do not forget.
You can not get much simpler than the method. You really should be able to even use volatile liquids w/ the freezing method & proper choice of solvent.
Salt bath probably quicker for the time being

Solid block @ bottom will make it more difficult to get proper melting point. Melt bottom w/ hand to clear out.

We have an estimated MP of 0.45. uncalibrated. The mean actual MP $\cong$ 0.45°C − 0.8°C = −0.35°C

Repeat measurement gives same result
MP $\cong$ 0.45°C $\Rightarrow$ 0.45 − 0.8°C = −0.35°C

therefore This is a significant measurement.

$MW = 1.82 \text{ gms} \left(\frac{1.86°C \text{ kg/mol}}{0.35°C\ (7.5E-3 \text{ kg})}\right) \cdot VHF = 1276 \frac{\text{gms}}{\text{mol}}$

Ok, there is discrepancy. This demonstrates the likely complexity of the molecule.

Rule of 13 above says Cys

We learn here when there is an ice block (whenever progressive freezing) we must agitate the solution vigorously and continuously to even out the melting of the crystals.
I believe that we have a good reasonably measurement. but we need to repeat a few times
Even if water is present, the proteins certainly dominate the solution.

1 dalton is 1 gram per mole.
1 kilodalton is 1000 gms/mole.
This we would be 1.3 KDa per mole which is very small.

The smallest protein known is
TRP-Cage, w/ only 20 amino acids
DERIVED from Gila monster saliva. 1L2Y
What is the molecular weight? Is the designation from PDB Protein Data Bank

It is artificial

Total Structure Weight = 2171.44

We are assuming no deletion of waters.
The may not be reasonable.
Quantitation would be helpful here.

—

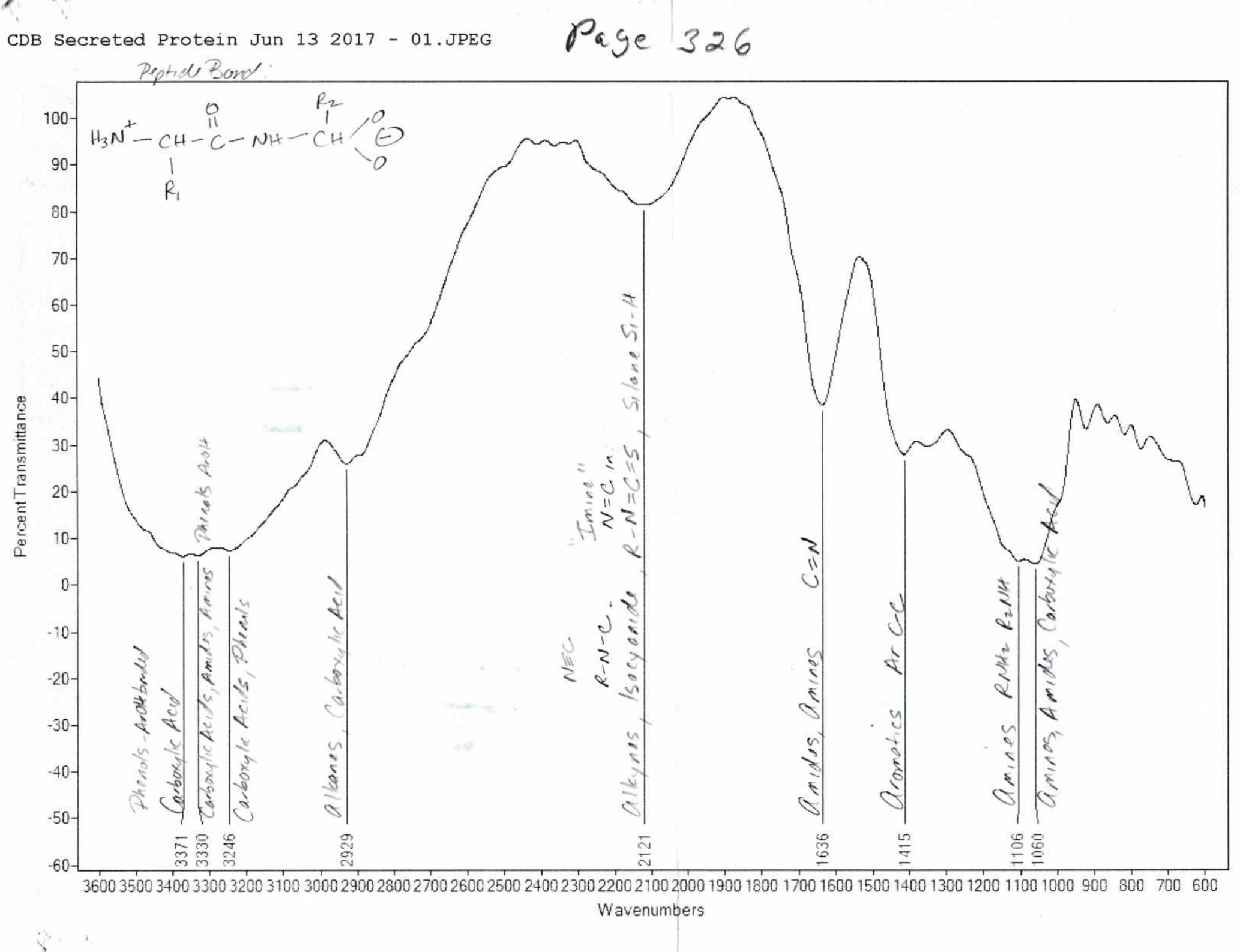
We could easily have a very small protein here. And sign point that direction

—

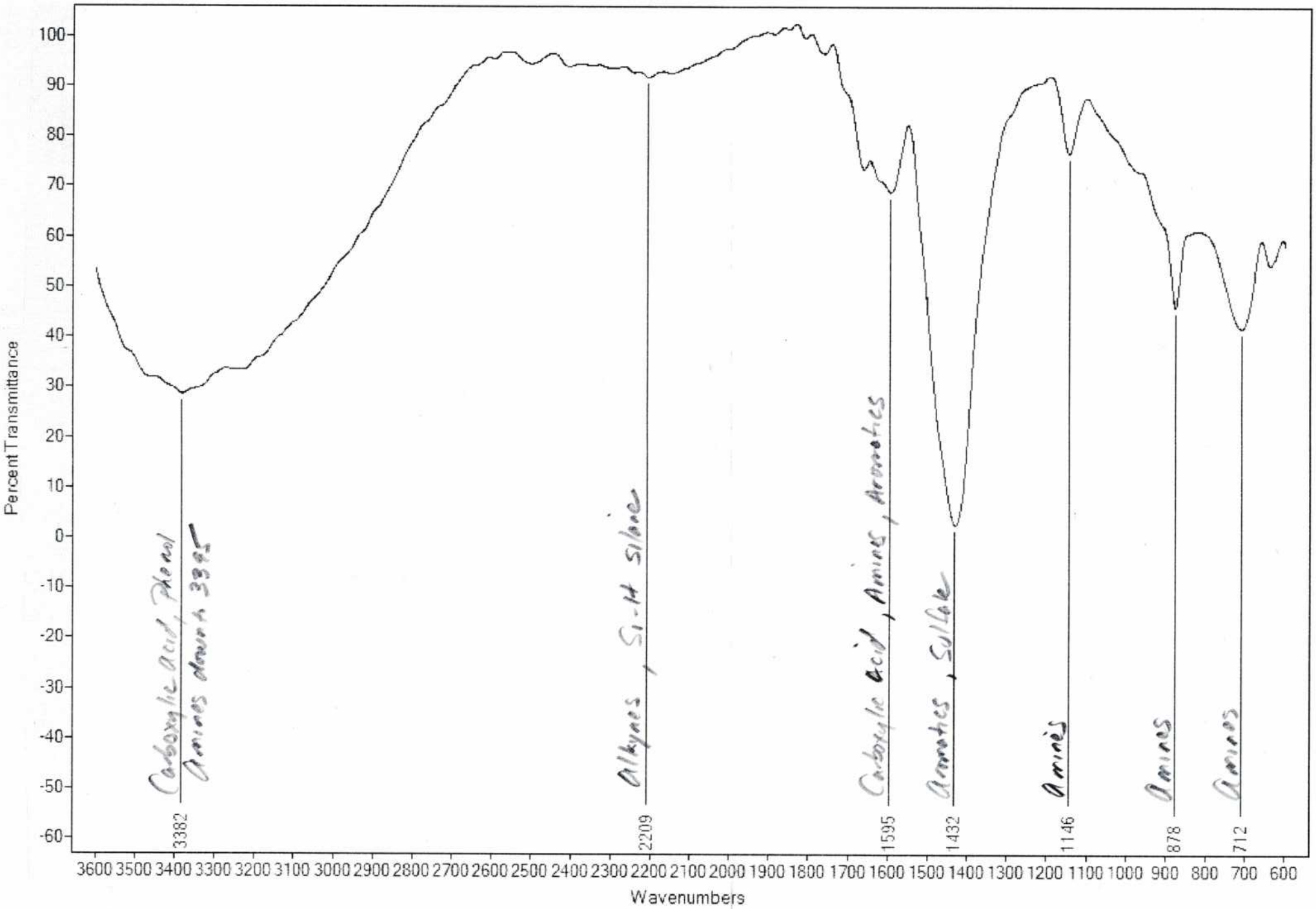
We have succeeded, to a fair degree with MW of

1. sugar (sucrose)
2. salt (NaCl)
3. glycerine
4. COB secreted protein

CDB Secreted Protein Jun 13 2017 - 01.JPEG



HEPA Air Filter Protein Jun 13 2017 - 01.JPEG



HEPA Air Filter Protein - CDB Secretion Protein Comparison Jun 13 2017 - 01.JPEG