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A Release of Internal Original Research Documents

Authored
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3 SUBJECT / À SUJETS

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LIGNES MOYENNES

10.5 in x 8 in / 26.7 cm x 20.3 cm

120 SHEETS/ FEUILLES

Chemistry – Lab Notes

Jul 2017

Vol ~~XX~~

Jul 23, 2017 – Wallace ID

A 2" skin rash area on neck today – first time of any kind. Under observation. Otherwise feel ok and ultrasound will be applied in an exploratory sense. Also sodium chloride applied w/ — ultrasound gel.

A candle as an effective means to produce CO_2. A pint size jar is the right size, a quart jar is too large. Collect w/ some O_2 required for combustion, seal w/ saran wrap, angle extract into syringe. I have a good signal and reading on Hayes dep D @ 150°C.

Paper into GC today via pyrolysis —

We have an important unknown peak of paper @ 3.93 min. Magnitude is ~ 1.0. Can we trap this?

We see:

Methane	C_1
Ethane	2
Ethene	2
Propane	3
Propene	3
Unknown?	
Butane	4
Butene	4
Pentene	5
Hexane	6
	$\Sigma \sim C_{30}$

What is CN of Cellulose?

It is a polymer also

Cellulose is

$(C_6H_{10}O_5)_n$

Not the Oxygen and the H parts in that is of interest here.

Starch is a polysaccharide of glucose units.
So we are dealing w/ "many sugars" here.
Molar mass is 162.1 gms/mol — this will be for the monomer.

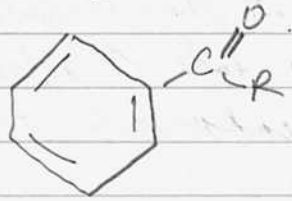
I would like to try to trap the unknown @ 3.93 min.
This peak starts @ 3.5 min
ends @ 4.3 min.

I have injected 1 ml of gas into GC instead of 1/2 ml for the first time. I have had to inject into GC. I have acquired two more signals in the fingerprint region. No alkane or alkene.

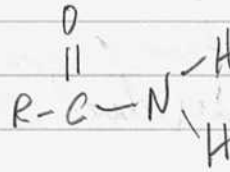
We do have two identifiable + defined signals.
The trap process has worked for the weakest peak.

Our peaks are at only 1693 & 1532.
No hydrocarbons seen.

The unknown peak @ 3.93 min appears to be either an

aromatic ketone 

or

an amide 

This will be it.

You need a further test to separate between these.

The trap process has worked remarkably well. The is superb.

Aromatic ketones and amides are strongest candidates. Amide appears to be the most likely.
It will be the amide.

Amides can also be polyamides. Nylon and Kevlar are polyamides.

Amides are derived from either acids or amines. We do, by all appearances, have the peak identified and it would have been impossible here without trapping & sending into IR. Good work.

The presence of the amide group tells us that we have oxygen and nitrogen in the original compound (or the case, paper).

We all that cellulose definitely has oxygen. The role of nitrogen in the sample (paper) is unknown @ this time.

1 ml injection of gas into GC worked fine by the gas chromatograph that all is volatile.

The amide peak @ 3.96 has a magnitude of ~2.5 mV w/ a 1 ml injection from paper pyrolyzer and you have successfully picked it up on IR gas analyzer

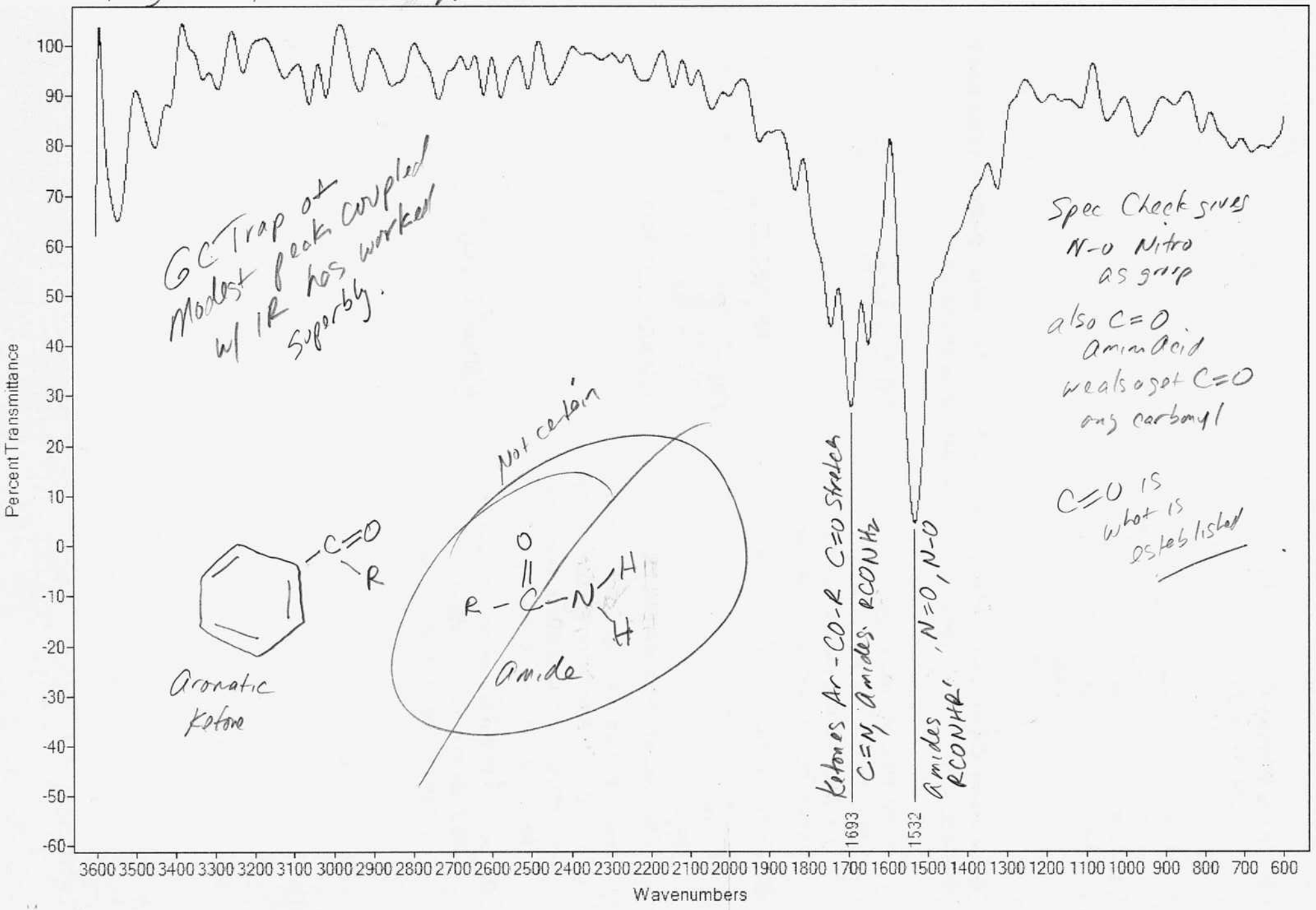
A successfully trapped GC compound and identified by IR analysis. ~~An amide~~ Carbonyl group only. ~~Good work.~~

page 4

Paper Unknown Peak at 3_93 min at 150D Jul 23 2017 - 01.JPEG

IR gas analysis of GC trapped peak.

Carbonyl group only is established here.



OK, here is the value of the work that we just accomplished.

The CDB charred protein sample shows the presence of this same peak. This shows that an amide is a pyrolysis product of the secreted protein sample.

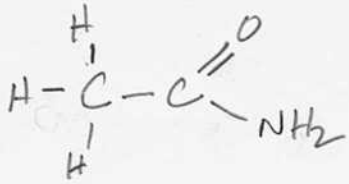
A perfect application of the method that has been developed w/ GC trapping coupled w/ IR analysis.
Now notice the magnitude of the peak w/ the charred protein was only 0.2 mV so you never would have identified it w/ IR by this sample type. Processed paper, 2 ml injection, WAS sufficient.

Dried milk also shows this peak. Magnitude is ~ 5 mV so it would be detectable w/ IR.

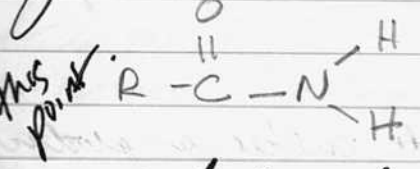
Tea Tree oil does not have the peak.
Yeast does not have the peak.

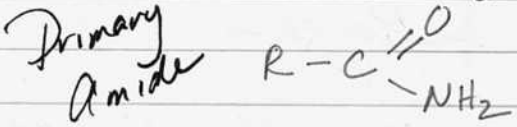
Sugar shows this? Does this make any sense? Sucrose does not have nitrogen in it.
Test this.

I do indeed have an amide peak, albeit small, forming under sugar pyrolysis.

Ethanamide is 

We know now that it is real, but how does it form?

Another way to draw this is 

Primary Amide 

This can only be formed at this point

Only the Carbon can be calculated at this point

It must therefore be formed from the presence of a Carbon Compound in the presence of O_2 & N_2. Not every thing does this, but some things definitely do. Proteins seem to form it as well but the sample compound does not require N in it. Just maybe it is required to have Oxygen in it, as sucrose has that. Keep an eye on Compounds with the oxygen in them to form the compound.

Notice that both sucrose & cellulose both have Oxygen within the Compound. This may be a precursor to amide formation under pyrolysis.

Styrofoam has a small peak but resembles that of a loaded w/ air.

The main hot plate has burned out. The alternate hot plate is heating to 350°C, however, vs 320° for the older one. This is an improvement.

—

We are getting higher gas production in the COB cultures by allowing nitrogen into the picture.

1 ml COB gas injected.

—

Remember how gas active the alternative culture was w/ detergent. We must trap that gas.

Notice that the COB secrete culture is producing the amide peak from the original gas without any pyrolysis involved. It is a clean well formed peak.

The other choice was an aromatic ketone. ketone. We will need to distinguish between an aromatic ketone and an amide.

The production of this peak by more than one method but not all is a curious situation.

It is also interesting that hexane is showing up, with not much in between.

Test for amide: Add NaOH + heat → gives ammonia (smell) and turns red litmus paper blue.

Small amides are soluble in H_2O.

Dried milk will produce the amide peak.

Try to trap the peak in water.

It is one thing to have an amide (or ketone) form as an act of pyrolysis.

But it is another thing entirely for a gas secretion (ie COB incubated secretion) to form an amide (or ketone) natively, without any other reaction involved, including pyrolysis. What exactly is the peak?

First of, is it a ketone or is it an amide? We need to know the difference. Would UV show such a difference? What chemical test can be used to distinguish these from one another?

The idea of trapping the peak from the GC and feeding (bubbled) it into the UV spectrometer via syringe (as opposed to IR gas sampler) has succeeded flawlessly. I have bubbled the trapped gas into a UV cuvette and I have positive UV absorbance up to a max of ~ 0.6. The problem is that there is no dip in the peak. It is monotonically increasing from around 306 nm to 220 nm, with a sharp rise around 230 nm. There is a very slight bump around 272 nm. Lets compare w/ the literature.

242 nm & 300 absorbance is listed for an unsaturated ketone or aromatic ring. I do not say we have this.

Now for C=O bonds alone, a carbonyl has an absorbance of 275 nm as listed. Thus has a possibility of overlap w/ US. This is a $n \rightarrow \pi^*$ transition.

We have found a UV spectrum of a carbonyl (acetone) that has a relatively good match.

So what does seem to be confirmed is that we have a carbonyl group (but whether we can say an amide or not & still do not know.

Significance of the carbonyl group:

1. Causes the molecule to be more polar
2. Causes greater solubility in water
3. Increases melting & boiling point
4. It is likely the most chemically reactive portion of the molecule

We have evidence for a carbonyl group only
Not a ketone
Not an amide. A carbonyl group only.

I think the carbonyl group presence in GC-MS tells you that you have a Oxygen bond (C=O) likely involved. Pyrolysis seems like it is relatively next to a more reactive carbonyl group. The CO2 gas production has the group relatively by no heating required. What does this mean?

Examples of gases with carbonyl groups in them:

1. Formaldehyde
2. Carbonyl sulfide

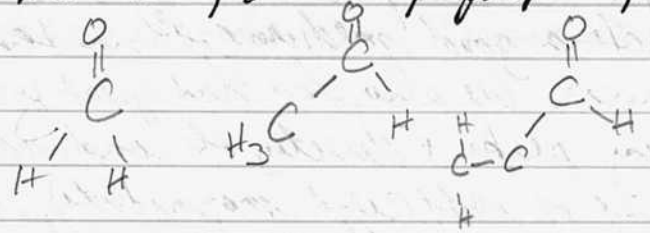
Amides have the highest boiling point (> aldehydes or ketones) so our case is not likely to be an amide.

Ok, this is what we needed to know for now.

Many other candidates in the aldehyde and ketone categories

Aldehyde

methanal, ethanal, propanal, methylbutanal



Ketone

propanone
butanone
pentanone

Many gases, therefore, exist w/ a carbonyl group.

Jul 24 2017
Reestablished Projects.

[redacted]

2. Progress w/ combustion analysis has been made. You all dy faculties w/ GC C-H ratio determination is, real time vs the integral of combustion. Stoichometric methods appear superior for C-H ratio. Nevertheless good progress has w/ additional capability of direct O_2 measurement now in placed.

3. Great progress has been made w/ GC.
   1. Hayes Sep D is far superior. We now have up to C12 compounds being detected, 150°C acts a good medium Coleman temp compromise. We also have done great work w/ trapping peaks & directing the individual peak (if of sufficient magnitude), into either IR or UV instruments. This is a great achievement & helps to both separate & elucidate structure. You have also extensively calibrated the Hayes Sep D column @ 150°C as well as having developed the 150°C to 220°C relationship. You would like very much to increase the diversity of GC operation and extend gamut into solvents as well as derivitization. These are extensive fields of study.

   Through peak trapping - IR you have also identified a carbonyl compound which remains important to identify more completely.

You have managed your traps to include:
1. Transferring a gaslove trapped sample to the IR gas sample tube
2. Bubbling the trapped gas into a solvent (eg H_2O) for use in the UV.

Both methods have worked extremely well and have allowed you to identify SPECIFIC & RESTRICTED functional groups, such as alkanes & Carbonyls.

Your pyrolysis work w/ GC has also been excellent & repeatable w/ temperature control also reasonably established.

You are also beginning to investigate headspace GC as an adjunct to the pyrolysis work.

4. DNA production is of course on the list. I can probably proceed w/ this project at my choice.

5. Citizen sample remain - volume is problematic.

6. Games is interesting but does seem fraught w/ calibration difficulties. Direct lake identification seems preferred by the locals & is too bad that NMR instrumentation is not available to me, I would know how to use it now.

7. ICMP release is still on tap for the summer.
8. Davis courses abound.
9. Proline - glutamic acid, tryptophan simulation remains.
10. Brain wave study - best for field project.
11. We now have successful Cd production by candle w/ lead. → Check this
12. Electrochemistry in future - likely mainstay of wonderland.
13. Can we GC fish oil in acetone, for example? How do ya safely test that remote?
14. Ya have learned ya can safely inject 1 mL of gas if ya only have volatile trace.

Questions:

1. What is the highest hydrocarbon reached thus far direct w/ headspace, w/ NO pyrolysis?

2. Under what sample type(s), exactly, does the Carbonyl group show up?

3. What do we see when we take CDB headspace directly into IR and how is this reconciled w/ the numerous HC's identified w/in GC? What is the highest level HC in GC that comes from CDB headspace?

4. What Carbonyl group is actually present? How would we determine this past IR & with highly limited gaseous sample to work with?

—

Gas production has definitely improved & increased since permitting nitrogen to enter into the CDB trap culture. Could we theyre have a Carbonyl - nitrogen based gas here? It seems to be.

CDB trapped gas run is in place @ 150°C. A great question is that we have Ethene or CO in place - which is it? There is an important distinction. Peak is @ 15 mV as it should be sufficient to trap.

We also have the carbonyl group showing up but the magnitude is only 0.15 mV.
The is not sufficient. You must use milk a alternate sample & produce & test then.

CO has maja IR activity @ 2100 - 2200.
It should be easy enough & distinguish between ethene & CO. Sample one will be an issue

The answer to our CDB gas question is:
1. A minimum of hexane is reached within the CDB gas sample.
1. We have presumably
   1. CO_2 (Fairly large)
   2. Likely some methane
   3. Presence ethene or CO, to be determined
   4. Carbonyl group
   5. Insignificant hexane presumed.
   6. Butane also.

Our ethene - CO question runs from
1.30 min to 2.5 min.
Let's see if we can capture it.

I picked up some of the previous peak on run. Let's see.
The previous peak is assumed to be ethene so there is no major problem there.

You are simply trying to discern between alkanes/alkenes vs CO.

The results are simply not clear.

1. There is no explicit signal of either alkanes or alkenes.
2. There is no explicit signal of CO @ 2100 - 2200 cm^{-1} either
3. There is a discernible signal of the carbonyl group again, with peaks at
   1692 cm^{-1}
   1541 cm^{-1}.

These the same as before, and now we see that it is not reproducible at the t=3.9 section.

We did not, therefore, settle the issue by any means. The signal is ~~not~~ strong enough.

Try again. ~~Absolutely no alkane/alkene evidence~~ NOT TRUE.
No CO evident. Carbonyl is evident.

Our signal is weak, but we do have evidence to suggest the following groups:

Alkenes - mildly probable
Aromatics - modestly possible
Alkanes - slightly possible
Certain Carbonyl — ketones Ar-CO-R - mildly possible
Amides $RCONH_2$ - slightly possible
Nitroso or Nitro (N=O or N-O) Seems specific & unique

For the COB gas peak, therefore, we assume that $t \cong 1.5$ min

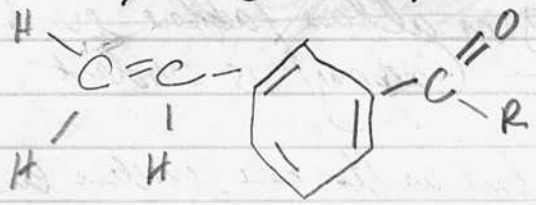
1. We do not have CO
   (The potential overlap with more than one group will be examined further.)

2. ~~Ethane~~ Ethene is consistent w/ this retention time.

3. We may have an aromatic carbonyl w/ethene upon ketone, occurring deri

4. We appear to have nitrogen involved but not sure how.

So we are looking for an aromatic ketone joined w/ ethene in the region.
(ie possibly two separate groups)?



C_9H_8O

This is ethenylbenzaldehyde MW = 132
It is also called vinylbenzaldehyde BP = 232°C
$10R = 1.608$ (high)
UV spectrum looks reasonable

CO_2 absorbs IR strong @ ~ 2360
CO " " @ ~ 2100-2200.

We have created a candle sample of CO_2 & CO.
GC picks up both peaks strongly.
But IR only picks up CO_2, not CO!

Instrument Control laptop computer crashed quite badly & destroyed background IR file. No idea what happened there - & have seen similar behavior on other laptop before - hard drive has block errors somewhere. I have recovered use of the machine but have lost my sequence.

Why had a good IR plot of CO_2 but it did not detect CO? But why since GC did quite easily w/ the candle. What was the magnitude of GC CO detection?

You may have a problem here.
With candle, how do you know you are not producing CO_2 & H_2O instead of CO_2 & CO?

Some things are confusing here, even w/ candle.
Test CO_2 w/ breath and hold breath.

Human breath: no hold:
Why does human breath, not held, show so many components, eg 5?

How can you have a carbonyl group and Ether comp. for direct human breath?

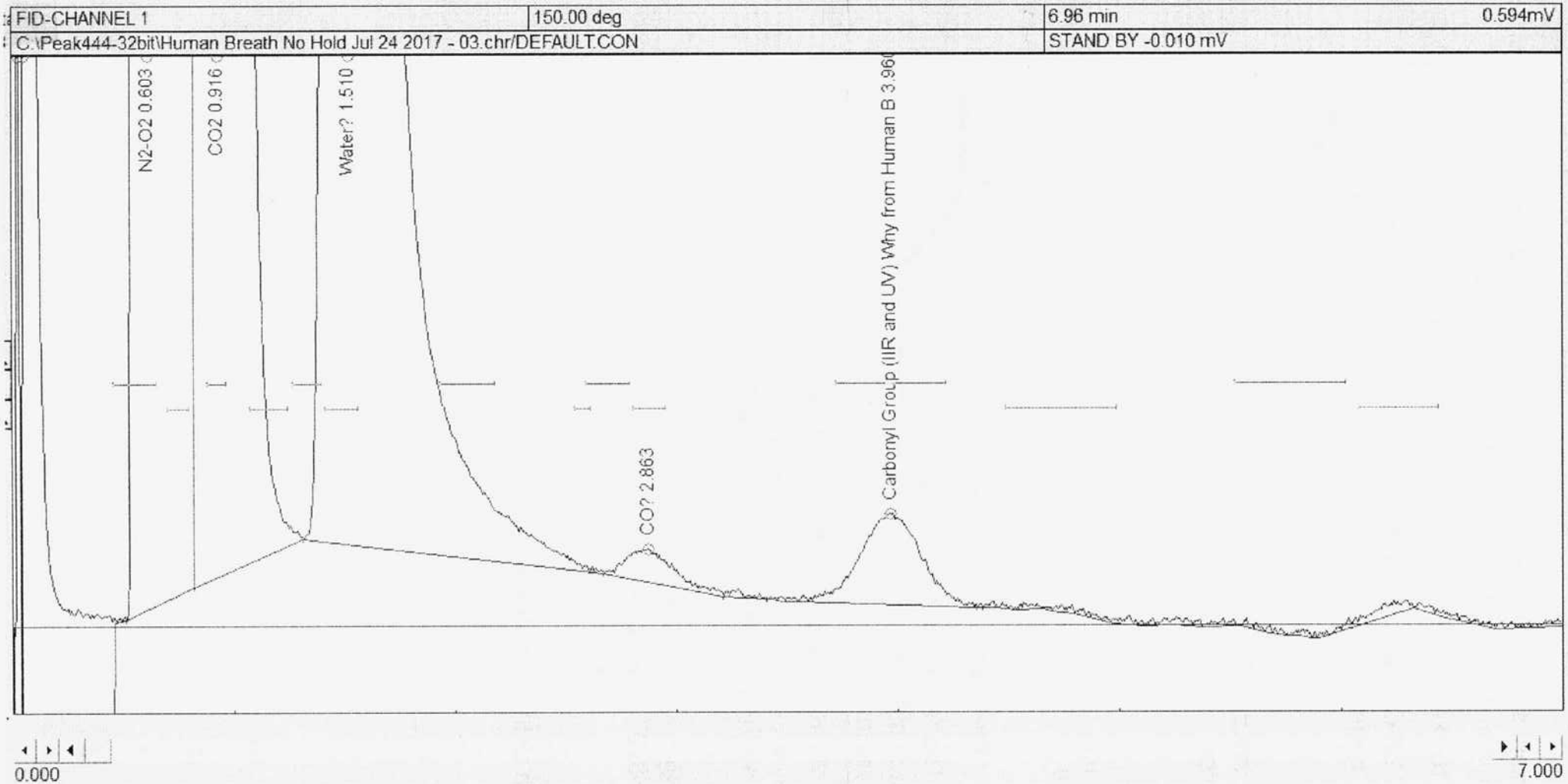
Collaby the computer product as a jar might solve the GC-integral problem.

Why so many components showing up in Human Breath not held over?
Test water CO_2 thoroughly.
Now hold breath
Also test room air.

Why are there so many components showing up in human breath? Sample size 1 mL.

Contamination in Syringe CEC 10-03-2017

Human Breath - Not held - why additional components? Jul 24 2017 - 01



The rash did better all day today but it has flared up @ night. It may also have acted up during nap today.

I have repeated a round with
1. Tea Tree Oil
2. Sodium citrate added to gel
3. on ultrasound probe for about 15 min @ 5 MHz.

We will keep an eye on it to see how it behaves. Tea Tree & salt are both slightly aggravating to the itch.

—

Ok, we have some big questions on breath samples. Why the additional components?

We see that exhaled air does indeed contain a variety, but the concentrations are not matching:
1. 5-6.3% water vapor
2. 74.4% nitrogen
3. 13.6% - 16% oxygen
4. 4% - 5.3% Carbon dioxide
5. 1% argon
6. ppm of hydrogen & CO
7. 1 ppm of ammonia
8. < 1 ppm of acetone, methanol, ethanol and other volatile organic compounds

TCD: Thermal Conductivity detector

TCD Cannot detect down to 1 ppm, we are reading much higher than that.

Investigate: $.0096\% = \frac{.0096}{100} = \frac{x}{1E6}$ $x = 96$ PPM

but we are only supposed to have several ppm, Not 100.
Butene come out @ 100 PPM.
Then we all are to high.

Our results:

	But Without Holding Breath	Holding Breath
N_2O_2	92.4	89.4
CO_2	3.4 <= Should not be this high	6.4
H_2O	1.9	2.0
CO?	~80 PPM	~100 PPM
Carbonyl	~300 PPM	~400 PPM
Butene		~100 PPM

19% N_2
21% O_2
~100%

Why is O_2 so high?

Exhaled breath contains:
- methanol
- isoprene
- acetone
- ethanol
- other alcohols
- ketones
- water
- hydrocarbons
- CO_2, CO, ammonia

But, why are the levels so high?

Clean the syringe needle first!

Jul 25 2017 Page 23

The first requirement to analyze any gas injected into the GC is

to have a clean needle!

There can be significant contamination in the needle. You can get false readings, and it certainly appear that you will if your needle is not very clean.

We have have questions of H_2O, CO, CO_2 that need to be settled.

Our chromatogram is quite simple compared to that before cleaning the needle.

The entire COB gas analysis will need to be repeated w/ the clean needle.

We do have 3 significant peaks. Why?
CO_2 & H_2O is OK.
but what is No 3?
CO should not be high.

We are clearly getting a different result w/ a clean needle, however it remains equally interesting. We have 4 peaks - why
CO_2
H_2O
CO
H_2O - alcohol?

You do not have to use a balloon - you can be careful w/ the needle as one alternative.

The questions therefore are:

X	±	Component	
0.91	0.92	0.91	CO_2 ?
1.50	1.50,	1.51	H_2O ?
2.03	2.03,	2.03	CO
10.42	10.4,	10.45	H_2O? Alcohol?

CO_2 should be easy to distinguish w/ breath holding

Actually 10.65

We can see that the CO_2 peak is dramatically large w/ the held breath.

Held breath: $CO_2 \cong 5.1\%$
$H_2O \cong 1.4\%$
Unknown $\cong 21.8\%$
CO detectable

Original Breath: $CO_2 \cong 2.1\%$
$H_2O = 1.1\%$
Unknown $= 21.5\%$
CO detectable

The H_2O vs the Unknown is the issue. Now let's test H_2O. Notice the water sample is not entirely clean.

We will iterate w/ cleaning the tube as well as the needle. Continuing to clean the combustion tube.

Cleaning Tube: 18.8 mV → 9.6 mV so reduced in ½. Tailing is gone. Still a significant peak but it is well formed.

You see that you cannot get the combustion tube completely clean. This can cause a distortion in results. It is unclear why we have 4 peaks, three of them major.

Is it possible that one of them is water vapor and that the other is liquid water?

t	Component
0.91	CO_2
1.58	Water Vapor?
2.03	CO minor
10.65	Liquid water?

Now let's go back to CDB gases. We are now known to have a clean needle.

The needle may have heated up little time that you inject it, instilling hydrocarbons (eg hexane) as a false signal into the chromatogram.

The revised analyses of CDB gas w/ clean syringe & tube is intriguing. We do appear to have something in the instrument that we also share peaks w/ H_2O analyses. We need to trap & use IR & UV to clarify.

We see what should be a CO_2 peak @ ~0.9
But what happens @ 1.52 remains uncertain:
1 Ethene?
2. CO?
3 Water vapor?

We also have the argon peak @ 10.70 - same as H_2O.
The must be trapped also.
We have identical peaks w/ water @ 1.50 (1.3 - 1.75)
and ~~10.00~~ 10.75 (10.00 - 11.75)

You get nothing in IR
for the 1.50 peaks.
Why is this? The water no show.
Now try 10.75 I get nothing either in IR @ 10.75
How can this be? Try again.

Because it is Argon! See Jul 27 2007

The must also have no dipole moment!

See Jul 27 2007

Jul 26 2017

I am very curious about 2 strong peaks that come out in GC, even with water alone, that I cannot get a signal with in IR
H_2O + CO_2 should give a signal.

A couple of factors to consider.

1. We now will have a polar tube and a non polar tube to reduce bleed over.

2. The tube itself is plastic - could this be a source of contamination.

We now use the polar column w/ a new tube

—

Even w/ the polar column (new tube) and water sample, we still get the peak @ 11.00 min. What is this peak, then?

The magnitude of the peak w/ the clean combustion (polar) tube is ~ 1.0mV - This is too small to detect w/ in IR. I do not know what we have here - for now we will just need to keep it as a reference.

Put in small liquid water to settle on liquid peak.

Third about substrates a Alderney a baseline w/
both polar & non polar Combustion Chamber
CO2 Trap Analyses:
We see again very slight rise @ the 1R Carbonyl peak
and the Lactone peak. We have seen this twice now.
We also see a slight CO_2 peak and a significant
water vapor peak (1.52). There is no known reason to
identify this or these @ this point.

We also have the large peak @ ~11 min - this is a
presumed liquid water peak (3). No tailing, a
very clean peak.

We also seem to have a rise which is closest to plasticizer.
There are all other miniscule peaks.

—

I now run a liquid water control, 1/2 ul @ 150°C
Fascinating but we have the extended peak at the beginning.
Very strong tailing. Century tailed peak is
indeed over the water component.

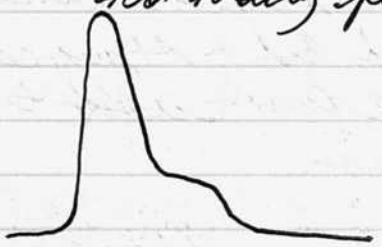
—

We will also run a control w/ air and water
mixed.

Lots of controls are needed:

1. Water
2. Air
3. Water & Air
4. Polar & Non Polar Combustion Chamber w/ no sample
5. Headspace Water - NO TUBE!!!

Ok, w/ straight liquid water injection (1/2 ul)
we DO NOT have the 11 min peak @ 180°C
There is highly significant.
With water alone we only have the early large
~~tea~~ tracky peak



1/2 ul of H_2O peak @ 180°C
has little charge.

There are no other peaks.
The peak @ 11 min is therefore significant but
unknown.

From water in the cleaned polar tube we
clearly have the addition of the 11 min peak.
Something appears to be coming from the column to be??

Now for air/water injected as 1 ul.
Very good. We have a clear separation between
the air peak + the water peak. Very distinct.
There is no secondary tracky peak on the
water peak. This also make perfect sense.

air water

The tube may be causing some problems. We may need to go back to foil. Or maybe the balloon! The is common to everything.

So we H_2O headspace w/ foil & H_2O w/ balloon! I am suspecting that the balloon is the issue. The balloon is likely not to be inert.

Uh oh! The tube w/ air & water mix we do have the peak @ 11 min & it is quite large!!

Can the be acetone from rinsing the syringe? Not really a hotspot. This says that it is NOT the balloon.

Air only now, I see. How can CO_2 be 1.38%!!?

Air from room is showing the peak @ 11 min! It should not be! @ .19%
Use a clean needle.

Try outside air. What about oxygen?
Ok, 2nd trial peaks @ 11 min is still there but smaller now w/ 2hr break from office
Now outside air.

Outside air is showing the peak also @ ~ 11 min and the CO_2 peak is much too high. What is going on here?

We now go to a brand new needle, w/ outside air.

All is pretty strange here. Clean needle not showing any signal.

Something is clearly not working correctly now.

Old needle gives very clean peaks. New needle no signal?
Ok, 3rd needle (untreated other than) has worked! Maybe I damaged needles by treating them.

We now have room air CO_2 down to 0.6% but this is still way too high. It should be .04%. 15 times normal.

No third peak with the clean new syringe. This tells us that CO_2 is higher than it should be but that third component is not there and it may be resulting from syringe contamination.

Now outside air. Fill syringe slowly from jar.
Outside air came in @ 0.5%. This is way too high. This is @ least 10 times higher than expected.

5000 ppm is the work exposure limit.

What is going on here?

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What are safe levels of CO and CO2 in rooms?

What are safe levels of CO and CO2 in rooms?

CO2

250-350ppm	Normal background concentration in outdoor ambient air
350-1,000ppm	Concentrations typical of occupied indoor spaces with good air exchange
1,000-2,000ppm	Complaints of drowsiness and poor air.
2,000-5,000 ppm	Headaches, sleepiness and stagnant, stale, stuffy air. Poor concentration, loss of attention, increased heart rate and slight nausea may also be present.
5,000	Workplace exposure limit (as 8-hour TWA) in most jurisdictions.
>40,000 ppm	Exposure may lead to serious oxygen deprivation resulting in permanent brain damage, coma, even death.

CO

9 ppm	CO Max prolonged exposure (ASHRAE standard)
35 ppm	CO Max exposure for 8 hour work day (OSHA)
800 ppm	CO Death within 2 to 3 hours
12,800 ppm	CO Death within 1 to 3 minutes

Jul 27 2011 Gas Control Work

Now we are going to look @ the CO_2 issue in more depth. Looking @ Human breath. There are still important uncertainties. Clean syringe.

Breath Sample

t		Measured	
0.61	N_2+O_2	92%	
.92	~~Labelled Methane~~ wrong	4.7% ?	4-5% O_2 in breath, 5-6.3 water vapor
1.52	~~Labeled~~ wrong CO_2	.94% ?	1% Argon?

no 11 min peak - good.

We all some serious questions here about potential mislabeling. Methane → actually CO_2?
CO_2 → actually argon.

Breath is %

Air is: %

74.9	N_2	78.08
13.6-16%	O_2	20.95
1%	Argon	0.93
4-5.3%	CO_2	.04
5-6.3%	H_2O Vapor	.001-5% ! (not included in most composition lists)
	Methane	.0002

There is very interesting and enlightening.

Now hold the breath:

t	%	Revised Components.
0.61	91.2	$N_2 + O_2$
0.92	6.92	CO_2 Notice increase from 4.7%
1.53	0.74	Argon

Ok, we have learned something big my, can't w/ the control work. I had no idea that can detect argon so readily. No wonder we were confused.

Now lets go back to H_2O vapor to distinguish from the labeled CO_2 peak. Use fork and no balloon, then use balloon.

You can start w/ liquid H_2O injection + air 1/2 ml
Ok, water presents a fairly peak. Use the center of the peak have an accurate for water.
Then actually took very good for now for acetone.

We have learned here that a clean needle is essential to reliable results. Hydrocarbons leave residue & strongly confuse the issue. You will need to pay much more attention to cleaning or use new needles more often. I recommend that you separate devoted Hydrocarbon needles & label them.

Watch out for fairly peak reference points.

Acetone has an unknown peak @ 2.81 min.

We are worky w/ a series of polar molecules.
Now lets pick up CO.

Back to Acetone. We learn that True Value Acetone is hardly just acetone.
It is only 60 ~ 100% Acetone
We see it is at 10.0 min is the primary peak.

—

on CO: There is no direct evidence of significant CO production w/ the Candle method.
We know now that we have
CO_2
H_2O (Because Argon - H_2O peaks > 1% (i.e, 1.7%)
Argon

There are some small peaks visible but you will have to produce CO in greater Concentration w/o Contamination in order to identify the Excitation.

We do not really know where CO is any more.
It may be near 5 min?

—

Now we are in better position to analyze COB gas trapped. We must still analyze the balloon.

Argon is a very significant peak that has been completely neglected and caused a great deal of confusion.

Lets analyze the balloon gases.

Air, Glass Tube, & Balloon only. We find N_2-O_2, slight CO_2 & Argon only. No Contamination from balloon.

Does argon absorb IR? NO Does oxygen? NO

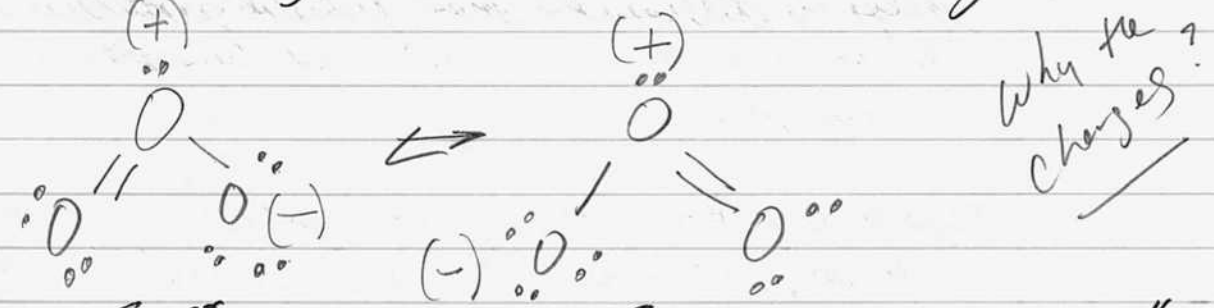
No d. pole moment

CO has absorption @ 2100
CO_2 has absorption @ ~2330.

Then explains how when you trapped the argon peak @ 1.52 min there was no IR signal.
We know now we are dealing w/ a lack of dipole moment of the IR peak, after trapping come out negative

Now we know how to more properly interpret the intriguing peak @ ~ 10.75 min w/ no IR absorption after trapping. We know therefore that it is a molecule w/ no dipole moment!

Let's also look @ ozone. There is no clear identifiable peak here, along w/ CO, for the time being. We have a small peak @ ~ 4.2 min but it remains ambiguous. What is the abundance of O_3?



Why the charges?

$\Sigma = 0$ $\Sigma = 0$

It is a resonant structure

minimizes overall formal charge

Let's look @ formal charge again.
It is stated that no Lewis structure is complete w/o an examination of formal charge.

Formal charge = # of valence electrons − (non bonded electrons + no. of bonds)

eg Oxygen has 6 valence electrons.
so in structure on the left, for central O atom:

Formal charge = $6 - (2+3) = +1$ OK

On structure to right:
Formal charge = $6-(2+3) = +1$ OK

Left structure, left oxygen:

$6-(4+2) = 0$ right oxygen $6-(6+1) = -1$

Ok, now you see how to get the formal charge.
What does it mean in terms of Lewis Structures?

You want to minimize the OVERALL formal charge

formal charge is certainly an ~~intellectual~~ topic;
it estimates the charge on a molecule, and helps to determine the most probable structure.

Now w/GC: Water in tube w/ foil.

Foil has worked really well with water samples.
Very limited testing.

What we see here is that H_2O vapor overlaps with Argon so there make it trickier to distinguish
We mean 3.9% - this includes BOTH argon and water vapor. There a critical observation.
Percentages are one of your main clues, argon should likely never exceed 1%.

—

Room Air:
Room air only hints at the presence of CO_2, the temp has to be lowered to pick that up properly. Argon is clear. Argon 0.94% percent vs 0.93 theoretical.

—

Glue the syringe handles, do not melt the Dremel on them.

—

Hot plate is going to 400°C now (new). For superior. Make sure you use ½ cc mL when H_2O gas is involved.

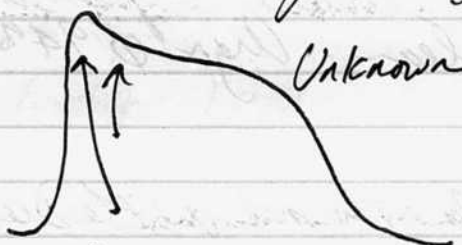
Now we look @ H_2O in glass tube w/ polar combustion tube + balloon.
We have
N_2-O_2
CO_2
A massively broadly wide peak @ the water - argon peak.

The activator is battery and much more complicated. The combustion tube and the balloon seems to have complicated matters considerably.

We seem to have:

N_2O_2
CO_2 - did separate (barely)
Massive H_2O fairly peak
Argon
An additional mass peak, currently unidentified @ ~ 2.0 min.

These 3 peaks seem to have combined into 1 w/ the following appearance:



H_2O
Argon
Combination

There are undesirable complications.

Next we need to separate the balloon influence from the plastic combustion tube

---

Now H_2O on Glass tube w/ balloon only. The balloon does get very hot this close to the tube so this seems to be a problem method. We have picked up

N_2O_2
CO_2 small
Argon inclusive
Significant fairly wide peak but manageable

Thereafter the combustion plastic tube seems to have introduced an additional complication that the balloon, and especially the foil alone, does not.

We may be headed toward foil, direct balloon w/out combustion tube may also be acceptable

We have some good controls in place now.
The foil alone w/o all possible - lead contamination.
Balloon is acceptable, especially w/out heat.

Now back to CDB culture - trapped gas
Very interesting development already - this is now w/ a clean needle and we should be free from some of the contamination that has affected us

We see:
N_2O_2
CO_2 - Ethene (a massive peak here) - Argon
Extends flat across propane peak
then descends back to zero level.
Then is an unusual peak structure

Repeat CDB trapped gas w/ clean needle
The second trial gives us
N_2O_2
CO_2 minor
Argon + Water Peak
We may have a Co peak

No additional significant or the peaks

Now the question arises, Can we do something w/ the protein itself?

Maybe before we go to that, we need to examine the contents of HC's and the state of the syringe.

We have a peak that shows up @ 10.8 mins. Is that hexane or our unknown peak? Something has happened here.

Maybe it makes more sense to use headspace = clean needle & air issue.

Now lets go back to the used needle which has now been cleaned w/ acetone.

We pick up the slight trace of acetone left in the syringe - other is quite good.
It was the Acetone 2 peak component.

We have injected air alone with the older syringe that has been cleaned thoroughly w/ acetone. We believed that everything had been completely evaporated, however we see

1. N_2O_2
2. Slightest hint of CO_2
3. Argon Peak > 1% (2.4%)
4. So we assume some water vapor
5.

5. However, we see the 2nd acetone peak (very small) @ 2.90 min

6. Then we have a major apparent acetone peak @ ~ 10.8 min & magnitude of 48.6 mV !

last we thought the syringe was evaporated.

Lets go again on this.

Jul 29 2017

Check the older needle again for acetone residue. If present, heat the needle.

Investigate the moisture needle w/ $MgSO_4$. This could be a factor in the elemental analyser - combustion analyser work.

Using about 1 gram $MgSO_4$

Dry (Room) $MgSO_4$: 0%
2 drops H_2O : 0.0%
+ 4 " : 0.2%
+ 6 0.5%
+ 10 Not a smooth surface so poor contact is made
0-50%

+ More water: It cakes up and is not homogenous It would have to be uniformly dissolved and evaporated, which is plausible.

On skin, the meter measures ~ 50%.
The meter will be useful with a homogeneous surface.
Wax Candle: 0%

Very interesting: 70% Isopropanol measures as 20% H_2O. There is a reasonable number and it is a whole lot quicker than distillation. This could be very helpful in many circumstances. Probably not a good idea to use w/ acetone, for example, however.

It could be useful, however, for many types of solutions to detect or determine the water content as long as you do not damage the probes or casing.

Electronic sensors are developing very well
1. Glucose
2. Oxygen
3. CO_2, maybe CO?
4. H_2O

H_2O detector could be especially helpful for IR work.

—

Acetone detection on older syringe
Now the acetone peak (presumably) is very small, ~ 0.5 mV so most of it now appears to have evaporated. What is interesting is that I can detect an odor from the older syringe and not the new syringes.

Let a tip to heat off the syringe.
It could be hexane residue vs acetone.

—

There is an odor. It is not acetone. All signs say that it is hexane. It is hard to get rid of completely, apparently.

—

Ok, the heat melted the needle - too hot.
The hexane peak is now very small. It could have been a combination of both acetone & hexane.

Now looky @ CDB top layer - scrubbed - by headspace @ ~350°C.

We only see
N_2, O_2
CO_2 (low)
H_2O & Argon combined

some colored residue remains. Thus indicate water has been driven off. No additional peaks showing up.

Now with light torching:
N_2-O_2
CO_2 (stronger)
Argon + H_2O (~8%!)
Still no additional peaks @ 15 min.

High torch level:
w/ water apparently evaporated & solid residue left
1. N_2O_2
2. High level CO_2
3. Ethane visible
4. Argon
5. Unknown @ 2.06 min
6. Propane
7. Propene
8. Carbonyl by IR
9. Butane
9. Pentene
10. Hexane

(Ethene?) OK now we have strong HCs

You should use an HC syringe now

Jul 29 2017 (Continued - Daylight session)

We are now ramping up the protein secretion production. Only Carbon, hydrogen, $FeSO_4$, & N_2, O_2 seem to be required to produce the protein. These elements are sufficient for tryptophan, proline and glutamine acid production. 8 pint jars set up.

We estimate that 90% of the secreted layer after 30 days incubation in water. Dubbelfast test on past produced an estimate of 70%. It is a highly water soluble protein 8.

We now have 2 pint jars of extracted dilute protein secretion. These will be treated w/ greater caution @ this point since previous session overheated and charred the protein (still useful for pyrolysis work). We will now evaporate small samples in a water glass to reduce water content — the process must be watched carefully.

We will be able to subject these concentrated samples to greater scrutiny, e.g. UV Concentration, pyrolysis, IR, electrolysis, etc; also IR simulation. GC, Trapping —

Moisture meter will also be helpful.
Meter still remains on overload after 2 evaporation sessions $\Rightarrow$ >50% H_2O, not subsiding.

However, after partial evaporation, and subsequently adding more dilute protein solution, considerable surface tension can be seen w/ the evaporated protein.

2 paper proposals:
1. Extended Poor Air Quality
2. Protein Characterized
   1. Pyrolysis
   2. IR
   3. UV

With GC inspect needle w/ room air @ any time to check on bleed over or contamination.

I have succeeded in evaporation of the protein. The moisture level is now down to 1.9%. The resulting solution is highly viscous.

At this point, it has failed the Bradford test and the ninhydrin tests, but likely is most certainly an IR response.

The material is acting like a plastic.

Remember that it has been heated.

Ok, What we understand now is that when we heat the protein (@ 100°C to evaporate) we denature and change the protein. Prior to heating it passes the Bradford test. Post-heating it does not.

We also have very certain repeatibility with IR. We also determine that the protein is highly acidic. We also see the protein peak.

The evaporated protein becomes very thick and viscous. No wonder it yields high level hydrocarbons under pyrolysis.

We have a highly acidic water soluble protein.

We have effective pyrolysis of the COB secreted & evaporated protein. We have a new peak @ $t = 25.3$ min

From the model:

$t_{150} \cong 2.74\,CN - 5.3$ $\qquad$ $t_{50} = 0.19\,MW - 5.6$

$CN \cong 0.36\,t_{50} + 2.0$ $\qquad$ $MW = 5.11\,t_{150} + 29.1$

Therefore the estimates are:

$CN = 0.36\,(25.3) + 2.0 = 11.1 \Rightarrow 11 \Rightarrow C_{11}$

$MW = 5.11\,(25.3) + 29.1 = 158 \Rightarrow C_{11}H_{24} = 156$ Excellent.

We therefore have also picked up C_{11} @ 150°D.

C_{10}: $t \cong 22.1$ min

C_9: $t \cong 19.4$ min

A comparison of the VISCAVS (secreted) vs the precipitated (separated) protein (KCl disk)

CDB Precipitated vs Viscous Protein KCl Jul 31 2017

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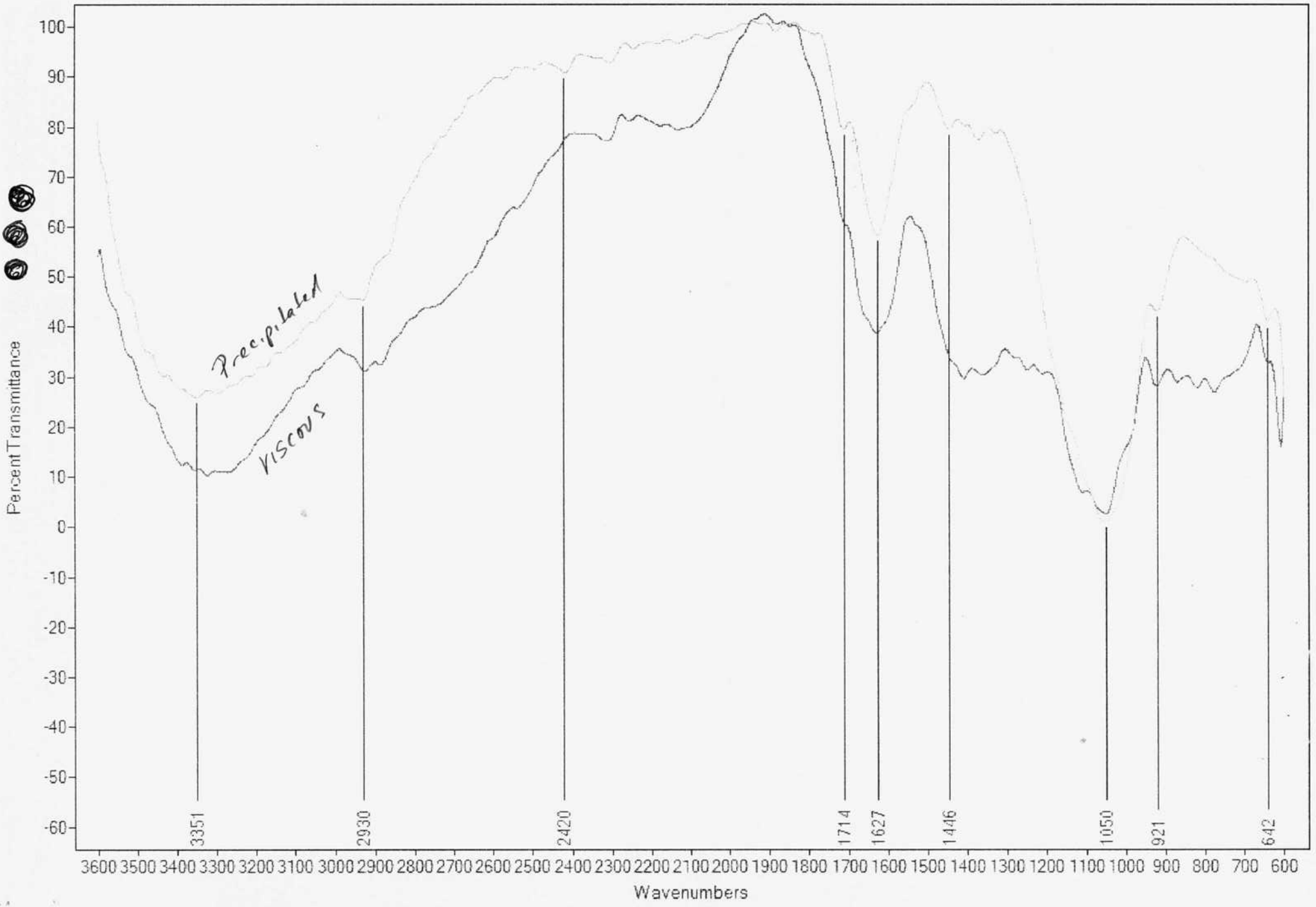
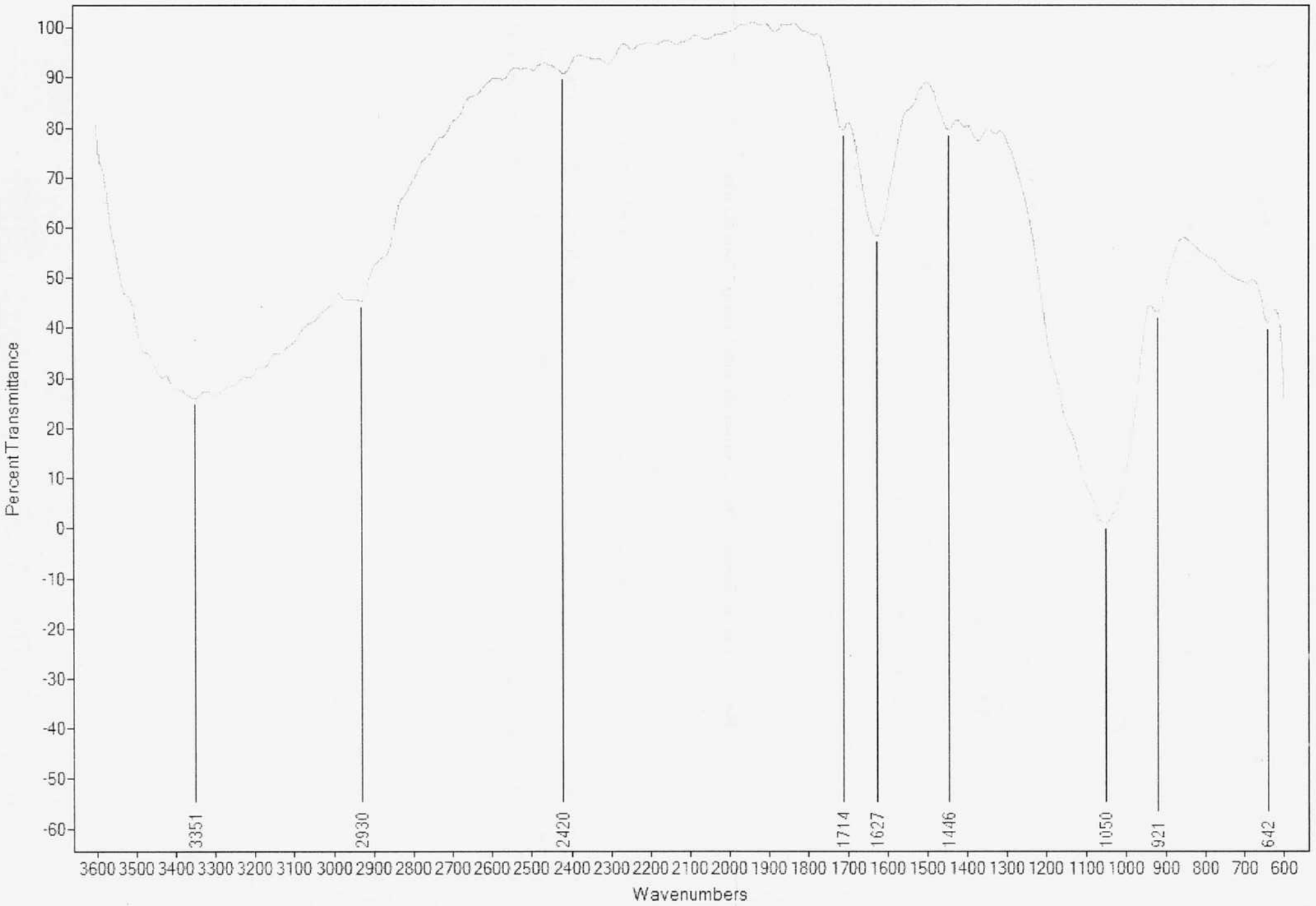


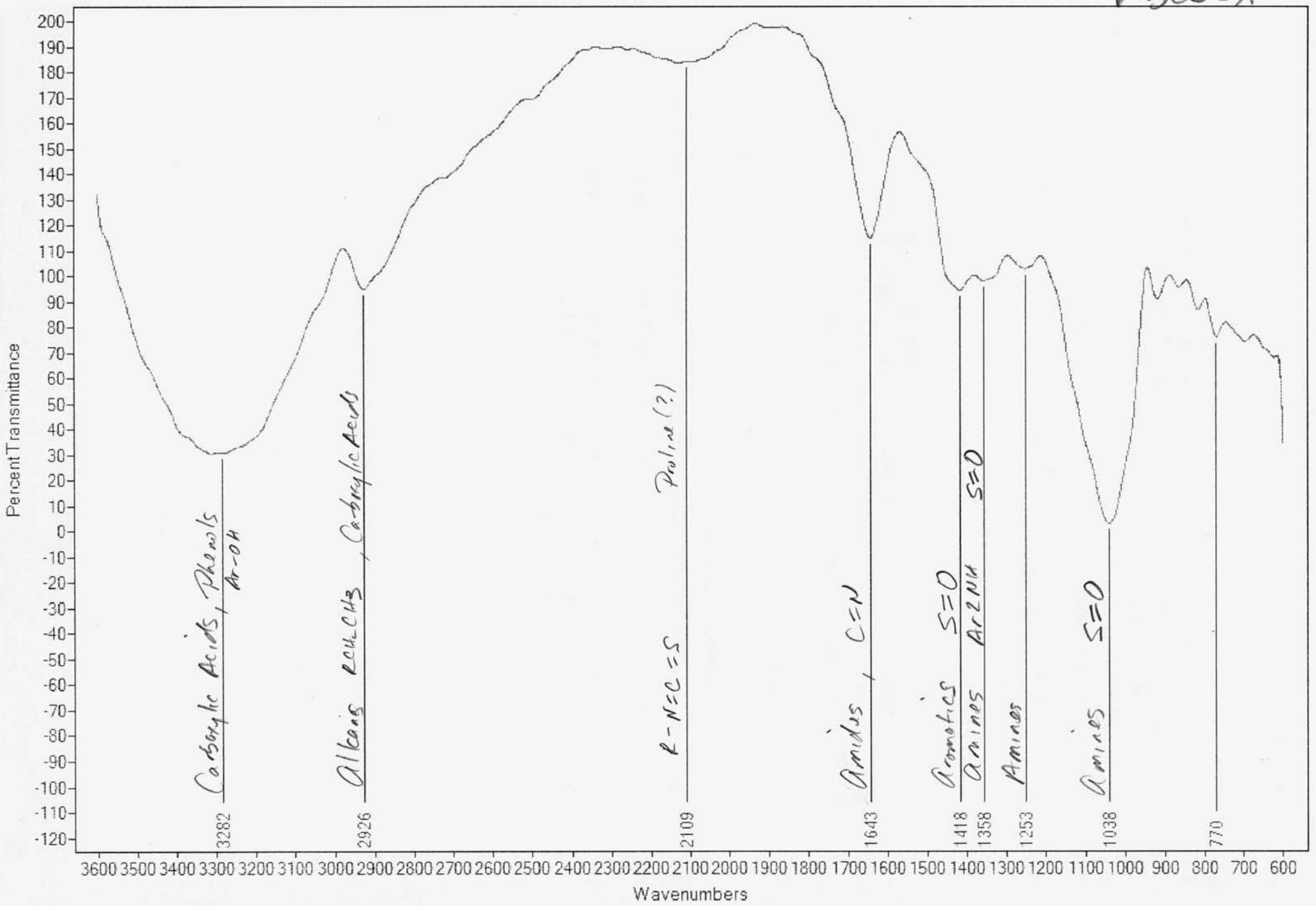
CDB Precipitated Protein KCl Jul 31 2017



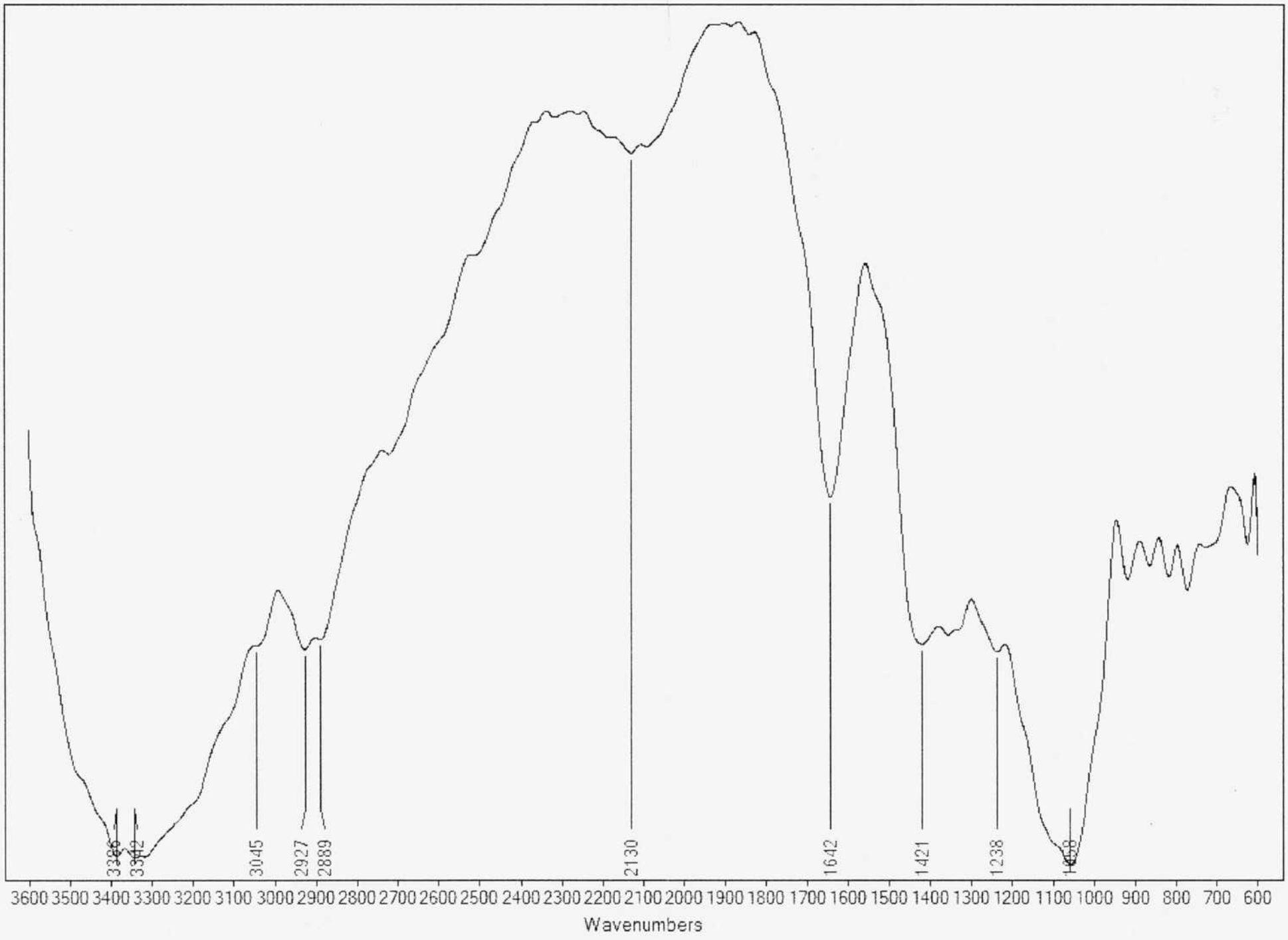
Proline
Tryptophan
Glutamic Acid
} Primary amino acid candidates

IR Analysis of Heated & Evaporated COB Seeded Protein

CDB ATR _KCl Merged - Protein Secreted - Evaporated - Heated - Denatured Jul 29 2017 - 01



CDB Protein Secreted - Evaporated - Heated - Denatured Jul 29 2017 - 01



Jul 30 2017

Today I would like to test the secreted protein for iron.

We have a very strong positive test for Fe^{+2} with the secreted protein. This is not a result of the original $FeSO_4$ in solution, there is a new compound that has been formed after oxidation of the Fe^{+2} to Fe^{+3} has taken place. The green test to the secreted protein is further evidence of the Fe^{+2} form.

The test for Fe^{+3} fails completely.

We know, therefore in general terms that we have a highly water soluble, acidic, organometallic protein that has been formed. We also know likely amino acids that comprise its structure, at least in part.

* dots

The availability of sulfur in the culture leads us to wonder if cysteine has been formed also.

Remember the test for amino acids with ninhydrin must prior to acid and heating fails, but that the Bradford test for protein succeeds admirably

Let's test amino acid detection of dried milk.

Dried milk also fails the ninhydrin test so they demonstrate the separation between testing for protein and testing for amino acids. They are separate & distinct processes and conditions of positive results.

—

Now, lets talk about the rash for a minute.
The started on Jul 23 as a 2" area.
The chronology is:
Over the next couple of days the area enlarged to encompass the entire front of the neck. The area turned red in color. The skin of the lower neck swelled and became "flabby" and loose in nature. I could feel the neck skin jiggle when riding my bicycle over a bump. After originally trying sodium citrate, tea tree oil & ultrasound I have backed off the for a few days. Hydrocortisone 1% was used and seems to alleviate the itch to some degree. The swelling of the skin has subsided but it remains a strong red color. The upper skin of the neck is now more whitish in color (natural) and less irritated. A new generation of the rash now exists in a milder form on the upper chest. I continue w/ hydrocortisone and have renewed use of ultrasound, along w/ baking soda mixed in with the ultrasound gel. The entire affair is moderately irritating @ the point. It is difficult to say if there is an allergic reaction or old expression of contagion from the recent lab visit. The skin under the microscope shows no obvious alteration in form & structure; just increased redness. An allergic reaction of some sort does remain as a distinct possibility. There is a small patch also on the left arm.

We have a good method now of concentrating the secreted protein w/ evaporative drying on a watch glass @ ~ 110°C. It must be carefully monitored for moisture removal but if heated to properly will result in a thick viscous material @ about 2% of the original volume. It is in good form for testing w/ IR and can be dissolved in H_2O @ anytime, as for UV testing

I have started 6 new pint cultures. The should provide ~ 2 pints of dilute secreted protein w/ in approximately 30 days.

Let's look @ prospect of sulfur bonds in the IR plot.

There are 3 possibilities of sulfur bonds taking place in the IR plot

1418 cm^{-1}
1358
1038

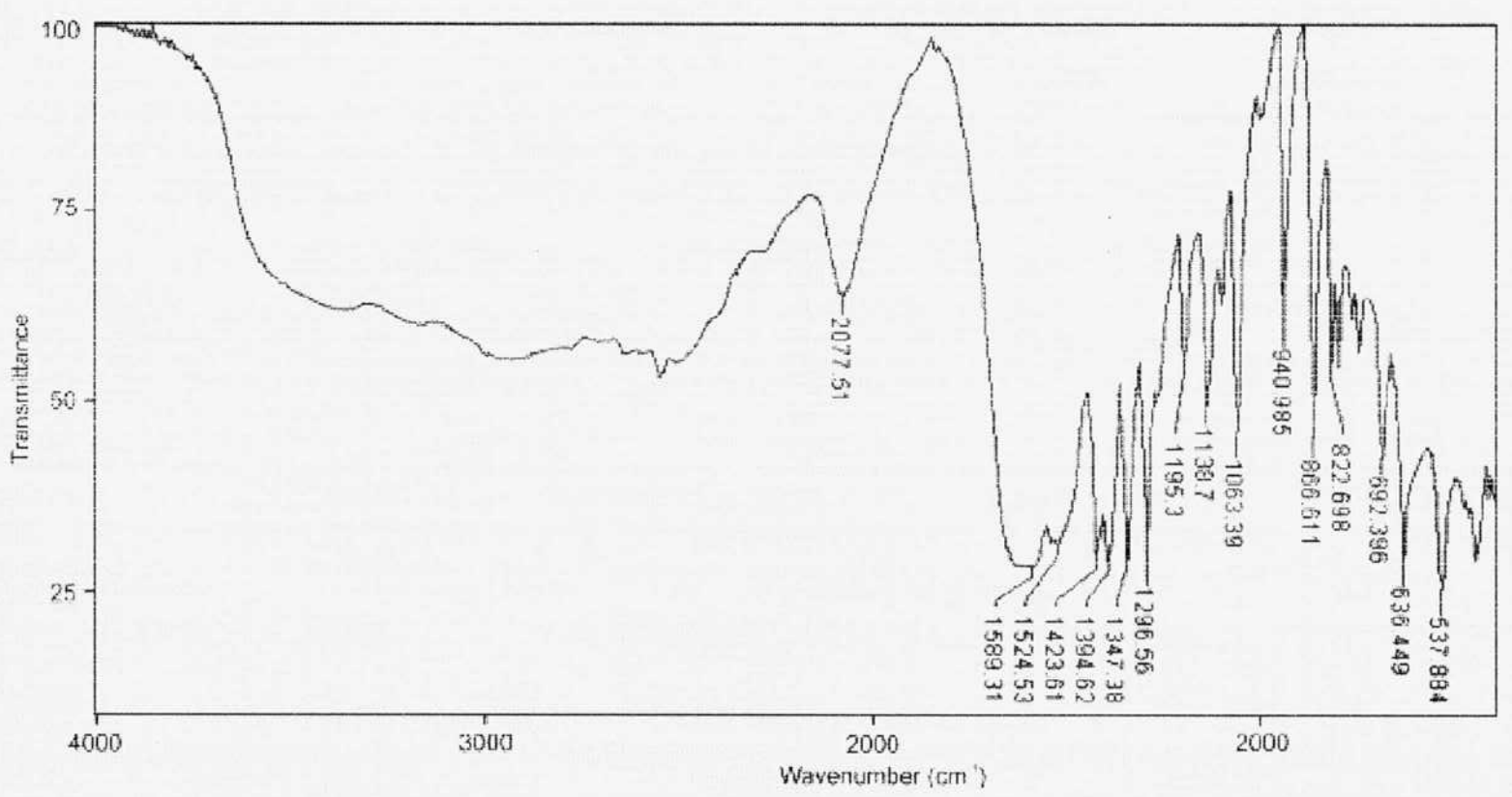
So the possibility cannot be discounted.

How would you test for cysteine? in IR?

Cysteine IR Plot - I see no obvious connection @ this point.

Cysteine IR Plot - Source: Research Gate

Figure 3. FTIR spectrum of L- cysteine



Ok, We have the protein and we know some things about it. What do we know:

1. Acidic
2. Fe^{+2}
3. Water soluble
4. Amino acid candidates
   Glutamic acid, proline, tryptophan
5. Bradford positive
6. UV analysis?

Molecular weight determination w/ accounting for moisture content would certainly be helpful.

Can we determine the current moisture content?

Our relationship determined is:

$$\text{Beginning Moisture in } \frac{\%}{100} = \text{Ending Moisture in } \frac{\%}{100} + \frac{\Delta H_2O\ \%}{100}$$

and $$\frac{\Delta H_2O\ \%}{100} = \frac{\text{Total weight of sample} - \text{Ending wt of sample}}{\text{total wt of sample}}$$

e.g. initial sample wt = 100 gms
ending sample wt = 76 gms
Ending Moisture in % = 21%

then $$\frac{BM\ \%}{100} = .21 + \frac{100\text{ gms} - 76\text{ gms}}{100\text{ gms}} = .21 + .24$$
$$= .45 = 45\%$$

Then should be possible.
Need 1. Beginning wt of Sample
2. Ending wt of Sample
3. Ending Moisture in %.

(Tare wt)
Delta weight. 22.54 gms → Beginning wt of sample

Our moisture meter has a max. reading of 60% H_2O.
Hypothetical Case. Assume orig. sample wt 22.54 gms
Assume our current sample weighs 12 gms. Assume
Ending moisture = 60%
then $\frac{BM\%}{100} = \frac{0.60 + 22.54 - 12}{22.54} = .60 + .47 = 1.07 = 107\%$ Not quite possible but close
Assume our sample weighs 16 gms @ the end.
$\frac{BM\%}{100} = \frac{0.60 + 22.54 - 16}{22.54} = .60 + .29 = 89\%\ H_2O$ Quite feasible.

When we get this value, we will be able to proceed w/ Molecular weight determination if our sample size is sufficient.

Container weighs ~~17.80 gms~~ 17.81 gms
Now it weighs 21.44 gms
I overheated it a little bit too much, but

21.44	22.54
− 17.81	− 17.81
3.63 gms left	4.73 gms original wt.

% Moisture $\approx \frac{22.54}{\text{~~4.73 gms~~}} - \frac{3.63 \text{ gms}}{\text{~~4.73 gms~~}\ 22.54} = 84\%$ original moisture.

I overheated the sample but we nevertheless have a good estimate of the moisture content ≈ 84%. This is good enough to start with.

Let's repeat the test.
Container wt: 17.81 gms
Container Wt of Sample: 38.07 gms
Initial Sample wt = 38.07 − 17.81 gms = 20.26 gms

Now water it carefully!

End Weight 19.07
Remainy Sample Wt: 19.07
− 17.81
2.06

~~38.07~~ $\frac{20.26 - 2.06}{20.26}$

= 90% Moisture.

Moisture meter shows 19% of remainy sample to be H_2O. This means the actual remainy sample weight is 2.06 gms − 0.19(2.06) ≅ 1.67 gms

Therefore: $\frac{20.26 - 1.67}{20.26} = 91.8 = 92\%\ H_2O$

This seems very reasonable. Fortunate to have not ~~overheated~~ the most recent sample. It was close.

This will help on molecular weight determination.

Jul 31 2017

We already have some data collected for MW estimation. We can improve it from the most type estimate per relative to the previous estimate of ~10%.

I see that on ~~Jan 15~~ Jan 15 2017 we have our first estimate of MW as 1276 gms/mole. But then assumed a 8% pure sample. We now estimate that 92% of the sample is H_2O, or that 8% is protein.

Therefore $\frac{1276 \text{ gms/mole}}{.08} = 15950 \text{ gms/mole}$ or $\sim 16 \frac{\text{KDA}}{\text{mole}}$

is now our most current estimate.

Gene size estimate (molbiol.edu.ru/eng/scripts/01_06.html)

The estimate given is that
Protein Size of 16 KdA $\cong$ Gene Size 0.432 kb $\cong$ 430 bp

No of amino acids in the protein $\cong \frac{16{,}000 \text{ dA}}{\sim 100 \text{ dA/amino acid}} = 160$ amino acids in the chain.

Bacterial genomes generally range from 130 kbp to over 14 Mbp.

People also ask

What is the average size of a bacterial gene?

A "typical" gram-negative bacterium, H. influenzae, has 1,743 genes each of **~900 bp**. So we can conclude that ~1500 genes are required to make a free-living organism. Bacterial genome sizes extend over about an order of magnitude, from **0.6 Mb** to **<8 Mb** (for review see 5863). The larger genomes have more genes.

First Estimate: Genome Size

We now have our first estimate of the genome size. The result is reasonable.

The human genome has ~ 20,000 genes.
A bacterial form (or a free living system) has a minimum ~ 1500 genes.

Our first estimate of the secreted protein gene size is ~ 4306 p.

Therefore $1500 (4306 p) = \cancel{\emptyset}\ 645000$ bp

$\simeq \emptyset.65$ Mbp. (Estimated minimum)

Bacterial genome size range from
~ $\emptyset.6$ to 8 Mbp
So our first estimate is a reasonable one, based upon field determination of molecular weight of the protein.

Genome Size - Reference Info

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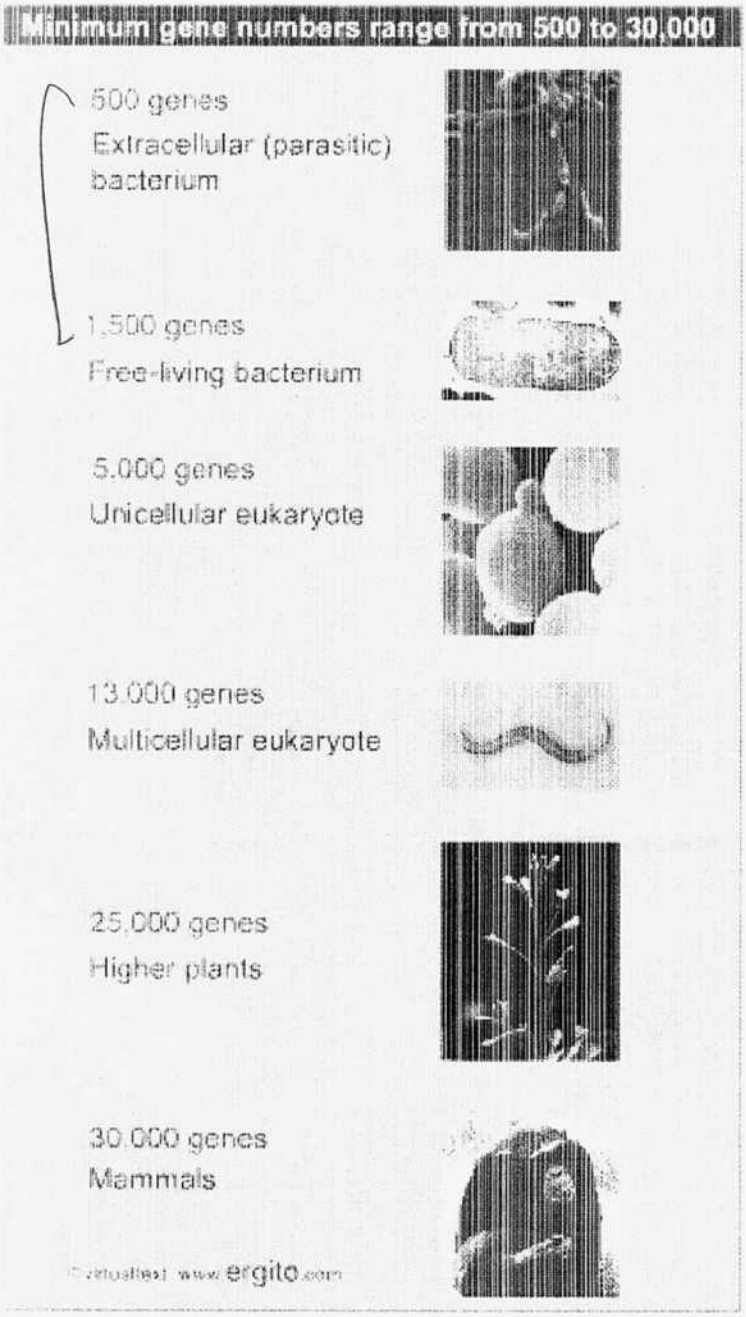
Minimum gene numbers range from 500 to 30,000



Humans ~20,000 genes

Sequenced genomes vary from 470-30,000 genes

Species	Genome (Mb)	Genes	Lethal loci
Mycoplasma genitalium	0.58	470	~300
Rickettsia prowazekii	1.11	834	
Haemophilus influenzae	1.83	1,743	
Methanococcus jannaschi	1.66	1,738	
B. subtilis	4.2	4,100	
E. coli	4.6	4,288	1,800
S. cerevisiae	13.5	6,034	1,090
S. pombe	12.5	4,929	
A. thaliana	119	25,498	
O. sativa (rice)	466	~30,000	
D. melanogaster	165	13,601	3,100
C. elegans	97	18,424	
H. sapiens	3,300	~30,000	

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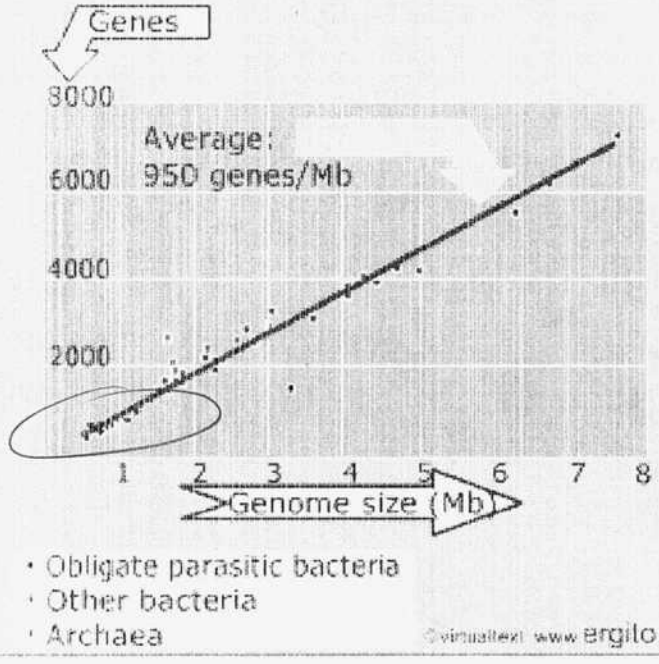
Bacterial genome size relates to gene number



Desires:

done 1. Repeat Pyrolysis of viscous protein sample.

done 2. Can we apply pyrolysis to the precipitate protein form?

done 3. Is the secreted protein optically active
Yes ϕ.95° - 1.00°

Yes, Preliminary Work Done 4. Can we repeat the MW determination of the secreted protein?

We know it is essentially the same protein. 5. Is it possible to determine the MW of the precipitated protein?

done 6. Set up tube cultures for gas production.
Do not fill completely ~~but more important~~ to allow for N_2 usage.

OK Better Acetone 7. FE crystal analysis

Lubricants, Fuel Cell Applications.

Tube cultures (~20) have been set up w/ balloon caps and about 20% air space in tube for N_2 availability. Also about 10 tubes have single needle punctures to the balloons to increase N_2 availability.

In pyrolysis we clearly have

C_{11}
C_9
C_5
C_4
C_3 } assumed
C_3 }
C_2 assumed

$\Sigma \approx 37$

The viscous material remaining after dehydration would appear to make a very good lubricant (polar lubricant).

Such are harder to come by, as in glycerin for example.

It also could easily be a source of fuels.

It is interesting to have a polar nature with so many hydrocarbons within the structure.

Jojoba oil is C40 - C44

"Polyol ester oil"

Polyolesters are polar lubricants (POE)
Pneumatic lubricants are polar (Air tool antifreeze)
Ester lubricants

Fuel cells may be an additional application.

The viscous protein is highly absorbant of infrared energy. Only a small sample will completely block the signal in a KCl disk.

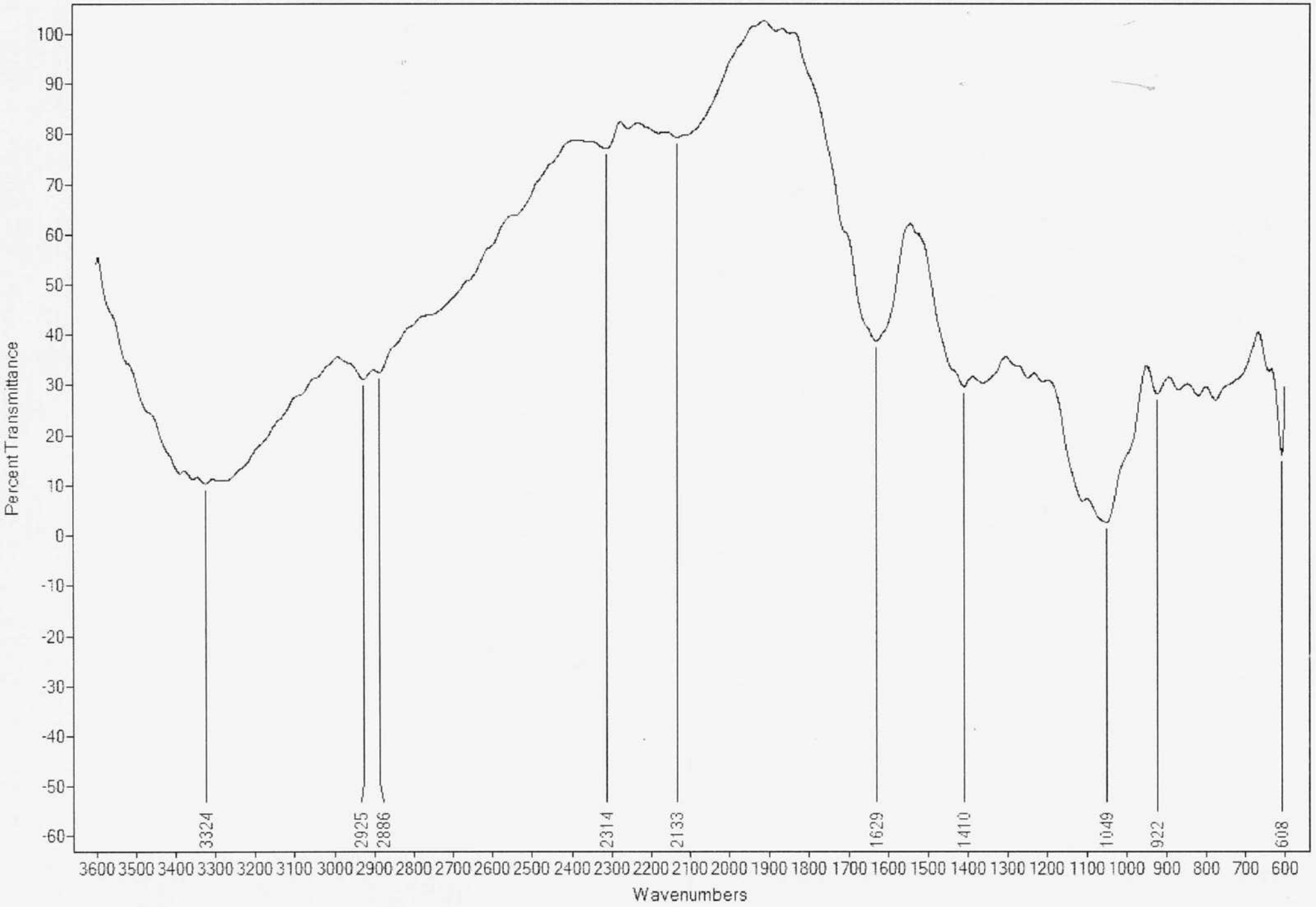
We now have comparison by IR of the viscous vs the precipitated protein. Indicates some of polarity and solubility here.

Some similarities, but differences not the same.

1. Precip is solid & non soluble in H_2O
2. Secreted in viscous & soluble in H_2O.
3. Developed by completely independent methods.
4. Pyrolysis shows some important differences as well.

CDB Viscous Protein - KCl disk

CDB Viscous Protein KCl Jul 31 2017



The mucous protein is optically active by 1°, n $\bar{x} = 0.975^\circ$.

Question: Is there sugar in the solution you are calling protein?

How do you know? Test positive for Fe+2
Test positive for protein
Is green in color

How would you test for sugar?
Benedicts.

Benedicts tests for reducing sugars.
Sucrose is not a reducing sugar.
Reducy sugar have a free aldehyde group or a free ketone group.

Some Color observations:

1. Protein + Benedicts reagent produce a muddy green color that causes a dark green precipitate to be formed upon heating

2. Protein + Sodium Citrate + $CuSO_4$ produce a clear light green color, distinctive from the yellow-green color of the original precipitate.

This is not a useful or valid test for sucrose.

False - Sucrose not required to this color to form.

3. ~~Sucrose + Sodium Citrate + only 1-2 drops of $CuSO_4$ produces a clear blue color, much darker than $CuSO_4$ would be alone!~~ This appears to be a useful color test for the detection of sucrose, which could be quite valuable in its own right. No heat required.

~~Benedicts~~ Biuret has a major disadvantage in that a clear colored solution is not produced

4. We do, however, have a diagnostic color test already for the presence of the protein via

Protein + Sodium Citrate + $CuSO_4$ → A clear light green solution - this is a useful color test.

It also shows that sucrose is apparently not present.

Aug 1, 2017

A determination (estimate) of the MW of the viscous protein is valuable by freezing point depression method.

The first step is to calibrate the thermometer w/ distilled water.

~~+3.6°C~~ ?

0°C = +0.8°C same as before on thermometer what ya want is uniform freezing. If you have an ice plug in the bottom, the melting of that plug distorts the results as the temperature is not uniform.

You are after the transition from crystals to solution.

7.18 gms H_2O
9.93 w/ protein added $\Delta = 2.75$ gms

The work was done on Jan 10 2017

$$MW(gms) = \frac{\text{mass of unknown in grams} \cdot K_f}{\Delta T \cdot \text{mass of solvent in kg}}$$

K_f for water $= 1.86 \frac{°C \cdot kg}{mol}$

Example

$$MW = \frac{10.95\ gms \cdot 1.86\ ^\circ C \cdot kg / mol}{1.55^\circ C \cdot 36.60E\text{-}3\ kg} = 359\ \frac{gms}{mole}$$

vs 342 actual for sucrose trial.

If you check it more often & stir a lot you can keep a large ice pkg from forming @ the bottom.

+ $\phi.45^\circ C$ first trial.

You need to have small crystal formation for melting point determination.

$\phi.8 - \phi.45 = -\phi.35^\circ C$

Therefore:

$$\frac{2.75\ gms\ (1.86\ ^\circ C \cdot kg / mol)}{\phi.35^\circ C \cdot 7.18E\text{-}3\ kg} = 2035\ gms/mol$$

However, this solution is 92% water & 8% protein.

Therefore $\frac{2035\ gms/mol}{.08} = 25,442.7 \approx 25500\ \frac{gms}{mol}$

This is our current estimate of the molecular weight of the viscous protein, or 25.5 KDA.

We had 16 KDA as first min on Jan 15

This brings us to a $\bar{X}$ of $\approx$ 21 KDA

and 21 KDA $\Rightarrow$ $\phi.568$ Kb = 568 bp per gene

The avg size of human protein (one source) gives ~ 50 KD.

Avg size of gene is therefore 1.35 Kb

If we assume 20K genes in human body then estimate of human genome is

(20E3)(1.35Kb) = 27,000 Kb = 27 Mb

What is actual estimate of human genome size?

3 billion base pairs are estimated!

Originally humans were thought to have ~100K genes. This has been revised downward to ~20K genes.

Ratio estimate by molbiol.edu.ru is that

1 Kb of DNA ≅ 37 KDa of protein

& that 1 KDa of protein ≅ 0.027 Kb of DNA

There must be some major problems here.

Another source, specifically for bacteria says

1 Mb Genome Size per 950 genes.

A reasonable estimate for the no. of genes in a bacteria is ~ 2000.

The MW of our protein is estimated @ 21 KDA.

Protein Size Estimate for CDB Viscous Protein

The mean size estimates that a protein size of
21 kDa ≅ 0.568 kb (base pairs).

Now we have ~2000 genes (0.568 kb) = 1136 kb
= 1.14 Mb ≅ 1.0 Mb genome type size.

This is not at all unreasonable.
Bacteria genome size range from 130 kbp to over 14 Mbp.

It would be good to repeat our MW estimate for the protein as that is our most important parameter thus far.

Initial Wt of H_2O: 9.19 gms Current estimate
With protein added: 14.67 gms Δ = 5.48 gms ~ 21 kDa
Melting point. 0.5° (21,000 gms/mole)

The median length of the protein (as measured in # of amino acids) for 67 bacterial types (groups in 267. Now we also know that there are an average of ~ 100 dA / per amino acid.

Therefore the average protein length for the group of 67 bacteria is
267·(100) = 26700 dA = 26.7 kDA

which is amazingly close to our most recent estimate of 25.5 kDA.

2nd MW estimate:

$$MW \cong \frac{5.48\ gms\ (1.86^\circ C \cdot kg/mol)}{(.8^\circ C - .5^\circ C)(9.19E\text{-}3\ kg)} = 3691\ \frac{gms}{mol}$$

But @ 92% H_2O & 8% protein:

$$MW = \frac{3691}{.08} = 46213\ gms/mol$$

This is considerably higher than the previous value, however the ~~Concentration~~ of sample size was much larger (almost double) so the results should be considerably more accurate.

There is no reason to doubt the estimate We could use a weighted average by the size of the sample, or ratio of sample to water:
First sample size to H_2O ratio = 2.75/7.18 gms = .38
2nd " " " " " = 5.48 gms/ 9.19 = .60

So we could form a weighted estimate of

$$\frac{.38(25.5\ kdA) + .60(46.2\ kdA)}{.38 + .60} = 38.2\ kdA$$

$$\cong 40\ kdA$$

Median Protein Length - Various Organisms

Average Protein Length of Various Organisms (No. of Amino Acids). Note: 1 amino acid = approx 100 dA

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organism	median protein length (amino acids)
H. sapiens	375
D. melanogaster	373
C. elegans	344
S. cerevisiae	379
A. thaliana	356
5 eukaryotes (above)	361
67 bacteria	267
15 archaea	247

Notice this does not actually vary this much in a relative sense.

If we were to project the genome size based upon the mucin site:

~ 40 KdA $\cong$ 1.081 kb
and if we assume 2000 genes in a bacteria
$2000\,(1.081\text{kb}) = 2162$ kb $= 2.2$ Mb

Thus our current estimate for the genome size $\cong$ 2 Mb

and our protein size estimate is $\sim$ 40 KdA

$= 40{,}000 \frac{\text{gms}}{\text{mole}}$

Average size of bacterial protein is 267 amino acids $\cdot \frac{100 \text{ dA}}{\text{amino acid}} \cong 27$ KdA

We will repeat molecular weight estimate w/ same tube.

Possible temp = 0.55°C $\Rightarrow$ $\Delta T = $ ~~0.35°C~~ $-.25$°C
This would make the protein size even larger.

$MW = \frac{5.40\,(1.86)}{.25\,(9.19E\text{-}3)} = 4436 \frac{\text{gms}}{\text{mol}}$

$= \frac{4436}{.08} = 55456 \frac{\text{gms}}{\text{mol}}$

So our estimates are $\frac{.30(25.5) + .6(46.2) + .6(55.5)}{.30 + .6 + .6}$

$= 44.1 \text{ kDA} \cong 45 \text{ kDA}$

We are going to hold to this value @ this time

If we accept Mussin DNA estimate

$45 \text{ kDA} = 1.215 \text{ Kbp}$

and if we accept 1500 genes as an "average bacterial one"

$1500(1.215) = 1.8 \text{ Mbp}$ as the genome size

which once again, we round to $\sim 2 \text{ Mbp}$.

Final estimates of viscous protein: Hold this

$MW \cong 45 \text{ kDA} = 45000 \text{ gms/mole}$

Genome Size Estimated for CDB @ $\cong$ 2Mbp.

Another estimate for bacterial protein size is ~ 330 amino acids

Other source had 267

Average amino acid wt is 110 daltons.

Therefore avg. bacterial protein size is on the order of

$330(110) = 36,300 \text{ dA} = 36.3 \text{ kDA}$

Your estimate is well within expected ranges.

We all now that the mutation rate is right on range. 36.3 KdA ≅ 0.98 kb (ie 980 bp)
(protein size)

We assumed a "typical bacteria. Can have ~1943 genes of 900 bp each)."

Therefore 1943 (900 bp) = 1569 kbp ≅ 1.6 Mbp

but the range is from 0.6 to ~8 Mbp.

So our estimate of ~2 Mbp is very reasonable and close to even an "average bacteria".

Very good work. We are right on target w/ expectations.

Aug 02 2017 - Repeat MW Determination

0°C = +0.8°C

Small Tube	Large Tube
Initial H_2O 6.32 gms	10.91 gms
H_2O + Sample 9.23	16.25 gms
Δ Sample = 2.91 gms	5.28 gms
92% H_2O = 2.68 gms	4.86 gms
8% Sample = 0.23 gms	0.42 gms
Revised H_2O = 8.91 gms	15.83 gms
Δ Sample = 0.23 gms	0.42 gms

TC:
Δ T .6°

+0.5
−0.3°

$MW = \frac{0.42(1.86)}{.3(15.83E-3)} =$

$MW = \frac{2.91(1.86)}{.2(6.32E-3)} = 4202$

$\frac{4202}{.08} = 53526$

$MW = \frac{5.28(1.86)}{.3(10.91E-3)} = 2984$

$\frac{2984}{.08} = 37300$

53.5

Lets use only large samples.. 46213, 55456, 37300

$\bar{X} = 46323$ (3 largest msmts)

25.5

Our previous estimate was 45000 gms/mole = 45 kDa
So we will hold this. This now has 4 msmts.

5 measurement average gives 43.6 kDa so hold it @ 45 kDa

Exfoliated Skin Sample examination via IR
Acetone Film Creation on ATR

We now see some of the properties of the skin exfoliation from FE sample.

Properties seem to combine hydrocarbon & acetamide.

Acetamide is a plasticizer

For a given hydrocarbon chain, viscosity increases w/ Chlorine content.

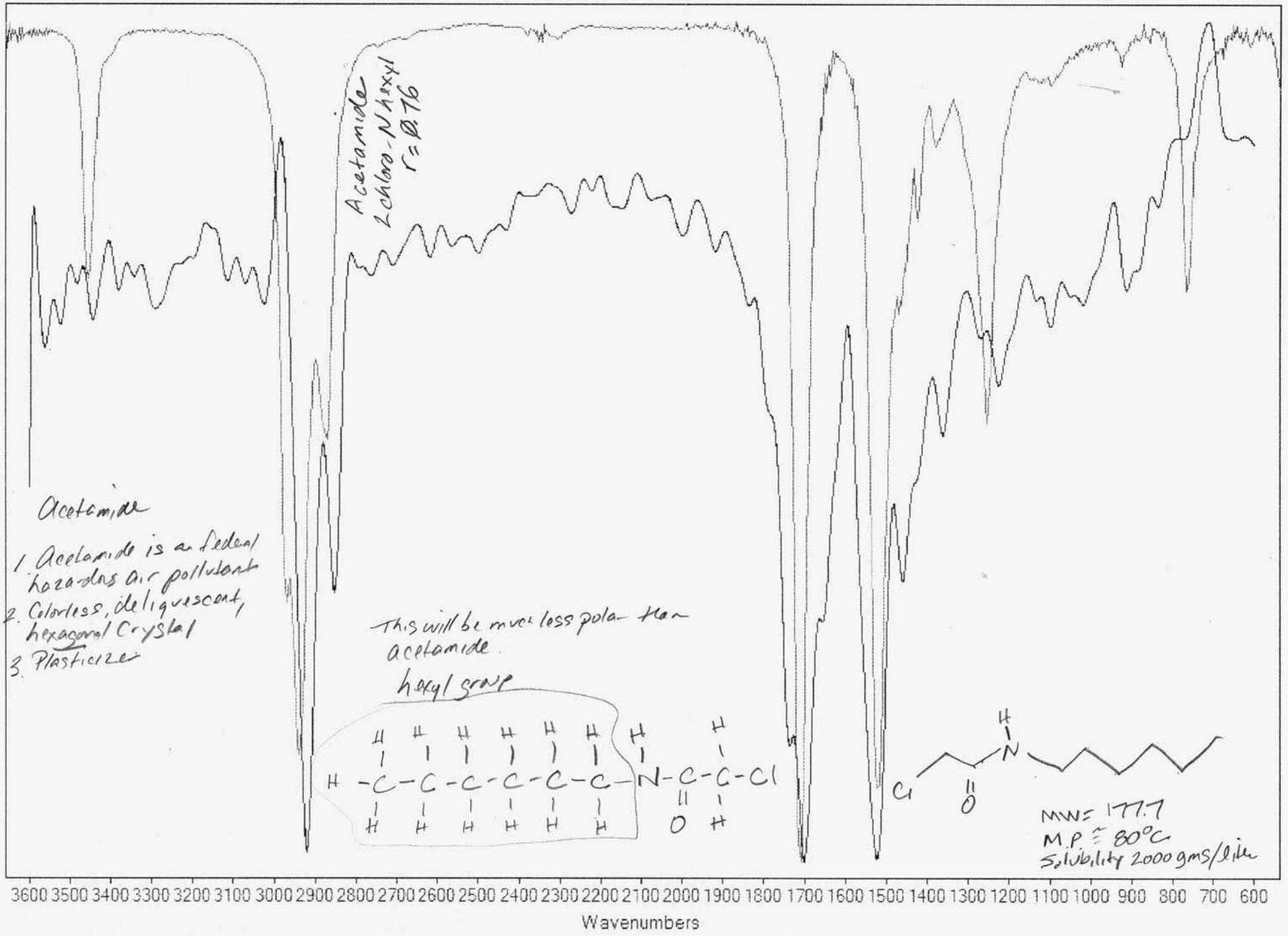
A hexyl group is C_6H_{13}, derived from hexane

Determining the MW of the precipitate protein is a real challenge - you have to get it into solution somehow. The precipitate was brought about by pH change but it does not appear to be reproducibly reversible.

Acetone - NO.
Isopropanol - NO
MEK - NO
Conc. HCl - YES - turns light green.

It does dissolve, but we all that the protein is not pure. But, how do we use this.

Conc. NaOH - KOH - NO.


Acetamide
2 chloro-N hexyl
r = 0.76
Acetamide
1. Acetamide is a federal hazardous air pollutant
2. Colorless, deliquescent, hexagonal Crystal
3. Plasticizer
This will be much less polar than acetamide.
hexyl group
MW = 177.7
M.P ≅ 80°C
Solubility 2000 gms/liter
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

Morgellons Skin Efoliation FE Aug 02 2017 ATR Acetone Film Layer - 02.JPEG

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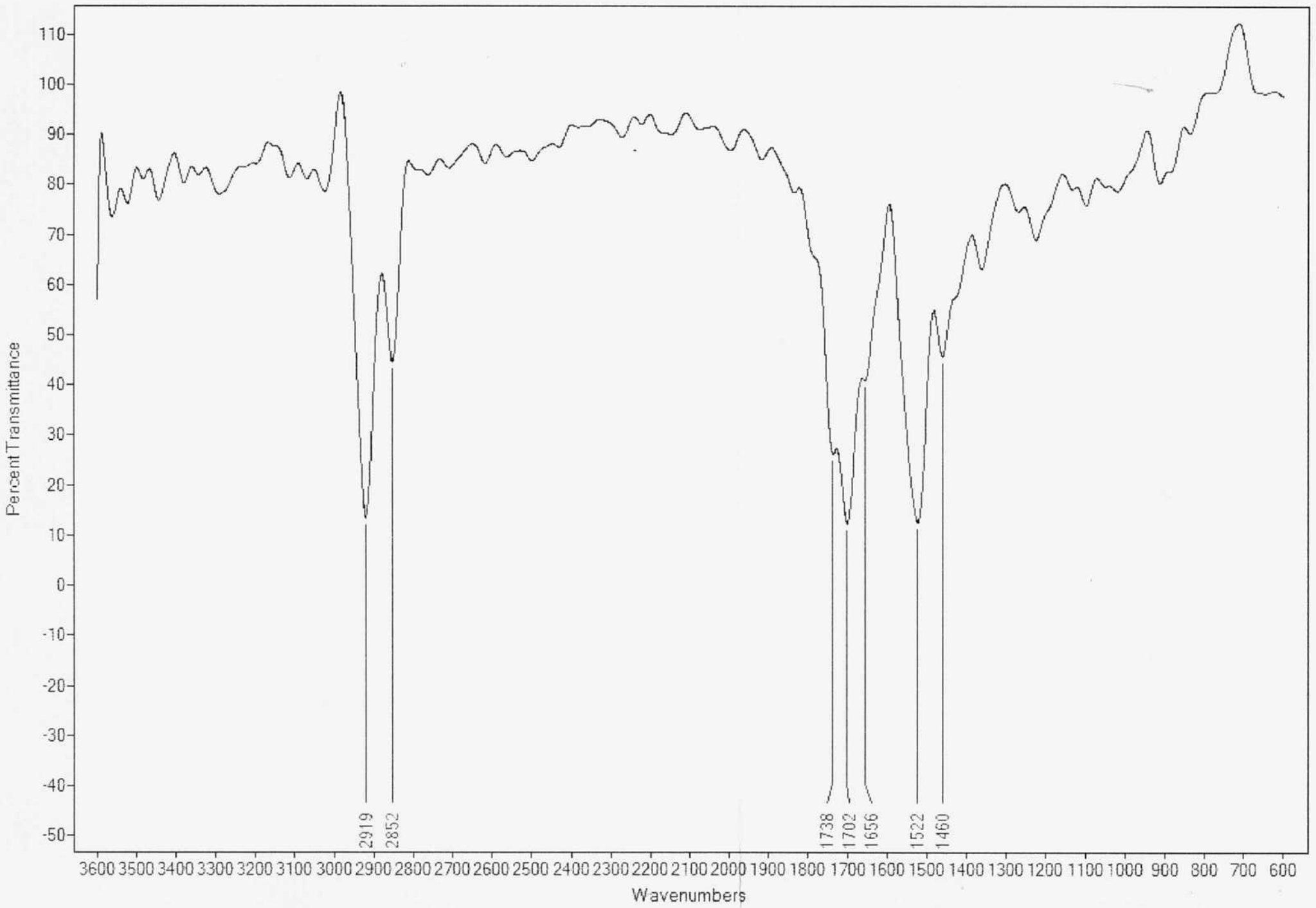

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Subject: Lab
From: Clifford E Carnicom <[redacted]
Date: 8/2/2017 19:52

[redacted]

[redacted] All,

I have established some procedures in the laboratory involving a protein that are undoubtedly of value and importance, if it can be brought into the proper environment. There will ultimately be numerous health and likely commercial applications from the work, again, if it can be brought into the right environment. [redacted]
two completely different methods of getting from point A to B. Previous IP methods use a difficult method, current methods are much much easier with the same but more productive end result.

[redacted] then the work goes to the [redacted] savvy members of the public that know how to use it.

[redacted]

Greetings and best from Clifford

comments from all always welcome.
This email should also be on record for CI [redacted] in case any one tries to claim knowledge or methods that will only be found in the lab notebooks.

[redacted]

Okay, really like the MW of the precipitated protein. Notice it is the same color as the secreted protein when it is acidified.

Well, well, well.

When you add a single drop of KOH - NaOH to the secreted protein it turns exactly the same color as the precipitated protein.

Guess what - the proteins must be the same. That is remarkable. Two completely independent methods of producing the same protein.

That is really quite astounding.
That is why the IR plot came out so similar.

We know many acid base chemistry take place w/ the protein therefore.

The protein, when dried, actually is quite pure.

Aug 03 2017

We have an interesting line of work ahead of us. We are ultimately after

1. The pI, i.e., the isoelectric point of the protein
2. A separation of the amino acids of/in the protein and potential identification of same.

In addition, we will now get involved w/ classical titration for the first time.
Later on, there is likely a course of action involving electrochemical titration but save that for later. For now, we will use the biuret collection and also learn to create some calculated acid-base solution in the process.

Another observation of importance, after showing the similarity between the secreted & the precipitated protein is the fact that under condition of alkalinity (i.e. the green precipitate of protein forms) it separates into two components after sitting overnight.

The tells us that the precipitate, after subject to extreme acid & base variation, is actually composed of two separate components that settle out overnight under alkaline condition.

1. a top layer (3/4 of total volume) is a brown precipitate

2. a bottom layer (1/4 of total volume) is a greenish precipitate.

These will need to be examined in more detail @ a later date.

For now, we work w/ classic titration development.

I would like to consider preparing standard solutions of
1. 1M NaOH MW = 39.997 gms/mole
2. 1M KOH
3. 10 M NaOH
4. 10 M KOH

1M NaOH: We dissolve 40 g of NaOH in 1 liter of H_2O.

The 40 gms needs to be dissolved in partial volume H_2O, then final H_2O added to bring to 1 liter.

Solubility is 111 gms/100 ml or 1110 gms in 1 liter. This means that you can easily make a 10M solution.

We will not use a liter. We will use 200 ml or 0.2 ml
Therefore 1M = (0.2) 40 gms = 8 gms within 200 ml.
10M = 80 gms within 200 ml

Wt of plastic jar: 177.10 gms
Add 8 gms to partial volume.
Partial volume = 298.00 gms Δ = 120.90 gms = 120.9 ml

Now here is what is interesting. If you add 8 gms of NaOH, then you must subtract 8 gms (8 ml) of water from the 200 ml level to keep the total mass of the water to 200 ml. This means your total volume of H_2O in the case is only 192 ml, not 200 ml.

We now have a partial volume of 120.9 ml
Now add 8 gms NaOH.

Weigh boat = 261 gms
Added 8 gms NaOH
Solution now measures 305.60 gms
We should have: 120.9 ml (gms)
8.00 gms NaOH
177.10 gms H_2O
Σ = 306.00 gms.
We actually have 305.60 so we are off by 0.4 ml.
This is certainly acceptable = 0.3% error.
Now dissolve and bring final volume to 192 ml or 200 gms.
Note: the NaOH generates a fair amount of heat in the solution. 10 M will literally explode so be careful there and add gradually!

200 gms + 177.10 gms (gain wt) = 377.1 gms final wt

200 ml of carefully calibrated 1 M NaOH is now prepared.

Now construct a 10M NaOH solution.

Wt of jar: 177.22 gms
Partial Volume H_2O: 290.61 gms ΔH_2O = 113.39 gms
Now add (0.2)(10)(39.997) = 79.99 gms NaOH
fraction of liters, M, gm/mol

This checks w/ 8 gms of a 200 ml of 1M NaOH
Wt of beaker (weigh boat) = 109.82 gms.

Now it is important to add this gradually.

Final wt will be 177.22 gms + 80 gms (200 gms) = 377.22 gms
~~251.22 gms~~
It is indeed very hot. Too hot too hold.
It is a good idea to use a Canning jar;
Heat production is a serious factor here.

Incidentally, on the rash on the neck, today is the first day I am not using the K4 - baking soda paste. All signs are to a subsiding condition. Neck and upper mid chest remain red colored, however. Show hydrocortisone @ approx 0300, ~10 hrs ago.

Final Weight = 177.22 + (80) (-80) + 200 = 377.22
We have a problem here. 80 gms of NaOH does not = 80 ml of water. We need volume to 200 ml.

200 + 80 + 177.22 = ~~457.22~~ This is not correct either. You must have volume total to 200 ml.

Ok, we see the problem here. The end product must be determined by total volume = 200 ml regardless of how solute dissolves into partial volume.

It is still to hot to handle but let us check volumes of 1M solution.

The mass of the final 10M solution of NaOH is 426.10 grams. This could not have been easily predicted. Dissolving is a complex phenomenon. What controls the solution is the mass of solute dissolved within a GIVEN volume. This is not an additive or replacement process w/ water, it involves the dynamics of dissolving.

Incidentally, because the 1M solution of NaOH is so relatively weak, that solution remains w/in error limits and reads fine @ 200 ml.

We now have both 1M & 10M NaOH Calibrated solutions @ hand.

We can now progress towards titration.

The 10M solution still remains quite hot!

I have 3 indicator solutions available & 2 pH meters.
1. Phenolphthalein changes to pink @ ≥ 8.2
2. Bogen universal indicator
3. Bromophenol Blue also! (Changes from yellow to blue @ pH 3.8)
Phenolphthalein turns pink in alkaline solutions
Bogen creates beautiful colors.

We should be able to titrate the HCl.
We estimate that it is approx 8.7 M.

We first will take 1 ml of 1M NaOH
Lets Calibrate a dedicated eyedropper.
We have plenty now.

$$\frac{105 \text{ drops w/ standard eyedropper}}{4 \text{ ml solution (H}_2\text{O)}} = \frac{1}{X} \qquad X = .038 \text{ ml}$$

$$= 38 \text{ ml}$$

This will be useful.
10 drops ≅ 0.4 ml.
So lets say we take 1 ml of 1 M NaOH
and add it to 99 ml of H_2O.. What is our molarity.
0.01 M NaOH.

This is probably far too weak. What we first use
1M NaOH. This sounds about right. Then we take our
8.7 M and dilute it by a factor of 20 (1 part + 19 part H_2O)

This should be about 0.4M and about 25 ml of 1M
NaOH should neutralize it.

~~Then we let go in reverse~~
No this is fine.

Let's take 50 drops of the acid and add it to 50 ml level by H_2O.

50 drops (.038 ml) = 1.90 ml

Now our bottle tells us that our muriatic acid is 31.45% by weight or by volume? MW of HCl = 36.46 gms/mol
It is by weight. It means:

$$\frac{31.45 \text{ gms HCl}}{100 \text{ g Muriatic Acid}} = 36.46$$

Oops, we do need to figure in density. Then on what happened w/ the NaOH calibration process.
The method is:

1. The density of HCl is 1.16 gms/ml

So

$$\frac{1.16 \text{ gms}}{\text{ml of HCl}} \cdot (100 \text{ ml HCl}) = \frac{116 \text{ gms HCl}}{100 \text{ ml of HCl}}$$

Now $\frac{116 \text{ gms}}{36.46 \text{ gms/mol}} = \frac{0.314 \text{ moles of HCl}}{100 \text{ ml}}$

However, the concentration of this commercial acid is only 31.45%
Therefore we actually have .314 moles (.3145) = $\frac{.099 \text{ moles}}{100 \text{ ml}}$

Let's go to liter reference:
Density of HCl is 1.16 gms / ml of HCl. so mass of 1 liter HCl = 1.16(1000) = 1160 gms / liter

but our concentration is only 31.45% so we have
.3145 (1160 gms / liter) = 364.82 gms HCl / liter. Commercial Purchase

Since the MW of HCl is 36.46 gms / mole
We have $\frac{364 \text{ gms HCl / liter}}{36.46 \text{ gms / mole}} = \frac{364.82 \text{ gms HCl}}{\text{liter}} \cdot \frac{\text{mole}}{36.46} = 10.01 \text{ moles / liter}$

We now know the molarity of the commercial muriatic acid and it is 10.0 moles / liter, not 6.7 as we had determined earlier.
This is certainly convenient.

Okay, we now have good standardized solutions of
1 M NaOH
10 M NaOH
10 M HCl.

Let's go to work with an example.
Let's add 1 / 10 drops ~~that~~ 10 M ~~10~~ HCl to 100 ml H_2O.
$= \frac{.038 \text{ ml HCl}}{100 \text{ ml } H_2O} \cdot \frac{10 \text{ M HCl}}{1000 \text{ ml}} = .380$ 10 M / HCl = $\frac{10 \text{ moles HCl}}{1000 \text{ ml}}$

$\frac{10 \text{ moles HCl}}{1000 \text{ ml}} (.038 \text{ ml}) = \frac{0.380 \text{ moles HCl}}{1000 \text{ ml}} = \frac{x}{100 \text{ ml}}$ add to 100 ml. $x = .038$ moles

So we have a 0.38 M solution of HCl.

The same to strong.
1 drop of conc HCl in 100 ml H_2O.

$10M\ HCl = \frac{10\ moles\ HCl}{1000\ ml} = \frac{364.6\ gms\ HCl}{1000\ ml}$

Error Here

No thus is OK

$\frac{1\ mole}{364.6\ gms} = \frac{3.46}{1000}$

That is certainly a lot of HCl.

Now we take 1 drop = .038 ml of this solution HCl

$.038\ ml \left(\frac{400\ gms\ HCl}{1000\ ml}\right) = .015\ gms$

Ok, and $\frac{.015\ gms}{.038\ ml} = \frac{X}{1000\ ml}$ $X = 394.8\ gms$

and @ 40 gms/mol; this is equal 9.87 M solution. Ok.

Now we are taking the 1 drop, which equals .015 gms and adding it to 100 ml H_2O

$\frac{.015\ gms}{100\ ml\ H_2O} = \frac{X}{1000\ ml}$ $X = 0.15\ gms\ HCl$

and $\frac{0.15\ gms}{40\ gms/mol} = .004\ M$ Solution then we go

Now I would rather have almost a 0.1 M solution so lets add 25 drops

Ok, again, We have a 10M solution HCl = $\frac{364.6 \text{ gms HCl}}{1000 \text{ ml}}$

We take 25 drops @ .036 ml per drop.

drops 25 $\left(\frac{.036 \text{ ml}}{\text{drop}}\right)$ = .95 ml

Now .95 ml $\left(\frac{364.6 \text{ gm HCl}}{1000 \text{ ml}}\right)$ = .3469 gms HCl

Now we add this to 100 ml H_2O

$\frac{.346 \text{ gms HCl}}{100 \text{ ml } H_2O} = \frac{x}{1000 \text{ ml}}$ $\quad x = \frac{3.469 \text{ gms}}{1000 \text{ ml}}$

and @ ~~364.6~~ 36.46 gms then $\frac{3.469 \text{ gms}}{36.46 \text{ gms/mole}} = .095 \text{ moles}$

$\frac{\text{gms}}{1} \cdot \frac{\text{moles}}{\text{gms}} = \text{moles}$

We therefore have a .095 M solution w/ 25 drops 10 M HCl added to 100 ml H_2O, This will be our reference solution & this does seem suitable.

Now add 2 drops phenolphthalein.

Ok, I added NaOH 10M by mistake, so it turned pink. Lets you redo that.

Also You must use the reference Eyedropper

NOT JUST ANY EYEDROPPER

Only one eyedropper is Calibrated!
This is not intuitive as it requires swapping the dropper.

There is a lot of attention to detail that is required here. Now add 1 drop of phenolphthalein (this is adequate).

It will remain colorless @ the point. You are using the phenolphthalein that has been mixed in isopropanol (strength unknown, likely 10% or 90%).

You can see in practice that you will perform an approximate titration w/ the calibrated eyedropper first as it will get you in range to set up the titrant apparatus properly, which is much more demanding.

Now I would like to run a titration which requires about 1/4 as much solution to equalize, eg about 25 ml.

To perform a titration you need the reaction first to anticipate the stoichiometric ratio

$$HCl + NaOH \xrightarrow{2OL} H_2O + NaCl$$

So 1 mole of acid + 1 mole of NaOH → 1 mole of Water + 1 mole of salt

So .095 (4) ≅ 0.38M ≅ 0.4M.
S. I would like to prepare a ~0.4 M NaOH solution.

(10 M) 100 ml (0.4 M) 1st Color Titration.

$V_1C_1 = V_2C_2$ Good work. Error in molar determination $\cong$ ~~0.9~~ % < 1%

$V_1 = \frac{V_2C_2}{C_1} = \frac{100\text{ml}(0.4\text{M})}{10\text{M}} = 4\text{ml}$ 0.1% error. Good.

$\frac{4\text{ml}}{.038\text{ml/drop}} = 105$ drops NaOH in 100 ml H_2O

You must keep track of the calibrated dropper @ all times.

1. Swapping.
2. Cleanliness
3. Know where it is @ all times

1 drop $\cong$ 0.038 ml

Next we must flush & prime the buret tube with the 0.4M NaOH solution.

We start @ 10.0 on the buret tube & now look for pink. We anticipate ~ 25 ml.

10 + 25 = 35 ml anticipated point.

We have it. 31.6 ml (32.05 − 10.0) = Δ 22.05 ml

NaOH turned the solution slightly pink.

Now we solve. $V_1C_1 = V_2C_2$

$(100\text{ml})(X) = (22.05\text{ml})(0.4\text{ml})$

$X = \frac{22.05\text{ml}}{100\text{ml}}(.4\text{M})$

$\Rightarrow C_1 = .088\text{M}$ (HCl)

and we know that theoretically it is .095M This look decent.

What is error? $= .007\text{M}$

$.009\text{M}\left(36.46\frac{\text{gms}}{\text{mol}}\right) = .328\frac{\text{gms}}{1000\text{ml}}$

~~.328 gms = x x = .03%~~

~~1000 gms 100 error~~

Error $= \frac{.328\text{gms}}{36.46\text{gms/mol}} = 0.9\%$ Error

Ok, you see that you do not need to be so delicate w/ the pink color. Even w/ only 1 drop of phenolphthalein it turns a brilliant pink @ the titration point.

Let it proceed to turn boldly pink.

Your error in molar gram determination is $\emptyset.7\%$.

Your final result mean that you would actually only have an error of .255 gms of HCl in 1 liter of solution.
So by molecular mass, you have an error of $\emptyset.7\%$
By volume of solution prepared your error is only

$$\frac{.255\text{gms}}{1000\text{ml}} = \frac{x}{100} = .0826\%$$

Thus an excellent .026% by volume is essentially error free. The first titration was, therefore an excellent success.

Lets test what the pH meter reads.
The pH meter shown the pink solution as measuring ~ 11.0 which I have a hard time accepting.

The pH meters are going to need Calibration.

What we are seeing here is that the newer pH meter seems to be relatively accurate.

We also see from wikipedia that phenolphthalein does not actually flip color until pH of 8.2, and not 7. We did seeing the same thing.

We also see that the pH of the old meter has a very slow response time, so this must be allowed for.

This means that if you chose a pH of 7 by the meter you would have considerable more error than you did by waiting until the indicator was solid pink. This may not, after all, have been appropriate.

This is somewhat of an interesting topic w.r.t. error reduction / and it is important, Calibrate pH meter

The pH meters actually seem to be approximate ± 1 unit no matter how you go about it. It is good but hardly as exact as you might think. Careful calibration w/ buffers is almost certainly required.

Bromothymol blue (not bromophenol blue) turns yellow to green @ pH 7.

We have learned that Bogen Univ indicator is far superior to Phenolphthalein. as it has much more sensitive around the neutral point. It turns green @ neutral, yellow below it, and blue above it.

Also I have diluted my supply with methanol and water and it looks like it will work fine. My supply is now quadruple of what it was. It should be plenty. Carolina Biological is restrictive on the sale to businesses & schools

But one source has it on Ebay. It is made by "Flo Science Company" They are on the net.

Carolina Biological is full of it. $
A standard sell on Flo Science Company 4oz 13.50.

Aug 04 2017

My first interest now is the repeatability of the strong acid - strong base titration results.

Recreate the "unknown" acid of .095 HCl
100 ml H_2O w/ 25 drops (.038 ml/drop) 10M HCl.

Add 5 drops Bogen indicator, (pink)

Recall that green is our neutral indicator
blue is alkaline
yellow & pinks are acidic.

.4M NaOH added to buret. looking for yellow, then green, on blue.

Buret currently @ 9.9 ml. (32.1-9.9) = 22.8 ml

Ok, it did change to yellow. At a green to green blue now so it has shifted. The color a very pale. Reading 32.1 ml.
Test for blue change w/ further addition:
Yes, just 2-3 more drops turned it blue
so I caught it @ just the right point.
Bogen is far superior. I suspect I caught it @ pH ~7.5

$C_1 = V_2C_2/C_1 = (22.8\text{ml})(.4\text{M})/100\text{ml}) = .091\text{M}$

This is excellent vs theoretical .095M Error = .004M ⇒ .146 gms
Error = .4% Molar error
Error = $\frac{.146\text{gms}}{1000\text{ml}}$ = .015% Error by Molar Volume

Superb Work Here.

We now have a very successful titration process in place for strong acid - strong base.

We know that we have found the titration pt. We found at ~ pH ~ 7.5. We expect pH of solution to now be ~ 8.0. Let us check out the pH meters.

Actually both pH meters seem to be exactly on target here. This is encouraging!
Older pH meter reads: ~~7.8~~ 7.7
Newer pH meter reads: 7.8
You could not expect any better result than this.

Now, weak acids & weak base titrations is a next topic of interest, however, let's estimate the precipitation point of the protein w/ the eyedropper.

With nitrogen now available, the large cultures are already showing initial signs of producing protein after about 5 days incubation.

There is also some initial gas being produced by 1-2 test tube cultures.

We now proceed w/ approximate precipitation protein titration. We will use

1. 10 ml of secreted protein (92% H_2O)
2. + 2 drops Bogen indicator - pink color as expected.

Titrate by eyedropper w/ 0.4 M NaOH

7 drops introduces green tint

9 drops introduces blue precipitate

I will choose 8 drops as the point @ which precipitation occurs as well as the color change to green.

Protein NaOH

$V_1C_1 = V_2C_2$

$C_1 = V_2C_2/V_1 = (\cancel{10ml})(8(.038\,me)/10\cancel{0}\,ml)(0.4M) = .012M$

Assuming a 1 & 1 stoichometric ratio.
Also there is 92% H_2O. 8% Protein $\quad .012M = 0.152M\ H^+$
Now the concentration would therefore be $.08$

This is interesting, since a equivalent is approx 1.5x the strength of the HCl we have been using in the titration testing. This is a fairly strong acid it would seem.

Isoelectric Point for Secreted Protein

Let's measure the pH of the now precipitated protein.

5.5

Measured pH: ~~6.6~~ old meter
5.9 ~~6.3~~ new meter

~~5.1~~

$\bar{X} =$ ~~5.9~~ Best estimate of isoelectric point of the protein.

See below

Now repeat

Indeed, 7 drops does begin to precipitate the protein. Therefore

$$C_1 = V_2 C_2 / V_1 = 7(.038\,ml)(0.4\,M) / 10\,ml$$

$$= .011\,M$$

and since we have 8% protein content

$$\Rightarrow \frac{.011}{.08} = 0.133\,M \text{ estimated}$$

assuming H^+ stoichometric equality.

Measure pH again: New meter = 5.5
Old meter = 4.5

$\bar{X} = 5.0$

Thus as a more refined trial. We now accept the best estimate of for the isoelectric point of the secreted protein as ~5.0 pH.

The isoelectric point of a protein is the pH at which the protein is least soluble. It is also the pH at which the protein is precipitated.

Casein (in milk) has an IEP of 4.6

Titration has been used to accomplish exactly what was needed, ie the IEP of the secreted protein.

In addition, reliable methods of titration for strong acid-base reactions have been established.

We can proceed as weak acid-base titration from a laboratory point of view, but that is a general topic @ this point.

The next topic of greatest advantage is to see if the amino acids of the protein can be separated

Enzyme approach: Approx 3 ml of secreted protein in tube staggered @ 5 min intervals. 5 tubes 3 drops of Ninhydrin added to each tube prior to heated water bath. General purpose enzyme solution (including some proteases) ~ 2 ml added to each protein tube.

We find no detection of protein digestion w/ the use of the general enzyme, even after 25 min of exposure to the enzymes.

What we do see, however, is what appears to be precipitation of the protein after they subject to the heat of the water bath.

This is certainly much simpler than salting out.

Because of this we can control of the problem by stability; no exposure to enzymes or dehydration, only water bath heat.

Ok, very interesting. Heat alone is not adequate to precipitate the solution. Now we need to test whether the enzymes or dehydration are involved in the heat precipitation process, or both.

Extended heat is precipitating the protein but it is not whole, it is damaged.

It is enzymes + heat + secreted protein that is causing the precipitation reaction.

We have, therefore, discovered an enzymatic reaction that may be of importance, via health issues. We will need to do this @ ~98°F (37°C) to see if the precipitation still occurs.

We are now running the test @ ~90°F.

The temperature may need to be lowered for the fast visible reaction to take place.

CDB gas production analysis:

Only CO_2 appeared in the screen. It is still very early in the culture screen that will be monitored as culture age.

Aug 05 2017

Enzymes (general set) + secreted protein
+ 85 ~ 90°F overnight

does precipitate out the protein. This means that it most likely has been denatured.

The has important health implications for changing the nature of the protein ingested to the body as well as its elimination from the body.

Bradford test to be used to verify protein concentration.

The precipitated protein is light in color, off white to yellow.

The enzyme + precipitated protein definitely passes the Bradford test.

I notice now that the Bradford test can take a while to develop fully (most likely as the protein continues to be hydrolyzed by the HCl). We know the reference value (w/out protein) is ~ 633 nm.

The existence of protein will drop the reference value to ~ 623 nm very quickly (immediate measurement).

However, we notice if the sample tube sets for a few minutes (e.g. ~15 min) the wavelength can be kept down to as low as ~605 nm.

Therefore if protein levels are marginal in concentration these along w/ proper reference controls may be useful in detecting lower limits of protein.

You also have your own colorimetric tests you developed this last winter which are highly sensitive (increased by an order of magnitude).

Then therefore (with the enzyme) a very effective means of denaturing and precipitating the protein. This shows great promise w/ health benefits.

We notice in our case, therefore, that the enzyme treatment did not hydrolyze the protein (as was somewhat anticipated) but that it precipitated the protein.

Now this is certainly interesting and likely of great value. We still have the problem, however, of how do we break down the protein into its component amino acids (anticipated to include glutamic acid, tryptophan, and proline)?

We also know that it can be precipitated by pH change but this is not likely to accumulate up in the body.

I would still like to acquire purified protease to attempt hydrolysis again.

We now try the same run w/ enzymatic detergent.

The trial is now run @ 5 min cumulative intervals w/ enzymatic detergent and ninhydrin.

Acid & enzymes seem to be the main methods. What about bile?

If protease does not break down the proteins what could be unusual.

old method: HCl method: Heat w/ 6M HCl @ 110°C for 24 hrs!!!

new method: Use 6M HCl in sealed container in microwave for 5-30 min w/ temps up to 200°C

i.e., microwave digestion.

That is now a possibility for us.

We are now trying a microwave digestion technique. Modest temperature, low power.

No sign of success @ this time. Trial will be completed.

We will now use the viscous sample w/ 10M HCl, water is therefore removed. The sample type is very limited, no opportunities will be limited. The question will be temperature and time.

We do have a reaction here w/ the viscous protein form in Conc. HCl under mild microwave use. We have a ~~ucl~~ amber color that has formed with the HCl liquid. Clearly it has caused a change of some form.

The max absorbance ~~is @~~ should be @ ~ 510 nm. ~~We have sig absorbance @ 510 nm~~. Negative.

Even though we know that we have had a significant reaction of some sort w/ the viscous protein & HCl & microwave, it fails the Ninhydrin test.

We have run a control test w/ Ninhydrin and it succeeded w/ max absorbance @ ~ 510 nm.

Our test fails @ this point.

Try again. 5 more minutes in microwave.

Good news is that the microwave digestion system is working, i.e., containing the solution under a power setting of 2 for 5 minutes. Small Carnsbury Jar w/ plastic threaded lid and enclosing container.

—

We need to prepare more of the viscous form of the protein. The evaporation process must be carefully monitored.

—

The second ninhydrin test (10 min microwave) has failed also.

Now increase heat to power level 2, 3.5 min Temperature monitoring

Ok, this was borderline to contain the solution. It has turned a dark brown. There were increased vapors. The run @ 3.5 min @ 20% power sealed container appears to be @ the borderline

—

It seems like, for some reason, our ninhydrin reagent is very weak??? It is working. It requires heat for color.

Ok, now we have a predicament.

Enzymes (therefore) did not break down the protein. It did however, precipitate the protein which has value in its own right.

Microwave digestion did not succeed either. Two different reaction levels also did not work both under reaction and the dark brown level.

The ninhydrin reagent is working properly.

We do have a problem here. Standard methods of protein digestion into amino acids is not working. Pure protease might be your next run.

How about if we try the digestion w/ dried milk?

The microwaved protein (now dark brown) even fails the Bradford test now so it has undoubtedly become completely altered. Interesting that the protein has been damaged but no detection of amino acids.

Conc. HCl did not damage the cannery lid.

Powdered milk in conc. HCl w/ microwave produces a slight pink color.

Ok, here is the big lesson and surprise for the day.

Powdered milk, per 5 min microwave digestion, DID NOT produce a positive Ninhydrin test.

This is an important finding and it does not mean that our Vitreous test has actually failed.

Add 5 min again.
If you cannot produce amino acids from dried milk, how do ya expect to produce from a new or unknown protein.

There is now a strong pink color after the 2nd 5 min session. It is actually more purple @ this time.

10 min microwave of dried milk also fails ninhydrin test, even though once again we have a elegante color reaction.

It is very unexpected to have the red purple color that has developed w/ 10 min microwave (mw) digestion @ 10 min.

The provide evidence that microwaving does change food. Who has heard of dried milk turning purple (admittedly w/ conc. HCl).

The dried milk ninhydrin test failed on the 3rd round. My only measurement is @ ~ 630nm.

—

We have protease on order.

I overheated the viscous sample.

—

Heated the dried milk solution again, 20% Microwave power, 3.5 min. It blew the Containing top, but the Canning Container did hold. Purple solution also turned dark.

—

Breaking down the protein into amino acids is not critical but it is highly desirable.

Aug 06 2017

[redacted] Release of notebooks

3. Of interest than amino acid digestion trials failed, even w/ dried milk.
4. Evaporation of viscous protein must be monitored carefully & conscientiously.

5. DNA lab research - candidate identification
There is the primary need @ this time. Also to become knowledgeable on the 16S sequence.

A trial run for identification of starter fluid. We quickly have:

1. IR plot
2. GC plot
3 UV Plot
4. Boiling point under examination.

Distillation Results:

37° 1st ~~estimate~~
38°C 2nd estimate.
42°
43°
47°
49°C

We have some range spanning 38°C - ~~58°~~ 52°C w/ majority around 44°C $\bar{x} = 45°C$

diethyl ether = 35°C
dimethyl ether = 46°C

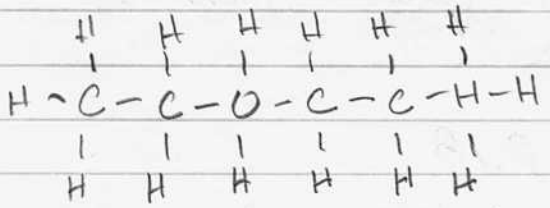
62°C is the next level.

It is 137°C @ base of flask.
Then must be watched carefully.
Base of pan is 370°C
The component is much more difficult to vaporize.

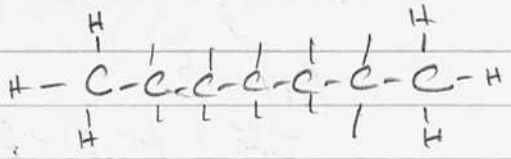
404°C base of pan
157°C base of flask
61° Thermometer

OK we need to quit.
We found an additional difficult boiling pt of 62°C.

Component are stated to be heptane & ethyl ether



Diethyl Ether (also called ethyl ether)
Boiling Point 35°C



Heptane (C_7) 98°C
Boiling Point

The IR plot nails this very well with use of only the major peaks: alkanes & ether.
GC nailed heptane but the issue of methane & ethane does remain uncertain for me in GC.
Remember that this was headspace / pyrolysis.

Aug 07 2017

Lets use our model to predict the most likely candidate, methane or ethane.

$t = \emptyset.82$

$t_{150} \cong 2.74CN - 5.3$ $\quad$ $t_{150} \cong \emptyset.19MW - 5.6$

$CN \cong \emptyset.36 t_{150} + 2.\emptyset$ $\quad$ $MW \cong 5.11 t_{150} + 29.1$

$CN \cong (.36)(.82) + 2.0 = 2.3 \quad \neq 2$

$MW \cong 5.11(.82) + 29.1 = 33.3 \quad \neq 33$

Methane: $CN = 1$

$MW = 16$

Ethane $\quad CN = 2$ $\quad$ C_2H_6

$MW = 30$

Clearly ethane is the most probable candidate

Ethane

$t_{150} \cong 2.74(2) - 5.3 = \emptyset.18$

$t_{150} = \underline{\emptyset.10}$ $\quad$ $\neq \bar{x} = \emptyset.14$

Model is not sensitive enough to distinguish here.

CDB gas analyses: Cultures are about 1 week old

We have a major peak @ 15 min.
Where is this coming from?
There is enough signal that timing could be off.
Is this could actually be @ the acetone level?

Test air control first.
We have the peak even w/ air, but it is a fraction of the size. CDB peak @ ~ 15 min = 4
Showed peak w/ air ≅ 0.6. What gives here?
Lets clear w/ acetone.

We have learned that it is difficult to clear residual effects from syringe.

Even after evaporating the syringe, acetone still shows up very strong. It is @ ~ 10.5 min, however, not ~ 15 min, so there is no confusion between the two.

The 15 min peak is now < 0.1 mV so it is ordered to inconsequential.

Now back to balloon culture - CDB.
Acetone shows up strong @ ~ 10 min. OK.
OK very exhilarating. We do again have a strong peak w/ the CDB tube culture @ ~ 15 min
This is very intriguing. @ 15.04 min

The CDB culture peak is by itself
quite strong
Magnitude ~ 2.0mV
It is a candidate for trapping.

Model ~~estimate~~:

$CN \approx 0.36(15.04) + 2.0 = 7.4$
$MW \approx 5.11(15.04) + 29.1 = 106$

$C_7H_{16} = 100$

$C_8H_{18} = 114$

Our closest match is heptane, however
since it does not match exactly we must
also consider heptene?

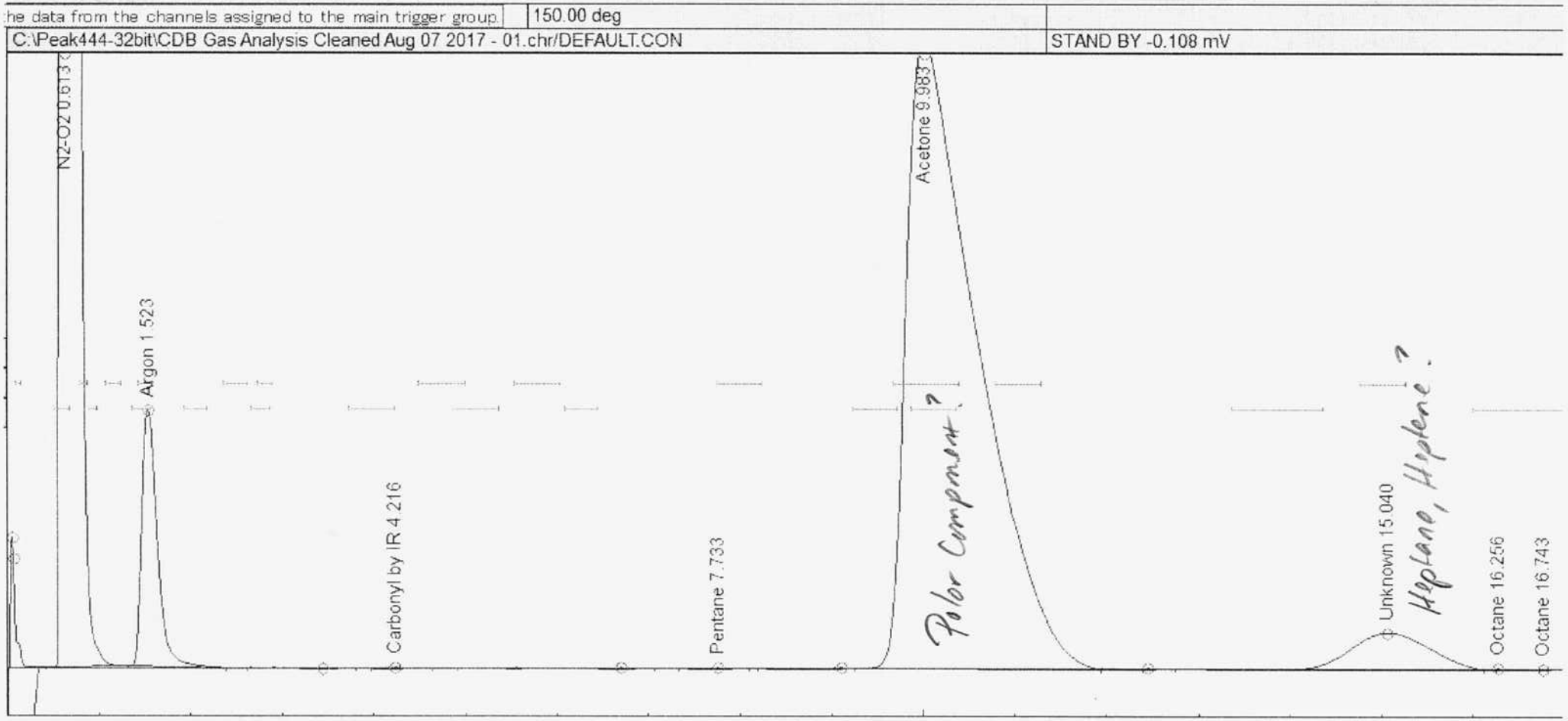
Heptene only has one double bond.

We also must study the peak about the very large
peak @ the acetone point (~ 9.03) Magnitude $\approx$ 40mV
This is much too large and too long after
evaporation & cleaning of the system.

This is a high level hydrocarbon and a
strongly polar compound. This certainly
matches the composition of the vacuum pollution.

Very unexpected however, these are very prominent
peaks.
These need to be trapped.

CDB Gas Analysis : Acetone like component (?) and Heptane or Heptene? Very unexpected 1 week culture.



Aug 09 2017 GC Controls - CDB Analyses

Concerning GC CDB gas analyses:

Air control: (4 syringe):

We notice that until instrument is thoroughly warm (~2 hrs) there is a drifty baseline down. I anticipate a mild ramp up (eg 0.2° per min) might be able to compensate for the until stabilization is complete.

We do have a peak @ 10.9 min w/ air-syringe control. May be ~ 0.8 mV. Residual Ethane?

We also have a small peak @ 15.2 min air-syringe control. May be ~ 0.1

The matches heptane.

Both of the peaks match CDB results. Now check may mistake.

With previous CDB analyses, these same peaks have magnitudes of 37.7 mV & 2.5 mV respectively.

So clearly there were very strong peaks in the previous CDB gas analyses & apparently we are dealing w/ residuals with syringe.

The part that is confusing is that the major CDB peak is actually @ ~ 10.5 min vs 10.9 min in the control but this may be in the range of variance.
What is most interesting is that the CDB analysis has a trailing peak and this suggests a more polar nature, which would make sense. It also does not make sense to have such high level hydrocarbons as C_6 + C_7 in a headspace analysis while having no predecessor HC's in the chromatogram. However, the fact that we have residuals in the syringe after 48 hrs indicates that they could indeed be high level HC's. The only answer to this is trapping, but only the 10.5–10.9 min peak is likely large enough to do this.

For now, we continue w/ controls. Re-cycling again. Lets also set mild ramp program.
We will experiment w/ 0.1 deg./min. This idea is works very well. This is a clever procedure to establish instrument stability during the extended warm up period. It should be @ 0.2°/min.

2nd air-syringe control: We do indeed seem to be picking up residual high level HC's: C_6, C_7, C_8.
The magnitudes are quite small but detectable.
Hexane is the largest @ ~ 0.4mV vs ~~1.0~~ 0.8 mV in 1st run!
Heptane ~ 0.03 mV
Heptene ~ 0.05 mV
Octane ~ 0.02 mV

Is there seems to be something to the fact that higher level HC's are coming from the COB GC analyzer. Sitting in the syringe for 48 hrs may have allowed hexane to volatilize in the syringe.

Either way, we will continue the controls until they are pinned down. We all now that the residual syringe HC's are quite small relative to original COB analyses. We can regard the effect as now quantified and under adequate control.

We will next rinse the syringe & see if we can remove these traces of potentially interfering an acetone residual check instead.

Set ramp to 0.2°C / min.
Dry syringe w/ hair dryer after acetone rinse. We see that the instrument is actually operating @ a very sensitive level (most likely down to a few ppm ~ 50 to 100?) and heating w/ the hair dryer & cleaning w/ acetone will be small steps.

We can actually see the color in the acetone rinse solution.

Remember the acetone is not a 1 component mix; it is proprietary and we can see the small alcoholay acetone peak @ ~ 2.8 min that is in the component file

The ramp is moving very well, ≈ 2 deg / 5 min now.

The argon % is now also more accurate @ ~ 1%.
When you get the baseline level you can pick up very small peaks.

Ok, we definitely pick up a large acetone peak, even after (~10.4 min) hair dryer w/ magnitude ≈ 40 mV. Definite tailing. There is an adequate distinction between acetone & Hexane.

Acetone is @ ~ 10.4 min
Hexane is @ ~ 10.9 min.] a degenate difference. But they are close!

Also we have the peak @ ~ 15.2 min so we know the syringe is clean except for residual acetone. I would like to eliminate the acetone peak since it is so close to Hexane & CDB & also apparently produces a similar peak in the system. Repeat until sufficiently small. Use the hair dryer again.

Shot to 0.3°C/min.
2nd Air run post acetone cleaning w/ hair dryer.
~ 8 mV
We have the acetone peak again @ ~~10.4~~ min, but now the magnitude is ~ 10 mV vs the 40 mV. So it has been reduced by 75% but it still exists. (80%)
Remember CDB magnitude was ~ 40 mV so we will soon be able to discern.

Also a very small heptane or heptene peak picked up, magnitude ~ 0.2 mV.

Let go again w/ the hair dryer.
Be careful do not get ~~...~~ it so hot that it melts the syringe.

Good level baseline now @ 0.3°C/min.
We have the peak again, exactly midway between acetone & hexane markers.
So it remains unclear what we do have. The peak magnitude is ~1.5 mV vs 8 mV vs 40 mV so it does continue to decrease.
The peak is not trailing, it is very symmetric.
We also see a heptane/heptene peak, magnitude ~ 0.08. It appears to me that the acetone peak has left quickly w/ the hair dryer and that what remains are gradually volatilizing HC's @ C_6, C_7 levels.

We are getting close to reevaluating CDB gas production.
The baseline is settling down after ~ 2 hrs.
We can drop to 0.2°C/min now.
OK, now CDB run w/ proper controls having been run.

~~We can~~ now drop to 0.1°C/min ramp.
Actually, still OK @ 0.2°C/min

Well, we definitely do have the peaks w/ CDB headspace.
We have

t (min)	Candidate	2nd trial	
10.56	Hexane	10.68	10.83
14.60	Heptane	14.80	15.12
	Propene		19.51
~24.6 min even	C_{11}	~24 min	

A very solid run here that demonstrates the presence of significant higher level hydrocarbons (HC) with the CDB headspace.

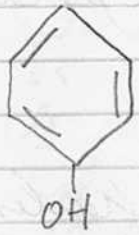
Repeat the run.
We can see that there are indications of a trailing argon peak, therefore this does indicate Methene.
We also see a small peak @ propane.
Hexane shows up.
Heptene shows up.
C_9 shows up.

We want to try & trap the C_8 peak.
It looks like the baseline is @ zero now.
Start @ 10.0 min, end @ 11.5 min.
Attempt to trap. Weak signal but detectable.
We have no match from the IR database.

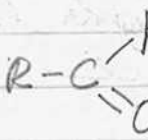
IR analysis of the trapped peak @ ~ 10.6 min reveals many interesting aspects. It is NOT an alkane compound.

Strong evidence of an aldehyde. ArCHO
Phenol can be entirely to pursue
Nitroso ^ nitro compound group seems likely
Ester group sounds possible also.

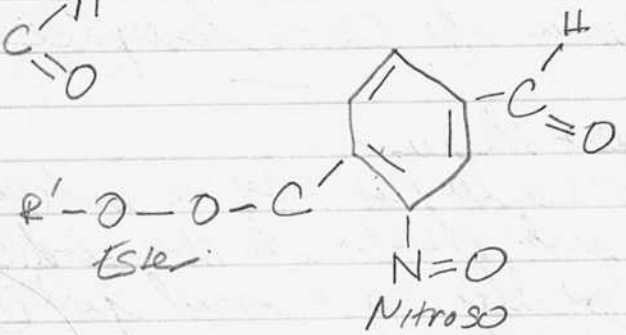
Phenol

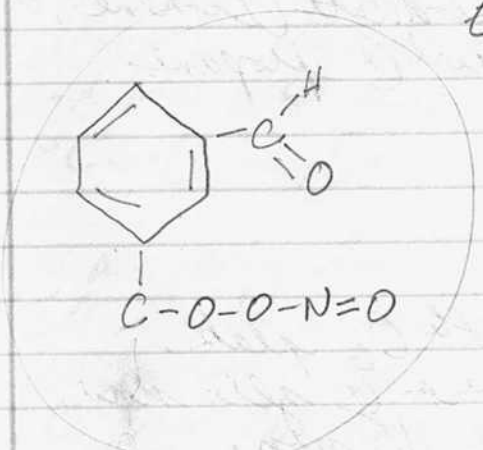


aldehyde



aldehyde ArCHO

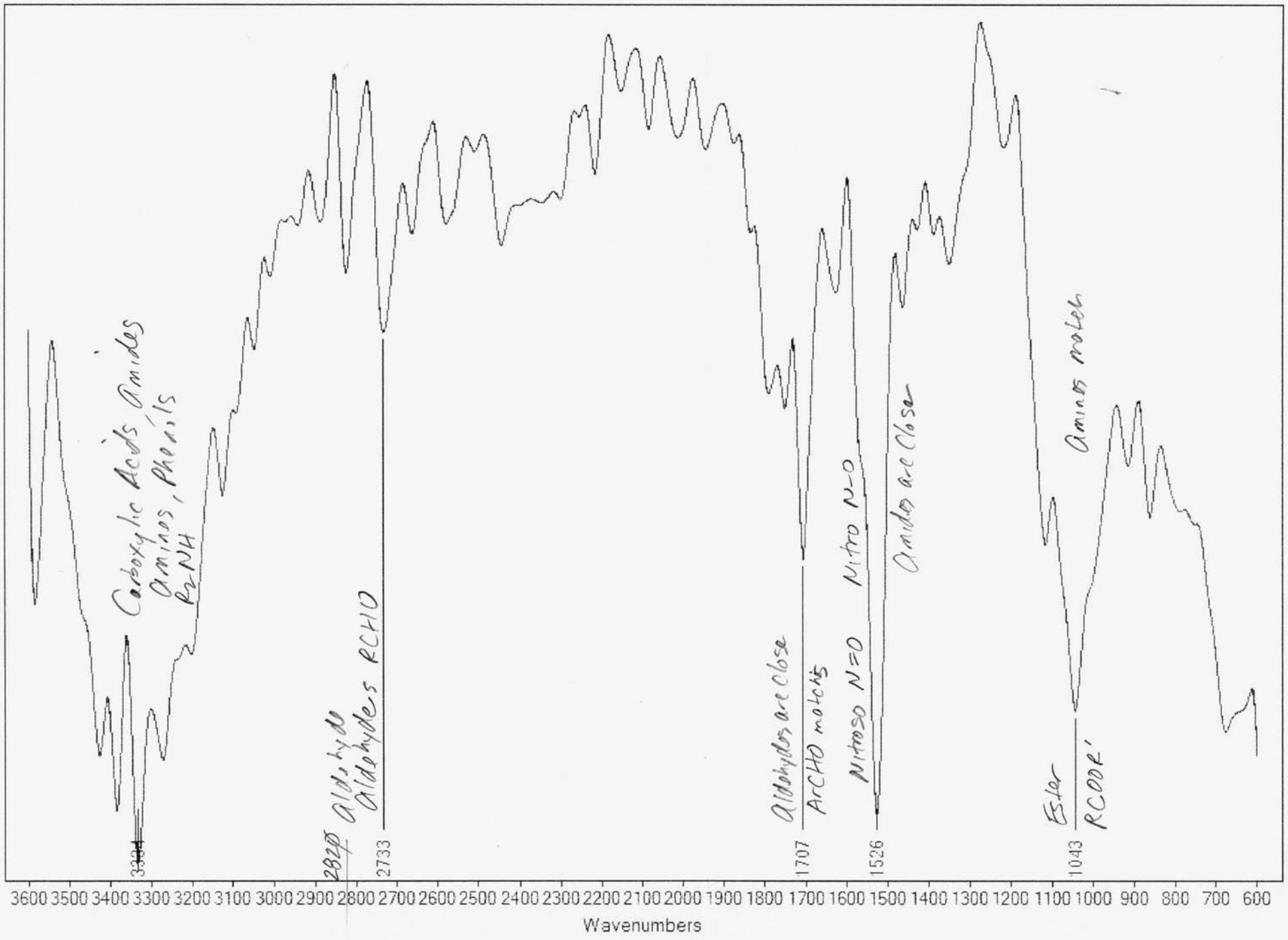




The compound is not an alkane. It is polar.

A name for this structure is
nitrosoperoxy methyl benzaldehyde

CDB Gas Analysis - Trapped Peak @ ~ 10.6 min


Carboxylic Acids Amides
Amines, Phenols
R-NH
Aldehyde RCHO
2820
2733
Aldehydes are Close
ArCHO matches
1707
Nitroso N=O
Nitro N=O
1526
Amides are Close
Ester
RCOOR'
1043
Amines matches
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

Molinstincts. com has given the most useful prediction of chemical properties.

An alternative name is formylphenyl methoxy nitrile ← Notice! This.

$\log S = -2.32$? ? ? ? ? ?

Intrinsic solubility $= -2.32 \log S$ S is in moles / litre

Log D is "the distribution coefficient"

Log P means optimal CNS central nervous system penetration. 2 ± 0.7

Top Log P = 2.13 We know this predicts

Very serious business here.

Aug 10 2017

Viscous protein Pyrolysis

Very thin pattern along w/ headspace.
Column set to ramp @ 0.4°C/min - quite stable & this does represent an improvement.

We must consider whether pyrolysis caused or contributed to the skin condition on my neck over the last 2 weeks. (Mostly healed now). Especially considering the body susceptibility (high log value w/ predicted properties). Central nervous system, etc.

It is one heavy pyrogram w/ the charred protein sample.

We have made it to C12

C13 C14
C12
C11
C10
C8
C7
C6
C6
C5
C4
C4
C4
C3
C3
C2

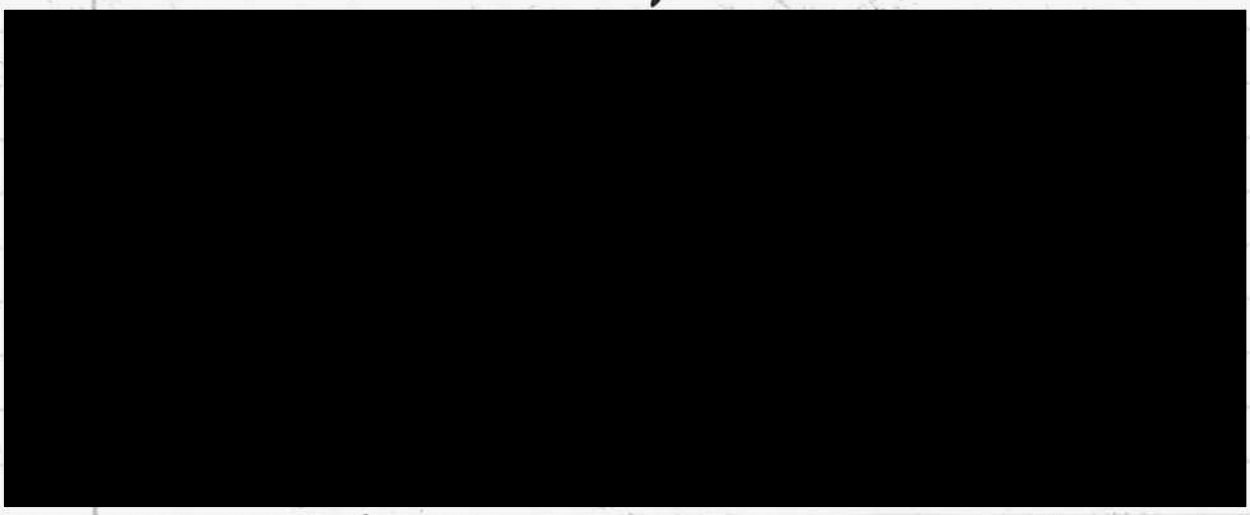
$C_{81}H_{165}$ ⇒ 1137 MW
$C_{94}H_{190}$ ⇒ 1318
$C_{108}H_{218}$ ≈ 1514 MW

$\Sigma = C_{81}$ max

Aug 11 2017 Page 127

1. We have enough secreted protein being formed now that we can begin to evaporate it in larger quantities. It is important to monitor it in an hourly basis. If only needs to be more concentrated, it does not have to have all water evaporated.

2. Today we will see if we can identify phenol content in the secreted protein.



4. I would like to evaluate the Log P, solubility predictions.

5. I am noticing that the secreted protein is turning turbid upon modest heat. This is the sign of denaturation.

Ok, we wrote & published the paper today.
Only fundamentals & uniqueness have been communicated @ this point.

—

We all now that too much heat will precipitate the protein. This is not what we wanted.
You set power @ ~ 2½ and it drove temp to 150°C & so. I believe that you should not go above ~ 65°C. You will need to keep heat down to power of 1 in the future. It is unlikely that you will recover the viscous form from this trial.

—

Really entertaining. The solution has now been evaporated to the final 10% level. The temp of the heating block now set @ 96°C on power setting of 2.

The solution has now become clarified again, ie, the turbidity has left!

Precipitation level has also decreased.
Something happening in the latter stages of evaporation that is unexpected.

—

You can indeed see that the solution is becoming more viscous and the precipitate also dissolves to produce a clear amber-green solution.
Very unusual.

CDB gas analysis of the balloon cal tube:

We now see two new peaks coming fast in the headspace process.

We interpret them to be

1. Propene
2. Butane
3. Vinyl benzaldehyde
4. Heptane

We do not have the C11 @ ~24 min on the run.

Bradford test on the heated protein comes out @ 631 nm.

This is telling us that sufficient heat is damaging the protein & I think that we ran into that before. The idea will need to be tested.

The ~~spectrometer~~ index of refraction for the 10% evaporated protein comes in @ 54.9 on the Brix scale. This is quite high.

Brix 54.9 $\cong$ IOR of 1.429

This is quite high.

The remains dilated so the final value is still likely higher.

I believe benzaldehyde is ~ 1.56 (?)

Yes, it IS 1.55 so this is another indication we are on the right track w/ this compound.

Benzaldehyde is soluble to level of 0.3 gms / 100 ml. Our protein is likely much more insoluble than this. We have our extract @ 8% by weight so I'm sure this is much much higher. Indicates molecule higher polarity than benzaldehyde.

We need to examine the properties of benzaldehyde combined w/ an ester.

Esters are less polar than alcohols but they are more polar than ethers.

The ester functional group has a similar character to the ketone & aldehyde functional groups.

Remember there is only headspace, NOT the protein!!
Naturally occurring esters are animal & vegetables fats & oils.

What about the phenol test?
The protein in H_2O does not react w/ ferric nitrate and neither does emulsion almond flavoring. Remember the color reaction w/ the protein that we did find? Protein + Sodium Citrate + $CuSO_4$ (a light green color)

The color reaction has been tested and indeed it is dependent upon citrate.

The term I used calcium citrate instead of sodium citrate. It has only existed also produced the green color. The green color does not arise from citrate and Cu^{+2} alone. It requires the protein to produce the green color.

Since the calcium citrate come from a supplement (ground to a fine powder) it must be centrifuged to clarify the solution color and to separate the filler from the solution.

Aug 12 2017

A very interesting observation today regarding the color of the moderately evaporated protein sample.

First, yesterday we noticed that the solution upon heating:

1. Turned turbid at the temp ~ 150°C
2. Precipitated also @ this higher temperature
3. Turned from the original slight yellow green to a muddy brown, as in iron oxide
4. Then we noticed as the water was removed more thoroughly, but not completely, that the solution became clarified (still brownish) and the precipitate dissolved.

The morning we notice that the protein solution, reasonably concentrated now (estimate ~ 70 to 80% of the H_2O was driven off) has turned GREEN as ark, as though Fe^{+++} is now prevalent.

So above 4 change of state have now taken place in the protein, all induced by applying heat to the protein. Recall yesterday that Bradford had failed. We will check this again for any change.

In addition, we surmise that we can more accurately determine the index of refraction by

1. Recording loss of H_2O against the index of refraction, especially if we also obtain a fixed moisture steady along the path of heating.

We have 3 ideas about what may have caused the neck reaction. It is clear @ about the 97% level now w/ some remaining discoloration on the lower neck & about 2-3 small sores left. At one time the neck skin was quite swollen and sharp red. Baking soda w/ KY gel seems to have been effective.

1. Unknown allergic reaction
2. A reaction to the fumes of pyrolysis of the charred protein. Many trials were run w/ this and we can see the complexity of the chromatograms in that case so there are numerous unknowns.
3. Contagion from an individual known to present the skin symptoms of Morgellons & analysis of such samples in the laboratory.

Of the three, I am prone to suspect option #2 as the strongest candidate.

An intro to genetic-DNA testing would be helpful today.

—

Ok, this seems really really strange:

I had conducted the Bradford test with the protein that has set out overnight (formerly heated to 150°C) and that has turned green again, as though Fe^{+2} is present.

IT NOW PASSES THE BRADFORD TEST w/ $\lambda_{max} \sim 615$ nm vs the reference 633 nm.

This indicates in two separate ways (color returns to green, ie Fe^{+2} presumed) and the now successful Bradford test

that Protein has now regenerated itself within the sample even after apparent * deformation, denaturation and precipitation from relatively high heat application.

This seems remarkable to me.

One must now wonder what the protein form is truly capable of, w/ or w/out the bacterial form present.

The next stage is to repeat the test. A question now will also be: Can the protein itself be used to create additional protein w/ suitable nutrients?

A sample tube has been set up with:
1. Water
2. Sugar
3. Fe+2
4. Protein (w/ new pipette)
NO CDB.

In incubator - what will happen here?

Notice that we do have several tube producing head space gas now.

~~to~~ today

Active
1. Condense any existing sample into an accumulator of ~~water~~ or concentrated form.
We do have an ample supply of protein now developing.

2. CDB gas analyses continues

3. Index of reactor model developed; required a dilute protein form to begin - we only have one container left right now.

Active

4. Molecular prediction -

5. It is interesting that amino acid digestion has failed.
6. HEPA study corrected.

Pwr Model: Brix ≈ 4280.6 mass$^{-1.417}$ $r^2 = 0.94$
Seems to experimental.

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Let's create a Controlled weight sample of the protein along w/ index of refractive measurement..

Constant
198.20 gms. Original mass of container
351.34 gms w/ Dilute Sample Added
Δ = 153.14 sample mass. Index of Refraction: 5.9 Brix.
Temperature = 85°C Pan-Setty #1

after approx 45 min the solution is turney stirred ~~the temp~~ ~ 85°C.

Total	Sample	Brix		Sample Brix
334.83	101.69	6.3	153.14 ~~198.2~~	5.9
			136.63 ~~101.69~~	6.3

Brix ≈ −.024 · Sample Mass + ~~10.70~~ 9.61

~~Sample Mass ≈ Brix − 10.70~~
~~−.024~~

~~If Brix = 0 then Mass =~~
10,000 ~ 0.

~ 367.2 Total Weight	Sample Wt	Brix	
351.34	~~198.2~~ 153.14	5.9	
(Container = 198.20) 334.83	~~101.69~~ 136.63	6.3	
320.35	122.15	7.0	~~Brix ≈ 4594 e$^{-.0146 \cdot wt}$~~
294.82	96.62	9.0	Brix ≈ 10229 e$^{-.022 \cdot wt}$
261.4	63.2	13.4	Brix ≈ 9.58E7 mass$^{-2.841}$
242.75	44.55	19.3	Brix = 8.15E8 m$^{-3.21}$ r^2=.98 r^2=.99
228.70	30.50 Solution is clearing now.	27.5	Br = 5.52E9 m$^{-3.54}$ r^2=.98
215.29	Solution clear 17.09	51.9	
	12.25	89	

~ ~~210.45~~ 220 8%
~~198.2~~ 214

~ 90
~~100~~

Brix = 1.105E13 m$^{-4.87}$ r^2=.89
3.25E13 m$^{-5.05}$ r^2=.85

T. ICMP data release?

With gas analysis, we have

1. Propene
2. Butene
3. Aldehyde group - benzylaldehyde
4. Heptane

The pattern has now repeated.

We can now increase the heat of evaporation to power level #2.

Two important projects:
1. Amino acid digestion
2. HEPA filter analysis & presentation.

So the question is, why were we unable to digest dried milk?

Very very Cool!!!

We have digested cheese into amino acids w/ a protease enzyme supplement!

Great! The Ninhydrin test has succeeded of Ninhydrin!

The muddy brown purple color is taking some time to develop, ~ 10 min, so this is important. Let's try Cheese by itself now.

It is taking up to 15 min. to develop the color. The cheese sample, by itself, is not bringing any color into the test tube fan.

—

Well, we have an interesting development here. The cheese, BY ITSELF, w/ NO ENZYME, is producing some purple color in the muddy brown test.

This may complicate the process considerably.

NOT TRUE — you mistook the tube. At this point the cheese tube w/out enzyme still does not show purple color.
Temp needs to be @ least 80°C in intention.

—

Power setting #2 is @ 105°C

—

The protein under evaporation @ 105°C is now showing the signs of precipitation and the muddy color as before.

—

The cheese sample WITHOUT ENZYME shows no purple color - this is complete success.

We also ran a control w/ the enzyme alone to make sure that there is no amino acid in that component.

Now, we also should observe a MAJOR reaction take place w/ the protease enzyme supplement and the dried milk. Much coagulation has taken place here. A MAJOR REACTION

OK, now for the powdered milk test a major reaction has taken place here w/ this particular enzyme supplement.

We will add 6 drops of ninhydrin instead of four.

Another observation: We see again a change of color. The tone from brown to green upon heating of the proteins. This mean that the protein, upon heating, is being reduced from Fe+3 to Fe+2. It seems to be very unusual behavior to me. This was a sample that has been sitting for a year and it was a combination of both brown and green solutions. So that cane brown has turned to green with the application of heat.

Good news: This enzyme, this particular enzyme is reducing the dried milk to amino acids - it is slowly turning purple. The product is called:

Protease IM by Transformation 60 capsules
Contains VIT A, C, Zinc
Astragalus root, Echinacea root, Thymus, Red Clover
Goldenseal, Raw Bone Marrow, Quercetin, Eleuthero root
&
Protease, Lipase, Phylase, Pectinase, Peptidase,
Cellulase, Hemicellulose.

So not all enzyme products are the same by any means

—

Powdered milk result in lighter purple but it is purple.

—

protease
The enzyme alone with ninhydrin is not producing product the purple color. Good, the enzyme product contains no amino acids as required.

—

Working on milk, now w/ the sealed enzyme product.

—

Milk, w/ the use of the sealed enzyme, produces a very WEAK purple color. Precipitation appears to be the primary influence from the sealed enzyme. The protease enzyme product appears to decompose more so into amino acids rather than precipitation.

I will maintain that the generic enzyme seems to be precipitating milk (and maybe proteins in general!) more than it is decomposing it into amino acids.

—

Strained & filtered milk w/ the generic enzyme did not produce the purple color. This means that it is the precipitate that is containing the amino acids.

This is an important observation. It seems that the precipitate w/ the generic enzyme is actually going to contain a complex that has decomposed the protein into its constituent amino acids.

—

OK, here is what we are learning.

The generic (walmart) enzyme supplement is very effective @ PRECIPITATION of the protein, and of potentially of other proteins as well, such as milk for study right now. It is this precipitate that contains the amino acids, and they are of weak concentration w/ a very pale purple color journey very slowly. THE CLEAR FILTRATE w/ the generic enzyme we DOES NOT CONTAIN AMINO ACIDS.

In contrast, the alternate enzyme compound (Protease, by the Company Transformation) filtrate DOES contain amino acids.

The enzymes are varying in their effect upon proteins but both supplements ARE having an effect and they ARE clarifying the nature of many proteins studied thus far.

Also you learned that you did not use enough of the generic enzyme in the original investigation. We now also know that there is a clear distinction between the filtrate and the precipitate when it comes to effect from the generic enzyme brand (Wal Mart)

ie,

Spring Valley Probiotic Multi-Enzyme (Generic, Wal Mart)
VS
Protease IM by Transformation

Ok, we have an basic idea. We have now transitioned to the COB protein to see if we can effect decomposition to amino acids as we have now demonstrated with both cheese and milk.

We cannot. However, . . .

We see that neither enzyme component seems to be especially effective w/ the ninhydrin reaction. Spectrometry UV shows no purple color w/ the use of the generic enzyme. The Protease IM does have a reaction but it is not purple.

We have also learned that precipitate vs filtrate is also a very important factor.

We also see, however, that the generic enzyme IS INDEED EXTREMELY EFFECTIVE AT PRECIPITATING THE PROTEIN

The Protease IM enzyme does not appear especially effective @ precipitating the COB protein

Precipitation of the protein still remains w/ the generic enzyme and a very important observation w.r.t. interaction w/ the COB secreted protein.

We now also have demonstrated effective decomposition of standard proteins such as cheese & milk into amino acids.

Estimated Index of Refraction ≈ 1.467
Based upon Measurements & Model Development

We have enough information now that we can Construct a Model of the index of refraction mass of the protein based upon the ~~moisture content~~.

Sample wt (gms)	Mass Percentage	Brix
153.14	100%	5.9
136.63	89.2	6.3
122.15	79.8	7.0
96.62	63.09	9.0
63.2	41.3	13.4
44.55	29.1	19.3
30.50	19.9	27.5
17.09	11.2	51.9
~~12.25~~	~~8.0~~	~~89~~
	10%	54.9

~~Brix ≈ 688.42 · Mass Percentage~~ ~~-1.048~~ ~~$r^2 = 0.995$~~

Final!
210.12 - 198.2 = 11.92 7.8%

So this is a good model.
Your estimate of the IOR of

Model based on actual data is: -0.991
Brix ≈ 545.78 · mass% $r^2 = 0.999$
This is inherently as it suggests Brix = K · mass^{-1}

Now we can legitimately predict @ a mass % of 8%
which has been created but IOR was not measurable
because it was too thick.

Estimated max Brix = 545.78 · 8% 1.8% -0.991 71.3 Brix
= ~~69.5~~

or estimated IOR = 1.467
~~1.462~~

Aug 13 2017

Yesterday was somewhat of a complex session. The interaction of proteins in a complex and potentially very important topic of research. The behavior of the protein with respect to changing color, variable response to the Bradford test, reaction to heat & additional regeneration of protein post denaturation are fascinating topics.

We also have an announcement paper published on the topic now.

We also have a method of monitoring concentration & H_2O Content @ any point now w/ the index of refraction model that has been developed

The timeline for the summer season is already shaping up. About 1½ months left. Goals include:

1. ICMP paper release
2. HEPA filter analysis
3. DNA production, testing in hand.
4. Write paper in Word / Markdown
5. Citizen samples
6. Skin exfoliate analysis

It appears that it will need to be in another lifetime...

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Someday you, as a gifted biochemist, will be in a position to possibly obtain a patent because you have isolated and characterized a new protein. Yes, one can obtain patents on biomolecules. One school of thought believes that allowing biochemists to patent biomolecules is similar to allowing physicists to patent gravity. However, the

???

knowledge of the structure and function of a protein can translate into a lot of money for someone—especially if the protein is important in human nutrition, disease, or in efficiency of production of a crop. The regulatory community has taken the position that such knowledge must be patentable to stimulated invest in this type of research and motivate researchers, investors, and corporations.

from
Biochemistry Demystified
Read & known many years ago.
Current paper is titled:
"Unique Protein Isolated & Characterized" Aug 2017

CDB culture headspace GC:
Again, we see directly:
1. Propene
2. Butane
3. aldehyde group - benzaldehyde
4. Heptane

These occurring repeatedly over the last week of several culture runs. Incubation @ ~~85°C~~ 85°F.

We learn today that headspace analysis was extremely effective in GC analysis of Tea Tree Oil.

No torching was required.
Numerous subcomponent determined.
Fingerprint analysis here would be easy to settle.

g Methane
Ethane
Propane
Unknown
Unknown
Pentane
Hexene

You notice some contamination in the tube as you will need to incorporate Control in the.

We have the same result after cleaning the tube of components.

Aug 14 2017

The ICP-MS paper has been prepared today. Seeking permission to post the data.
on Environmental Filament metals testing.

—

The protein sample from last year has been evaporated. The result is a dark viscous form similar to molasses. It also has a strong odor.

We have measured IR.
It matches the protein arrived @ by distillation extremely well.

—

Let's test the concentration of our active cultures with the index of refraction method.

Control Brix = 1.4 This is very weak.
1.3 also weak.
If you think about it, the ~~Brix~~ Brix level looks like it corresponds pretty closely to the level of protein concentration.

Watched over the next week or so.

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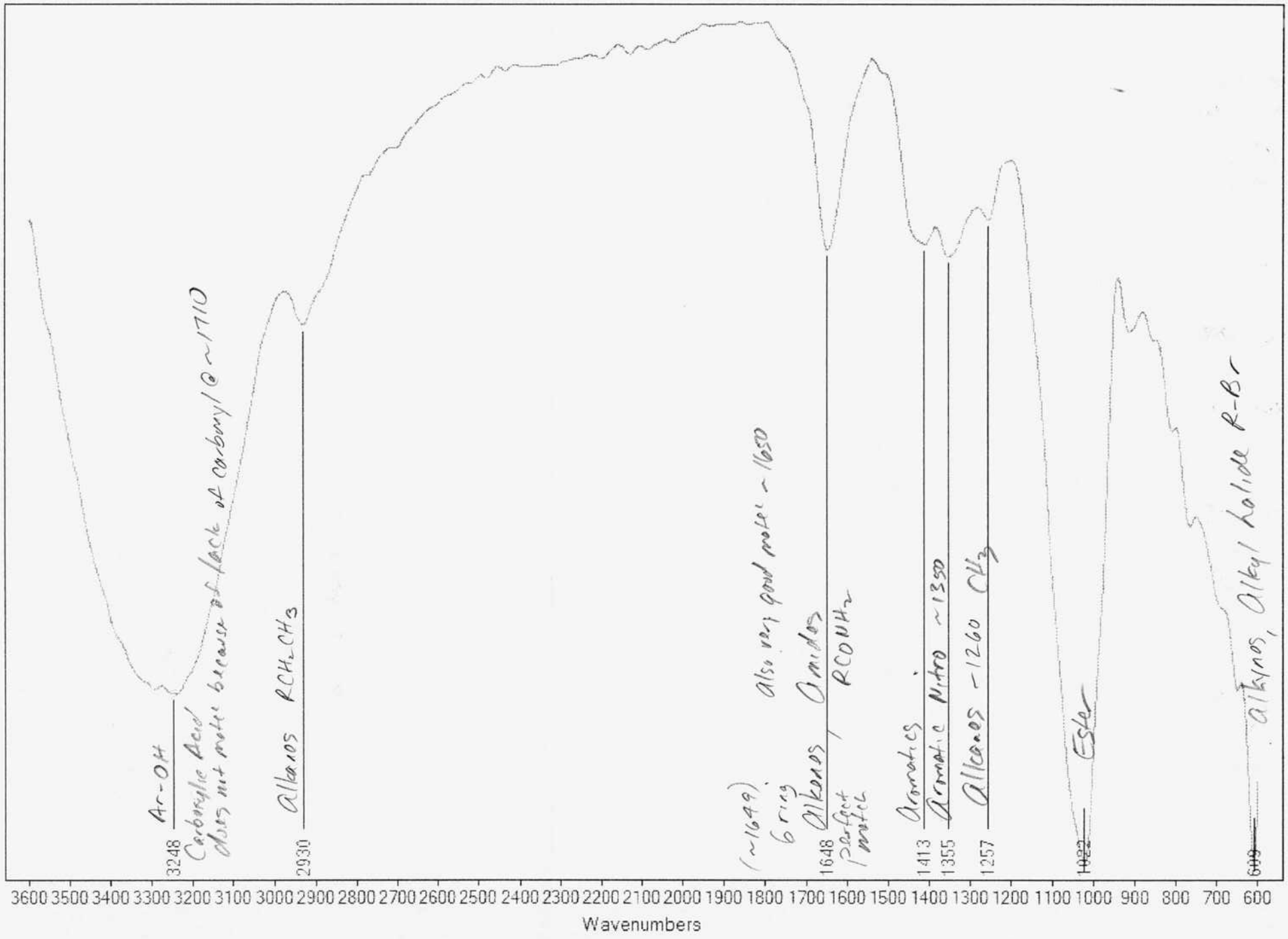
IR of VISCOUS Protein
Molasses style - evaporated - ATR mode

CDB Viscous Protein Dark Molasses Style Aug 14 2017 - 02.JPEG



Aug 15 2017

1. The protein announcement is in place
2. The ICP-MS paper draft is prepared and wait for permission.

3. Projects are
   1. HEPA filter analyses
   2. DNA production
   3. Text Markdown language
   4. Skeen oleate analyses
   5. Cotyledon samples

I am most interested in the HEPA filter analyses. I have done a lot of prepatory work, also LC work & I separated Humic Components.

This means that we know have @ least 3 components, 2 in the liquid form & sediment @ the bottom. The problem is that we have no control. We have purchased a control filter - we just need to use it now.

We can already see that the control filter is not creating a colored solution or a solid material that settles.

We analyze the HEPA filter.
We have a decent ATR plot.
Our closest match sample is the rainfall analyses.

We have some very interesting work taking place.
I have established a high level of equivalency
in organic IR analyses of the HEPA filter
extract and the rainfall concentrate. They
contain the same signature.

In addition, we have very clear 5000X microscopic
images of the filament network that
has grown in the rainfall concentrate.

Next, a UV analyses of the rainfall concentrate
for water soluble protein fails.

There is a case where the more sensitive
colorimetric test that you developed could
be helpful but before we do that look
@ the IR signature of the protein.

The IR signature is NOT the same
in any significant way. Therefore we do NOT
expect the rainfall concentrate to contain
the water soluble protein.

The dye not appear to be a fruitful lead.

We see that the HEPA filter contains a significant hydrocarbon nature.

Search for similar compounds characterized raw samples

Our closest match is decanoic acid, acetyl ethyl ester.
And it is a fairly close match.

Now a problem remains. GC showed that you had two components w/ in the HEPA extract, not one.

So it seems to me that you need to isolate these again. It would be nice if GC could analyze them but I am not sure how.

Could we compare headspace of the alcohol to headspace of the HEPA extract?

Headspace control on denatured alcohol tested. - Done
Headspace applied to natural extract, two GC difference seen

Ok, we have some interesting comparisons to make.

(1) LC work occurred around Jun 3 2017. Two different components were separated, and one of them appeared to be a protein!

(2) We ~~tested HEPA dx~~ Rainfall for protein and it failed. (Dilute test)

(3) But I believe HEPA test came out positive

(4) But IR of rainfall Julian & of HEPA are the same! ?? How can this be?

(5) Skin of leaf looks to have its own properties.

So well we have some real separation detective work to do here.

—

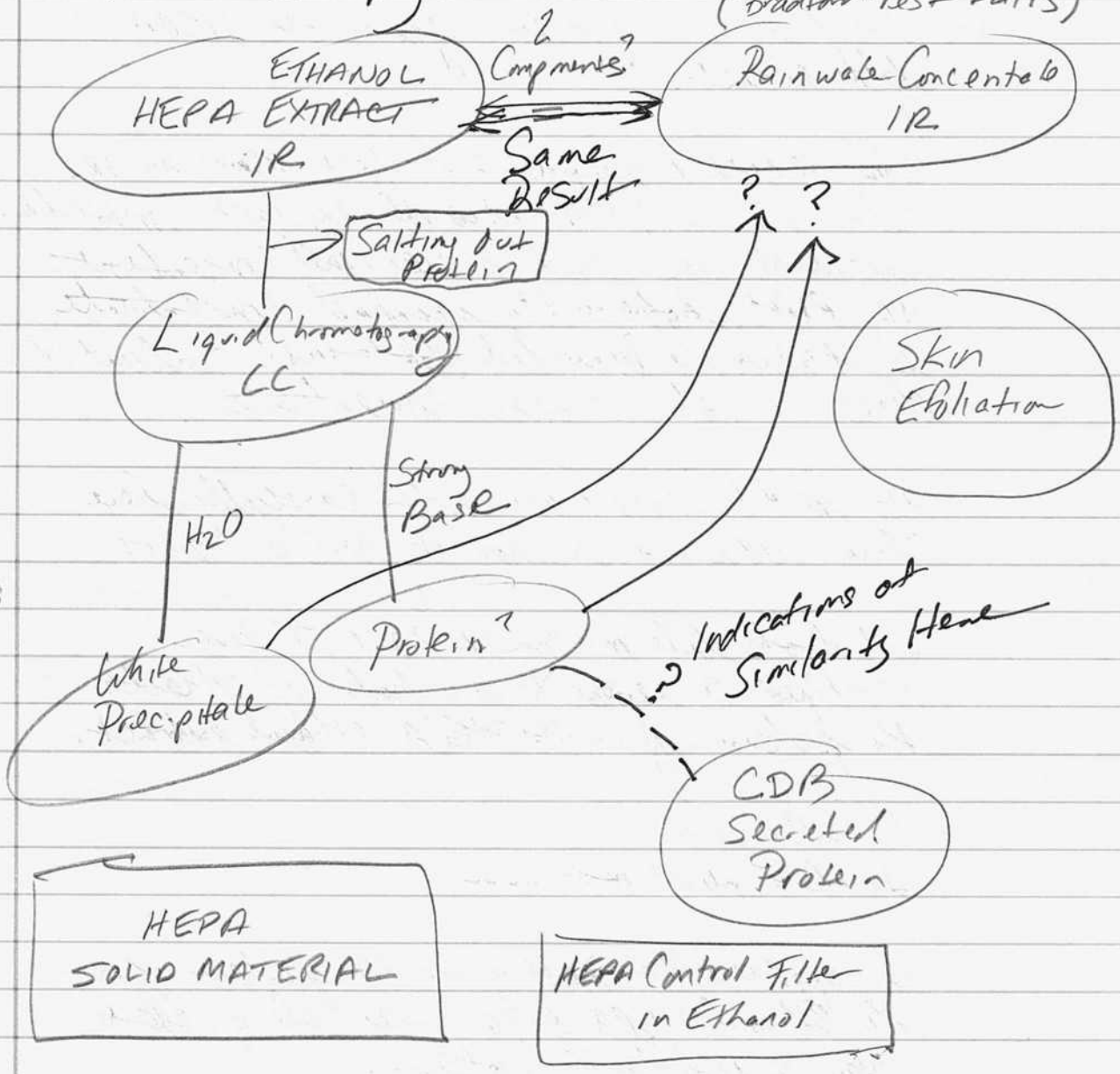
Additives to ethanol are 90%
1. Isopropanol
2. methanol 10%
3. acetone
4. MEK
5. Denatonium

Relationships Between These Entities is Needed

Aug 16 2017

The scenario developing is:

actually we do have a positive test on 6-13-17 (Bradford Test Fails)



You have a fairly Complex network here involving

1. HEPA Filter IR
2. Rainwater Concentrate IR
3. HEPA LC a) precipitate
   b) Protein?
4. CDB Secreted Protein
5. HEPA solid material
6. HEPA Control Filter
7. Skin Efficacy

Around June 08-09 we started some very important work that led to a partial isolation of a protein from the HEPA felt extract.

This occurred despite the fact that an IR plot of the HEPA ethanol extract matches essentially exactly with a rainfall concentrate IR plot, however the rainfall concentrate is failing the Bradford test when conducted directly w/ the rainfall concentrate.

We need to ensure our notes carefully here that my memory is now on Jan 06 2017

It looks like on Jun 12 2017 I have developed 2 separate methods of extracting the protein from the HEPA ethanol extract.

1. Salting out
2. LC separation

It appears that salting out the protein from the Ethanol HEPA extract was the quickest way to isolate the protein.

Actually we see that we DID have a positive Bradford test w/ the rainwater sample w/ λ max of 619 nm
(Conc = 14.55)

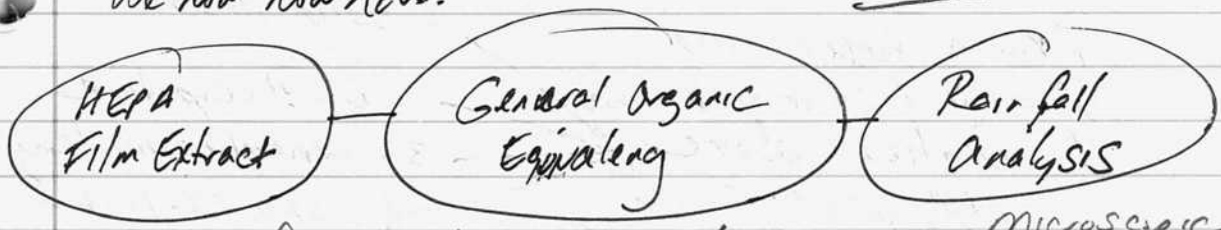
On 06-13-17 We have a very important statement.

1. CDB secreted protein.
2. HEPA LC isolated protein
3. Rainwater Concentrate protein

] are all stated to be the same.

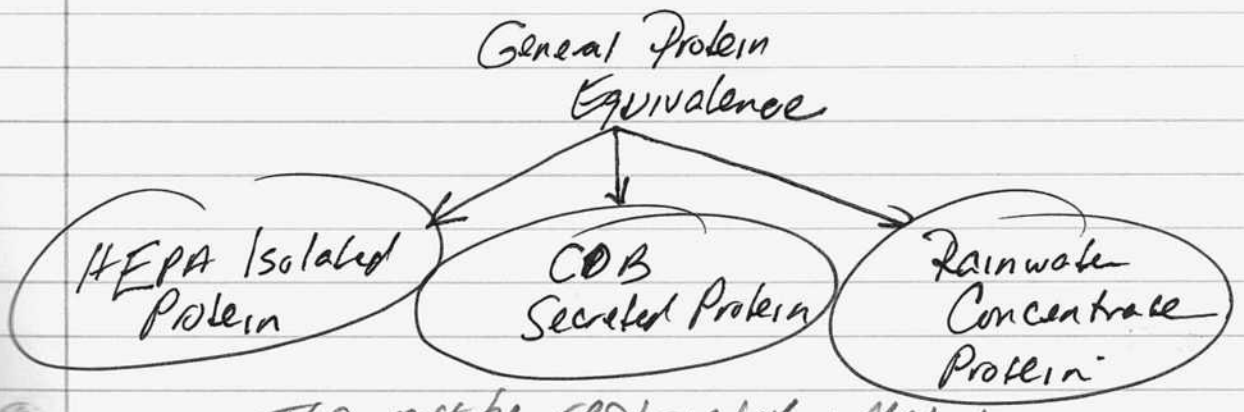
The fact is that are kept very complete notes and we have already proved these facts. What ga have done since the trial, among other things, is study the nature of the protein differences.

We have good IR plots of equivalency of protein.
Titled Report: A Point of Reckoning
We now now have:



This is free to be analyzed openly
IR

Microscopic IR



This must be restricted in detail.
Partial IR

We now have the crux of the maze sorted out.

Outliers are:

1. Solid HEPA filter material analyses
1.5 HEPA Control Filter Analyses
2. LC Precipitate analyses

3. Generalized organic equivalency between HEPA film extract & nasty all IR plot

4. Skin exfoliation

Priority Projects are:
0. Write A Point of Reckoning
1. Outliers above
2. DNA production
3. ~~Skin exfoliate analyses~~ Citizen samples

1. Solid HEPA
2. LC Precipitate
3. General Equivalency
4. Skin exfoliation

Protein Comparisons: CDB Viscous, HEPA Extract, Rainfall Concentrate
They are essentially the same.

Average Protein Plot CDB Rain HEPA FIlter Aug 16 2017 - 02.JPEG

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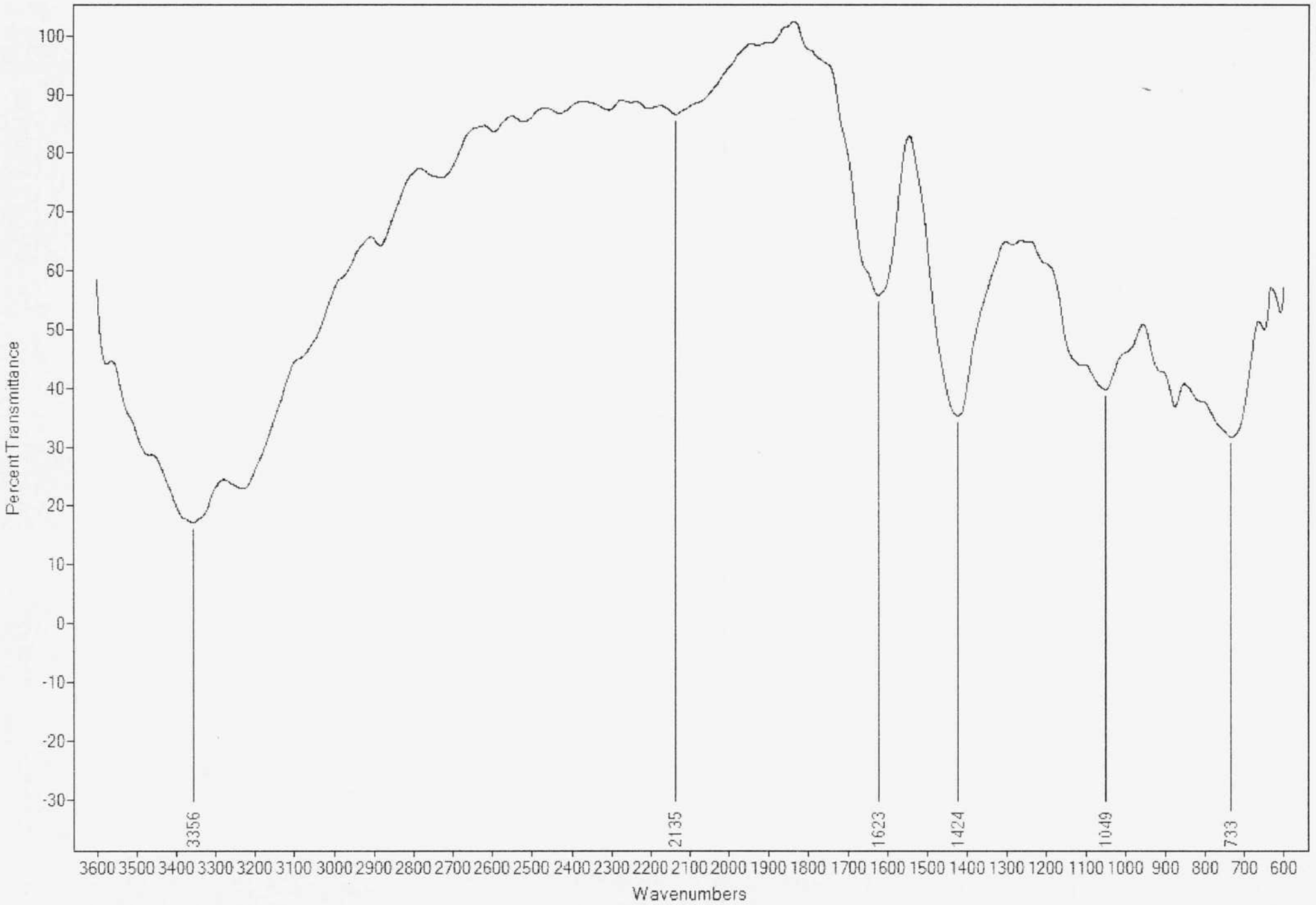
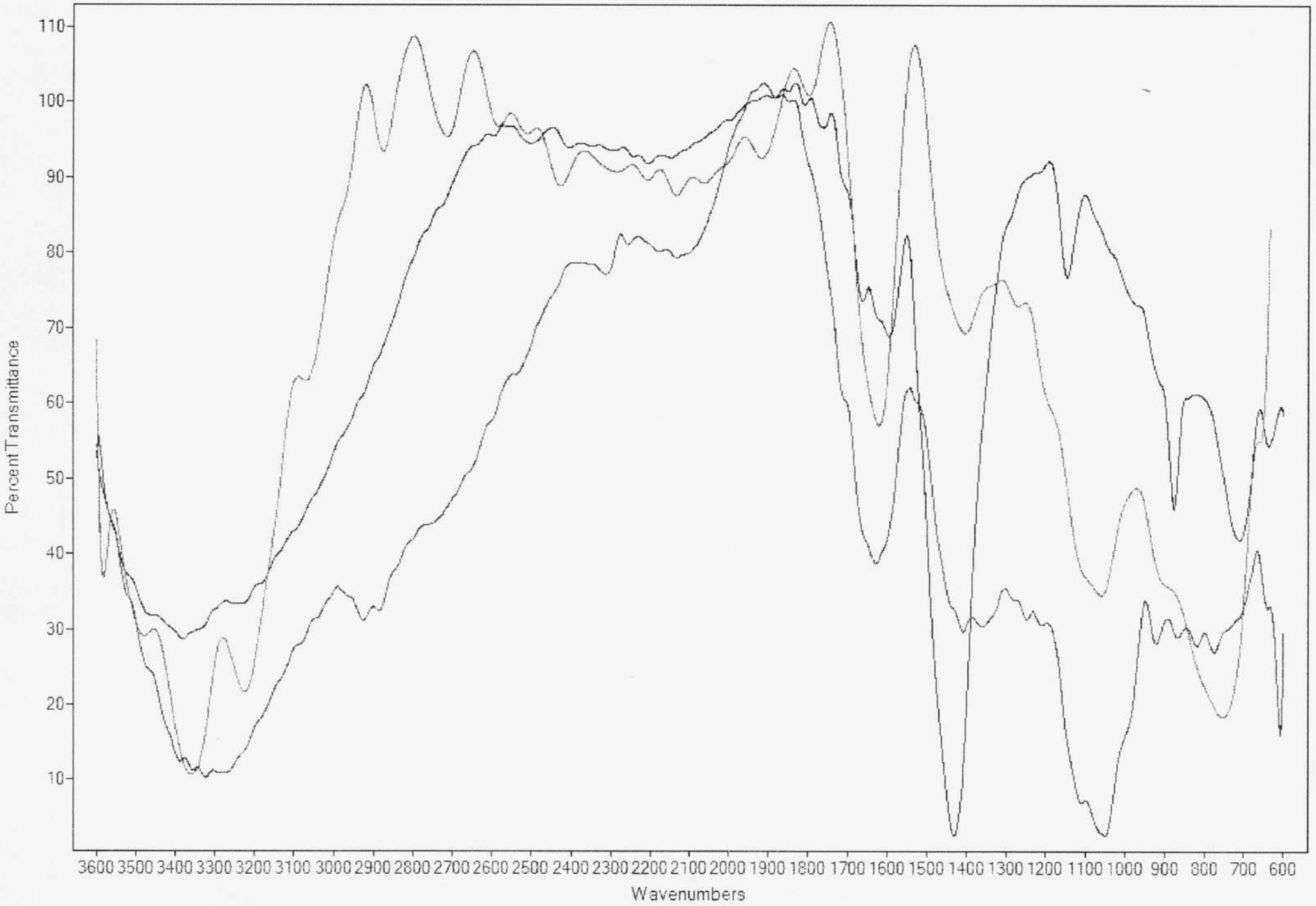


Protein Comparison CDB HEPA Rain Aug 16 2017 - 01.JPEG

Page 158B



What interests me now is the soluble HEPA material. It appear to be carbon based, & generally insoluble. ✗

Lets start w/ some solubility tests.

Notice w/ Crippens solubility flow chart on p42 that we now have
1 ether available! (starting fluid)
2. baking soda can be brought in also.

Conc HCl & Conc NaOH (10M) do not phase it. It acts like carbon black,
Bleach does not react in any way.
I do believe it is carbon.
4.5M H_2SO_4 has no effect either.

Then follows through on the flow chart as an Inert Compound

What is exactly what I anticipate w/ what would be carbon black.

The big question is, why would there be so much carbon black in the atmosphere in such a rural location?
There is a lot of material.

Carbon Black - Discovery & Analysis

Carbon black is the most solar energy absorbing component of particulate matter and can absorb some million more times energy than CO_2.

Carbon black warms the atmosphere.

Carbon black is a climate forcing agent.

Carbon black results from the incomplete combustion of fossil fuels, biofuels & biomass.

We have a significant climate effect expected from this, and it is a warming effect.

Our tests to substantiate the result include:

1. Calculating tests
2. Appearance
3. Microscopic photographs - Filament Network
4. Pyrolysis to convert - yes, good success here.

Also recall that we have equivalence w/ Coke.
Rainwater of the material also.

Aug 18 2017 Page 161

A Point of Reckoning - Part I

is now posted. It compares the organic signature of the HEPA filter extract against the concentrated rainfall sample.

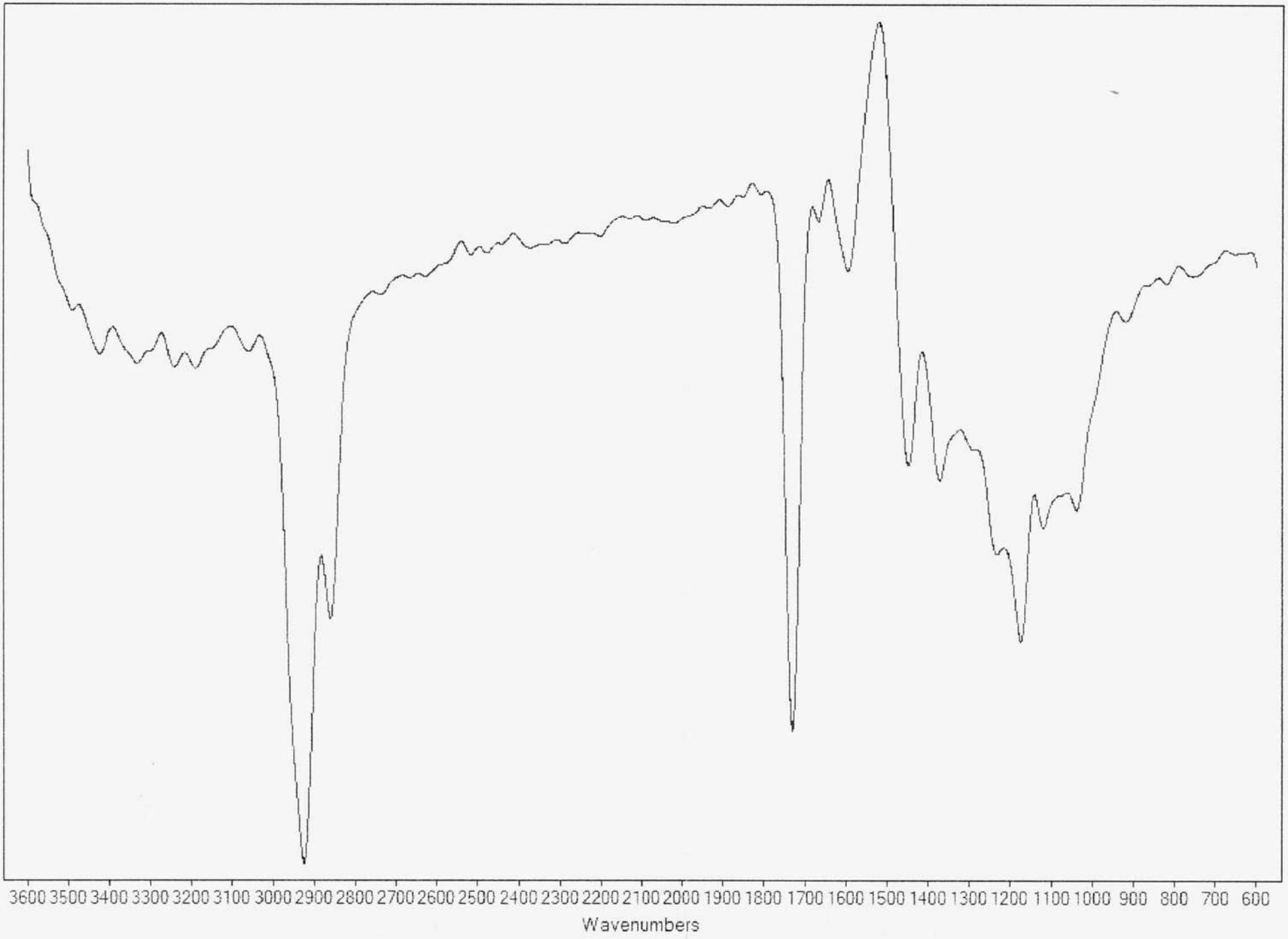
Also we have tested the tube to see if "protein begets protein"

This is a test that never should have been postulated however the grounds for it remains. Protein changed color after heating to indicate Fe+2.

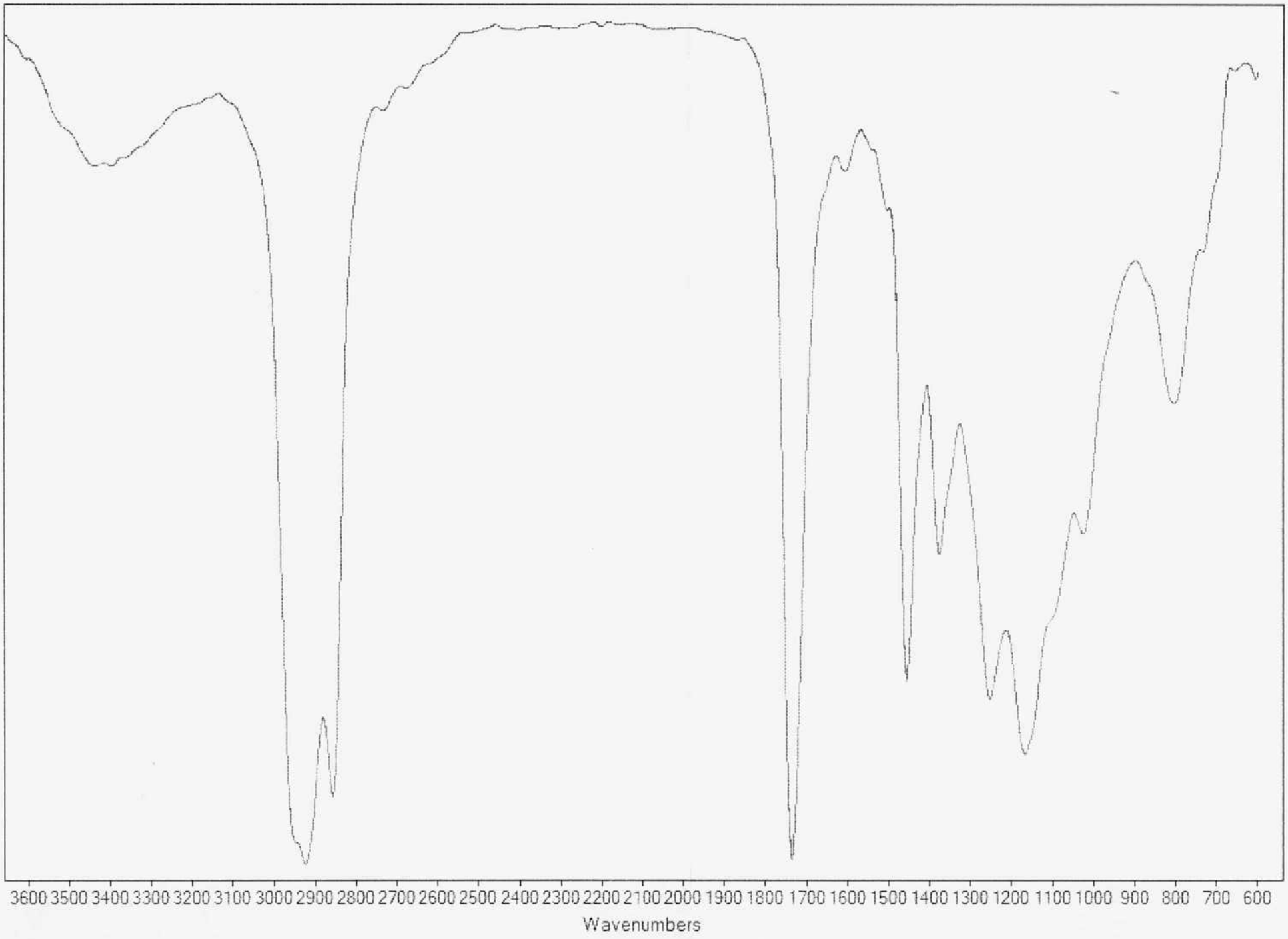
The particular tube test being run there for (protein, NO CDB! added to sugar iron culture) dove fail the Bradford test @ that point. This is what should be anticipated. But in this world of science fiction you never know.

You have verified these results again. The match between IR plots of HEPA & rainfall agree to R = 0.94 I had some questions about Control established w/ a clean HEPA filter in denatured alcohol but they have been resolved by sufficiently correlating the HEPA filter extract.

HEPA Air Filter Methanol Extract ATR Aug 15 2017 - 05.JPEG



Rainfall Extraction - KCl Disk - Clean - No Residual Solvent Jun 20 2016 - 04.JPEG



I have verified the conclusions of the A Point of Reckoning - Part I paper.

We may now proceed to:
A Point of Reckoning - Part II

which will show the relationships between the proteins identified in the

1. CDB Secreted Protein
2. The HELA extract (LC)
3. The rainfall concentrate.

The most difficult of these to verify will be a protein extracted from the rainfall but apparently we have done so.

Perform these steps carefully & completely.

We have 2 outliers left from the post of Aug 17 of 5 outliers.

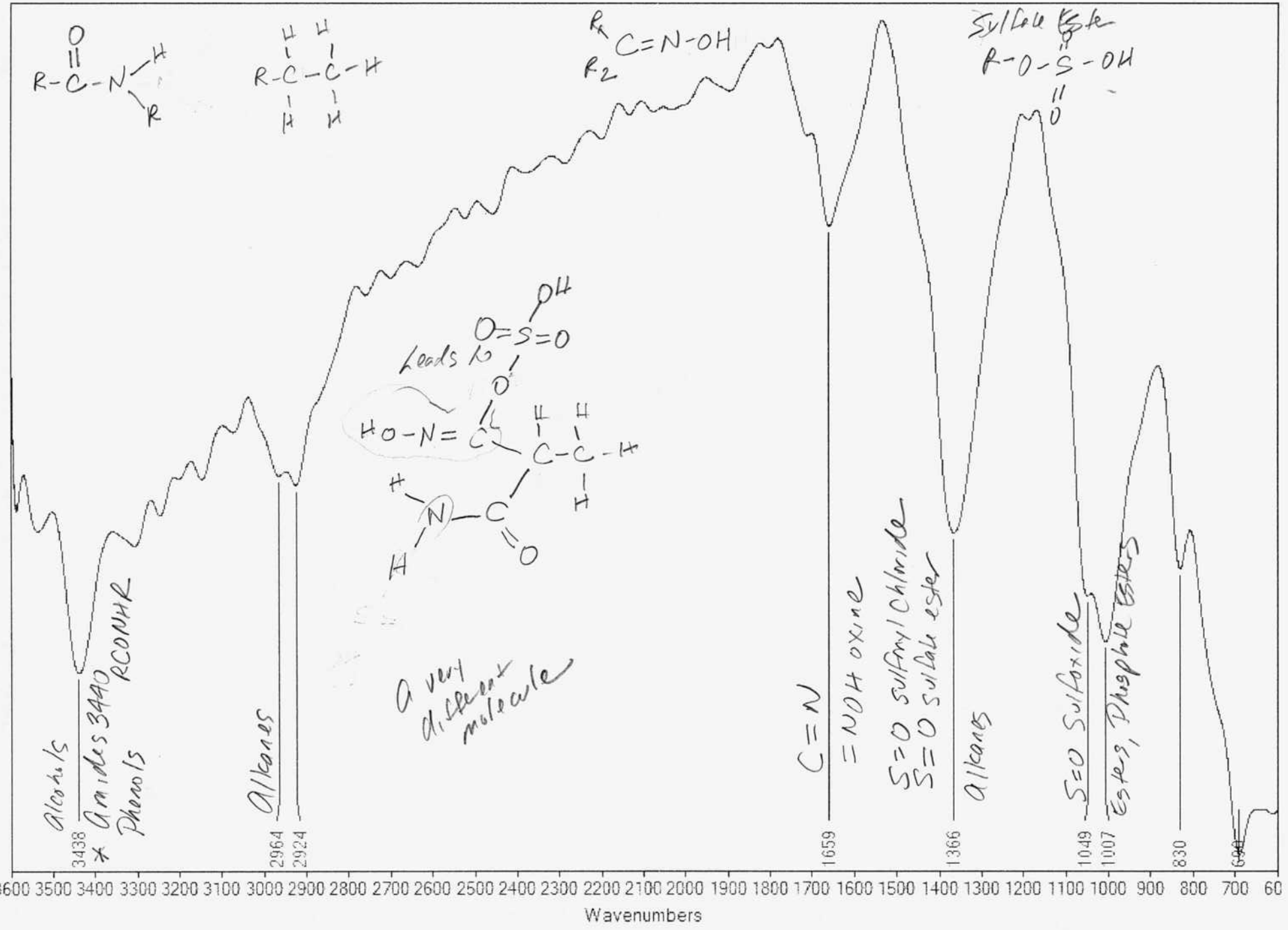
Remain as:

1. LC Precipitate Analyses
2. Skin Exfoliation analyses.

IR Plot of LC separatin of precipitate from HEPA ethanol extract. First ektrin w/ water.

The precipitate is in water and agitated easily. Tray infused w/ micropipette.

LC Precipitate Aug 19 2017 - 01.JPEG



Aug 20 2017

The LC separated precipitate from the HEPES ethanol extract seems to have some unusual structure to it, with nitrogen & sulfur likely.

I think that solubility is the next most important piece of information.

Chemsketch 2015 (the full version) is very easy to use to construct a model and will save in a .mol format.

The newer version (2017) is no longer free so never get rid of the version that you have.

Avogadro will import the 2D file and create a 3D file from it,

Then go needed when Avogadro 1.1 crashes, which it sometimes does.

Avogadro 1.2 is nothing but trouble w.r.t. geometric optimization.

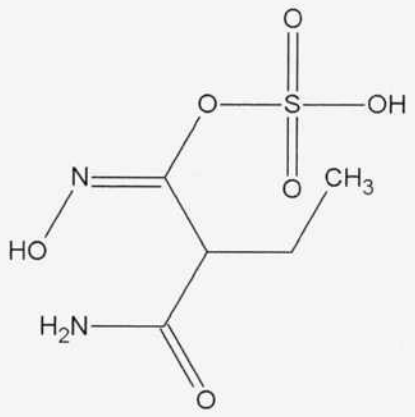
I do like Chemsketch also as it is constructing a Lewis structure - very easy to interpret.

In the Gamess module @ chemcomp.ucf.edu turn the "Guided Molecule Creation Mode" off in order to be able to upload a file.

Avogadro is good for creating the Gamess input file.

Gabedit is best for reading an output IR file from Gamess. Remember to get file suffix in Gabedit as an .out file.

Very tentative structure proposal for IR plot of GC LC HEPA extract.



(1*E*)-2-carbamoyl-*N*-hydroxybutanimidoyl hydrogen sulfate

Let this set right now & go for solubility.

Solubility exploration on the LC precipitate.

We know that it is insoluble in water.
It also appears to be insoluble in ether.

There is some kind of reaction w/ 10M NaOH but it does not dissolve the precip.
It does turn the solution darker but it may actually increase precipitation.

Acid may dissolve it to some extent.
We are testing in murky dirty glacial acid - base reactions.

Scattershot. We are getting a strong reaction of color @ room temperature (to a blue color) with:

1. Add weak base. No real reaction
2. Add strong base. A reaction of some sort.
3. Added acetone
4. Added murky dirty acetone
   A blue color forms
   (NOT PURPLE & w/ OUT any heat added).

Absolutely no idea what happened here.

Another series:
1. add Ammonia, no reaction
2. add strong base, no strong reaction
3. add ninhydrin in acetone
strong blue layer forms @ top.

622 nm on Bradford w/ thin tube, 1

Tube no is 01-08 0045 on 06/13
Protein Confirmed.
Then a strong protein concentration

Ok, the biggest news here is that we a positive protein LC HEPA separation that has been saved. You should be able to get the IR plot again.

I have been able to get Chemsketch 2016 working - the company had given me a hard time about a corporate license. The method was to sign in using the CEC102@USA.com address.

It looks like it may even be able to search for compounds, at least w/ Chemspider

We are preparing for A Point of Reckoning - Part II

We should get back to the CDB secretion IR Plot.
It would be great to condense, but we already have this w/ the VISCous form.

Right now the precipitate (a small amount that has settled in the protein tube from LC HEPA separation) is a lower priority.

The protein is clear and strong in that tube (it was separated using NaOH).

I recommend that you try to reacquire an IR plot. Water will be a problem of course and your heat may also be a complication.

Start w/ the reference CDB Vincow protein.

Aug 21 2017

[redacted] witnessed the eclipse today on top of Lookout Pass, ID & MT boundary. 90% sun coverage here - a grand and rare lifetime opportunity. I have witnessed 2 major eclipses in my lifetime, a several minor. Also several lunar eclipses.

I am becoming more strongly convinced that the major skin issue on my neck was a result of pyrolysis & fume from the CDB secreted protein. It was a fairly serious effect for about 2 weeks. The problem just stabilized w/ irritation to the nose tip.

After clearing, a few days ago I ran a minor short trial w/ CDB viscous protein. I have consequently had another, fortunately, minor flare up w/ the nose tip again. Exposure was minimal vs the extensive original tests, and it seems to be recovering fairly well w/ petroleum jelly alternated w/ hydrocortisone.

Pyrolysis / fumes of the CDB viscous protein is increasingly suspect on the problem. I believe the risk should be taken quite seriously and not to be repeated.

Fume Control is required.

1. Today I will post the metals lab report and
2. add the outdoor HEPA filter photos to the meat paper.
Note: filaments captured & visible after 24 hrs exposure to outside air.

Questions:

1. Can we truly identify proteins a rainfall concentrate sample? Do we want a blast to try this? Most important to repeat LC?
2. Do we want to repeat HEPA LC separation of protein? How does that affect the protein if we attempt to concentrate it for LC work?
3. Biker glucose analysis?

4. DNA collection
5. Cotozen samples
6. Point of Reckoning - Part II
Protein equivalency @ functional groups level.

So here we go again. The rainfall tests positive for protein again w/ Bradford @ 608 nm!

How can this be? How can you have a film IR plot which shows HEPA (air) and rain (water) functional alkanes very strong and then show a protein which matches the COB viscous & HEPA extract @ the same time?

Notice the rainfall film also showed strong alkanes.

I think you need to repeat the rainfall film but you are running out of material.

Maybe just maybe with the rainfall and used a solvent! The work was done on June 20 2016. I bet this is what you did.

The alkanes are coming from solvent effects!

You have no entry on Jan 20 for this work. However your memory now advise you that you DID use a solvent, you considered it an extraction problem.

You then, in your title also indicate that this is what you did with:
"RainFall Extraction - KCl Disk - Clean — No Residual Solvent" And now we see what happened.

We used a solvent to try & extract organics from the water (samples)

I recall we must have used xylene because you would not have wanted any polarity in the extract.

Now I ever recall my test tubes, 50 mL. I had an entire set of them and I even took photographs of them.

There was definitely organic matter that extracted into solution, the color was quite distinct. You actually had very successful extraction take place.

You then stated that all solvent had been evaporated which we certainly known to be true. Whether there still was a residual component from the solvent is still a legitimate question, however now we know that we used

1. An alcohol extraction method for the HEPA filter
2. An xylene extraction method for the samples.

Since we have two different solvents and we still have "General Organic Equivalency" w/ the IR plots we know that it is due to the extracted material.

Yesterday we tested the signal of alkane (and organic signal in general) from an denatured filter AIR evaporation alone. The signal was very weak.

The r all telling us that the IR plots of HEPA (air) and rainwall Concentrate (water)

ORGANIC EXTRACT RESULTS ARE A LEGITIMATE RESULT & the alkane as well as the general signal is quite real.

Remember we also applied mild heat yesterday to the HEPA extract & it helped tremendously to Concentrate the sample.

Our difficulty now is that we have located rainwall Concentrate as our most promising Candidate. We will hope to see others.

The r also telling us that our positive test for protein in the water sample is also quite legitimate. What we need to do is to see if we can replicate these w/ other samples and can we get a sufficient IR plot of protein signature to Compare against CDB viscose & HEPA extract LaC protein???

This is a crucial step.

We now understand what we have & how we developed it and understand that a level of matches @ the functional level of 3600 to 1300 cm^{-1} is occurring.

Three Totally Different methods & medicine of seeing a protein:

1. Culture development.

2. Liquid Chromatography applied to HEPA ethanol extract

3. Rainwater - dissolved in native form & detectable w/ sufficient concentration of rainfall.

There is confusion here

The actually quite profound and is why

A Point of Reckoning - Part II

Comes into existence.

We must add the organic extraction process to the rainfall portion of A Point of Reckoning - Part I or will and add short term HEPA Capture.

We definitely have protein congruence between the three sample types & mediums

1. COB Viscous protein
2. HEPA LC extract protein
3. Rainfall Concentrate separate.

Then a very good week.
When we can get the net back we need to

1. On Point of Reckoning Part I
   ✓ 1. add HEPA short termphotos
   ✓ 2. Change to Organic Extract on rainfall
2. ✓ Add metals laboratory report summer analysis
3. Prepare Point of Reckoning Part II based upon above, Make possible reference to show description.

Aug 23 2017

Projects:

✓ 1. Metals testing laboratory report has been posted. ICP-MS

2. Prepare: A Point of Reckoning - Part II

3. Skin folate analysis

4. Citizen samples

5. DNA collection

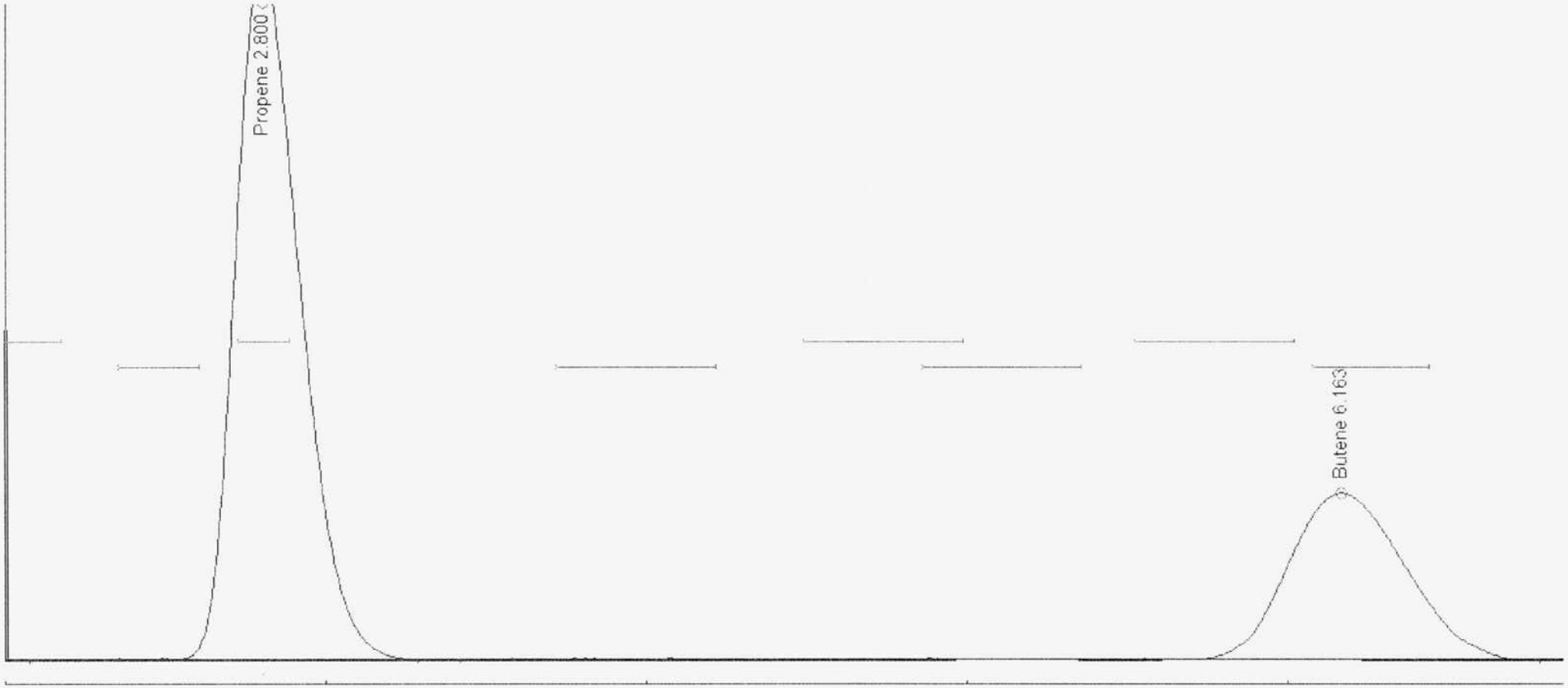
6. Env Analysis - repeat investigation?

✓ 7. GC Analysis - any changes?

✓ 8. Amino Acid Combination project

Significant Alkene hydrocarbon production from CDB GC Analysis = - Propene & Butene indicated.

CDB Headspace Analysis - Significant Alkene Production - Aug 23 2017



We see that Proline is highly soluble in ethanol. Like so great.

—

We now have a very decent plot of proline powder w/ IR.

We also have a comparison against average CBB Viscous - HEPA - Rain concentrate isolated protein.

Closest match to proline plot is actually a hair sample from a young male, treated w/ KOH. Does hair have significant proline w/in it?

Proline is definitely one of the higher concentrated amino acids w/in hair, but it is hardly the highest.
"Half-Cystine", Serine,

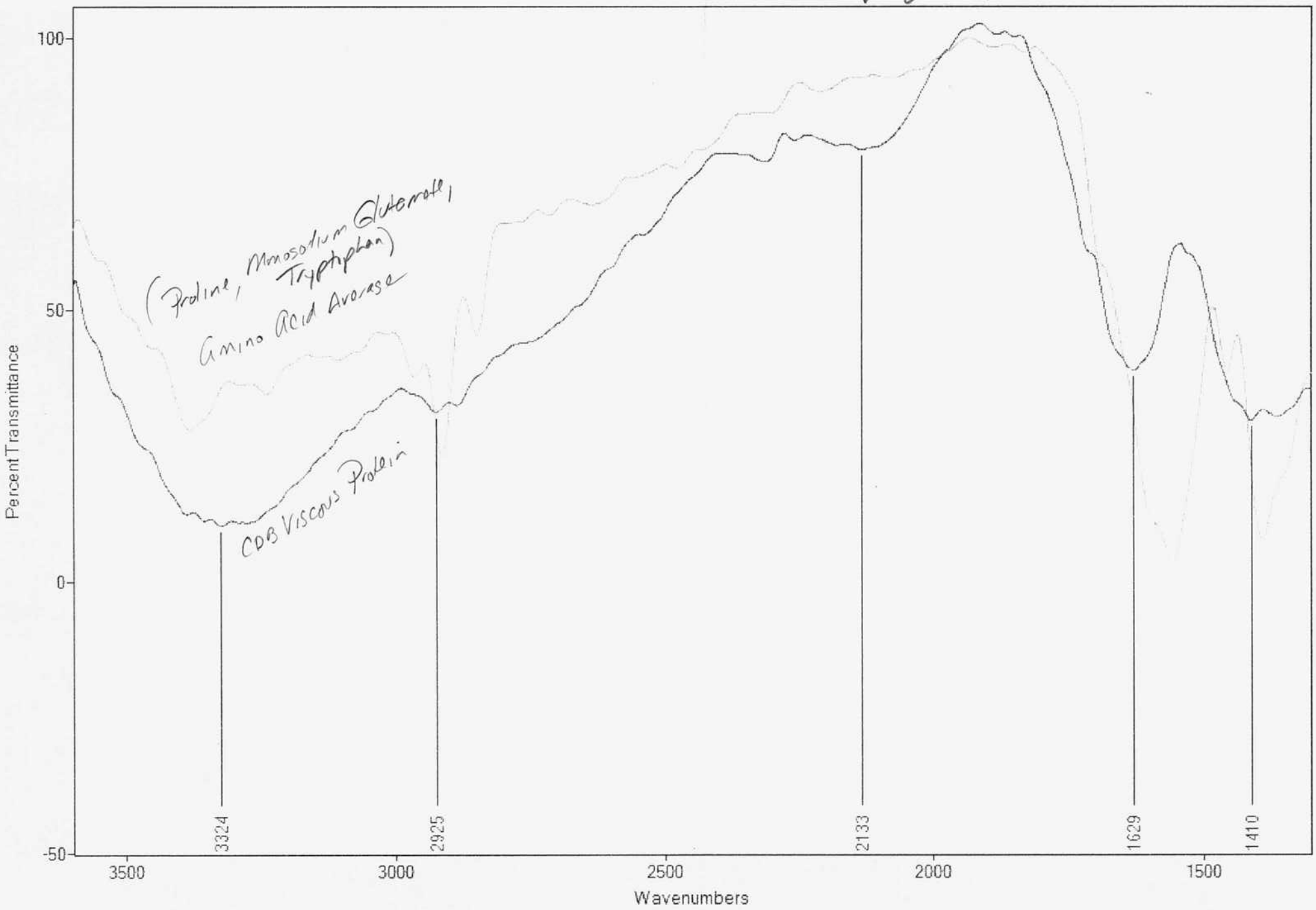
I have attempted to form the average of the three candidate amino acids:
1. Proline
2. Tryptophan
3. Glutamic Acid (Sodium Glutamate)

There is certainly a level of similarity but to what level is unfortunate. What would other amino acids do? Why no strong OH group w/ the tryptophan?

Aug 24 2017

Amino Acids vs CDB Viscous Protein Aug 24 2017 - 01.JPEG

Page 180A



Aug 25 2017

Our projects now are:

1. Prepare a Point of Reckoning - Part II
2. Shen Jolicate analyses
3. Citizen samples
4. DNA collection
5. Env. Filament Analysis - Repeat?
6. Electrochemistry?

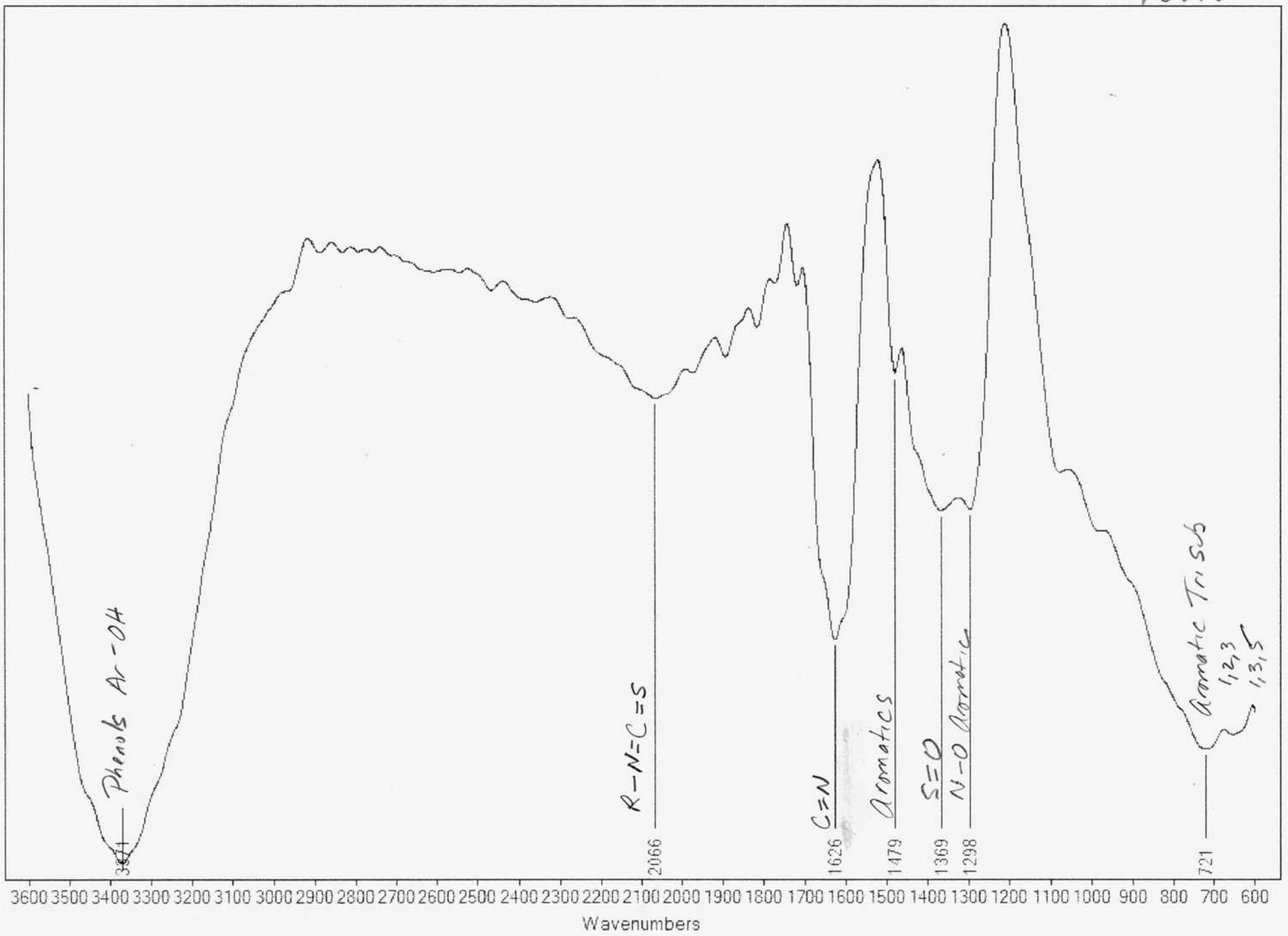
We have a major accomplishment tonight. The has been in pursuit for several years. I now have "broken into" the EPA environmental filament form in an effective IR fashion.

The method finally developed is that of microwave digestion for strong NaOH.

Specifically:

1. Only a very small sample was required.
2. 10M NaOH (~2 ml)
3. Microwave @ lowest power setting.
   Approx 5-6 sessions
4. Very slight color tint visible
5. Neutralized pH & tested w/ pH paper to seal solution
6. Air Dried on ATR & KCl plates
7. Synthesized ATR & KCl IR spectrums for final

Environmental Filament ATR KCl Average NaOH Microwave Digestion Aug 25 2017 - 01 trn.JPEG



Aug 27 2017

There has been great progress made w/ a repeat investigation of the EPA Env. Filament material. We have

1. Broken into the filament via Conc. NaOH & microwave digestion

2. Obtained a usable IR plot for the first time

3. Matched the IR plot w/ the rainwater sample & the highest level amongst approx 6500 IR spectra.

4. Verified via Bradford the existence of protein w/in the sample (660 nm vs 635)

The has happened all very quickly after several years of attempt on this front.

A Point of Reckoning now has four parts, only one of which has been posted:

done Part I – Confluence organically, & microscopically w/ the HEPA filter extract, the rainwater concentrate residue and the skin sample of an affected individual.

done Part II will cover the confluence of protein between
1. The CDB viscous sample protein
2. The HEPA LC separated protein
3. The rainfall concentrate water soluble protein

Part III will cover the presence of black carbon in both the HEPA filter extract & the rainfall concentrate.

This should go last

Part IV will cover entry (IR) into the EPA filament material & the identification of protein via Bradford w/ the samples. Confirmed match to core rainwater sample $r = 0.92$.

Probably part IV

Maybe combine w/ part III?

Maybe separate, not a problem

V Urine Sample vs EPA filament?

The projects now are:

1. Write additional Point of Reckoning papers — II, III, IV
2. Skin foliate analysis
3. Citizen samples
4. DNA collection
5. Electrochemistry (want EIS books)
6. Crystallization techniques
7. Time to collect protein + malt culture

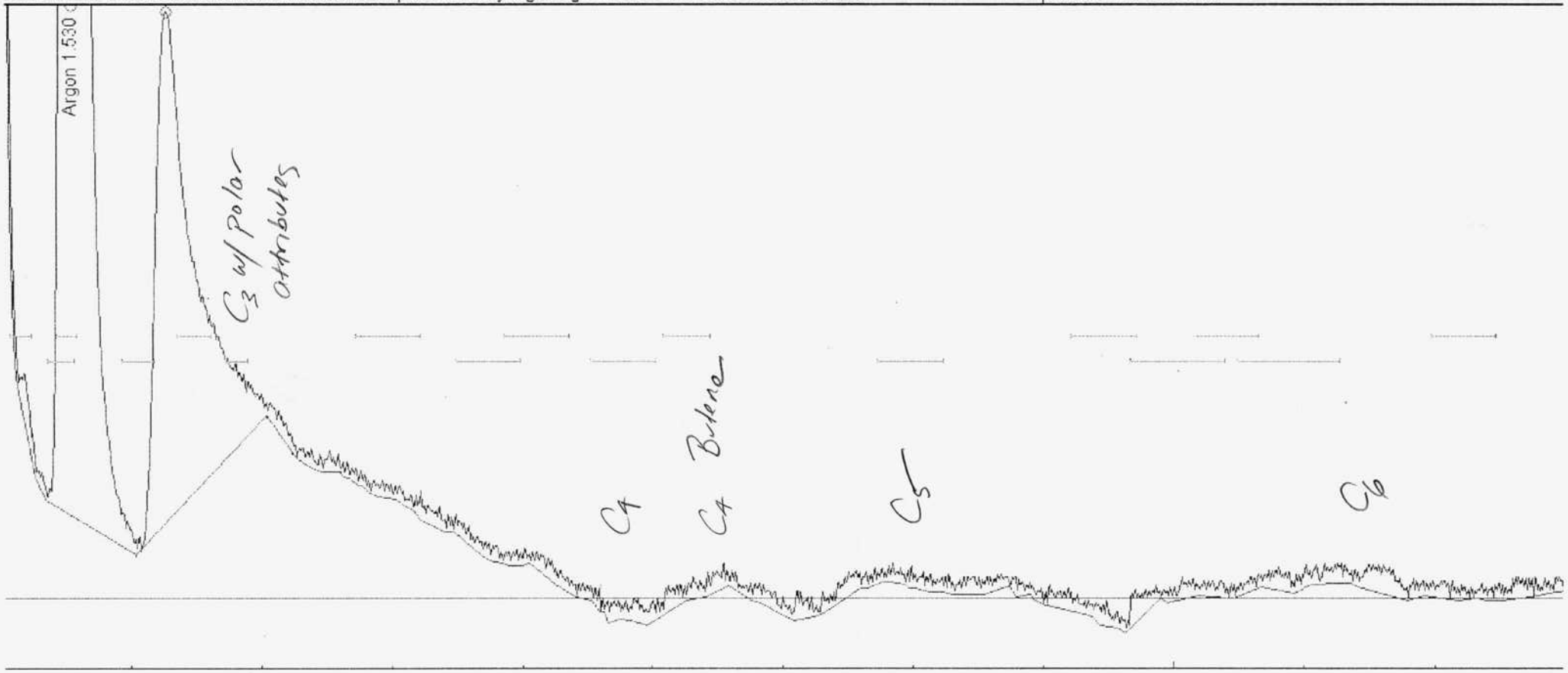
Done 8. Pyrolysis of the env. filament, if you can get enough sample material, is a very intriguing prospect.

Minimal sample material available for analysis attempt.
C_3-C_9 appears to be apparent.
Polar attributes appear to be present in C_3-C_4 area.

9. Isothiocyanate investigation.

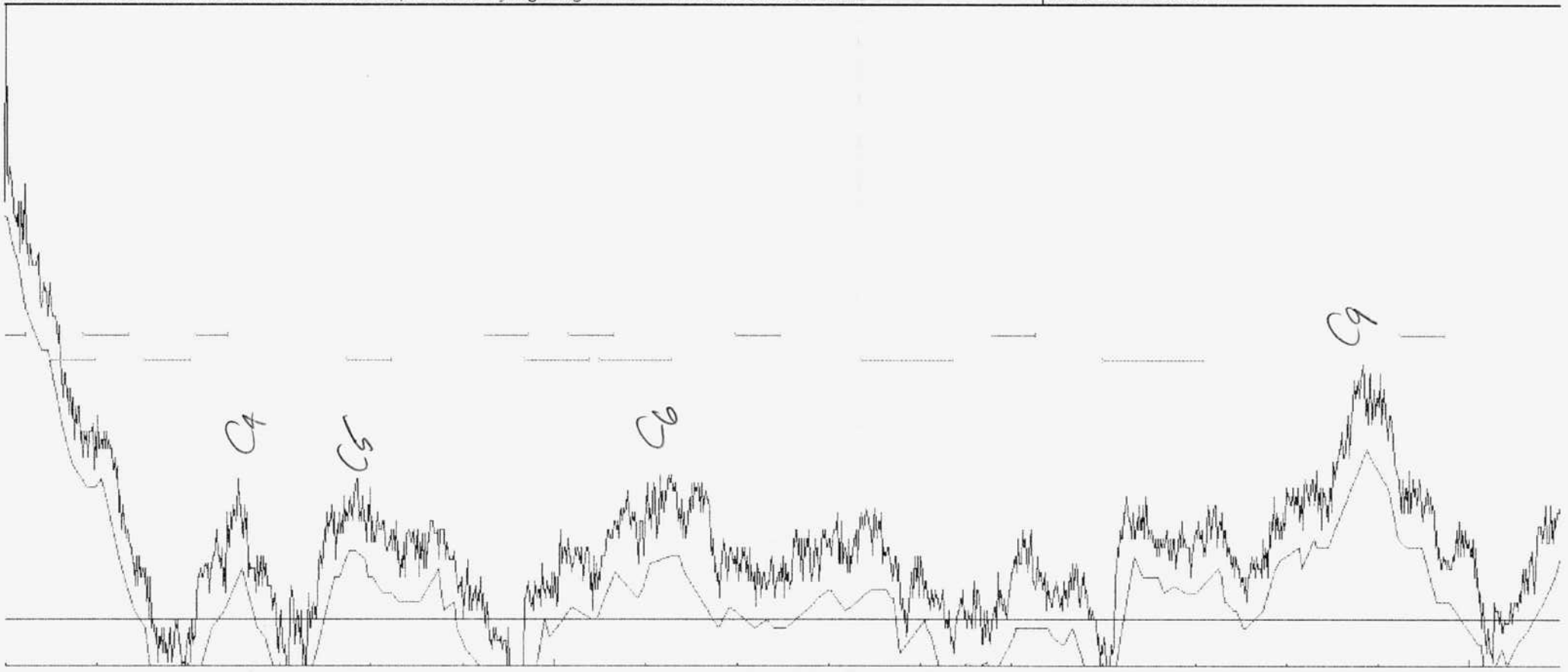
Environmental Filament Pyrolysis Aug 27 2017

FID-CHANNEL 1	150.00 deg	12.46 min [Hexene ?]	0.377mV
c:\Peak444-32bit\Environmental Filament Headspace New Syringe Aug 27 2017 - 03.CHR/DEFAULT.CON		STAND BY -0.899 mV	



Environmental Filament Pyrolysis Aug 27 2017

FID-CHANNEL 1	150.00 deg	
c:\Peak444-32bit\Environmental Filament Headspace New Syringe Aug 27 2017 - 03.CHR/DEFAULT.CON		STAND BY -0.918 mV



Env. Filament Protein ⇔ Conc. Rain Fall Protein ⇔

Aug 23 2017

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[redacted] rejected [redacted]

[redacted] "delusional paranoia"

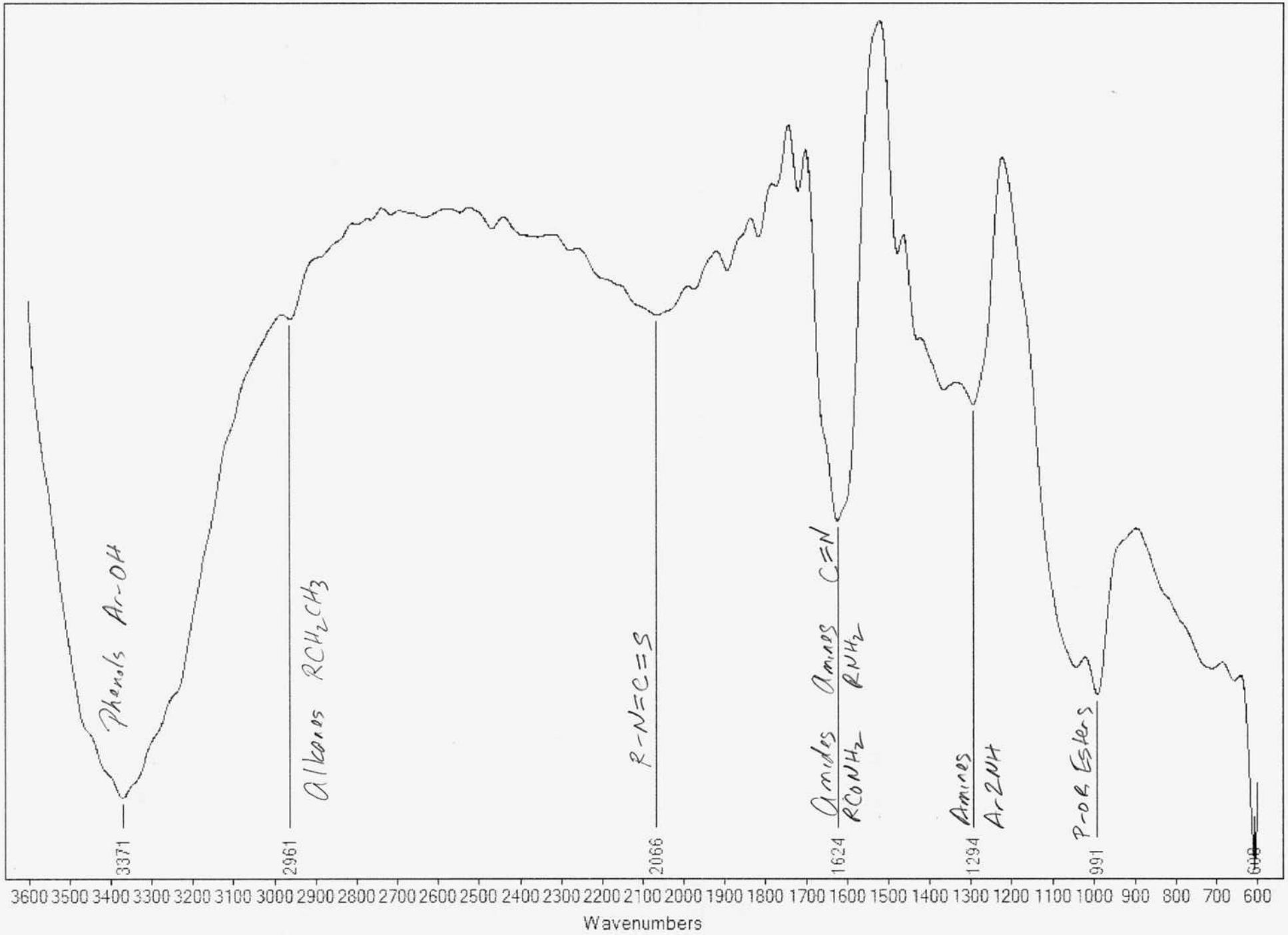
[redacted] — The patent application IS now online.

Today we have our best yet composite IR spectrum of the "Environmental Filament". The best match for it come from the Concentrated Rainfall sample.

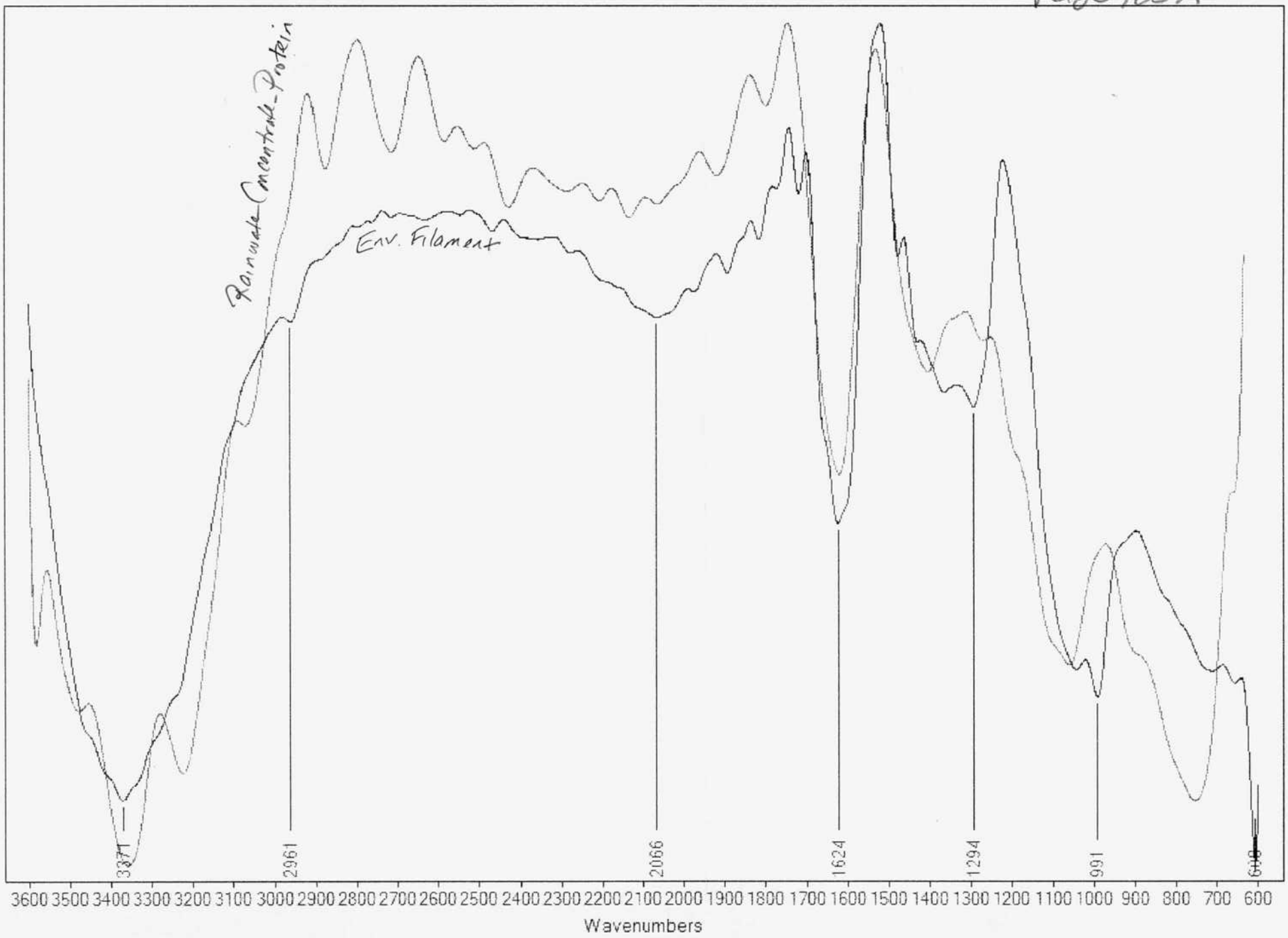
This is as we may surmise a remarkable finding. It has taken 20 years to get to this point.
The rainwater Concentrate is also confirmed to contain verified protein via Bradford.

We have also verified protein via Bradford on the Environmental filament sample (via NaOH microwave digestion pH neutralized, crystallized, and dissolved in H_2O).

Environmental Filament ATR KCl Average Digestion-Catalysis Aug 28 2017 - 02.JPEG



Environmental Filament ATR KCl Average Digestion-Catalysis Match to Rain Concentrate Aug 28 2017 - 02.JPEG



We now work w/ the oleic palmitate.
Denatured alcohol has minimal dissolving
power, some filament breakage observed.

Shifting to microwave digestion technique
w/ 10M NaOH. 5 min @ power 10^{20}.
Double sealed container. Inside container
is canning jar (small) w/ threaded plastic caps

Most of the material does look dissolved
after 5 min. Will repeat another segment.

The second run - total run 10 min @ 10^{20}
has definitely dissolved the material.
Now will neutralize the pH.
pH paper strips are 1/16" x 1/4" - this is all
that is required.

(HCl)
OK, I have the pH neutralized. Now
evaporate.

The method seems to be unintentionally very
favorable to IR analysis, esp. ATR IR analysis.

$NaOH + HCl = NaCl + H_2O$

The means that we are essentially making a
non absorbing (IR) salt plate w/ the
organics (hopefully) dissolved within.

Fundamental Findings *

Air = Rain = Env Filament = Skin Foliate

The is extremely similar to make a salt pressed disc.

The main question would seem to be the sufficient concentration of organics w/in the evaporated salt film on the ATR plate or on a KCl plate if you so that route.

We have done a good job. The signal is weak but sufficient. Microwave digestion has also worked upon the skin foliate.

*

The closest match to the IR spectrum of the skin foliate (known to contain the filaments) IS the environmental filandent (ie EPA) itself.

We do have a lock. Everything is the same

Air = Rain = Env Filament = Skin Foliate

Everything has opened up simultaneously to reveal the presence of the life form and the protein in the general environment.

This is A Point of Reckoning - Part V

1. FE photo
2. FE IR Skin Foliate
3. Env Filament - IR.

Skin Foliate IR plot:

Closest match: Env. Filament (EPA)

$r = 0.94$

Concentration of skin foliate is weak but sufficient.

Viscous CDB Protein

Air = Rain = Env. Filament = Skin Foliate

August 28 2017

Skin Foliation NaOH Microwave ATR Aug 28 2017 - 01.JPEG

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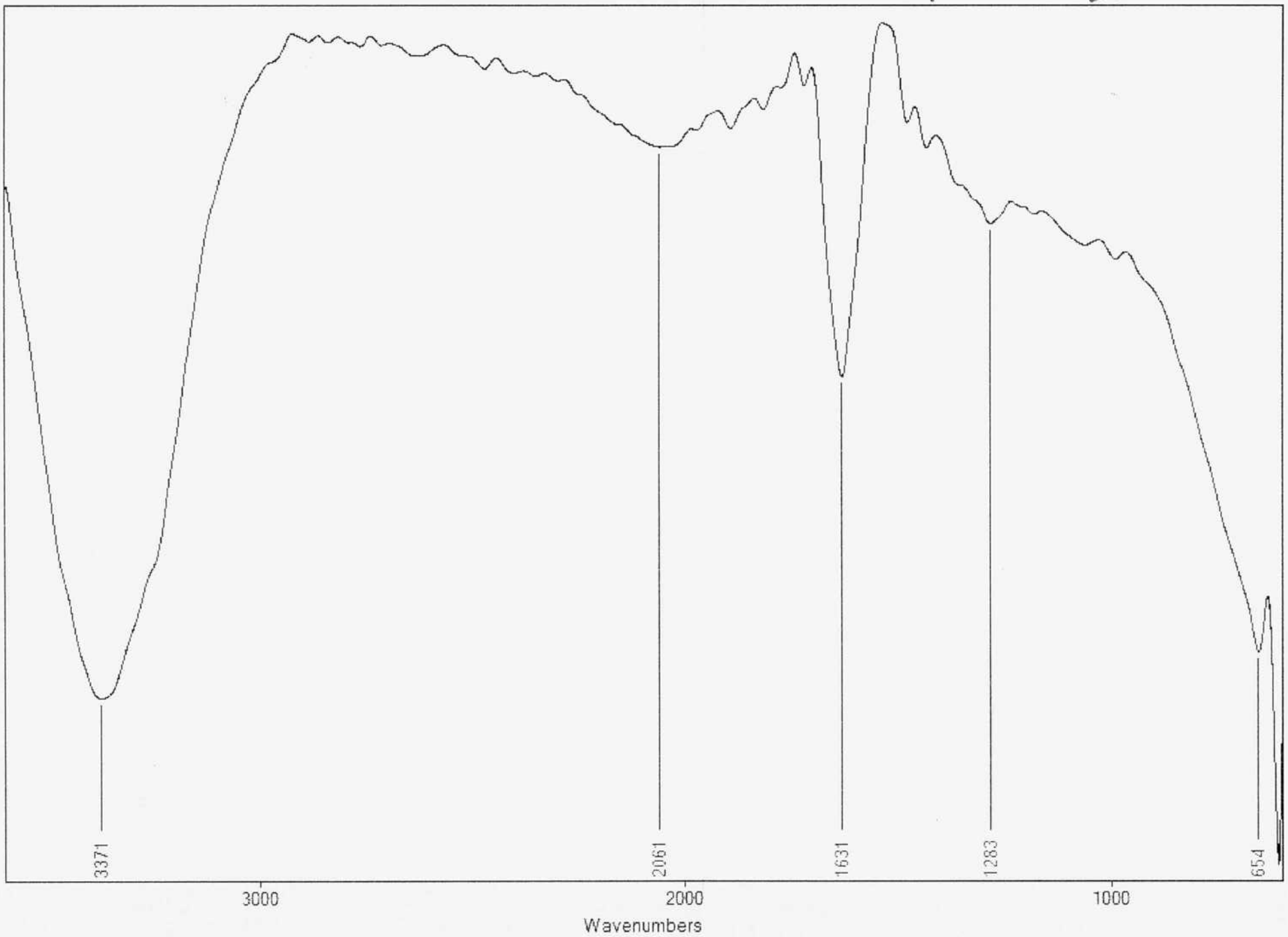

Skin Foliation NaOH Microwave ATR Compare to Env Filament Aug 28 2017 - 02.JPEG

r=R94

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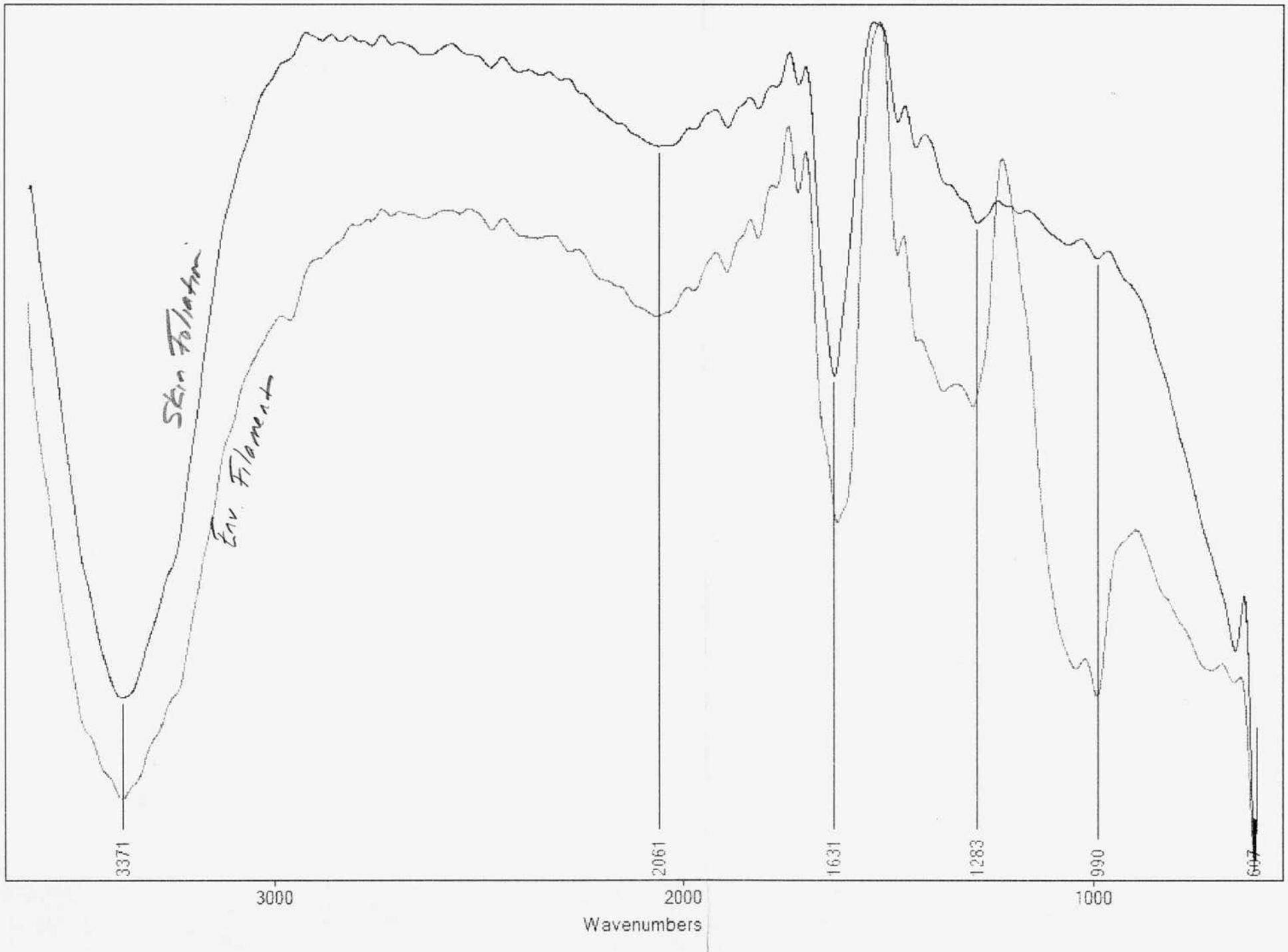

Aug 29 2017 Page 193

There is undoubtedly a strong interest in the R-N=C=S group. This is

isothiocyanate

Horseradish, mustard & radishes & watercress are examples of plants that contain isothiocyanates. Plant order is Brassicales.
They produce glucosinolates & an enzyme called myrosinase. The combination produces isothiocyanates.

* Brassicaceae vegetable interfere w/ iodine uptake

* There may be genotoxic effects from isothiocyanates & glucosinate precursors.

Certain isothiocyanate compounds may act as antioxidants & reduce tumors.

Isocyanate absorbs @ ~ 2222 to 2260
We are @ 2066
Isothiocyanates are @ ~ 2125 Not really
We are therefore dead center. see → (1990-2140) $\bar{X}$=2065

Goitrogens are substances that disrupt the production of thyroid hormones

Examples:

thiocyanate (smoking produces this)
broccoli & cabbage

The sulfur in Brassicas competes for iron leading to goiter and anemia.

What is the difference between thiocyanate & isothiocyanate?

Thiocyanate is $[SCN]^-$ Lewis: S-C-N

Isothiocyanate is R-N=C=S

Isothiocyanate apparently is mustard oil pungent & repellant
They have many physiological actions

It looks like we might be able to test for isothiocyanate in the urine.

It also is now how ground mustard seed for testing.

Page 195

Chandra Amar, Biochem Pharmacol (Los Angel) 2015, 4:3
DOI: 10.4172/2167-0501.1000171

Biochemistry & Pharmacology: Open Access

Research Article Open Access

Iodine, Thiocyanate and the Thyroid

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Introduction

Thiocyanate is a ubiquitous metabolite in man and animals consuming plants containing cyanogenic glycosides and thioglycosides (glucionates) while iodine is present in the earth crust since its origin and is an essential constituent of thyroid hormone requires in trace amount. Iodine is essential for thyroid hormone synthesis while thiocyanate prevents the synthesis of thyroid hormone. As a result the physiological rather functional status of thyroid is very much dependent on the balance between these ions because of their similar ionic volume and charges and competition at different steps in thyroid hormone biosynthesis. Both iodine and thiocyanate enter in the body / thyroid gland through food and water. Thiocyanate in relatively higher concentration regulate the uptake, efflux, organification of iodide, thyroid peroxidase activity and biosynthesis of thyroid hormone. In addition the retaining capacity of iodide in the thyroid gland and body also depends on thiocyanate concentration or in other words the excretion of iodine is related with thiocyanate concentration. In the semi-arid region of earth, the consumption of cyanogenic food (thiocyanate precursor) is relatively high and many regions are environmentally iodine deficient therefore the people are at the risk of iodine deficiency disorders (IDD). The pregnant and lactating women and the women of childbearing age group are the most vulnerable group of IDD because the neuronal development of the fetus and neonate are greatly affected even in mid to moderate iodine deficiency. This article reviews the sources of thiocyanate and iodine in food and thyroid gland physiology in relation to thiocyanate and iodine based on experimental and epidemiological evidences.

From available literature along with our observations, thyroid gland morphology, iodide uptake, iodide influx, iodide organification, activity of thyroid peroxidase, thyroid hormone synthesis and the excretion of iodine in relation to thiocyanate concentration including thiocyanate metabolism have been discussed based on experimental and epidemiological evidences from available literature along with our observations.

In the semi-arid region of earth, the consumption of cyanogenic food is the cause for the development of goiter and associated iodine deficiency disorders (IDD). The pregnant and lactating women and the women of childbearing age group are the most vulnerable group for IDD because the neuronal development of the fetus and neonate are greatly affected even in mid to moderate iodine deficiency.

General Consideration

In thyroid gland iodine is an indispensable constituent for the synthesis of the thyroid hormone, thyroxine (T4) and triiodothyronine (T3) which are essential for normal growth, physical and mental development in man and animals. The most familiar effect of iodine deficiency is goitre (enlargement of thyroid gland) with a number of physiological disorders on the foetus, neonate, the child, adolescent and the adult in the whole population collectively termed as iodine deficiency disorders (IDD). The role of iodine deficiency as an environmental determination in the development of endemic goitre is established. However many agents in the environment interfere with thyroid gland morphology and function acting directly on the gland or indirectly by altering the regulatory mechanism of thyroid gland. The uptake and utilization of iodine, by the thyroid gland is impaired by the pseudo halide thiocyanate (SCN^-). Thiocyanate is formed from cyanogenic substances. It is metabolized in thyroid gland. The role of thiocyanate ion in the homeostasis of thyroid is a provocative issue where IDD persists inspite of adequate iodine intake and consumption of cyanogenic plant food is relatively high. The article reviews the physiology of the thyroid gland in relation to iodine and thiocyanate metabolism.

Bioavailability of Iodine and Thiocyanate

Iodine

There is a cycle of iodine in nature. Most iodine is present in oceans. It was present during the primordial development of earth, but large amounts were carried by wind, rivers and floods into the sea. Iodine occurs in the deeper layers of the soil and is found in oil-well effluents. Water from deep wells can provide major source of iodine. In general, the older and explored soil surface the more likely it is to be leached of iodine [1].

The dietary source of iodine is the food crops grown in the region and drinking water. Meat, fish and dairy product are also the main source of iodine. In sea fish and seaweeds contain high amounts of iodine. Supplementations of iodine through salt, water, bread are the additional sources of iodine specially in iodine deficient area.

Thiocyanate

Cyanide in trace amount is almost ubiquitous in plant kingdom and occurs mainly in the form of cyanogenic glucosides and glucosinolates (thioglucosides); both are nitrogen containing secondary metabolites share a number of common features. They derive biogenetically from amino acids and occur as glycosides which are stored in vacuoles. They function as prefabricated defense compounds that are activated by the action of a β-glucosidase in case of emergency, releasing the deterent: toxic cyanide from cyanogens or isothiocyanates from glucosinolates [2].

When the cyanogenic plants are wounded by herbivores and other organisms, the cellular compartments are broken down and the cyanogenic glucosides come in contact with an active β-glucosidase having broad specificity, which hydrolyses them to yield 2-hydroxynitrile (cyanohydrin) that is further cleaved into the corresponding aldehyde or ketone and HCN by a hydroxynitrile lyase.

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HCN is highly toxic for animals and microorganism due to its inhibition on enzymes cytochrome oxidases (respiratory chain) and its binding to other enzymes containing heavy metal ions. The lethal dose of HCN in man is 0.5-3.5 mg/kg after oral administration and death of animals or man reported after the consumption of plants with cyanogenic glycosides, whose concentrations can be upto 500 mg HCN/100 g seeds. Normally 50-100 mg HCN/100g seeds and 30-200 mg/100 g leaves have been reported [3].

Animals can rapidly detoxify small amounts of HCN by rhodanese. A number of herbivores can tolerate HCN at rest in lower concentrations [4]. Cyanogens are active and potent chemical defense compounds. HCN is toxin for plants which synthesize them. To prevent autotoxicity, a detoxification pathway exists - HCN combines with L-cysteine to yield 3-cyanoalanine by β-cyanoalanine synthase, cyanoalanine is hydrolyzed by β-cyanoalanine hydrolase to L-aspargine. β-cyanoalanine synthase occurs in all plants but likely to be more in strongly cyanogenic species [2] shown in (Figure 1).

Glucosinolate

Glucosinolates are similar to cyanogens in many respects, but they contain sulphur as an additional atom. Under hydrolysis, glucosinolates liberate D-glucose, sulphate and an unstable aglycone, which may form isothiocyanate (common name mustard oil) as main product under certain conditions, or a thiocyanate, a nitrile or cyano epithioalkane.

All plants which sequester glucosinolates also possess thioglucoside glucohydrolases (commonly known as myrosinase) that can hydrolyze glucosinolates to D-glucose and an aglycone, spontaneously rearranging to isothiocyanate. These hydrolases are stored in the cell wall, in endoplasmic reticulum, Golgi vesicles and mitochondria.

When the tissues are wounded or disintegrated, the enzyme and its substrate come together liberating the pungent and repellant isothiocyanate. Depending on the environmental condition, enzymes and other compounds, present, the aglycone can rearrange to isothiocyanates as the most common product, or to nitriles, thiocyanates or cyano-epithioalkanes or oxazolidine-2-thiones (Figure 2).

A number of isothiocyanates are liophilic, volatile with a pungent smell and taste, while others are not volatile and pungent smelling, but have otherwise similar properties. Isothiocyanates can penetrate

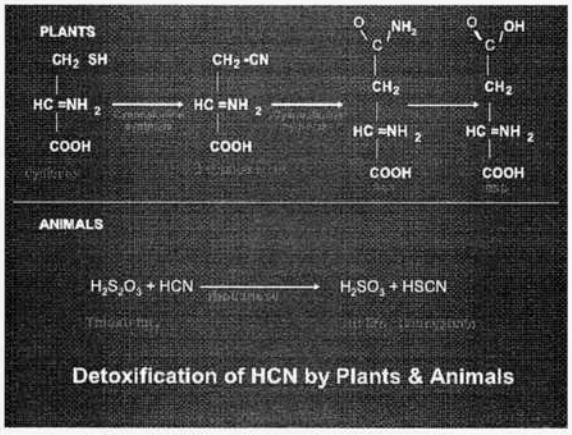

Figure 1: HCN detoxification in animals and plants.

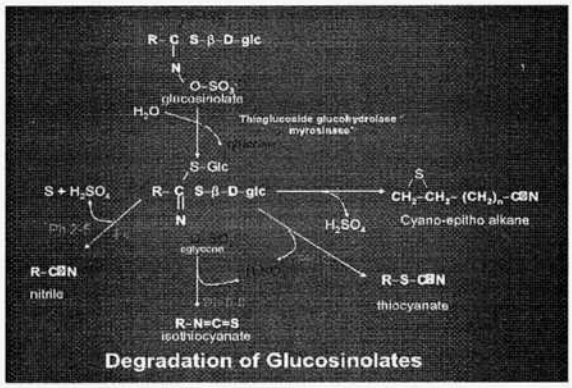

Figure 2: Degradation (defense activation) of Glucosinolates.

through biomembranes and they have many physiological actions.

Glucosinolates are thus considered as preformed defense chemicals which are activated during emergency. They have a wide range of activities and important specially in plant-harbivore but also in plant-plant and plant-microbe interactions.

Thyroid Physiology

Thyroid morphology/histology

The morphological profile of thyroid cells can be altered by dietary iodine. A low iodine diet causes distinctive functional alteration in thyroid cells. Some of the effects are direct result of iodine deficiency and the others are the secondary by elevated serum TSH level. In contrast high doses of iodine cause various responses depending on the dose of iodine given, the duration of the experiment and the route of administration [5].

Feeding of thiocyanate in rats at relatively high dose showed increased weight and abnormal histology of the thyroid. The histological features of the thyroid of animals from iodine non-supplemented thiocyanate added groups (-KI+SCN) indicated hypo functioning of the thyroid with a marked decrease in colloid containing follicles and significant proliferation of new follicles with indistinct lumen (hyperplasia) in contrast to the iodine supplemented control animals. The thyroid of rats deprived of KI (-KI) also showed a decreased in mature follicles and mild follicular hyperplasia. Colloid content of the follicles was however unaltered [6].

Cruciferous plants viz., cabbage, cauliflower, mustard (seeds and leaves), turnip, radish, brussel sprout, sprouts of Brassica family, bamboo shoot and cassava from non-Brassica family constitute a major portion of human diet contain naturally occurring goitrogenic substances or thiocyanate precursors (glucosinolates and cyanogenic glucoside). Extreme differences in the goitrogen content of these plants belong to same family and same taxonomy owing to their genetic and ecological backgrounds have also been reported [7]. Besides, the goitrogenic / anti-thyroid potential of the plant foods not only depend on their relative concentrations of the goitrogenic constituents as found in fresh plants but as also on their processing as foods [8]. The histological status of thyroid after prolonged feeding of cyanogenic plant foods e.g., fresh and cooked radish, turnip, cassava, bamboo shoot etc. by replacing 1/3 portion of the diet with and without iodine supplementation was investigated [9]. The thyroid follicles were lined by cuboidal cells with distinct nucleus showing hypertrophy and

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hyperplasia filled with less homogeneous colloid; some follicles were invaded by epithelial cell. Increase in the number of comparatively small follicles was one of the characteristic features. On the contrary, in the control rat, thyroid follicles were almost equal in size, lined by follicular cells filled with colloid. Alterations were found in the thyroid structure between the fresh and cooked cyanogenic plant fed rats. Variation in the number and size of follicular cells and colloid content was observed in KI- supplemented and non- supplemented plant fed group of rats. In addition, colloid stained more with eosin in the experimental group (-KI) as compared to control and KI- supplemented (+KI) group of rat for the variation of concentration of iodine [10,11].

Consumption of excess cyanogenic plants in relation to iodine is considered as an etiological factor for the persistence of iodine deficiency disorders in many regions. Moreover, in spite of salt iodization, residual goiter still persists [12-14].

Therefore moderate intake of iodine could be sufficient to meet the requirement but may not ensure normal thyroid in the presence of goitrogens like thiocyanate that come through cyanogenic plant which contributes a major portion of human diet of the people living in semi-arid region of the world.

Iodide uptake

Thiocyanate has been considered as a possible cause of thyroid disorders because it inhibits iodide transport [15-17]. The effect of thiocyanate on iodide transport in different TSH concentrations were studied in porcine thyroid cultured cells and found that SCN concentration >10 μmol/ L inhibited iodide uptake in a dose response manner regardless of TSH concentration. It has also been reported that follicles without preincubation and with 24hr preincubation by thiocyanate showed identical inhibition [18].

cAMP production and Na^+K^+ - ATPase activity

cAMP production and Na^+K^+- ATPase activity were measured in thyroid follicular cells to investigate the mechanism of inhibition of iodide uptake by thiocyanate. The presence of 200μmol/L thiocyanate for 24hr did not inhibit TSH mediated cAMP production in the cultured thyroid follicular activities of Na^+K^+- ATPase in the control and the thiocyanate group were almost same [18].

All these results indicate that inhibition of iodide uptake by thiocyanate is independent of TSH concentration and that thiocyanate does not affect cAMP generation or Na^+K^+- ATPase activity. Then the question arises how thiocyanate inhibits iodide transport. [19] have described thyroid iodide translocator, a Na dependent iodide transport protein different from Na^+K^+- ATPase, in the phospholipids vesicle of plasma membrane; and speculated that thiocyanate inhibits the system [17]. Their model is also convenient to explain competitive inhibition of iodide transport as thiocyanate and iodide are common substrates for the iodide transporting protein. Competitive inhibition is important to understand the action of thiocyanate *in vivo* because iodine deficiency may facilitate action of thiocyanate or an excessive iodine intake may diminish the action of thiocyanate in thyroid gland.

Metabolism of thiocyanate in thyroid

The concentration of thiocyanate in thyroid is fairly constant. There is no concentration gradient for thiocyanate between the thyroid and serum, the ratio (T/S) varying from 0.29 - 0.34.Thiocyanate ion is metabolized by the thyroid of rat. Sulphate is the major sulfur product; iodine is a potent inhibitor of the metabolism of thiocyanate. On the contrary there is a maximal inhibition of the concentration of iodide by the thyroid at time when the serum level of thiocyanate ranged from 180 – 286 μmol/ml. It would appear that there is narrow ranged between the level of thiocyanate in the serum of rat and the level at which there is an inhibition of the concentration of iodide by the thyroid. Thus the role thiocyanate ion if any in the homeostasis of thyroid was found important [20].

Monovalent anions with a molecular size corresponding to that of iodine viz. SCN are concentrated in the thyroid and inhibits the normal metabolism of iodine [21]. Thiocyanate not only interferes with the uptake of iodide but also on the iodination of thyroglobulin. Thiocyanate is therefore, a potent antithyroid substance and may cause hypothyroidism at high concentrations [22]. However, short term administration of thiocyanate, serum levels of thiocyanate < 18mg/ L. did not suppress thyroid function. Because thiocyanate is largely exerted through kidney and can therefore, be accumulated in the body, results from short – and long term – experiments are however, not fully comparable [22].

Iodide efflux

Thiocyanate has been reported to increase iodide efflux [23] and in vivo model thiocyanate is more potent for iodide efflux than for inhibition of iodide transport. In culture thyroid follicles thiocyanate at 10μmol/L or greater increased iodide efflux from the thyroid follicles. The degrees of iodide efflux by 10μmol/L and 100μmol/L thiocyanate were almost the same. Even the increment of iodide efflux was minor at 200μmol/L in comparison with that of 10μmol/L thiocyanate [18].

Iodide organification

Thiocyanate decreases iodine organification in a dose response manner. The decrease in iodine organification occurred in parallel to the decrease in iodide uptake. To study whether thiocyanate has an independent inhibitory action on iodine organification, thyroid follicles were incubated with the mixture of Na ^{125}I and NaI for 2 hrs to load iodide; then 50 μmol/L thiocyanate was added. After the addition of thiocyanate, iodide uptake becomes plateau and iodine organification decreased significantly indicating that thiocyanate inhibits iodine organification independent of iodide uptake [18].

Thyroid peroxidase (TPO)

Cyanogenic constituents affect hormone synthesis in thyroid gland either by inhibiting iodide uptake or interfering the activity of thyroid peroxidase (TPO) i.e., by inhibiting the organification of iodide (I$^-$ leads to I_2) or iodination of tyrosine in thyroglobulin followed by coupling reaction [24-28]. The goitrogen content of a number of cyanogenic plant foods of Indian origin and their *in vitro* anti thyroid activity in raw, boiled and cooked extract s with and without extra iodide have been studied by Chandra et al, (2004). The relative anti TPO potency of the studied plants and PTU equivalence was also determined by estimating the amount of plant food or PTU capable of producing 50% inhibition (IC_{50}) of TPO activity. The IC_{50} was highest in bamboo shoot, followed by cassava, mustard, cauliflower, radish, turnip and cabbage. This observation was confirmed by PTU equivalence of the studied plants

After the feeding of the cyanogenic plants in experimental animals for a prolonged period, the TPO activity of thyroid of the treated animals was reduced markedly [29-42]. As mentioned cyanogenic glucosides are readily converted into active goitrogenic agent thiocyanate by glucosidases and sulphur transferase enzymes present in the plant or in the animal tissue. Thiocyanate or thiocyanate like

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compounds primarily inhibit iodide concentrating mechanism of the thyroid, however at high concentration thiocyanate (SCN) inhibits the incorporation of iodide into thyroglobulin by competing with iodide at the thyroid peroxidase level [43] and forming insoluble iodinated thyroglobulin in thyroid [44]. High concentration of thiocyanate is also responsible for inhibition of TPO catalyzed oxidation (I leads to I_2) [28] while glucosinolates undergo a rearrangement to form isothiocyanate derivatives [26]. Isothiocyanate reacts spontaneously with amino groups to form thiourea that interferes in thyroid gland with organification of iodide and formation of thyroid hormone and this action cannot be antagonized by the iodide [43].

T3/T_4 synthesis

At low doses, thiocyanate was shown to inhibit the uptake of iodide; in addition it also affects the organic binding there by influence in the thyroxine synthesis. The latter effect of thiocyanate cannot be counteracted by excess iodide. Further, thiocyanate causes depression in protein bound thyroxine levels with concomitant increase in free thyroxine levels [45]. The reduced total circulating thyroxine levels could be a consequence of reduced thyroxine synthesis, depression in protein bound thyroxine levels and the utilization of free thyroxine levels by the peripheral tissues. Feeding of cyanogenic foods viz., bamboo shoot [39-41], radish (*Raphanus sativus Linn*) [34-36], cassava (*Manihot esculata crantz*) [34-36], maize (*Zea mays Linn*), cabbage, cauliflower, mastered etc. decreased the serum total T_3 and T_4 levels significantly. As mentioned, reduced TPO activity may be responsible for decreasing thyroid hormone levels because it regulates the synthesis of thyroid hormone.

Iodine/ thiocyanate ratio

The studies in Zaire have shown that cassava a staple diet in the region has definite antithyroid action in man and animals, resulting in the development of endemic goiter and cretinism. This action is due to the endogenous release of thiocyanate (SCN) from linamarin, a cyanogenic glucoside contained in cassava despite the fact that the cassava is consumed a large scale within tropics, however goiter and cretinism are not found in all population, where staple food is cassava. One possible explanation for the lack of goitrogenic action of cassava in some populations may be that they have a high iodine intake [46].

The development of goiter is critically related to the balance between dietary supplies of iodine and SCN. Under normal conditions, the urinary excretion of iodine (UEI) and thiocyanate (UESCN) or UEI/ UESCN or I/SCN is higher than 7. Endemic goiter develops when it reaches a critical threshold about 3 and becomes hyperendemic, complicated by endemic cretinism when it is lower than 2. The validity of this ratio as an index of the risk of development of goiter has been demonstrated by comparative studies conducted in different regions of Zaire [46].

In Manipur and Tripura of north- east India and Siddhartha nagar in the foot hills of Himalayas, universal salt iodization is in progress and the people consume adequate iodine, they also consume cyanogenic plant foods regularly in relatively considerable amount. Goiter is prevalent in the areas as mentioned and the UIE is almost adequate. In India, mean urinary thiocyanate level from non-endemic population is 0.504 ± 0.19 mg/dl [14]; the SCN levels in all those areas is much more. Therefore the contribution of SCN in the persistence of residual goiter during post salt iodization phase may not be ruled out. However the validity of I/SCN as mentioned (i.e., below 7) is not universal. This is consistent with earlier studies / report [8]; I/SCN ratio was a useful indicator in the epidemiological studies in Zaire that elucidated the goitrogenic effect of SCN from cassava consumption [46]. However the proposed use of a defined threshold of 3 for this ratio for prediction of goiter frequency has several limitations. First it requires that the ratio is not clearly defined. It has been used as both the mean of individual I/ SCN ratio, and as the ratio between mean I/ mean SCN. As there ratios are mathematically different, they yield different results. Secondly, the distribution of individual I/SCN ratio is much skewed and that if any summary statistics is to be used the median and mode are preferable to the mean. A third limitation with the use of this ratio is that very high thiocyanate load will yield serum levels that exceed the kidney threshold. Urinary excretion of SCN is therefore not linearly related to the serum levels that exert the effect on the thyroid. Finally the often pronounced seasonal variation of cyanide exposure from cassava can result in 10 to 15 fold variations of SCN and this must be considered when estimating goitrogenic effect [47].

In the semi-arid region where the agricultural production of cyanogenic plant food and consumption of thiocyanate are more, the balance between the dietary supplies of iodine (I) and thiocyanate (SCN) play important role in the etiopathogenesis of endemic goiter and associated disorders but for the prediction of this ratio is yet to be determined.

Excretion of Iodine and Thiocyanate

The concentration of iodine in urine is the most widely used as biochemical marker of nutritional iodine deficiency as most of the body's iodine is excreted in the urine, usually over 90%. For surveys it is to collect 40-50 samples from an area and express the iodine as a concentration (µg/dl urine). A median urinary iodine concentration of 10 µg/dl in an area indicates no iodine deficiency [48].

Ingeston of the Brassica vegetables in human causes a rise of thiocyanate ion in blood followed by its appearance in urine. The thiocyanate level was found to drop as soon as the eating of the plants was discontinued [49]. As mentioned the urinary excretion of SCN (after a very high thiocyanate level) is not linearly related to the serum levels that exert the effect on the thyroid [50,51].

Inspite of adequate iodine nutritional status, endemic goiter is prevalent in many regions because the urinary iodine does not always truly reflect the iodine nutritional status in an environment where consumption of food containing thiocyanate precursors is relatively high [31-33]. Excess thiocyanate thus not only inhibit the iodine concentrating mechanism by inhibiting unidirectional clearance iodide from the thyroid gland but also the iodine retaining capacity of thyroid and body is also dependent on the consumption pattern of cyanogenic plant food [34-36].

Closing Remarks

In the semi-arid region, the cyanogenic plant food is grown and commonly consumed by the people and thus regular exposure of thiocyanate or its precursor is relatively high. It is also higher in cigarette smokers. When the dietary supplies of iodine and thiocyanate reach a critical point, endemic goiter and associated iodine disorders develops. On the other hand, iodine enters in the body through food and water but its availabilities vary on the geographical location. Therefore dietary supplies of iodine and thiocyanate vary from region to region depending on the availability and consumption patterns. The intake of iodine in relation to SCN and vice versa is a determinant for the causation of thyroid disorders viz. iodine deficiency disorders (IDD).

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- Iodide itself is goitrogenic when it is presented in excessive in serum. Indiscriminate consumption of iodide salt regularly in environmental iodine sufficient region may be a risk factor for the development of autoimmune thyroid disorders, thyroid carcinoma, iodine- induced hypo and hyper thyroidism in long run. Conversely, intake of high thiocyanate is also goitrogenic if the intake of iodine is not satisfactory.

- Thus the question arises what should the 'adequate' or 'optimum' level of iodine? Will this remain at uniform level all over the country irrespective of its geographical distribution of iodine and consumption pattern of dietary goitrogens as cyanogenic plant foods.

- Experimental observations revealed that thiocyanate feeding inhibits iodine absorption by the mammary gland as well as by the thyroid. Such an effect on mammary gland conserves the iodine for the lactating animals but lowers iodine content milk for his young. Therefore lactating mothers ingesting thiocyanate ion could possibly cause goiter in her young affecting the development of foetus.

- Mild and moderate iodine deficiency due to thiocyanate overload associated with iodine deficiency affects intelligence, fine motor skills, problem solving capacity etc of the children and thus evaluation of their brain damage is important.

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Sulfur - Thiocyanate - Thyroid Relationships

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Health Foods that Lower Thyroid Function

by Michael King December 27, 2016

The most common foods that reduce thyroid hormone production (when consumed in excess), and what you can do for your thyroid.

The most common foods that reduce thyroid hormone production (when consumed in excess, especially if raw) belong to the mustard family of cruciferous vegetables known as brassicas (kale, maca, broccoli, cabbage, mustard, etc.) due to their higher than usual levels of sulfur-containing compounds (which provide the pungent taste characteristic of the mustard family).

Brassica foods have health benefits for the immune system and with detoxification due to their sulfur compounds, yet sulfur compounds, when taken in excess, and not counterbalanced by iron-rich or iodine-rich foods, lead to a reduction in thyroid hormones. Other non-brassica foods also lower thyroid function (both are listed toward the bottom of this article) like estrogen promoting soy products, stimulants, alcohol, concentrated sugars, certain grains, and various common foods, due to their tendency to compete with iron and iodine, deplete minerals, or disrupt hormone reception in the body.

The largest food group with thyroid lowering influences are the brassicas which offer both benefits and detriments to overall body health depending on the existing mineral balance in your body at the time of consumption.

Here is what you need to know in order to balance the good and bad among common health foods in your diet today:

Sulfur in Brassicas

Brassicas contain a sulfur compound called isothiocyanates (mustard oil) which block the production of thyroid peroxidase (TPO), the enzyme that transfers iodine to the thyroid hormones and to mother's milk. The net effect is a reduced production of thyroid hormones due to the absence of the fundamental building block for thyroid hormones – iodine (a characteristic which may actually have its benefits in hyperthyroid conditions).

Brassica isothiocyanates have also shown to disrupt signaling across the thyroid cell membranes thereby reducing hormone transportation to other parts of the thyroid.

Isothiocyanates, (as members of the glucosinolate family of compounds) are associated tohyperplasia (enlargement due to increased cellular replication) and hypertrophy (enlargement due to increased cellular size) of the thyroid gland inruminantanimalsby inhibiting the uptake of iodine.https://journals.uair.arizona.edu/index.php/jrm/article/view/9648/9260

Brassicas also contain a sulfur amino acid, SMCO. The sulfur in brassicas compete for iron leading to goiter and anemia. Reduced iron leads to a reduction in oxygen to the cells and thereby a disruption in the production of cellular ATP (required for energy). Feelings of lethargy and chronic fatigue are the common result.

Iron deficiency impairs selenium utilization, yet selenium is essential for the proper utilization of iodine. (Zimmerman MB and Kohrle J. The impact of iron and selenium deficiencies on iodine and thyroid metabolism: biochemistry and relevance to public health. Thyroid 2002;12:867-78).

Consumed in excess, the high sulfur levels in brassicas (also in eggs, onions, garlic and Black Salt/Pink Sulfur Salt) will compete for iron and iodine and thereby reduce the absorption of selenium in the body which is iron dependent. Yet these conditions exist only where an insufficient mineral/nutrient support exists in sufficient quantities to offset the iodine/iron drain from the brassica sulfur compounds.

Seaweeds (in Thyroid Balance, Earth & Sea Greens, and Vital Cleanse & Nutrify) for iodine, and Sacred Clay for iron, provide usable forms of offsetting nutrients required for optimal functioning of the thyroid when consuming moderate amounts of brassicas and other sulfur-rich foods.

While an excess of sulfur can cause anemia, a proper balance of sulfur, iron, selenium and iodine (along with numerous other mineral ratios that depend upon each other in relationship to these) can provide the body with powerful health-building properties.

Categories

The Truth About Building Physical Health

Recent Articles

Mustard seed (ground) does not dissolve readily in ethanol.

We are trying microwave digestion w/ Conc. NaOH

You passed the titration point - repeat.

1. We have a good Conc. urea IR spectrum.

2. Microwave digestion of mustard seed is in place.
   Now I have the pH neutralized.

We have a very important observation on the presence & absence of Isothiocyanate in urine between 2015 & 2017 analysis.

The hypothesis of transfer of Isothiocyanate in the protein of various samples into the body (by urine) appear to be correct.

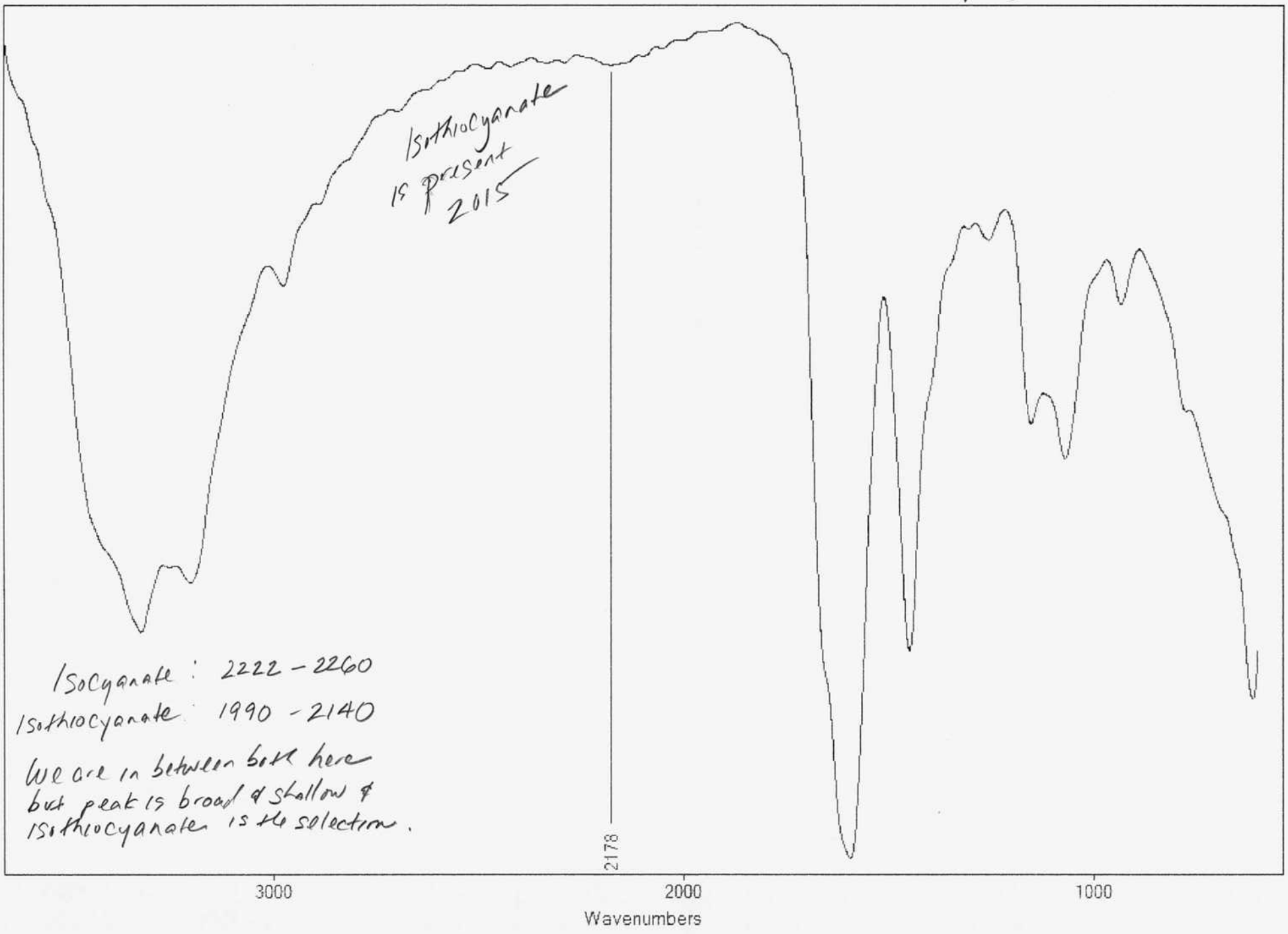
I believe it was in blood also & this will need to be checked. I believe Vit B-12 was the focus of that strategy to remove thiocyanates.

Urine Isothiocyanate Comparison 2015 - 2017 CEC

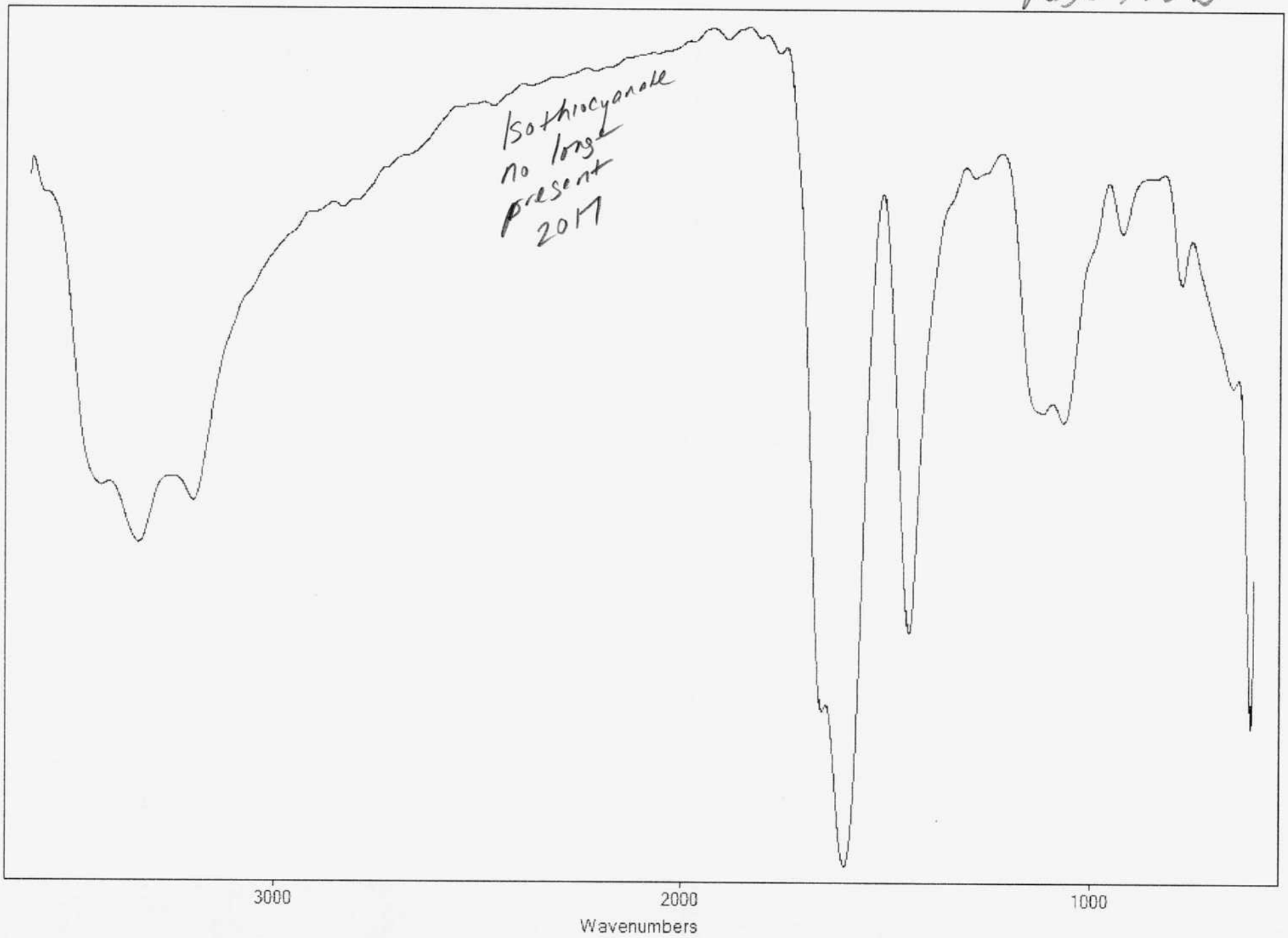
2015 [redacted] Urine Sample: Isothiocyanate IS present

2017 [redacted] Urine Sample: Isothiocyanate IS NOT present.

Urine CEG Paste ATR Nov 07 2015 - 01.JPEG



Urine Concentrated Evaporated Aug 29 2017 - 01.JPEG



Lets work on to mustard + blood.

Ok, Isothiocyanate has been determined and verified in ground mustard seed with IR.

This is a good reference plot, therefore.

Notice that the signal is weak, but nevertheless detectable @ 2062.

Isothiocyanate test on blood.

No significant difference with Isothiocyanate detected in 2000 [redacted] blood samples

It is detectable to similar levels in both samples. The question is: Should it be there?

It can be detected in the blood after eating broccoli. therefore the interpretation is that it should not be resident permanently in the blood.

3rd source IR Spectrum

Consumed by second source: it should be eliminated from the body 24-48 hrs after oral administration, i.e consumption

Conclusion: Isothiocyanates should not be resident within human blood but they appear to be.

Hair Testing should also be investigated

Isothiocyanate - Mustard Testing

Isothiocyanate Investigation - Ground Mustard - Microwave NaOH Digestion Aug 29 2017 - 01.JPEG ATR

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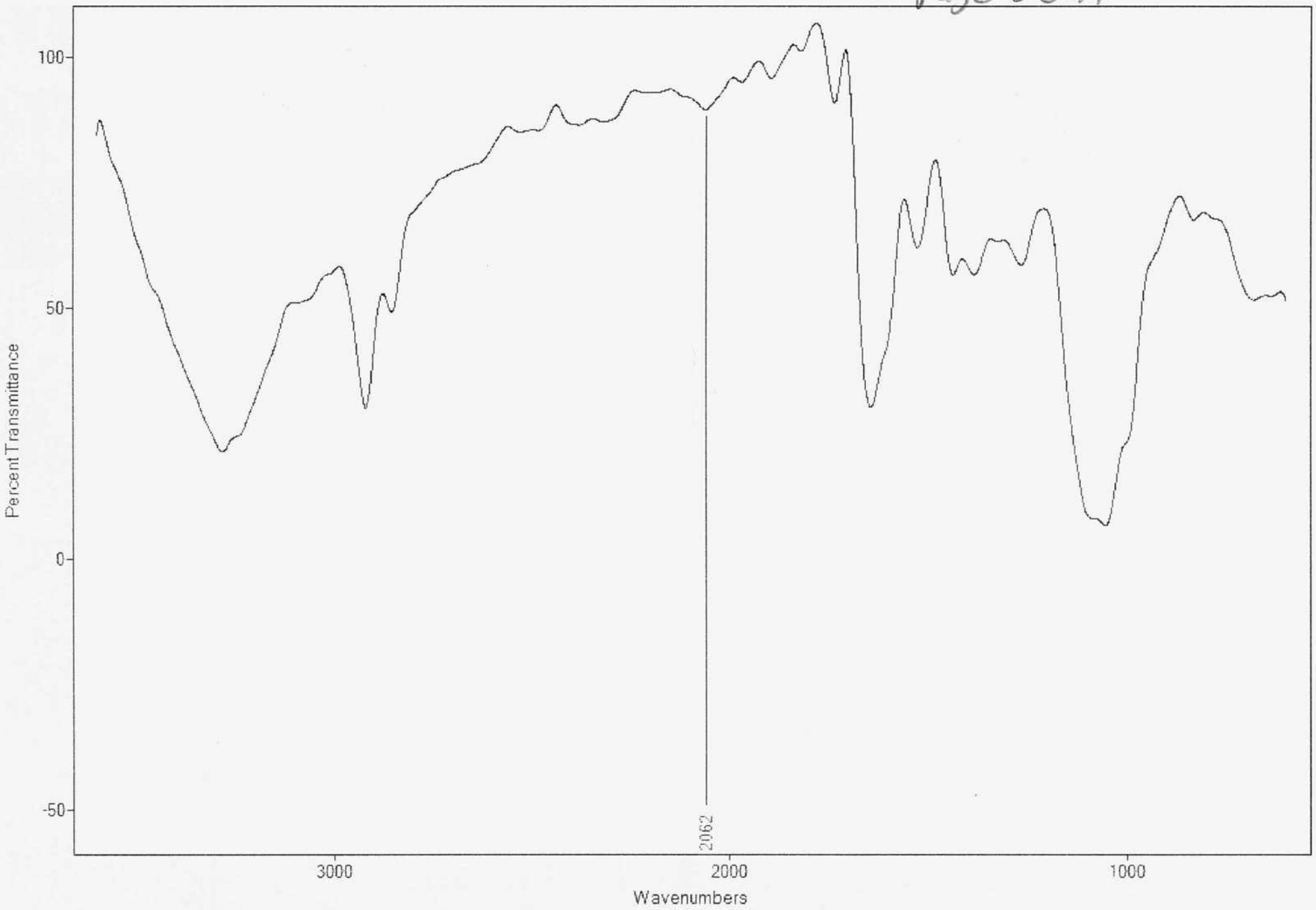
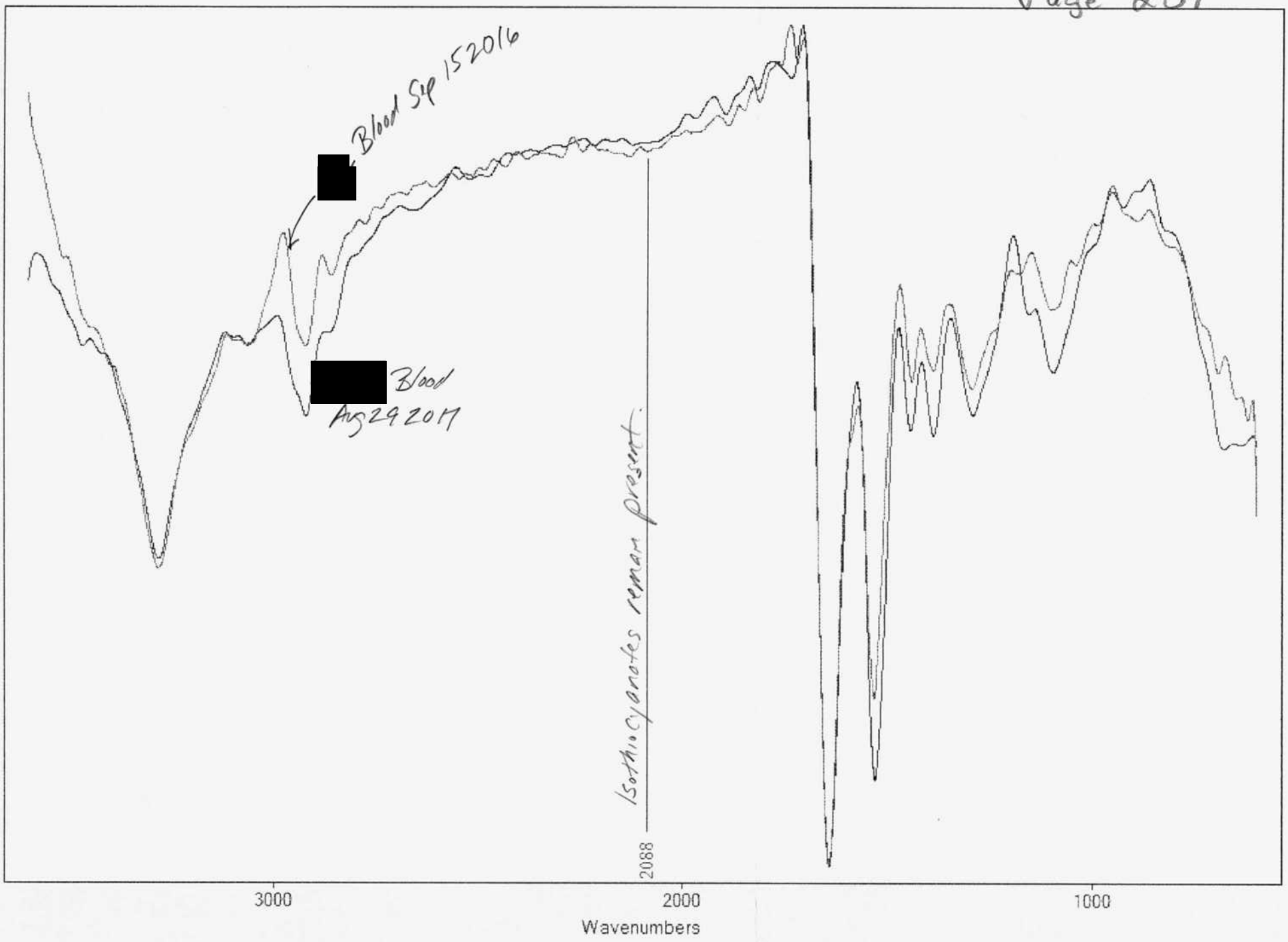


Blood ATR [redacted] Comparison to 2016 - 01.JPEG

No Significant isothiocyanate reduction apparent.



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PMCID: PMC5469854

Isothiocyanates are detected in human synovial fluid following broccoli consumption and can affect the tissues of the knee joint

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Abstract

Go to:

Osteoarthritis is a major cause of disability and there is no current pharmaceutical treatment which can prevent the disease or slow its progression. Dietary advice or supplementation is clearly an attractive option since it has low toxicity and ease of implementation on a population level. We have previously demonstrated that sulforaphane, a dietary isothiocyanate derived from its glucosinolate precursor which is found in broccoli, can prevent cartilage destruction in cells, in *in vitro* and *in vivo* models of osteoarthritis. As the next phase of this research, we enrolled 40 patients with knee osteoarthritis undergoing total knee replacement into a proof-of-principle trial. Patients were randomised to either a low or high glucosinolate diet for 14 days prior to surgery. We detected ITCs in the synovial fluid of the high glucosinolate group, but not the low glucosinolate group. This was mirrored by an increase in ITCs and specifically sulforaphane in the plasma. Proteomic analysis of synovial fluid showed significantly distinct profiles between groups with 125 differentially expressed proteins. The functional consequence of this diet will now be tested in a clinical trial.

Introduction

Go to:

Osteoarthritis (OA) of the hip or knee is ranked as 11th of 291 conditions that contribute to global disability; and the consequent years lived with disability (YLDs) are estimated to have risen by 61% from 1990–2010[1]. There are no disease-modifying OA drugs (DMOADs) currently available, and pharmacological interventions provide symptomatic relief only, which is frequently insufficient[2, 3].

The National Institute for Health and Care Excellence (NICE) and the American College of

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Metabolism, kinetics and genetic variation

Humans

Ingested isothiocyanates are metabolized principally through the mercapturic acid pathway and excreted in urine as dithiocarbamates, mainly in the form of *N*-acetylcysteine conjugates. The initial reaction with glutathione (GSH) may be either spontaneous or may be catalysed by GSH transferases (GSTs). The role of GST polymorphisms in exposure of tissues to isothiocyanates and excretion of isothiocyanates remains unresolved. Analytical methods, especially the cyclocondensation assay, have enabled quantification of isothiocyanates in cruciferous vegetables and of isothiocyanates and their dithiocarbamates in human urine. Isothiocyanates react with GSH and excretion via the mercapturic acid pathway, but minor pathways, such as hydrolysis, oxidation–reduction, ring hydroxylation and alkyl-chain degradation, may be used, depending on the structure of the compound. Analysis of urinary metabolites has shown that there are species differences in the metabolism of isothiocyanates. Studies with radiolabelled isothiocyanates show that these compounds are readily absorbed into blood and tissues and are eliminated almost completely within 24–48 h of oral administration.

Little information is available on the metabolism and distribution of indoles in animals fed cruciferous vegetables. The fate of purified indole-3-carbinol has been examined in rats and trout, whereas the fate of ascorbigen has been studied only in mice. Purified isothiocyanates are potent inhibitors of cytochrome P450 enzymes. Studies of structure–activity relationships have shown that aromatic isothiocyanate compounds with longer alkyl chains or greater lipophilicity have enhanced inhibitory action against these enzymes. These studies have resulted in identification of some isothiocyanates that are remarkably powerful inhibitors of cytochrome P450 enzymes.

Cancer preventive effects

Humans

Studies were considered in this evaluation only if the reports provided estimates of risk along with statistical confidence intervals for estimated consumption of all cruciferous vegetables or for specific cruciferous vegetables.

Whole blood and semen identification using mid-infrared and Raman spectrum analysis for forensic applications

Check for updates

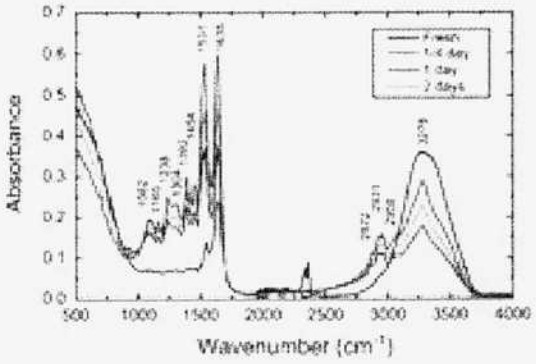
Page 203 A

Yun Zou[a] Pan Xia[b] Feiyu Yang[a] Fangqi Cao[a] Ke Ma[a] Zhongliang Mi[b] Xiaochun Huang[c] Nengbin Cai[b] Bei Jiang[c] Xuejun Zhao[a] Wenbin Liu*[a] and Xianfeng Chen*[c]

Author affiliations

Abstract

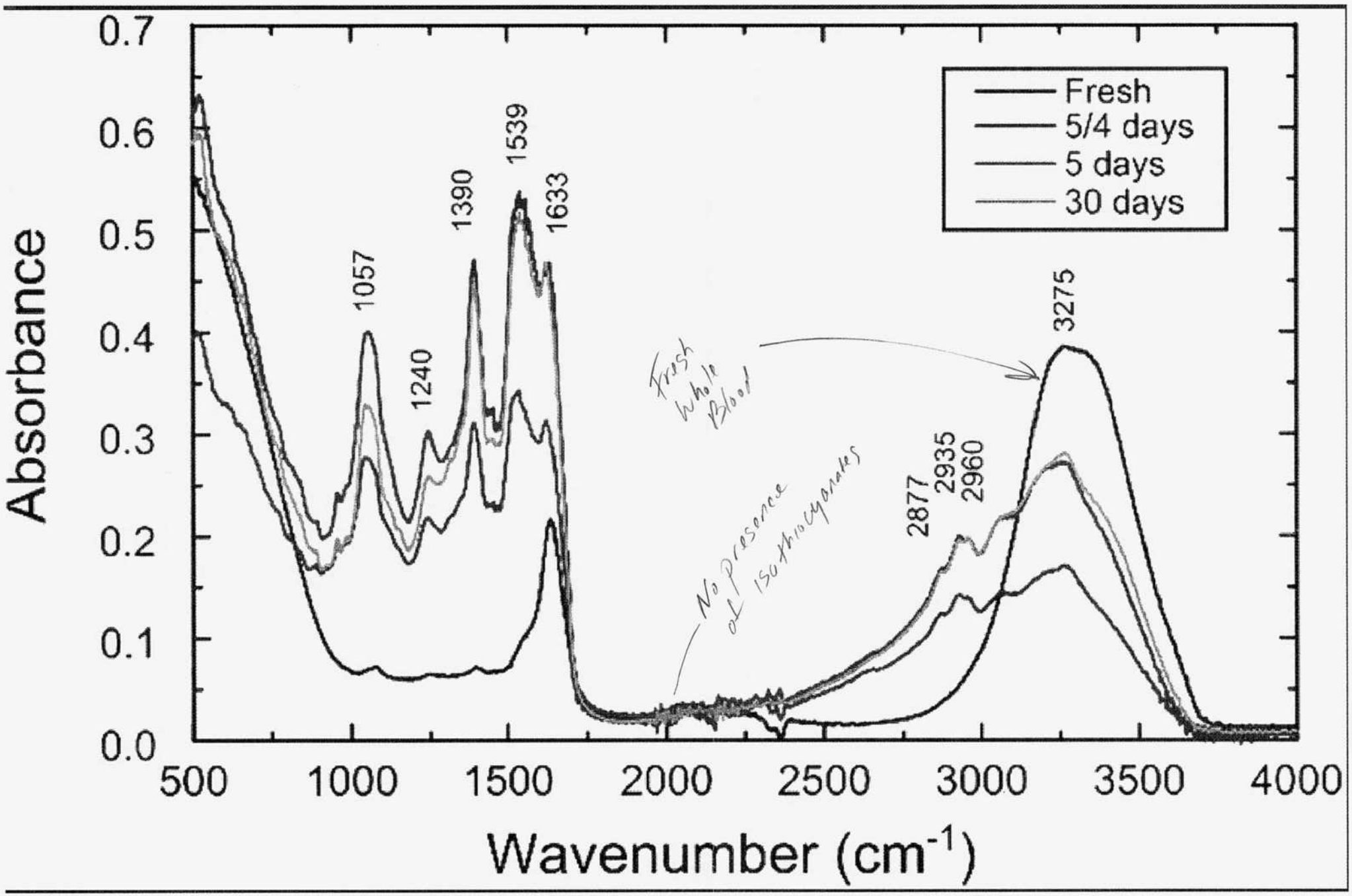
The identification of body fluids is important in forensic science. This paper describes the application of mid-infrared and Raman spectroscopies in the non-destructive identification of human blood and semen, where other detailed information can also be obtained in one single measurement. Samples of human blood and semen were probed and characterized utilizing Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) and confocal Raman spectroscopies. The result shows their ability to identify an unknown substance to be human blood or semen without the use of chemical reagents. Age determination of dried blood and semen spots through their mid-infrared spectra was investigated, which could probably be used during forensic casework. Furthermore, the origin of the Raman scattering peaks of human semen at 2907 and 2968 cm^{-1} is detailedly analyzed, which has not been studied in previous literature. Overall, this optical detection and identification method exhibits advantages over conventional chemical methods in terms of non-destruction, high sensitivity, rapid detection and direct confirmation.



Thyroid (hypo) & joints are two hallmark symptoms of Morgellons

Isothiocyanates should not be resident in the blood but they are. This is affecting thethyroid & joints.

Black Line with Peak at 3275 is Fresh Whole Human Blood



Aug 30 2017

With Dy vulgaris / hair, we definitely have a strong signature of isothiocyanates.

1. Does burning hair normally produce isothiocyanates?

2. Can we produce isothiocyanate in a digested hair sample?

3. Can isothiocyanate be a gas?
   Yes, the NIST IR spectrum is in gaseous form.

Allyl isothiocyanate is an oil w/ a boiling point of ~ 150°C so indeed it can form a gas @ that point.

Hair can have hydrogen cyanide in it, I recall that, that not isothiocyanates

Smoking, etc?
Yes, it is a by product of cigarette smoke

Hydrogen cyanide can react w/ sulfur to produce thiocyanate. $(SCN)^-$ $R-N=C=S$

What does it take to change thiocyanate to isothiocyanate?

Isothiocyanate is R-N=C=S and is formed by substituting the Oxygen in Isocyanate w/ a sulfur

HCN hydrogen cyanide

$[SCN]^-$ thiocyanate

R-N=C=O Isocyanate

R-N=C=S Isothiocyanate

So:

R-N=C=O → R-N=C=S

Isocyanate — Isothiocyanate

HCN + S → $[SCN]^-$

hydrogen cyanide — sulfur — thiocyanate

I do not see a route of going from HCN to R-N=C=S right away.

Lets look @ digested hair

We have excellent success in the microwave - NaOH (10M) digestion of hair w/ 15-20 min @ power 10%.

Hair changes color (the NaOH solution) @ the neutral pH point. Interesting that some filamentous material formed @ the same titration point.

You are going to need to look @ the ~~formed~~ formed filaments under the scope. One of them is red, a classic Morgellons formation.

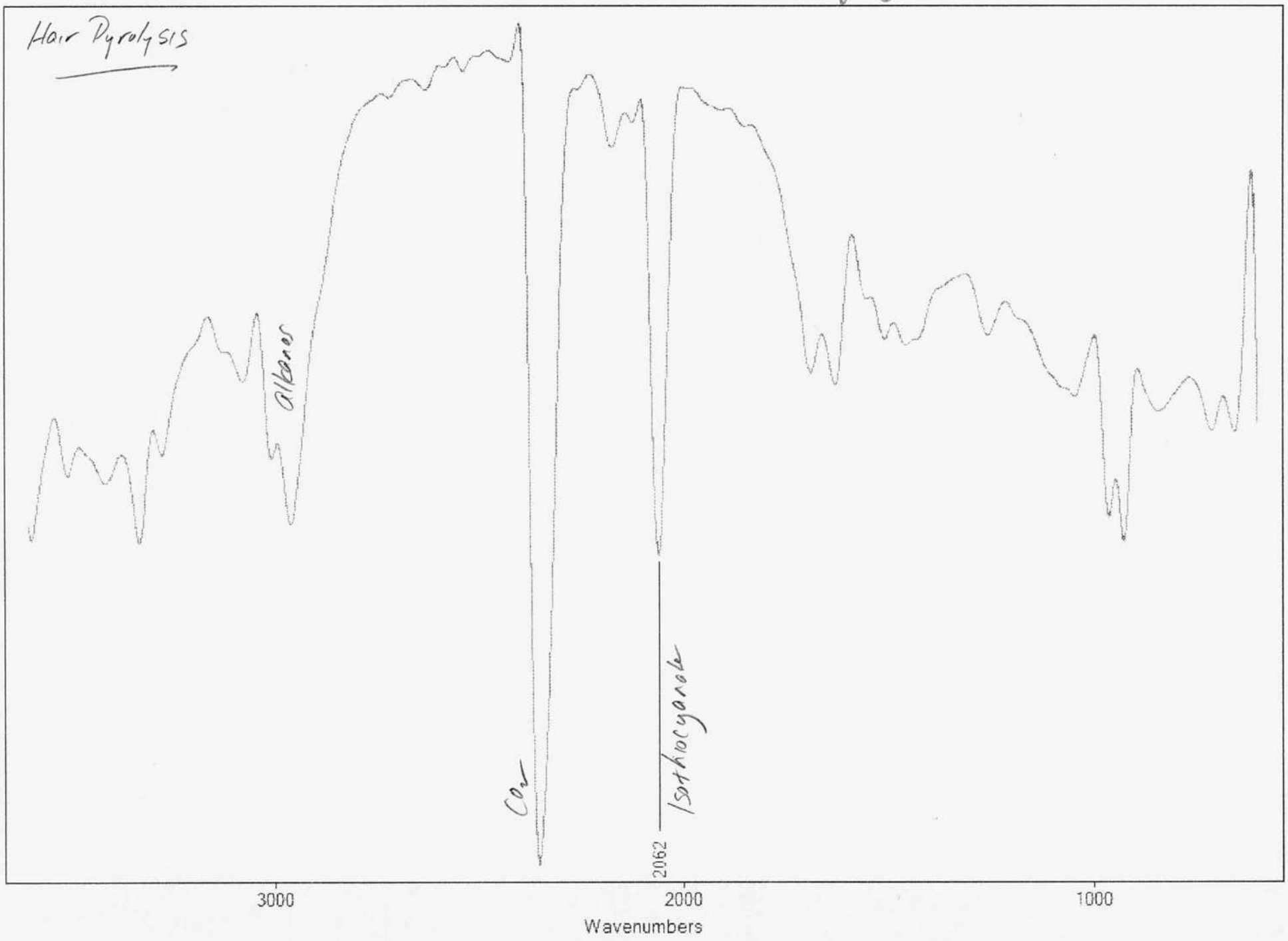
But the main fibers are definitely hairs. I wonder why these did not dissolve & only show up @ the equivalence point. There are only 2-3 of them in my 3 ml titrated sample.

You have become quite skilled @ making salt films for ATR IR analysis.
$NaOH + HCl \rightarrow NaCl + H_2O$

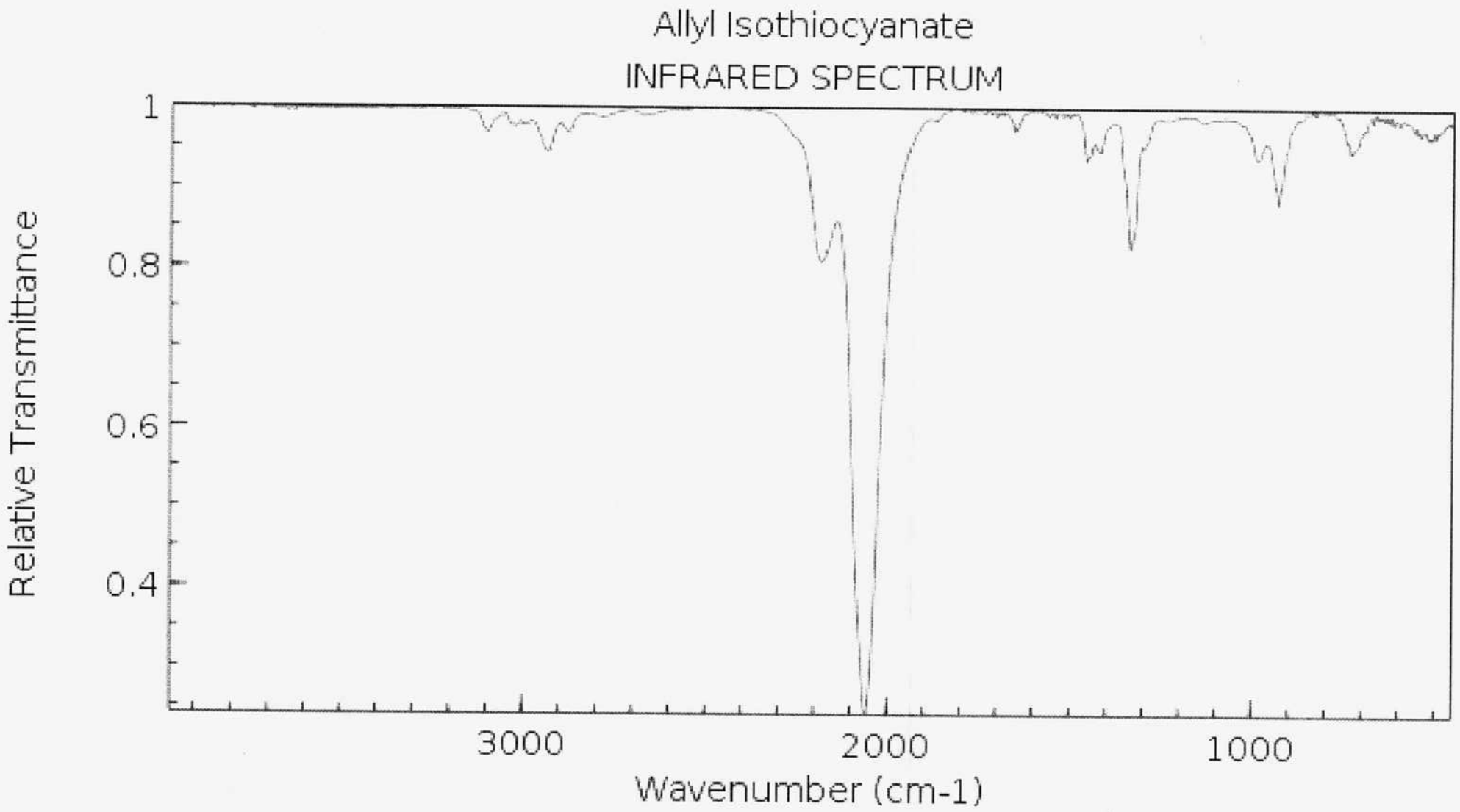
A good solid film presents a very clean signal. Approx 20 ul is required to develop the film.

[The isothiocyanate issue will need to be added to the Supplemental Discussion paper]

Hair Pyrolysis - Gas Cell [redacted] - Aug 29 2017 - 01.JPEG

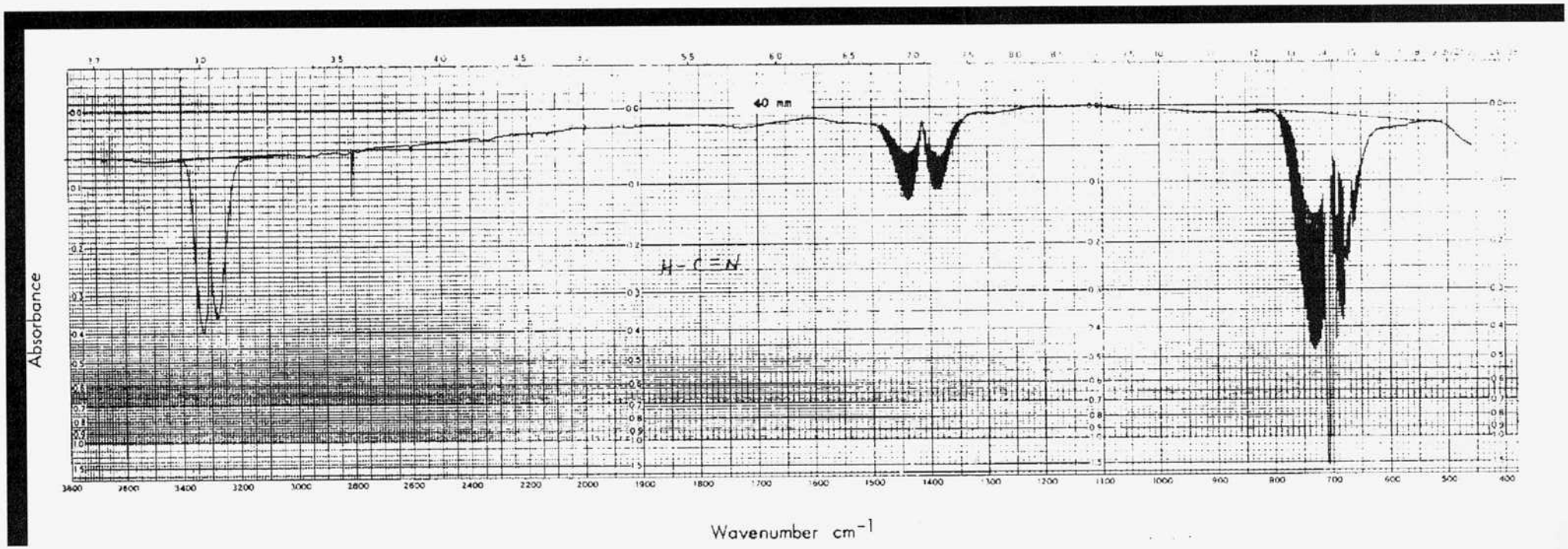


Isothiocyanate IR Spectrum



NIST Chemistry WebBook (http://webbook.nist.gov/chemistry)

Hydrogen Cyanide IR Spectrum



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Cyanide Chemistry

Cyanide Species

The term cyanide refers to a singularly charged anion consisting of one carbon atom and one nitrogen atom joined with a triple bond, CN^-. The most toxic form of cyanide is free cyanide, which includes the cyanide anion itself and hydrogen cyanide, HCN, either in a gaseous or aqueous state. At a pH of 9.3 - 9.5, CN^- and HCN are in equilibrium, with equal amounts of each present. At a pH of 11, over 99% of the cyanide remains in solution as CN-, while at pH 7, over 99% of the cyanide will exist as HCN. Although HCN is highly soluble in water, its solubility decreases with increased temperature and under highly saline conditions. Both HCN gas and liquid are colorless and have the odor of bitter almonds, although not all individuals can detect the odor.

Cyanide is very reactive, forming simple salts with alkali earth cations and ionic complexes of varying strengths with numerous metal cations; the stability of these salts is dependent on the cation and on pH. The salts of sodium, potassium and calcium cyanide are quite toxic, as they are highly soluble in water, and thus readily dissolve to form free cyanide. Operations typically receive cyanide as solid or dissolved NaCN or $Ca(CN)_2$. Weak or moderately stable complexes such as those of cadmium, copper and zinc are classified as weak-acid dissociable (WAD). Although metal-cyanide complexes by themselves are much less toxic than free cyanide, their dissociation releases free cyanide as well as the metal cation which can also be toxic. Even in the neutral pH range of most surface water, WAD metal-cyanide complexes can dissociate sufficiently to be environmentally harmful if in high enough concentrations.

Cyanide forms complexes with gold, mercury, cobalt and iron that are very stable even under mildly acidic conditions. However, both ferro- and ferricyanides decompose to release free cyanide when exposed to direct ultraviolet light in aqueous solutions. This decomposition process is reversed in the dark. The stability of cyanide salts and complexes is pH dependent, and therefore, their potential environmental impacts and interactions (i.e. their acute or chronic effects, attenuation and re-release) can vary.

Metal cyanide complexes also form salt - type compounds with alkali or heavy metal cations, such as potassium ferrocyanide ($K_4Fe(CN)_6$) or copper ferrocyanide ($Cu_2[Fe(CN)_6]$), the solubility of which varies with the metal cyanide and the cation. Nearly all alkali salts of iron cyanides are very soluble, upon dissolution these double salts dissociate and the liberated metal cyanide complex can produce free cyanide. Heavy metal salts of iron cyanides form insoluble precipitates at certain pH levels.

The cyanide ion also combines with sulfur to form thiocyanate, SCN^-. Thiocyanate dissociates under weak acidic conditions, but is typically not considered to be a WAD species because it has similar complexing properties to cyanide. Thiocyanate is approximately 7 times less toxic than hydrogen cyanide but is very irritating to the lungs, as thiocyanate chemically and biologically oxidizes into carbonate, sulfate and ammonia.

The oxidation of cyanide, either by natural processes or from the treatment of effluents containing cyanide, can produce cyanate, OCN^-. Cyanate is less toxic than HCN, and readily hydrolyzes to ammonia and carbon dioxide.

Cyanidation

The process of extracting gold from ore with cyanide is called cyanidation. The reaction, known as Elsner's Equation, is:

$$4\ Au + 8\ CN^- + O_2 + 2\ H_2O = 4\ Au(CN)_{2-} + 4\ OH^-$$

Page 208 D

Although the affinity of cyanide for gold is such that it is extracted preferentially, cyanide will also form complexes with other metals from the ore, including copper, iron and zinc. The formation of strongly bound complexes such as those with iron and copper will tie up cyanide that would otherwise be available to dissolve gold.

Copper cyanides are moderately stable; their formation can cause both operational and environmental concerns, as wastewater or tailings from such operations may have significantly higher cyanide concentrations than would otherwise be present in the absence of copper.

High copper concentrations in the ore increase costs and lower recovery efficiencies by requiring higher cyanide application rates to compensate for reagent that complexes with copper rather than gold.

Cyanidation is also adversely affected by the presence of free sulfur or sulfide minerals in the ore. Cyanide will preferentially leach sulfide minerals and will react with sulfur to produce thiocyanate. These reactions will also enhance the oxidation of reduced sulfur species, increasing the requirement for lime addition to control the pH at a sufficient level to avoid the volatilization of hydrogen cyanide (HCN).

Cyanide chemistry is complex, and those seeking additional information may find the list of reference materials found at the Code's website helpful: References.

*

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We now have a very clean & simple IR plot of hair digested in NaOH, microwaved & neutralized

The isothiocyanate absorption is visible. This confirm isothiocyanate in hair in both solid & gaseous form (w/ pyrolysis).

We also have it in blood. Formerly in urine, but not now.

There is no evidence that it should be there in this form.

Affects:

1. Thyroid
2. Joints
3. Mineral utilization

Interesting that no Hydrocarbons show up in the IR plot.

*

I have to say that we have an incredible result. The hair digestion leads to a closest match, amongst ~ 6500 spectra in the library to:

1. Skin foliation from individual w/ strong Morg. Symptoms
2. Rainfall concentrate extracted into acetone,
3. The environmental filaments

We also have the isothiocyanate structure in each of the four spectra.

Oh my, oh my...

Hair Microwave Digestion NaOH [redacted] Aug 30 2017 - 01.JPEG

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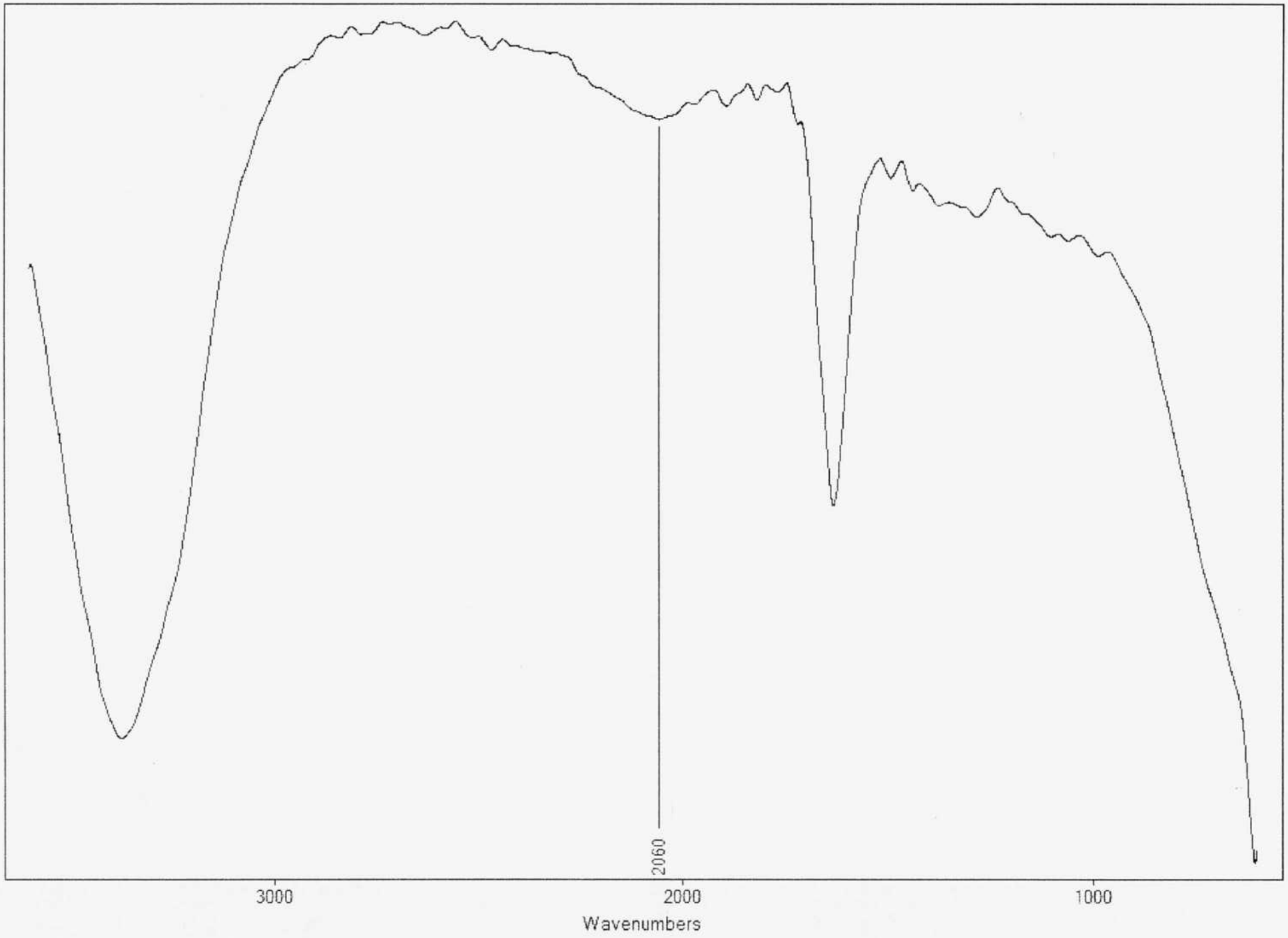

Hair Microwave Digestion NaOH [redacted] Compare to [redacted] Skin Foliate Aug 30 2017 - 02.JPEG r=0.98

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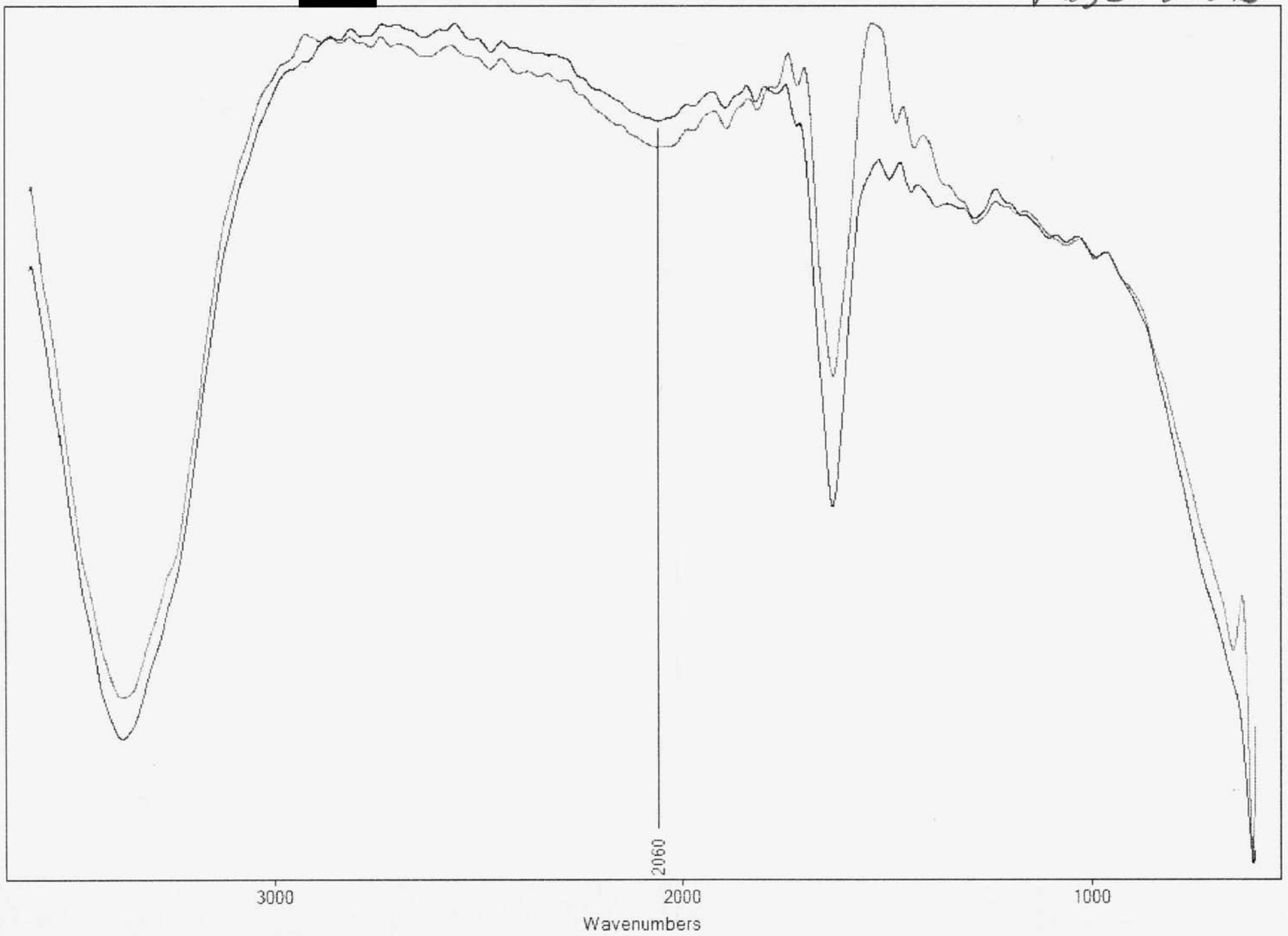
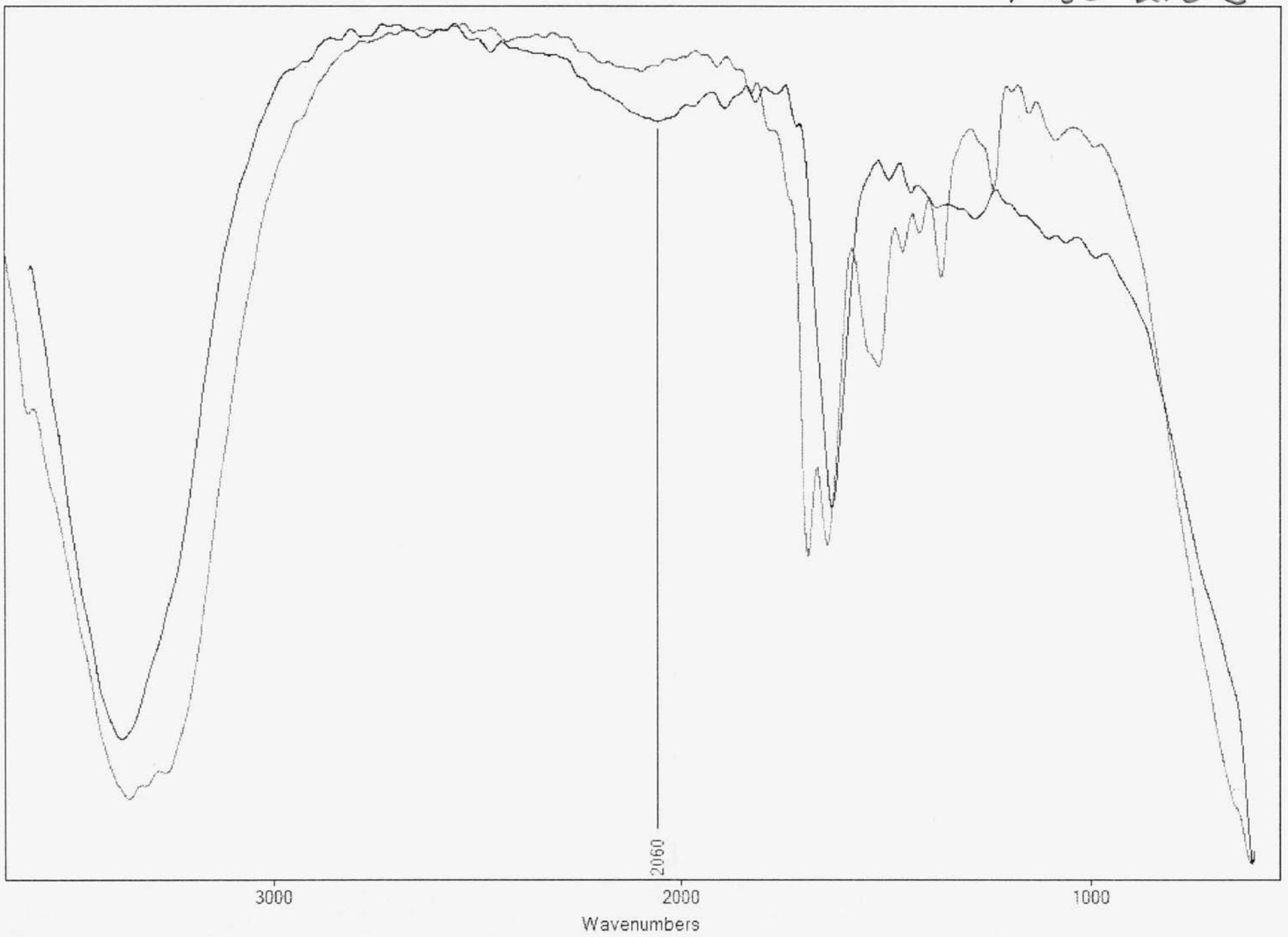
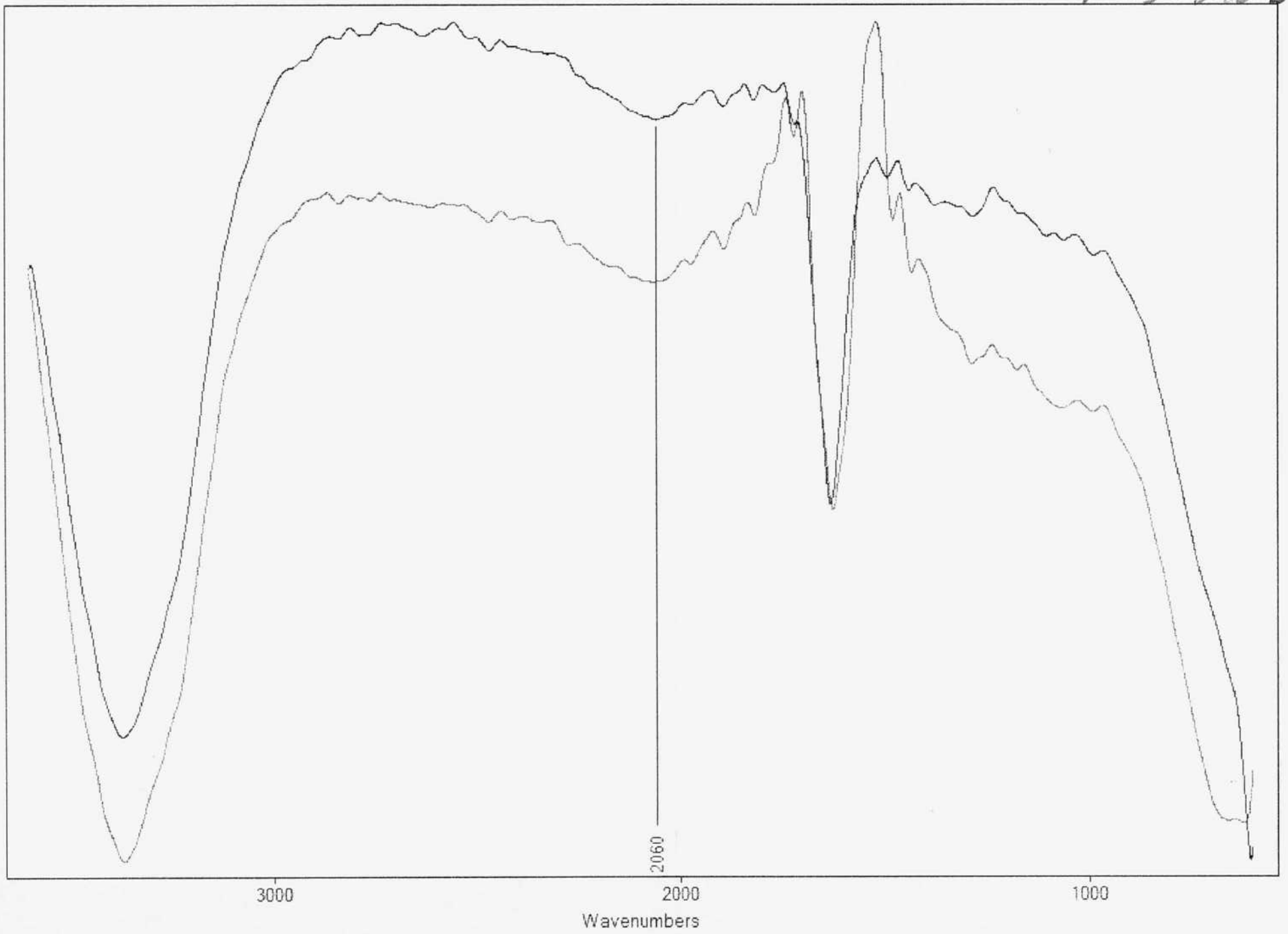


Hair Microwave Digestion NaOH [redacted] Compare to Rain Concentrate Aug 30 2017 - 03.JPEG r=0.97

Base 210 C



Hair Microwave Digestion NaOH [redacted] Compare to Env Filament Aug 30 2017 - 04.JPEG r=0.94



Aug 31 2017

I am interested in the difference between the IR absorption peaks of proline vs isothiocyanates.

Saliva (Proline known)
Blood
Hair
Urine
CDB Viscous Dokin
(Isothiocyanate known)

There is question whether the interest in proline is actaly a interest in isothiocyanates.

Ok, we how good IR information on saliva & blood.

In forensic but

Saliva has a strong peak @ 2057 which is classified as a thiocyanate,

but IR Pal gives the range of 1990-2140 ($\bar{x}$ = 2065) as R-N=C=S which is an isothiocyanate.

We know ~~three~~ two types of thiocyanate

$[SCN]^-$ thiocyanate

R-N=C=S isothiocyanate

Now the peak in saliva is known to come from proline.

We now have a very important graphic that has been constructed that has:

1. Proline Lewis structure
2. Blood IR
3. Saliva IR.

Now, there are some interesting issues here.
Saliva absorbs fairly strong @ 2057
2057 is classified as a thiocyanate by the forensic thesis
2057 is classified as a <u>iso</u>thiocyanate by IR pal

but Proline <u>does not</u> have sulfur in it!

So there are two problems here:

1. Is 2057 thiocyanate or isothiocyanate?
2. How can it be proline when proline does not have sulfur in it?

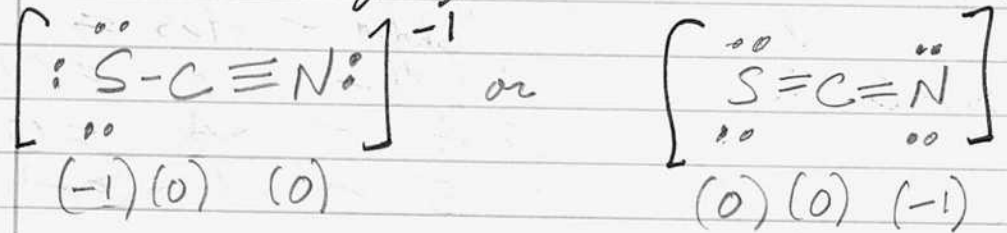
Thiocyanate $[SCN]^-$

Maybe the difference is not as great as you think.

Isothiocyanate $R-N=C=S$

Electronegativity
Sulfur: 2.58
Nitrogen: 3.04 ← More electronegative

Then ends up being a very interesting topic involving resonance & formal charge determination (Remember that?)



because of electronegativity of nitrogen vs sulfur Preferred Resonance Structure

Formal charge of both forms is -1, but structure to right is preferred.
Great yt tube video on this topic

I have found a very smooth method to determine formal charge.

You go through each atom one at a time using the following relation:

Formal Charge of a particular atom

Should (have a certain number of valence electrons)	−	Has (actually this no. of valence electrons

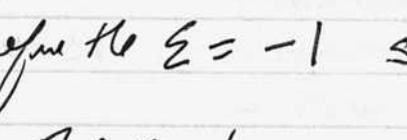
You must then add up each particular atom to get the total formal charge @ the end.

Example:

(−1) (0) (0)

from periodic chart:

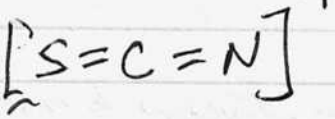
	Actually Has	
Sulfur should have	6 − 7	= −1
Carbon should have	4 − 4	= 0
Nitrogen should have	5 − 5	= 0
		Σ = −1

and therefore the Σ = −1 so 

Voila! This is why $[SCN]^{-1}$ has a formal charge of −1

But because of electronegativity of N > S, we have

	Should	−	Has	=
S:	6	−	6	= 0
C:	4	−	4	= 0
N:	5	−	6	= −1



Formal Charge Determination:
A Simpler Method - Very good

Proline Lewis Structure - Blood and Saliva IR

Formal Charge = Should – Has

Formal Charge Formula and Shortcut
Definitely faster, right?

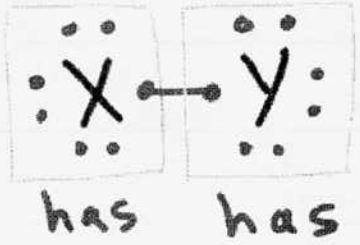
This shortcut is guaranteed to save precious seconds on your exam IF AND ONLY IF you understand how to apply it.

But when you understand it you'll be able to solve formal charge in your head, in under 8 seconds per atom.

Let's make sure you understand this shortcut

Should = the number of valence electrons that a neutral atom SHOULD have.

Has = the number of electrons an atom HAS directly attached, touching the atom in question.



Lone pairs represent 2 electrons sitting on the atom so that Has = 2

Each bond only counts for a single electron since the second electron in the bond is touching the other atom.

Now we understand what the thiocyanate ion looks like.

And now, because of this we see that thiocyanate and isothiocyanate are not really any different @ all other than the presence of the R group. And in IR we always have the presence of the R group anyway! So as far as IR goes, they can essentially be regarded as equivalent and therefore the previous assignment to thiocyanate is all fine and good.

Now, the remaining question is: How can sulfur be designated for problems which accompanied demonstrated by IR cyanate present when Problem does not have sulfur in it?

A very important graphic on the distinctions between blood, saliva & proline

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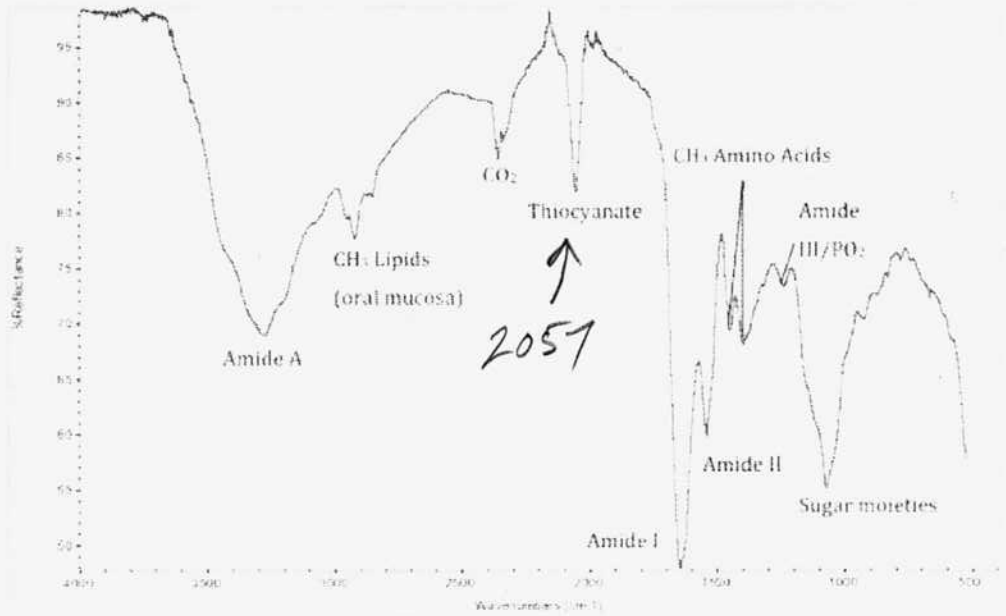
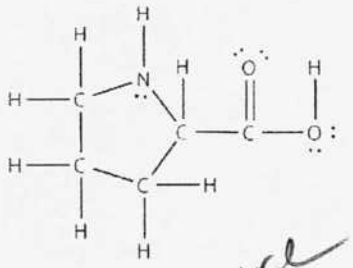
Proline Lewis Structure - Blood and Saliva IR

Saliva IR



Lewis Structure for Proline



Proline Lewis Structure

Figure 3.15. ATR-FTIR spectrum of neat saliva air dried in situ at 5 hours.

Blood IR

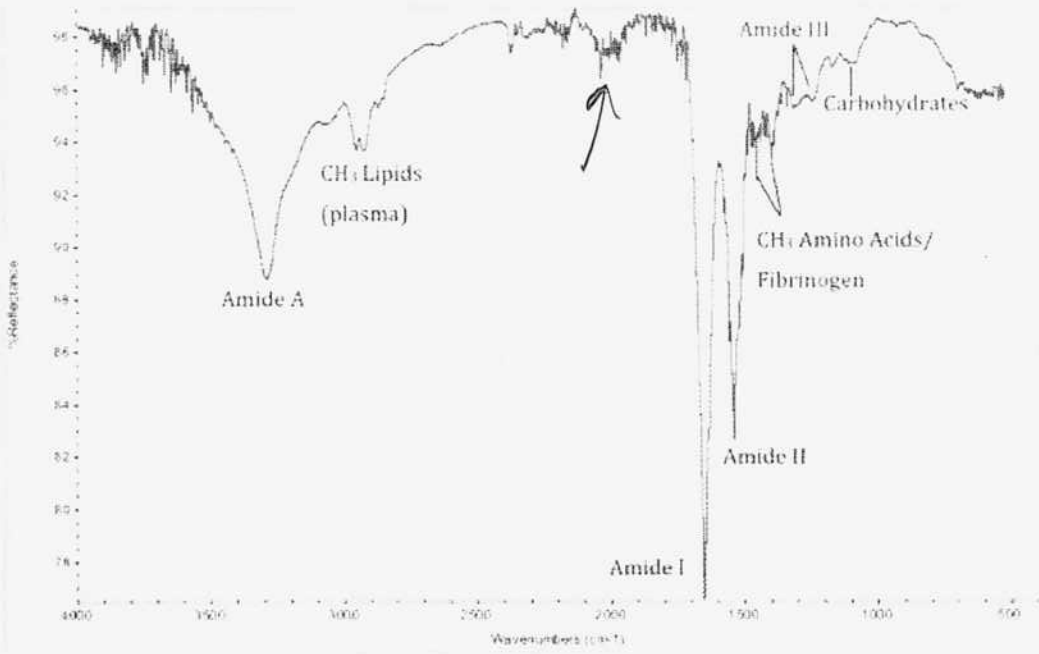


Figure 3.4. ATR-FTIR spectrum of neat blood air dried in situ at 5 hours.

Now the question is, why does proline show a thiocyanate peak when it does not have sulfur in it?

Does saliva have thiocyanates in it? Why?

There is a great color test method that we know of. Fe^{3+} + Sodium Thiocyanate = RED Complex

Smokers have thiocyanates in saliva, but do most of us?

Yes, thiocyanates can be detected by simply adding Fe+3 to the solution

Saliva (w/ thiocyanates) + Fe+3 = Red Color

So the answer is, it is indeed thiocyanates in saliva that are producing the 205 peak, it is not proline, per se.

Now, the question is, does proline commonly react with sulfur to produce thiocyanates?

Iso this only means that there is an R group attached.

So now the question is, what is the relationship between proline & ~~the~~ thiocyanates, IF ANY?

Page 218A

Thiocyanate in plasma and saliva

Matrix:	Plasma and saliva
Hazardous substances:	Hydrogen cyanide, cyanides and cyanide releasing chemicals
Analytical principle:	Photometry in microtiter plates
Completed in:	October 1998

Overview of the parameters that can be determined with this method and the corresponding hazardous substances:

Hazardous substance	CAS	Parameter	CAS
Hydrogen cyanide	74-90-8	Thiocyanate	302-04-5
Cyanides	57-12-5		
Sodium cyanide	143-33-9		
Potassium cyanide	151-50-8		
Cyanogen chloride	506-77-4		
Oxalic acid dinitrile	460-19-5		
Acetonitrile	75-05-8		
Acrylonitrile	107-13-1		

Summary

Thiocyanate (rhodanide) is the main metabolite of cyanide and can thus be used as biomarker for exposure to cyanide or to cyanide releasing chemicals. Especially for chronic exposure to low cyanide concentrations, such as occur for example in smoking and at a number of workplaces, thiocyanate in plasma and saliva is a suitable biomarker. The procedure described here is based on a method published by Degiampietro et al. [1] and permits the rapid and reliable determination of thiocyanate in plasma and saliva. It is a photometric method performed in 96-well plates for high throughput using a plate reader. When Fe(III) ions are added to samples containing thiocyanate (SCN^-), a red complex is formed, which is measured close to its absorption maximum at 492 nm. After addition of mercury(II) nitrate, which forms a colourless $[Hg(SCN)_4]^{2-}$-complex, the sample blank value is determined and subtracted.

The MAK-Collection Part IV: Biomonitoring Methods, Vol. 13
DFG, Deutsche Forschungsgemeinschaft
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Page 218B

Thiocyanate in plasma

Within day precision:	Standard deviation (rel.)	s_w = 17.4% or 4.2%
	Prognostic range	u = 48.4% or 10.8%
	at a concentration of 1.33 mg or 12.4 mg thiocyanate per litre plasma and where n = 5 or 6 determinations	
Day to day precision:	Standard deviation (rel.)	s_w = 12.8% or 5.1%
	Prognostic range	u = 30.2% or 12.0%
	at a concentration of 2.49 mg or 11.0 mg thiocyanate per litre plasma and where n = 8 determinations	
Accuracy:	Recovery rate (rel.)	r = 114.8% or 98.4%
	at a nominal concentration of 2.71 mg or 12.1 mg thiocyanate per litre plasma and where n = 4 determinations	
Detection limit:	0.76 mg thiocyanate per litre plasma	
Quantitation limit:	2.28 mg thiocyanate per litre plasma	

Thiocyanata in saliva

Within day precision:	Standard deviation (rel.)	s_w = 1.4% or 2.9%
	Prognostic range	u = 3.9% or 8.1%
	at a concentration of 38 mg or 167 mg thiocyanate per litre saliva and where n = 5 determinations	
Day to day precision:	Standard deviation (rel.)	s_w = 2.7% or 1.2%
	Prognostic range	u = 6.4% or 2.8%
	at a concentration of 36 mg or 162 mg thiocyanate per litre saliva and where n = 8 determinations	
Accuracy:	Recovery rate (rel.)	r = 103% or 100%
	at a nominal concentration of 38.9 mg or 112.7 mg thiocyanate per litre saliva and where n = 4 determinations	
Detection limit:	0.76 mg thiocyanate per litre saliva	
Quantitation limit:	2.28 mg thiocyanate per litre saliva	

Thiocyanate

Detoxification of cyanide, a potent inhibitor of cellular respiration, occurs primarily through formation of thiocyanate. The major mechanism to form thiocyanate in the human body is the enzymatic transfer of a sulfur atom from thiosulfate to the cyanide by thiosulfate sulfurtransferase (rhodanase) [2] (Figure 1). Thiocyanate in body fluids (plasma, saliva, urine) can therefore be used as a biomarker of exposure to cyanides or to cyanide-releasing chemicals. On account of the relatively long half-life of 6–14 days in the mentioned body fluids [3, 4], thiocyanate is especially suitable for the detection of chronic low-dose exposure to cyanide, whereas the determination of cyanide in blood is mainly used for acute cyanide exposure [2].

Gaseous hydrogen cyanide is liberated from cyanide salts when in contact with acids or carbon dioxide and is thus occurring wherever cyanides are handled. This

Page 218C

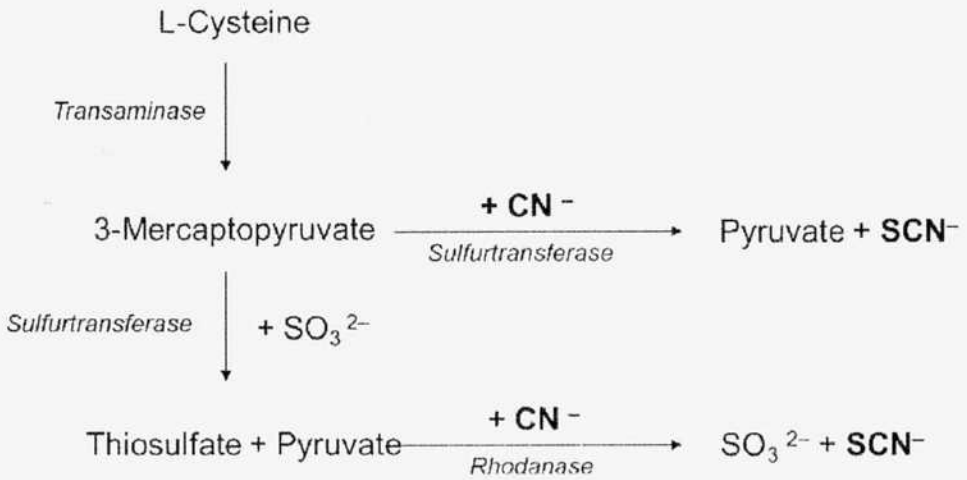

Fig. 1 Thiocyanate formation from cyanide acc. to [5]. Thiocyanate is formed by enzymatic transfer of sulfur from 3-mercaptopyruvate (via sulfurtransferase) or from thiosulfate (via rhodanase) to cyanide. The formation of thiocyanate mainly takes place via rhodanase.

is the case among others where galvanic baths are used. Hydrogen cyanide is in addition readily formed from nitriles as acetonitrile, acrylonitrile and cyanohydrins [6, 7] and is released from the combustion of nitrogen-containing plastics. In addition, hydrogen cyanide is used as a fumigant on ships.

Compared with non-smokers, smokers show 2 to 3 times higher thiocyanate levels (Table 1). The mainstream smoke of a cigarette contains about 50–200 µg hydrogen cyanide [8]. Up to the 1980s, thiocyanate levels were frequently used to differentiate between smokers and non-smokers, and as an objective measure for exposure to tobacco smoke. Today, cotinine is used as a more specific biomarker of tobacco smoke exposure [9]. The specificity of thiocyanate as a marker of exposure to low cyanide concentrations at the workplace or from active smoking is limited by the fact that cyanides or thiocyanate occur also in several foods. Cyanogenic glycosides occur in almonds, nuts, pulses, bamboo shoots, beans, linseed and beer.

Table 1 Thiocyanate levels in plasma and saliva of non-smokers and smokers (MV ± SD).

Non-smokers	Smokers	Reference
Thiocyanate in plasma (mg/l.)		
3.47 ± 2.39 (n = 6815)	9.09 ± 3.41 (n = 10377)	Bliss and O'Connell, 1984 [11]
3.16 ± 1.75 (n = 1356)	10.10 ± 3.22 (n = 5090)	Ockene et al., 1987 [12]
3.08 ± 1.59 (n = 3274)	10.04 ± 3.03 (n = 4553)	Ruth and Neaton, 1991 [13]
Thiocyanate in saliva (mg/l.)		
70.9 ± 44.2 (n = 242)	158 ± 64.5 (n = 287)	Bliss and O'Connell, 1984 [11]
75.5 (n = 100)	142 (n = 94)	Jarvis et al., 1984 [14]
97.0 (median) (n = 207)	170 (median) (n = 117)	Degiampietro et al., 1987 [1]

Page 218D

Furthermore cyanides are present in the seeds of pome and stone fruits and they are in that way also present in fruit brandies. Preformed thiocyanate (in form of glucosinolates) occurs in cabbage, root vegetables, mustard and milk [10, 11]. These sources make it generally difficult to evaluate thiocyanate levels as biomarker of exposure for cyanide.

Authors: *K. Riedel, H. W. Hagedorn, G. Scherer*
Examiner: *J. Angerer*

We now have a very good reference IR-ATR saliva spectrum.

We notice that our isothiocyanate peak in saliva seems to be considerably weaker than that shown in reference spectra. Recall toxicity measurements for smokers.

We also notice that our urine seems to have a reduced level

and that our blood level seems to match. expected reference spectra.

It would seem as though IR could be used to some extent for concentration work. (but volume & distribution of sample vary, not like solutions in a test tube)

Based upon a Colorimetric test w/ saliva & ferric nitrate, I also seem to show little to not detectable isothiocyanates in my saliva. This may well be why my teeth have been saved w/ ~~what~~ those that remain.

IR - ATR Saliva Plot - [redacted] - Good Quality

Saliva ATR [redacted] Aug 31 2017 - 01.JPEG

Page 2204

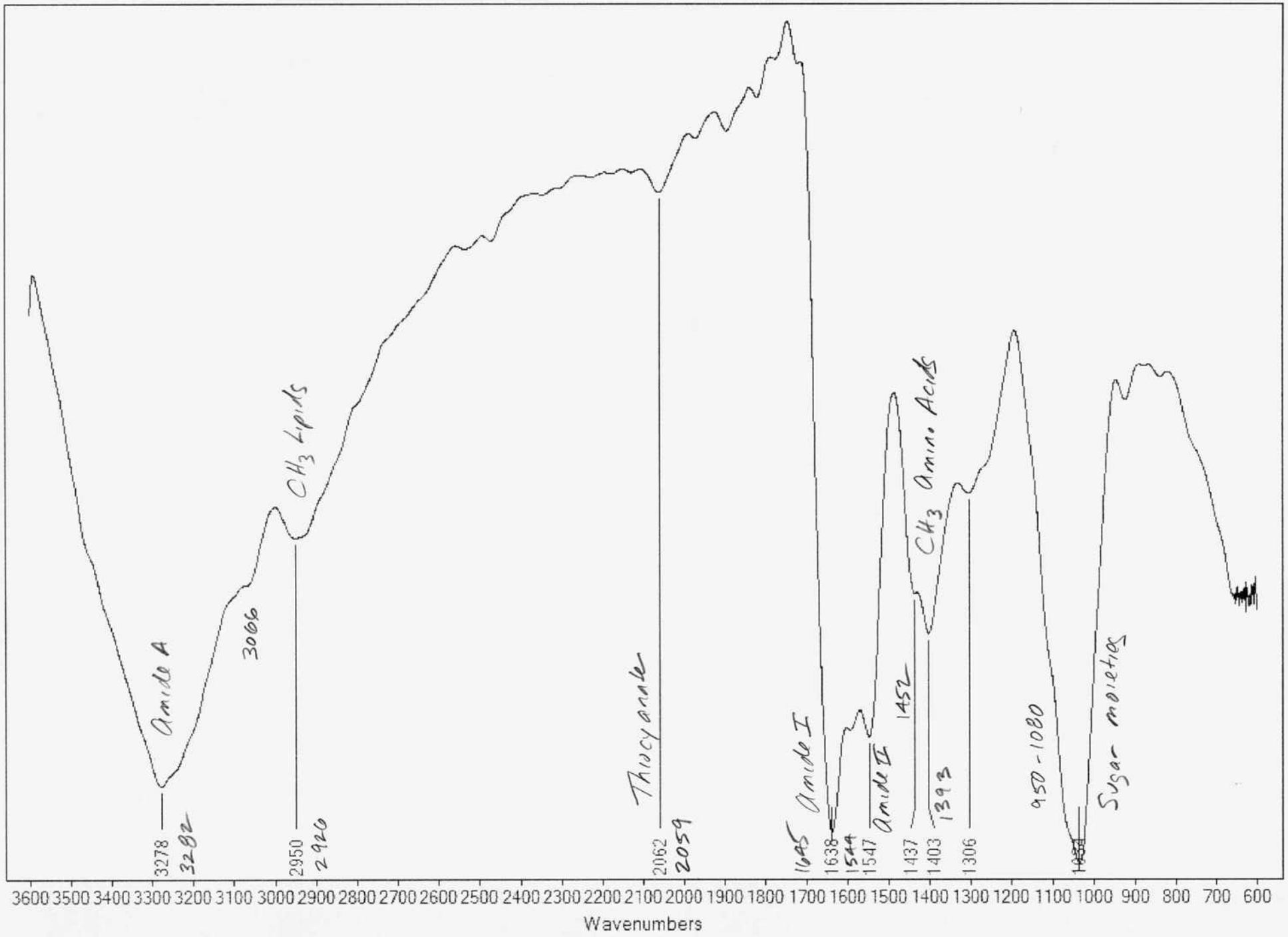

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Table 3.5: ATR-FTIR peak component identification for saliva.

Wavenumber (cm^{-1})	Component Identification	Vibrational Mode	Reference
3282	Amide A	H bonded OH stretching, NH stretching	Garidel & Schott (2006b); Khaustova et al. (2010)
2926, 2850	Methylene stretches of lipid acyls in oral mucosa	Asymmetric & symmetric CH_2 stretching	Khaustova et al. (2010); Scott et al. (2010); Yoshida & Yoshida, (2004)
2059	Thiocyanate anions (SCN^-)	CN stretching	Schultz et al. (1996); Scott et al. (2010); Shaw & Mantsch, (2006)
1645	Amide I (α helix)	C=O stretching	Garidel & Schott (2006b); Khaustova et al. (2010); Movasaghi et al. (2008); Scott et al. (2010); Sultana et al. (2011)
1544	Amide II	NH bending coupled to CN stretching	Garidel & Schott (2006b); Khaustova et al. (2010); Movasaghi et al. (2008); Scott et al. (2010); Sultana et al. (2011)
1452	Methylene bending of amino acid side chains of proteins & lipids	Asymmetric CH_2 bending	Ahmed & Mantsch (1994); Khaustova et al. (2010); Scott et al. (2010)
1393	Amino acid protein side chains	Symmetric CH_2 bending	Khaustova et al. (2010); Scott et al. (2010); Sultana et al. (2011)
1239	Amide III/Phospholipids	CN stretching, asymmetric PO_2^- stretching	Arrondo & Goñi (1998); Movasaghi et al. (2008); Sultana et al. (2011)
1080-950	Sugar moieties (glycosylation)	CH_2OH groups, CO stretching and bending of COH groups, symmetric PO_2^- stretching.	Ahmed & Mantsch (1994); Khaustova et al. (2010); Scott et al. (2010); Sultana et al. (2011)

Whole blood and semen identification using mid-infrared and Raman spectrum analysis for forensic applications

Check for updates

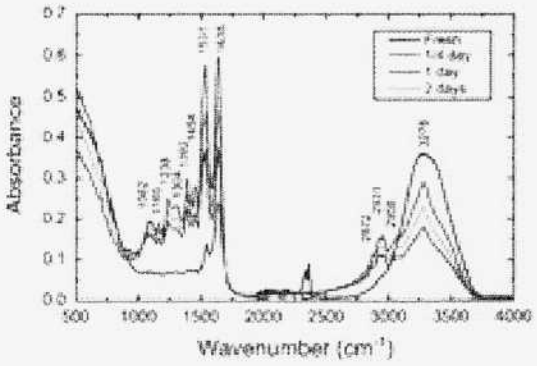
Page 203 A

Yun Zou[a] Pan Xia[b] Feiyu Yang[a] Fangqi Cao[a] Ke Ma[a] Zhongliang Mi[b] Xiaochun Huang[c] Nengbin Cai[b] Sai Jiang[c] Xuejun Zhao[a] Wenbin Liu*[a] and Xianfeng Chen*[c]

Author affiliations

Abstract

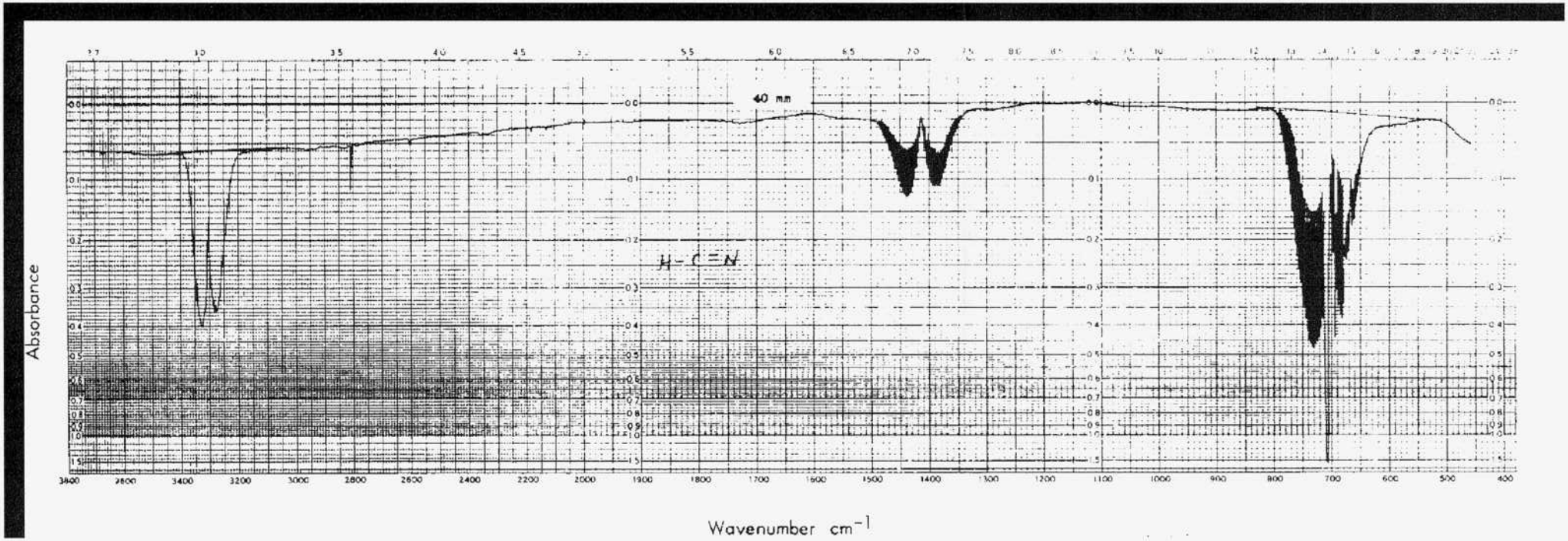
The identification of body fluids is important in forensic science. This paper describes the application of mid-infrared and Raman spectroscopies in the non-destructive identification of human blood and semen, where other detailed information can also be obtained in one single measurement. Samples of human blood and semen were probed and characterized utilizing Attenuated Total Reflection Fourier Transform Infrared (ATR-FTIR) and confocal Raman spectroscopies. The result shows their ability to identify an unknown substance to be human blood or semen without the use of chemical reagents. Age determination of dried blood and semen spots through their mid-infrared spectra was investigated, which could probably be used during forensic casework. Furthermore, the origin of the Raman scattering peaks of human semen at 2907 and 2968 cm^{-1} is detailedly analyzed, which has not been studied in previous literature. Overall, this optical detection and identification method exhibits advantages over conventional chemical methods in terms of non-destruction, high sensitivity, rapid detection and direct confirmation.



Thyroid (hypo) & joints are two hallmark symptoms of Morgellons

Isothiocyanates should not be resident in the blood but they are. This is affecting the thyroid & joints.

Hydrogen Cyanide IR Spectrum



Proline Lewis Structure - Blood and Saliva IR

Saliva IR

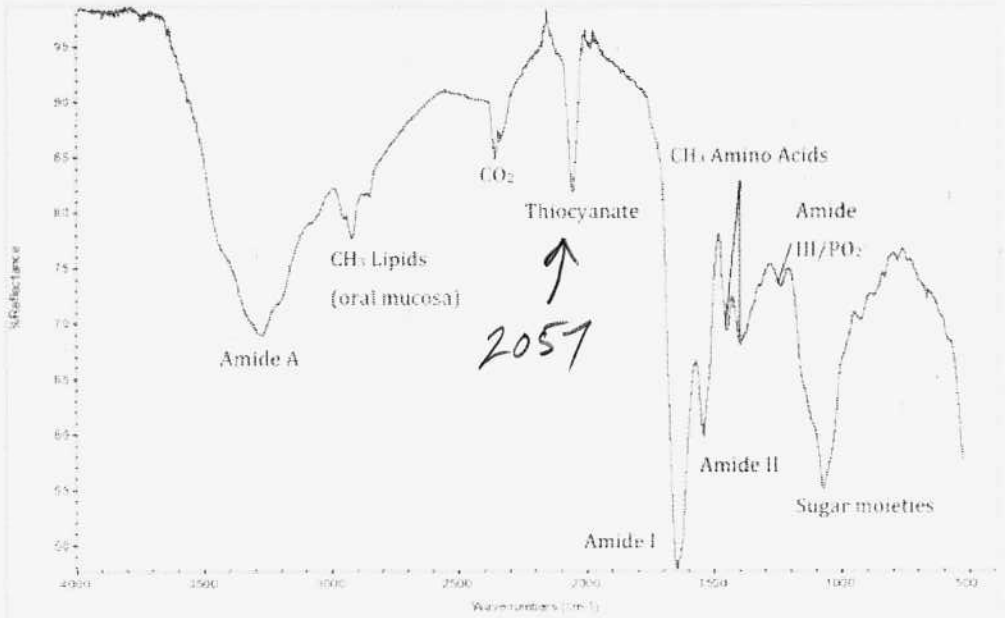
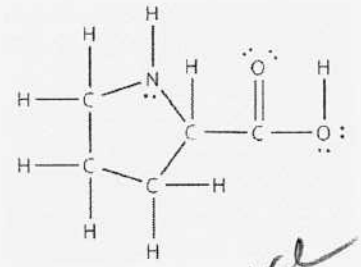


Figure 3.15: ATR-FTIR spectrum of neat saliva air dried in situ at 5 hours.

Lewis Structure for Proline



Proline Lewis Structure

Blood IR

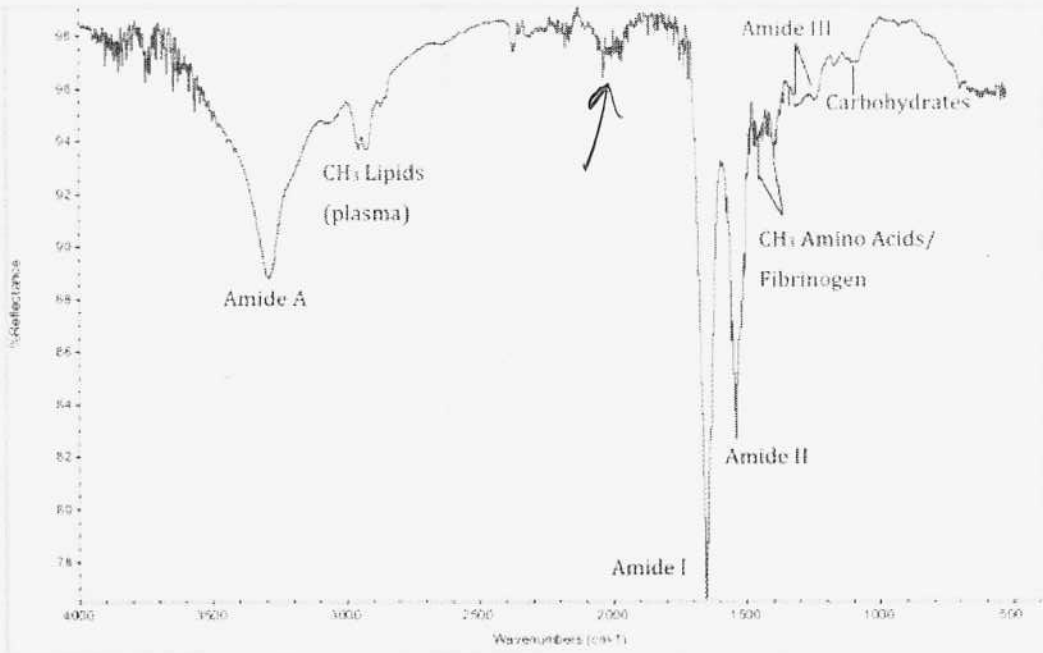


Figure 3.4: ATR-FTIR spectrum of neat blood air dried in situ at 5 hours.

[redacted] isothiocyanate - ferric color test does indeed produce a stronger color reaction than [redacted] My result matches the control of $H_2O + FeCl_3$.

Thus is my hypothesis. Isocyo Isothiocyanate production will be an additional biomarker.

Sep 12, 2017

There are some interesting things taking place here.

First, we now have pure mustard oil available. The IR plot by ATR showed no thiocyanate presence. The IR plot by double cell KCl does show the weak presence of thiocyanate. Mustard seed powder produced the same weak response, but nevertheless detectable.

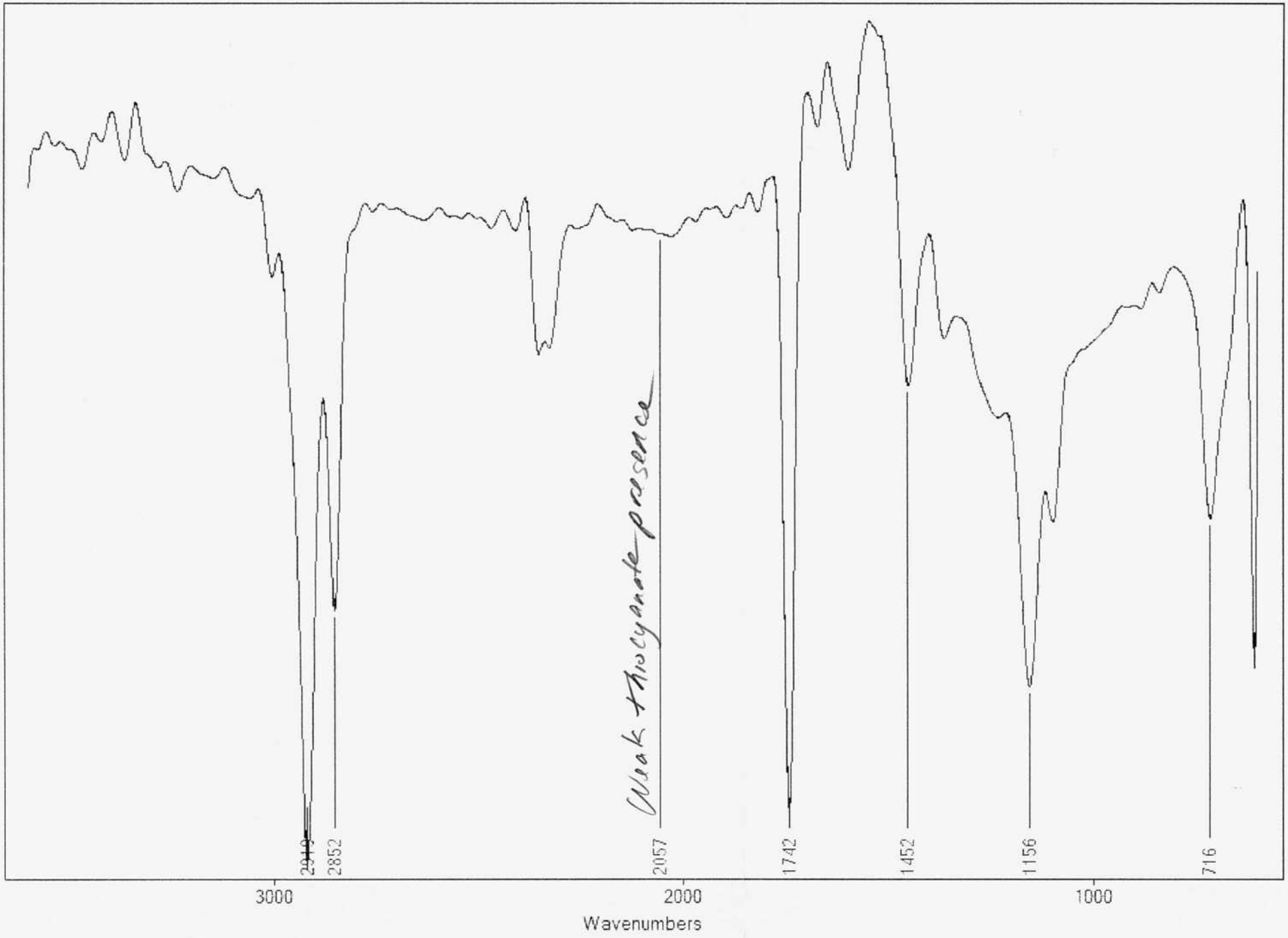
This tells us, that while mustard seed and mustard oil (the classic natural source for thiocyanates) contain thiocyanates, the amount actually seems quite low compared to our human and CDB biological study IR comparisons.

We therefore postulate that the concentration of thiocyanates (isothiocyanates) in some biological (human) samples of the CDB protein, as well as environmental samples (e.g., HEPA filter and Conc. rainfall) is actually quite high, relatively speaking. This would be a cause for much concern.

We see one case (ie, myself) where the thiocyanate concentration in the urine has been significantly reduced over the last couple of years. Conc. bio-available B-12 may have been an agent in the reduction.

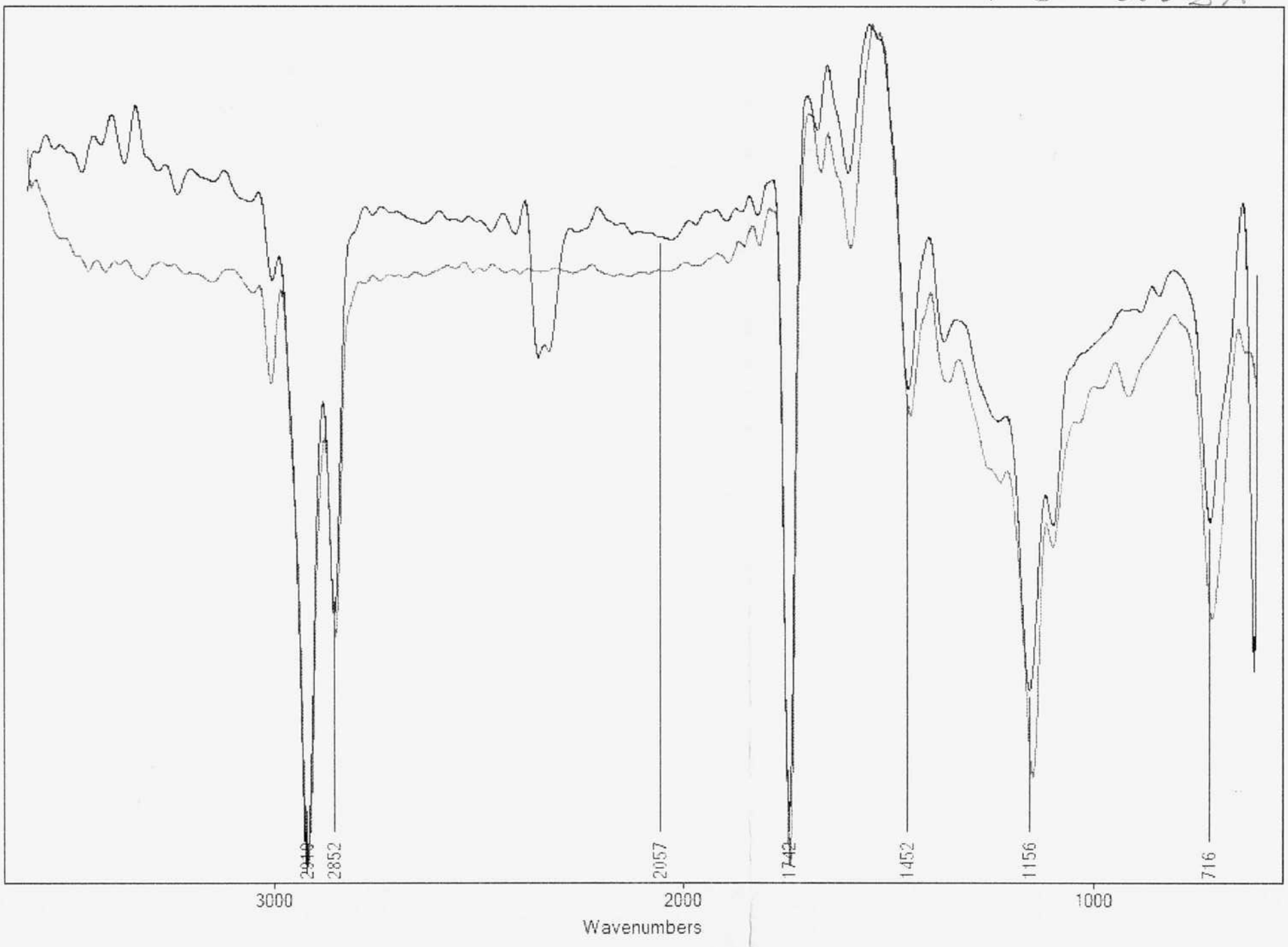
IR plots on next page.

Mustard Oil KCl Double Smoothed Sep 11 2017 - 01.JPEG



Mustard Oil KCl Double Smoothed Best Match Fish Oil Sep 11 2017 - 01.JPEG

Pase 223A



We also see that in comparison to the IR database (~ 6500 spectra) that the closest match is to ~~FISH OIL~~ FISH OIL. (ie, to mustard oil). Quite close, as a matter fact, r = 0.95.

The primary difference is indeed the lack of thiocyanates in the fish oil, all other absorptions are reasonably similar. This says that a comparison of chemical structure of mustard oil and fish oil would be a most interesting project, ie plant & vertebrate oil similarity.

The next thing observed of huge interest is that [redacted] when sample produces a relatively huge amount of ferric hydroxide complex when ferric nitrate is added to a urine sample. This is in contrast to my urine sample which produced almost no reaction. The hypothesis was that the ferric ion could be used to detect a thiocyanate existence, but the proposal has failed. It was expected to fail in my case, but not in the one that should IR thiocyanate present.

What did happen, however, in the urine sample that shows thiocyanate present is the formation of a presumed ferric hydroxide complex, stated to be essentially insoluble in both water, acidic & basic solution.

Now is where it gets even more interesting.
It has been learned that ferric hydroxide is more soluble in concentrated alkaline PLUS NaCl. This indeed has worked when the salt concentration is high enough & the pH is alkaline enough (high in both respects).

Dissolution to a large degree has taken place.

However, what is important is that the particular urine sample is ALSO PRODUCING A LIPID LAYER AT THE TOP OF THE SOLUTION. It floats to the top, has an emulsion quality to it and can be seen adhering to the test tube wall.

We appear to have definite lipid production with this particular reactive urine sample.

WHY and WHAT is the nature of this lipid?
Why this particular lipid sample that also shown a thiocyanate presence?
DOES urine normally have substantial lipids in it?

The conc. NaOH + NaCl solution is what has made the presence known in conjunction with a significant ferric ion Complex formation.

IT IS NOT A LIPID
IT IS A MODERATELY NON POLAR COMPOUND THAT FORMS AN EMULSION.

The is most certainly a candidate for IR, with removal of any water.
Na, Cl & Fe will be transparent, in general to the IR analyzer.

Let's go with this.

We now know that

"Human urine usually contains only very small amounts of lipids".

"However, under certain nephrotic syndromes excretion of cholesterol, triglyceride, free fatty acids & phospholipids is considerably increased".

So what is a "nephrotic syndrome"?

Lipiduria is the presence of lipids in urine

Nephrosis is any form of kidney disease.

We need new crystals (KCl) ~ 24mm x 3mm or thicker.

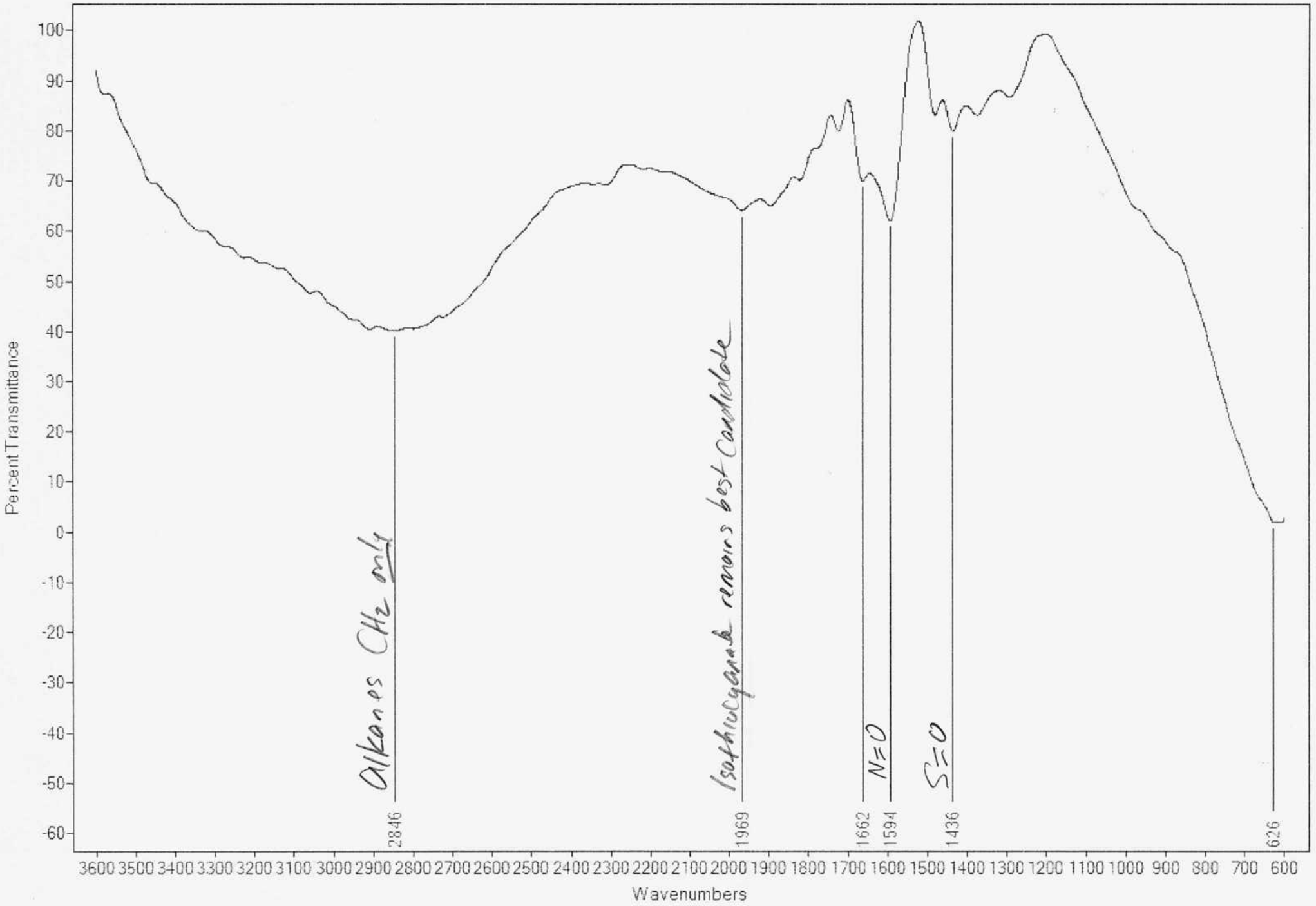
We see that the material is highly IR absorbed

This has been examined by IR, NIR, and UV. IR & UV Plots to right.
No significant NIR absorbance.

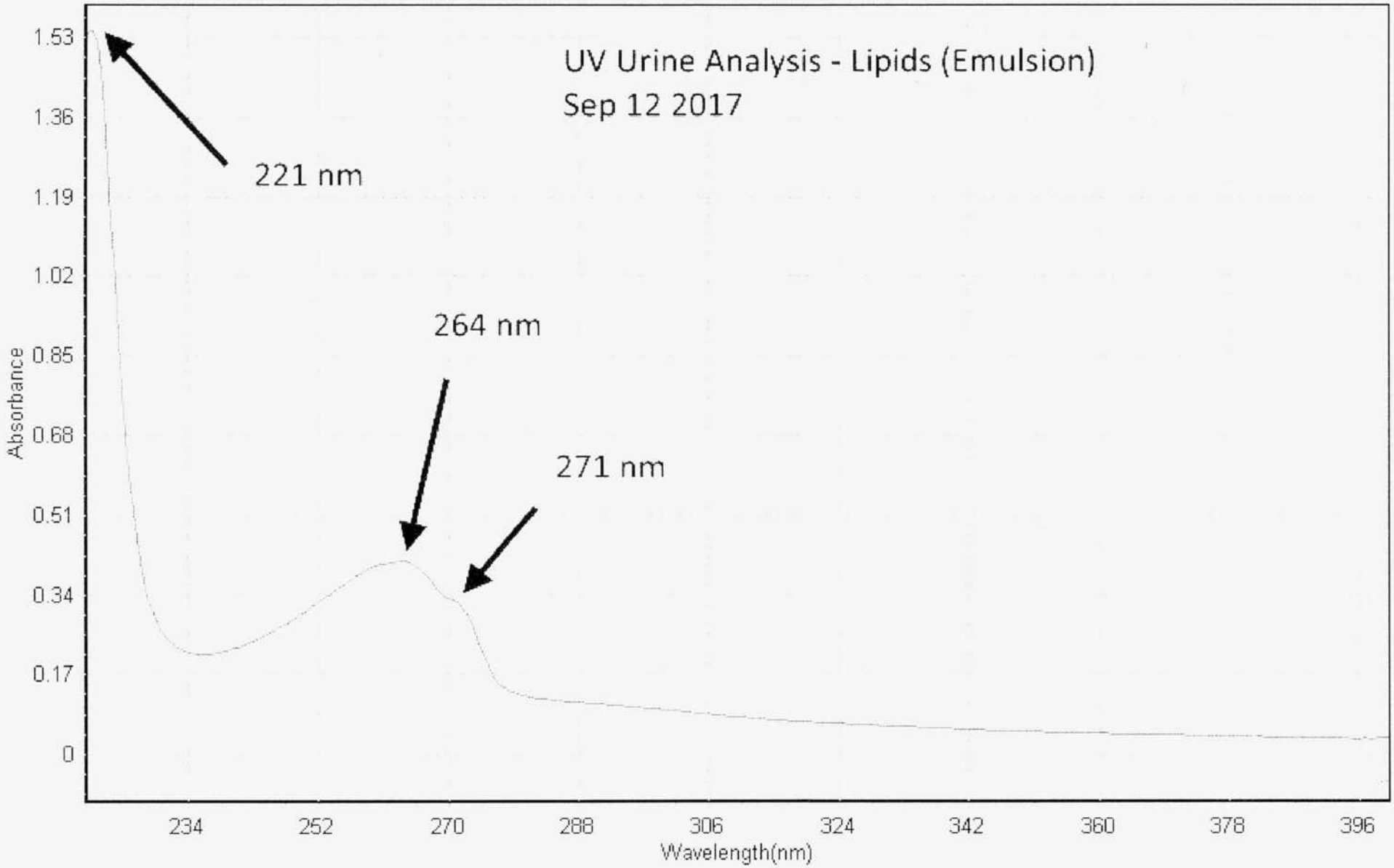
IR & UV Absorbance of Urine Lipids (Emulsion) Extraction

Urine Analysis Lipids ATR [redacted] Sep 12 2017 - 01.JPEG

Page 227A



Urine Analysis - Lipids Sep 12 2017 - 01.jpg



We have some interesting things going on with these absorption plots.

First, NIR has no significant absorption 700-1100 nm. We therefore do not learn anything there other than it indicates a lack of predominance of Carbonaceous Compounds.

IR: We have very detectable Thiocyanates @ the absolute border of the IR Window.
Recall:

R-N=C=S Isothiocyanates from 1990 - 2140 cm^{-1}
C≡N ~~Nitrile Isocyanate~~ 2222 - 2260

We now have a more explicit table available for IR absorption in this region.

On most occasions our IR absorption is @ ~2060 cm^{-1}
This would seem to clearly be isothiocyanate.
N=C=S On the ATR Wheel occasion, we are @ 1960 cm^{-1}
This suggests we may still be in range @ the edge of isothiocyanate, but we may also have altered the range of an allene. However we have no further evidence of alkene (above 3000) to support that contention. Isothiocyanate remains a primary candidate here.
C=C=C

Incidentally, only ATR produced a suitable plot here w/ an evaporated film. KCl evaporated did not produce a suitable IR plot.

2000-2400 Explicit IR Table

2400-2000 cm^{-1}						
2349	strong		O=C=O	stretching	carbon dioxide	
2275-2250	strong	broad	N=C=O	stretching	isocyanate	
2260-2222	weak		C≡N	stretching	nitrile	
2260-2190	weak		C≡C	stretching	alkyne	disubstituted
2175-2140	strong		S-C≡N	stretching	thiocyanate	
2160-2120	strong		N=N=N	stretching	azide	
2150			C=C=O	stretching	ketene	
2145-2120	strong		N=C=N	stretching	carbodiimide	
2140-2100	weak		C≡C	stretching	alkyne	monosubstituted
2140-1990	strong		N=C=S	stretching	isothiocyanate	
2000-1900	medium		C=C=C	stretching	allene	
2000			C=C=N	stretching	ketenimine	

Condensed IR analysis.

IR gives us the following proposed functional groups:

cm^{-1}	
2846	CH_2 (only, not CH_3)
~1970	N=C=S (absolute edge of window +)
1594	N=O, RNH_2 (amine)
1436	S=O

Now, lets look @ UV, Colby:
We have peaks @
271 weak
264 moderate
221 strong

There are 29 matches to this.

This looks hard to work with.

(X) The first proposal there for is for a moderately non polar hydrocarbon chain — Isothiocyanate Condensation or ring?

Focus on 264 nm + S & N.
Best candidate there is Chlorobenzenesulfonamide
Interesting on benzene ring, sulfur & amine group and S=O group.
This has some similarities.

There is an aromatic odor to this detectable in the test tube from the emulsion.

This further supports the hydrocarbon aspect. This is an interesting find

It has required ferric iron, strong alkaline, & salt to produce it. IR analysis reveals a rather unique plot that has not been seen before.

Later also perform IR database search.

Well, we have another interesting find here. Guess what the closest match is within the total IR database library that is available?

An extraction from the environmental filament using, no less, strongly alkaline w/ an alcohol

We are not dealing w/ a "lipid" here.

We are dealing with a mildly non polar compound in combination w/ isothiocyanate (most likely) that forms an emulsion in water

---

It appears to be a biomarker of the "environmental filament" expressed within the urine. We expect individuals to show this.

What, of course, are the health implications of these compounds?

They would certainly seem to be detrimental.

There was never a "lipid"! It is

a mildly non polar compound similar to mildly non polar solvents or even an aromatic alcohol (such as a phenol) likely attached to an isothiocyanate group.

*

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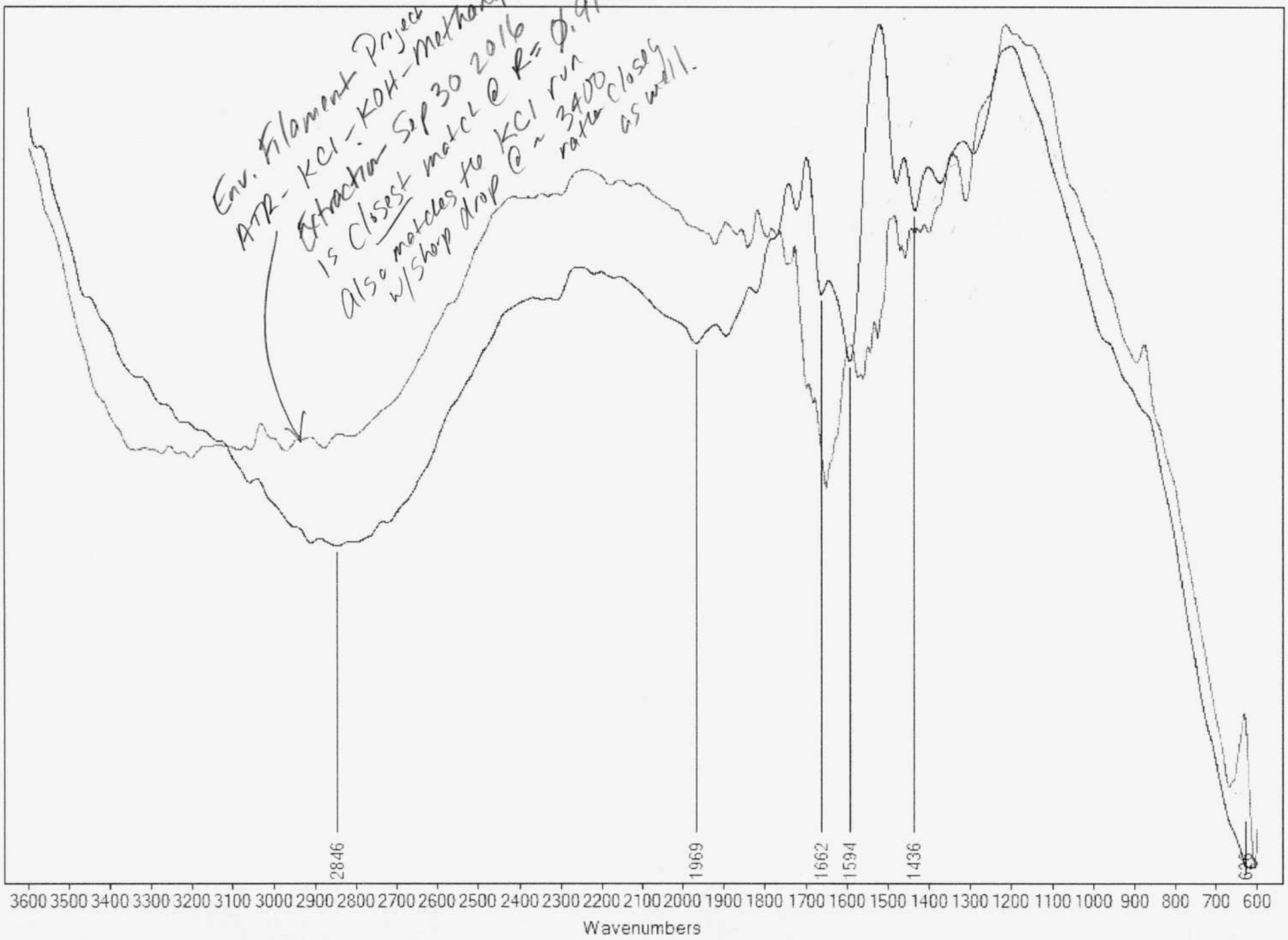
SEP 20 2016

"Mildly Extractive

Closest match to urine "Non Polar - thiocyanate"
Compound is FROM THE ENVIRONMENTAL FILAMENT!

Urine Analysis Aromatic Isothiocyanate Comparison ATR [redacted] Sep 12 2017 - 01.JPEG



* That is certainly important enough to end this notebook with.

The emulsion forms a very strong & copious iron oxide complex when added to H_2O w/ ferric nitrate.

The emulsion is highly concentrated. The ferric oxide complex (precipitate) is bright red orange and can be purified easily w/ centrifugation.

The emulsion is important for its purity. It will be important to work w/ from a diagnostic effect for its health effects with the study.

The nature of the emulsion requires deeper study. The precipitated ferric complex is the key to understanding its nature. UV & IR analyses are also helpful @ a preliminary level.

Incidentally, I have little doubt now that the fumes of pyrolysis of the COB viscous protein caused my extreme skin reaction on my neck and nose.

Sep 12 2017:

This is a crowning achievement of approximately 20 years of work today, dutifully noted and appropriately noted in the 20th volume of the laboratory notebooks.

It can now be stated that common elements of chemical and biological contamination, i.e. "assault" have now been isolated, identified, and linked between:

1. The Air
2. The water
3. The Notorious "Environmental Filament"
4. A specific microorganism, its culturing, and its metabolic products

AND:

5. OUR BODIES

AND

6. Essentially all of life on earth, including our food supply.

C. Carrico