CARNICOM INSTITUTE LEGACY PROJECT

A Release of Internal Original Research Documents

Authored
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CHEMISTRY VOL XXI

Mead

Chemistry - Lab Notes

Vol XXI

Sept 2017 –

Sep 14 2017

There may be a move to UT coming forth, so be ready for lots of change & a bit of chaos to that end.

—

Back to the urine analyses. This is becoming an interesting topic already. A significant precipitate is forming, now with two individuals, with the addition of ferric nitrate to the urine sample. It makes the solution very cloudy but it can easily be centrifuged out to produce a precipitate mass at the bottom and a very clear solution again on top.

We already has a suspicion that this involves a ferric oxide formation of some sort, but the question is why. I see no record of urine tests involving the use of ferric ions yet.

By seeking to dissolve a ferric oxide we see that it is generally not fairly to come by. We did, however, find that in strong alkaline solution w/ fairly strong NaCl will accomplish this to good end.

What we did not expect was to see an emulsion formed, which is what happened in the previous trial.

Furthermore, the closest IR match to this emulsion was the "Environmental Filament" – the notorious EPA "Environmental Filament". This against a database of ~ 6000 spectra.

It appears that we may have a very significant finding here.

The entire session has been motivated by solely the ferric-cyanide reaction, in pursuit of thiocyanate complexes observed in blood, urine, saliva, and the CDB protein, and rainwater & the HEPA filter. Come on, come all....

So, as well repeat the alkaline-NaCl approach to the two precipitate samples that have been collected.

We are dealing w ~ 5 ml urine
100 mg ferric nitrate solution (unknown concentration) has been added.
Lets add
10 drops 10M NaOH
We see that the NaOH is turning the light colored precipitate a bright rust color, but it does not dissolve it.
Now add 6/32 tsp salt. Excess salt visible on bottom of tube + 10 more drops NaOH.
No emulsion seen w/ the sample.

Next one: Same treatment.
There is no emulsion formed in either case.

But guess what?

There is a very significant reaction w/ the ferric nitrate ALONE.

Forget the NaOH, forget the salt.

There is a major reaction taking place w/ the ferric nitrate alone in sufficient quantity. You keep adding it until reaction stops and a significant change in color (muddy brown) fully develops. One sample accepted far more ferric nitrate than the other and yet produced a much stronger reaction (my urine).

Three layers are formed:

1. A substantial tan precipitate on the bottom.
2. The clear portion (urine colored in one sample, almost clear in my case)
3. A foam or suds or emulsion layer @ the top.

My sample
1. accepted far more $Fe(NO_3)$ in the reaction
2. Produced 2-3 times as much precipitate
3. 5-10 times as much foam / emulsion on top

We desperately have something to look @ here.

Here is a very unusual plot (IR) of the purported "emulsion" (top layer) from the CEC urine sample.

The most starkly striking feature is actually the LACK of absorbance @ ~ 1215 cm^{-1}.
This corresponds to many functional groups, such as: Amines, P-H, P=O, N-O, Esters (RCOOR')

The plot does not match anything well, other than the environmental filament plot recorded @ the end of Vol XX does show this same feature.

The "emulsion" production does not seem to be the same this time. I do not know why @ this time. On the last trial, it seemed as though dissolving of a precipitate took place. Not @ all this time. ??

The precipitate has NO significant absorbance of any kind. We do not have the same reaction as occurred on Sep 12 2017. ???

We cannot reproduce the reaction (emulsion). Why? Was there heating a concentration of the urine involved?

Actually, if you remember correctly, you acidified at first w/ 10M HCl and it made no difference, so that when you shifted to NaOH. And then you added salt.

I have just tried this and the chemistry does seem entirely different. The switch from acid to base also generated heat. Remember the aroma!
It was quite real.

Sep 15 2011 Page 5

Continue w/ urine analysis.

Then Jan + I cannot reproduce the aromatic / emulsion reaction. It was dependent upon:

1. acid-base chemistry
2. NaOH - NaCl solubility of ferric hydroxide complexes.

What we do have, however, is the interim of a definite precipitate reaction between urine and ferric nitrate.

Well guess what? Ferric nitrate will react w/ NaOH to produce $Fe(OH)_3$.

However, we are not using NaOH to start! We are using urine, which is normally acidic! So we can acidify to eliminate the question.

The difference this time seems to be that after added ferric nitrate to urine (+ having the strong precipitate form) that when you add conc. HCl everything clarifies immediately. This is not your recollection w/ the first trial.

So the question is, if urine is not alkaline, when it normally is not, how do we have such strong precipitate formed w/ ferric nitrate?

OK, we actually have produced a reaction to some extent. It is w/ my urea sample, not the other one.

Our closest matches are indeed w/:
1. Rainwater residual Nov 02 2015 - 01 r = 0.74
2. Environmental Filament Catalyzed - Aug 15 2015 r = 0.72

It has used some extreme chemistry. The recollection is
1. Urea (~ 3ml) w/ 3-4 drops ferric nitrate
2. Make extremely acidic to clarify precipitate.
3. Let set for a few minutes.
4. Added NaOH & NaCl in ratcheting amounts.
5. Centrifuged. Large amount of precipitate at the bottom.
6. Very small foam layer on top which would not dissipate.
7. ATR the layer w/ minimal intrusion into primary urea layer
8. Heavy salt content shown up in ATR

There may be risk of ATR damage here with high alkaline pH. Be very careful here!
Turn to a rapid salt film layer as rapidly as is possible w/ ATR.

Compare this to regular urine to make sure it is a different compound.

There may be our 2nd level of success.

We have the indication of an alcohol w/ peaks @ ~3400 & @ ~1050.

It would also make sense that the density is lighter and that it is slightly less polar than water. These attributes are fitting.

—

We are
1. trying to reproduce the chemical reaction (ie, $Fe(NO_3)_3$, strong acid, sit ~15 min, lots of NaOH & NACl, added HCl @ end, layer on top, no Shaking!
2. Determine the spectrum of Control urine from two individuals & variance
3. Comparison of control urine to supposed extracted less dense layer.

Urine control does have some similarity indeed to the presumed extraction but it really is not the same. The closest match is in fact another urine sample from 2016 ($r=0.714$) but even it is not fantastic. The current spectrum seems to be the strongest and most clear of the two.
(Urine Male Removed CEC Sep 16 2016-03.SPC)
We seem to have a very good urine ATR Control spectrum here

—

I cannot, thus far, identify the exact chemistry that is producing the alcoholic emulsion compound in urine, but it has been accomplished twice now to some extent.

The two urine samples are not exactly the same. Upon concentration, my sample produces a much higher level of solids in it than the second one does. We are now comparing IR plots.

Regardless of difference of physical appearance upon concentration, the ATR plots of urine are almost absolutely identical.

This means that we have extracted a unique compound from actually both individuals (separately), each time which matches the environmental pollutants or the rainfall rendered the closest. We do have a match of significance here.

The thiocyanate levels in both individuals appear to be almost nil; possibly slightly detectable. Saliva & blood are of greatest interest after this.

We do know that the urine + $Fe(NO_3)_3$ is producing a large amount of precipitate even though the urine sample is acidic. This seems to be unusual.

NaOH + water + $Fe(NO_3)_3$ does produce the bright orange precipitate. The precipitate is of no value to us, it is not organic.

Many concentrated urine samples seem to be producing a little bit of foam by adding ~2 drops of $Fe(NO_3)$ alone.

This could be important. It does not appear to be acting like water does. No, it matches urine control exactly.

It looks like we may have something.
Forget acid-base - NaCl for now.

1. Concentrate the urine
2. Add $Fe(NO_3)_3$ gradually & shake thoroughly & you will see an emulsion form. It is white in color.

Nothing else was needed. This looks pure & avoids the acid-base-salt chemistry.

Yes, we have it! The defining property is the appearance of absorption @ ~ 1050 cm^{-1}.

This is the secondary alcohol peak!.

Control urine does not have this.

The addition of $Fe(NO_3)_3$ to urine is producing an alcohol which is, at this point, unexpected & unexplained.

Ok, this is not exactly true. We definitely do have the appearance of an emulsion when we shake the tube w/ CCl$_4$ urine + $Fe(NO_3)_3$. We also have confirmation of an alcohol.

Nevertheless, our closest match to the emulsion IR is: without question, other urine samples.

IR strongly suggests that the emulsion that we have made, although real & distinct, IS NOT the same emulsion that came out on the examination of the presumed "lipids" on Sep 12 2017. That sample remains quite distinct w/ an extremely strong thiocyanate presence and NO strong alcohol content. Those reactions and emulsions ARE DIFFERENT.

You still have not reproduced the original "lipid", ie aromatic emulsion (recall the odor?) even though you have produced "something" which has altered urine to produce an apparent alcohol functional group (~3400) and a possible sulfur group (@ ~1350) and a stronger secondary alcohol peak @ ~1050.

Your first reaction was unusual but I cannot reproduce it then or now. Back to acid-base chemistry & salts???

What would bring out a thiocyanate group in urine the way that sample did?

Now, the sulfur group appearance is still very interesting as it may indeed correlate w/ the thiocyanate groups.

Should sulfur be in urine?
Guess what? Nitrites are an indication of bacteria, a waste product of bacteria. "This Causes the sulphur-smell"

OK- we have a Connection to pursue.
"Sulfur reducing bacteria".

Sep 16 2017

I have made MEK & ethanol extractions of urine passed into IR. They are to no avail. Only urine shows up in the output.

Even an attempted xylene extraction, which definitely produces a "foam layer" after shaking & centrifugation, still only produces urine spectrum in the IR.

You definitely do not seem to be able to reproduce the reaction. The question now is, what is in the precipitate that forms under mildly acidic and alkaline conditions? It should not form @ neutral pH but it does.

Both $FeCl_3$ & $Fe(NO_3)_3$ produce the same precipitate so it is the Fe^{+3} that is causing the reaction.

The pH of one urine sample is 5.2 so it is definitely acidic. This should not produce a precipitate normally. Test this w/ a control solution of H_2O @ pH of ~5.2.

Ok, here is a color chart from 1902 that shows many reactions w/ urine. Most notable is the reference to phosphates, and that a precipitate will normally form.

Let's try a control on this. Done! It is quite true.

It appears that we have explained the precipitate. Also, we do not seem to be able to reliably reproduce the thiocyanate reaction. We have done it twice to some degree but exact conditions remain unidentified.

Ok, it looks like we have identified a major component in the reaction. Ferric ion does indeed form a major precipitate w/ the phosphate ion. $FePO_4$

Ferric Chloride with Urine

Ferric chloride test solution give a number of...

... very characteristic color reactions with urine containing derivatives of many of the synthetics. The test is applied by adding a few drops of ferric chloride solution to about 15 cc. of urine in a test-tube and noting color of precipitate. Usually a precipitate will be formed with the phosphates; the color reaction will appear best by precipitating phosphates first. This test is very useful, and I believe it has wide applications; I hope to take up the line in the near future to work out a line of reactions with urinary constituents. The colors given with ferric chloride solution are:

Salicylic acid	Violet color.
Gallic acid	Green-black color.

Notice this comment w.r.t. phosphates!

Tested & this is quite true

phates first. This test is very useful, and I believe it has wide applications; I hope to take up the line in the near future to work out a line of reactions with urinary constituents. The colors given with ferric chloride solution are:

Salicylic acid	Violet color.
Gallic acid	Green-black color.
Benzoic acid	Pinkish precipitate.
Carbolic acid	Deep blue color.
Alumnol	Blue color.
Antipyrine	Red color.
Aspirin	Red-violet color.
Arbutin	Deep blue color.
Hydroquinole	Blue, turning yellow.
Kairine	Violet-red color.
Diaphthol	Green color.
Phenocoll	Cloudy red-brown.
Pyrocatechol	Green, violet by ammonium hydroxide.
Resorcinol	Dark violet color.
Salol	Deep violet color.
Salicin	Red-violet color.

We can now move on.

Conclusion.

The could be a very good test (phosphate concentration or urine) to apply to the biochemical tester.

We do indeed have a very sensitive phosphate test kit 0-3 ppm

We have another that range from 0-100 ppm.

The ferric test produces a precipitate, which is turbid.

Guess what, a .NIR CAN be used for turbidity measurements. Recommended @ 860 nm & ISO 7027
The opens up the problem of concentration a quite a bit.
Journal of Physics 2016 / Conf Series - open access paper

The gets you through a very common problem.
Even sugar at Benedicts may work here.
Remember that precipitates settle, however.
Stated to be good up to 50 NTU
NTU = Nephelometric Turbidity Units

Guess what: $FeCl_3$ is used to treat turbidity.
Why -? It makes a precipitate that can then be filtered out!
Clear water apparently should be less than 1 NTU
and ideally < 5

It actually looks to be most sensitive @ ~ 360 nm.
We could try @ @ 860 nm and see what it looks like.

Sept 17 2017

1. Turbidite example - exploration
2. ~~HHFC Briefing papers posted?~~
3. ~~Chest & Lymph~~ - ~~Ultrasound~~ & Electromagnetics?
4. ~~IMEDW Papers summarized~~
5. Point of Reckoning - Part III?
   Ed v filament with: ? Skin, rain, urine, blood, saliva? air?
6. Crystallization Project.
1. ~~Collect protein samples~~ from cultures

Approx 3000 ml of dilute protein CDB has been collected and is now under evaporation/concentration. Hopefully the process will be safe and not produce any health effects as the pyrolysis does. High caution fields to be exercised here to avoid fumes, hopefully only water vapor is being removed here. Temp of protein bath is ~ 65°C.

Rainfall collection has also started; a fair amount of rain is expected to come in the week.

Sep 18 2017 Functional group study, it seems...

An interest in the crystallization process.
With the proper solvent, it can be used to purify many solids that are solid @ room temperature. If ya had a mixture of some kind that was partially soluble in a particular solvent, this would be a perfect application of purifying it.

A steep solubility curve with the chosen solvent is desired.

There is a great condensed functional group list:

		Example
H_2O	Water	
RH	alkanes	
ROH	alcohols	
ROR	ethers	(diethyl ether)
RNH_2	Amines	
RCOR	aldehydes, ketones	(acetone)
RCOOR	Esters	(ethyl acetate)
RCOOH	Organic acids	
$RCONH_2$	Amides	(dimethylformamide)
ArH	Aromatics	(benzene)
RX	Halide	CH_2Cl

You should learn these functional groups but once & for all.

ROR Ether. Starting fluid is an ether.
Starting fluid goes ROAR!! R-O-R
ROR goes roar, roar means starting fluid,
starting fluid means ether. Oxygen supports combustion

RCOR ~~Aldehyde~~, ketone (acetone)
This is saying that an ether w/ some Carbon attached
(and a bond to a double bond w/ oxygen) creates
an ~~aldehyde~~ or a ketone.

this is actually a ketone

one aldehyde is actually when one of the R's is a hydrogen. $R-C(=O)-H$

So our basic level of recognition is actually a ketone
So a "roar" w/ Carbon attached becomes a ketone (eg acetone)

You could say a "roar" w/ a C (Ek, Eh) is a Ketone
(hard C, as in K)

so a "roar" is an ether. ROR
An ether w/ a Carbon (hard C) is a Ketone. RCOR
an aldehyde puts one of the R's as an H.

Next we double up the O. This changes to a ketone
to an ester RCOOR

So ROR ether R-O-R
RCOR ketone $R-C(=O)-R$
RCOOR ester $R-C(=O)-O-R$

With exception of an amide, you already have what you wanted:

ROR ether
RCOR ketone
RCOOR ester.

Now an amide just take an amine, which you know quite well as NH_2
and adds an oxygen to it, but also a carbon

$RCONH_2$ is an amide

So our primary new candidate are

ROR	ether	R–O–R
RCOR	ketone	R–C(=O)–R
RCOOR	ester	R–C(=O)–OH
$RCONH_2$	amide	R–C(=O)–N(H)–H

Notice ester is a ketone where R becom

Notice in all cases the presence of an oxygen is involved.

oxygen oxygen, carbon two oxygens, carbon

Ether, ketone, ester

Amine – Amide
carbon, oxygen

Notice all groups involve the addition of an oxygen, carbon, both or a multiple of oxygen.

The COB viscous protein has some unusual properties to it. When you heat it moderately, ie ~ 65°C, it forms a brown precipitate, as if oxidation & denaturing take place.

My collection, however, is that as final H_2O is driven off, it once again turns to a clear green solution as it turns viscous
The would imply a reduction @ the final moments of evaporation.

What are the properties of these functional groups?
You have a sense of an ether and a ketone
R-O-R RCOR
but what about an ester and an amide?
What are these properties ↓ ↓
RCOOR $RCONH_2$

We are adding an oxygen to a ketone. What effect does this have?
Esters become the fats and the oils.

and they build up to get there.

Crystallization of a mixture is a very interesting and practical topic. It is discussed in some detail in Introduction to Laboratory Techniques by Pavia. p 650 - 651.

Plant experiment developed:

Wgt of Spoon + Viscous Protein 17.60 gms
Wgt of Spoon Alone 5.82 gms
Wgt of Viscous CDB Protein = 11.78 gms

Volume of Plant Nutrient = 600 ml

Control Tap Water.

By weight, protein solution 11.78/600 (100) = 1.96% solution.

At 2% CDB Viscous Protein solution has therefore been applied, with H_2O controls, to four different seed types:

1. Blackeye Peas
2. Dill seed
3. Mustard seed
4. Sesame seeds.

Sep 19 2017

1. Rainwater collection is in place.
Continues
Mass of empty jar is 141 gms
Mass w/ rainwater added is 1349 gms
Mass of original water sample is (1349-141) = 908 gms.

Evaporation is in place.

2. Protein concentration is in place @ ~ 65-70°C
Failed
The solution is now clarifying as it becomes more concentrated. Clear to begin, then turbid, now clearing. Also of interest is that upon heating a precipitate forms that appears visually identical to the most colored cocoa form. I believe that will reform again to full clarity @ the close of evaporation.

3. Plant trial w/ blackeye pea, sesame, dill & mustard are all being subjected to 2% protein solution w/ H_2O controls for sprouting runs.
Continues

4. I will attempt a yeast run. Optimum temperature is 32°C = 90°F.
Continues
Recipe 240 ml H_2O use ~ 0.5% protein
5 ml yeast (1 tsp.) solution vs control
5 ml sugar (1 tsp)

With exception of an amide, you already have what ya wanted:

ROR ether
RCOR ketone
RCOOR ester.

Now an amide just take an amine, which ya know quite well as NH_2
and adds an oxygen to it, but also a carbon

$RCONH_2$ is an amide

So our primary new candidates are

ROR	ether	R–O–R
RCOR	ketone	R–C(=O)–R
RCOOR	ester	R–C(=O)–OH
$RCONH_2$	amide	

Notice ester is a ketone where R becomes O

R–C(=O)–N(H)–H

Notice in all cases the presence of an oxygen is involved.

oxygen — oxygen, carbon — two oxygens, carbon

Ether, ketone, ester

Amine – Amide

carbon, oxygen

Notice all groups involve the addition of an oxygen, carbon, both or a multiple of oxygen.

Adding protein to solution. Time of Mixing: 1340 MT
Sept 29 2017

Weigh Boat: 3.15 gms
Toothpick 0.8 gms
3.23 gms

We want ~ 1 gm → 4.25 gms
① 4.03 gms actual = 0.4% Protein by wt.
② 4.40 gms = 0.6% " " "

Toothpick added to each culture (10, VISCous protein transfer & weigh)
Toothpick subsequently removed from each culture.
Full dissolution obtained in each culture.
Now incubate @ 65°F

Budding yeast are visible in control sample by 2000.

The final culture subjected to the COB VISCous Protein @ a ~ 0.5% Concentration is budding perfectly normally. You see no destruction or oily radiation. If anything, the protein culture - yeast may be budding more than the control solution.

We had a little problem today w/ the protein concentration. The glass dish shattered during final stages of concentration. Two months of cultures was lost, along w/ breaking of the hot plate & the broken glass dish. Temp was in 65-70°C. So much for Chinese pyrex glass.

We have sufficient protein from previous trials to meet current requirements!

We continue to concentrate the rainwater.
Heating will always need to take place within a containment dish, as in a baking pan.

UV analysis of rainwater, even prior to concentration, is showing the presence of organics.

Sep 21 2017

The mustard seed fermentation project is showing significant interference from the 2nd Protein solution.

I have now diluted the solution to 0.5% concentration

It seems quite apparent to me that the rather extreme skin reaction that I had was a result of pyrolysis fumes from the protein concentrate.

I will not be repeating that process w/out more adequate safeguards.

I have had an upper right lung problem for several years now, and it creates a low level chronic cough. It is immediately adjacent to the brachial lymph nodes that have been a source of chronic shoulder interference also for several years. It has taken some time to discern that the problem appears to originate w/ the lymph nodes rather than presumed bone problems.

Ultrasound has been applied to the lung location (extreme upper lung area, adjacent to the shoulder) for approximately 20 to 30 minutes last week. Frequency is 5 MHz. I was able to feel the exact location of the problem w/ application by ultrasound, it was a dull pain upon ultrasound application.

I have been convinced for some time (~ 1 year now) that the source of the cough is a resident infection of bacteria (namely the CDB of the mycoplasma). It has been impossible to affect in any way this for w/ the use of supplement strategies.

The important finding is that ultrasound seems to have had an immediate and beneficial effect. There has been a noticeable improvement & I have felt no sense of resident infection at the same level since the application of ultrasound. The shoulder continues to have effect, although it too seems to demonstrate w/ the use of ultrasound.

I am watching the lung-shoulder situation very closely.

At this point, it appears that ultrasound may become an incredibly effective method for disruption of resident CDB infection locations. The hypothesis has been under development and testing for 6 months but it was never previously applied to the lung/lung location, only the shoulder region.

All applications of ultrasound appear to have been beneficial, the latest incredibly so.

Rainfall Concentration in Progress.

We are getting some interesting results even very early in the game w/ Concentration, w.r.t.

UV
VIS } spectrometry.
& NIR

Preliminary concentration already shows NIR peak @ 962 nm.
This corresponds to ArOH.

We also have a definite peak @ 265 nm.
Can be protein or . . . ?

Now with $FeCl_3$ added we have some additional interesting results. We set a $FeCl_3$ control solution as the reference blank.

We show peaks @

981 nm ArCH

874 nm ArOH

582 nm Visible is purple (I cannot see it but VIS detects it).

We have now have strong evidence for a phenol group in the rainwater.

Phenols form a violet complex w/ Fe^{3+}

We therefore have confirmation by both NIR & VIS of a phenol group w/in the rainwater.

It should only be easier to detect w/ further concentration.

The 2430 osmometer has arrived. Signs are encouraging. A $15,000 instrument, acquired for $500 from Ebay & manufacturer to include instruction, manual, and sample tubes. The startup passes the system test without error and the temperature probe is an accessory (it may not be a requirement). We will see how it behaves with the manual & tubes arrive.

Meal Worms coming
Proto 20a Coming
KCl Crystals for IR Coming
2430 manual & sample tubes Coming

A film is not noticed developing on the surface of the yeast cultures that have the phosphate solution added.

Yeast culture:

At this point it appears that the yeast culture w/ the protein added might be more productive than the control yeast culture. It appear that shake may be successively or each day more dense.

The film layer on top interests me.

We have an interesting event taking place here. Many of the cells on the surface layer are highly elongated

I do not know the significance of this yet but the protein solution

1. is creating a floating layer on top of the culture
2. The surface layer contains many elongated cells, unusually so.

We will continue to monitor the growth.

Sep 22 2017

The yeast culture is "deformed" by the presence of the protein.
A more appropriate word choice might be "mutated".
The article has been posted. A deliberate low key approach has been taken.

The change is that of commonly producing doublet & triplet cells vs separated, singular spherical cells. The change is taking place primarily within a surface layer that forms @ the top of the culture. The control culture does not form the layer.

It has taken 72 hrs to verify this although it was observed @ 48 hrs.

—

The rainwater continues to be concentrated. We have:

867
908
842
454 ml
Σ = 3071 ml

Lets try to concentrate this by a factor of 20.
This requires final to be ~ 150 ml.

—

The bean seeds are starting to germinate. The mustard seeds show a radical difference.

—

My shoulder seems to be improved w/ stationary application of the ultrasound @ 5 MHz for ~ 30 min yesterday.

Plans include:

1. Thiocyanate comment
2. Ultrasound comment
3. Point of Reckoning - Part III - filament
4. Shen Carlson

The work is of great consequence during this period. There is a convergence of many different lines of research that have occurred over extended time.

Sep 23 2017

We are doing some background analysis of ethanol (denatured alcohol) on IR ATR.
Some interesting results: Test material is an evaporated ethanol film:

1. The CO_2 peak @ ~2360 has a negative absorption from the air reference. We noticed this problem during the HEPA extract.

2. Anything less than 680 is no longer usable on the instrument. I am not sure why but we have seen this over and over.
We must reset limits of operation now from 3600 - 680. Let us do that.

3. A background of evaporated ethanol ATR will indeed be recorded and saved and used as a background ATR reference.

4. All new backgrounds must include the region from a 3600 - 680 ATR ± Crystal until you learn if it can be eliminated.

We are now working w/ the HEPA extract.

First run is w/ the ethanol film ATR as a background reference. It produced some unsteady results. CO_2 peak remains troublesome as a negative absorption. Also the lack of absorption @ 1204 cm^{-1} is quite pronounced.

Recall our extract is more concentrated now from evaporation by a factor of ~2.

Then scan is troublesome w/ CO_2. We must eliminate 2400 - 2300 and scan it in 2 sections. Ethanol film reference background is not making any significant difference.

What else is in this extract is completely blocking CO_2 absorption - how & why?

Test ATR in air as reference
CO_2 in room is @ ~550 PPM. We have a meter.

We have a problem w/ the ATR air background.
We are getting bogus data.

Revising the limits from 3600 - 600 has caused some problems. A reset of the software and instrument was required. We seem to have it back now.

ATR plot is now satisfactory of air reference.
Ok, we have a good HERB spectrum again, that took some doing to get everything reset again from 3600-680. It is great to no longer have a spike of data @ 680 now.

When you reset the range, make sure that you reset both the instrument and the software.

Computer has now acted up again - hard drive problem.

The KCl disk picks up absorption on the high end (3600-3000) that the ATR simply does not pick up. A significant absorption @ 3380 has been lost w/ ATR but retrieved w/ KCl single disk.

We know from LC that there are two primary components in the extract.

Ok, it is of interest that our closest matches are:
1. A step 15 Repeat IR analysis; the signal is stronger in this case
2. Tea Tree extracted from Gas Chromatography! Jun 2016!
* 3. Rainfall - Xylene Extraction - clean book r=0.92
4. Hexadecanoic acid, dodecyl ester R=0.92

Since LC produces two compounds, we look at functional groups w/in hexadecanoic acid.

We have a very good + clean HEPA extract spectrum here.

1. Best matches are rainfall extraction w/ xylene & hexadecanoic acid.

2. LC previous analysis shows 2 component compose

Next let's look @ UV analysis:

Extraction sample: NIR
UV
VIS
$FeCl_3$ – VIS

Rainwater concentration:

3071 ml
– 10 ml estimated loss
= 3061

265 ml left

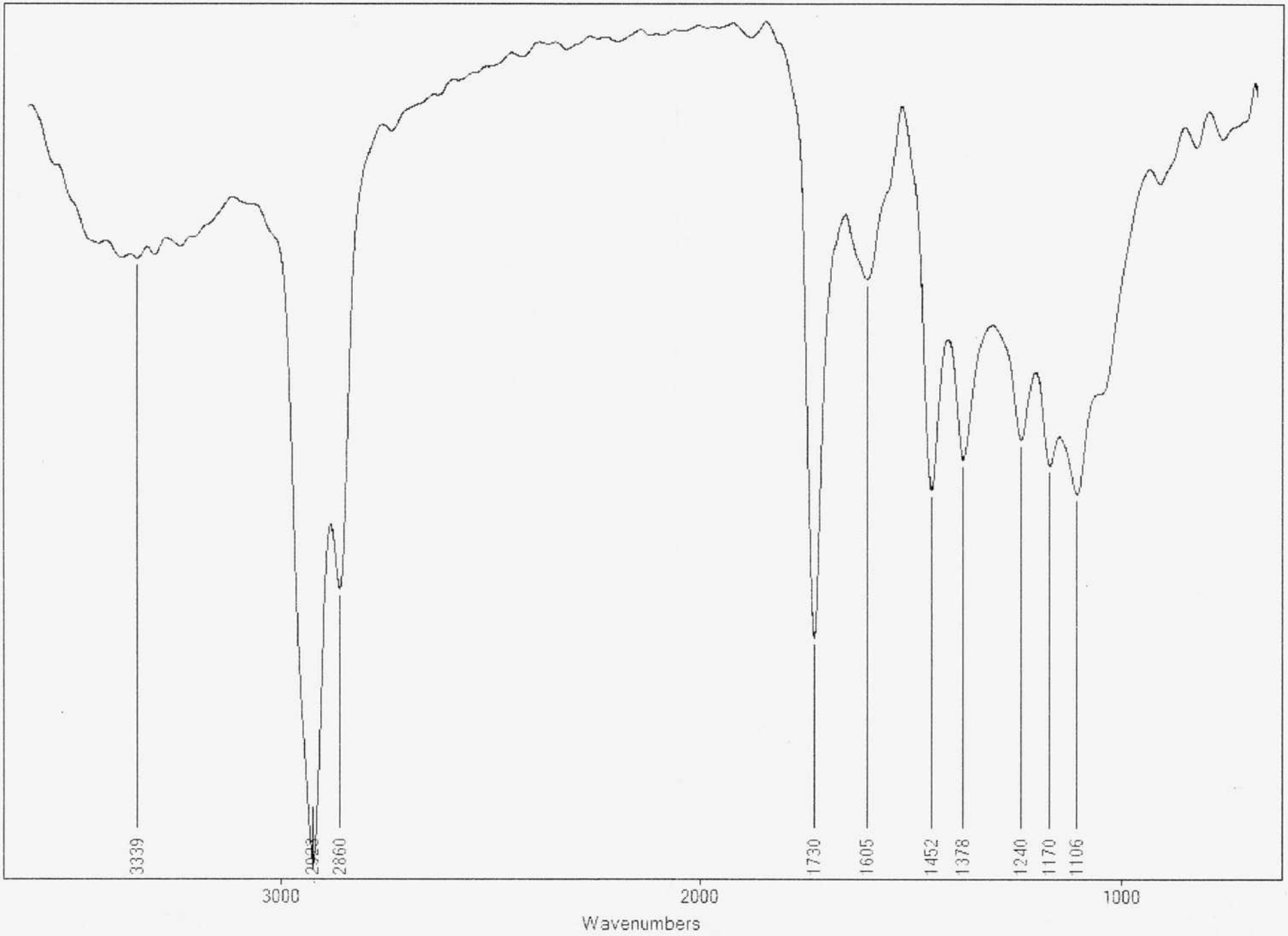
Concentration factor = $\frac{3061}{265} = 11.55$

There is a lesson taking place w/ ethanol as a solvent in UV. For some reason we have a sharp discontinuity @ ~ 293 nm even when trying to just zero it as a reference.

Question: What is the cut off point for ethanol?
205 nm. I do not know why we have the discontinuity.

Very clean HEPA extract spectrum. Best match is Rainfall extraction w/ xylene. From LC analysis, two compounds comprise. additional match hexadecanoic acid.

HEPA Air Filter ATR-KCl Average Clean Spectrum Sep 23 2017 - 03.JPEG



You could tell that something w/ the gain was off trying to set ethanol as the reference, & the change occurred @ ~ 293 nm. This meant the gain was out of range for the instrument.

Lets try a 1/2 H_2O 1/2 ethanol solution as a reference. Ok. That worked, and it was close. The discontinuity was moved over to 222 right next to the end of the scan.

There is a lesson here. You cannot use ethanol (or at least denatured alcohol) full strength as a reference. But you can if it is diluted ~ 1/2 to 1/2. It looks like 2/3 H_2O & 1/3 ethanol would be even better.

So now you know how to construct your reference solution that involve ethanol - dilutions are required. As long as you subtract the background properly, you are fine.

So our background here will be:
H_2O + 200 ml ethanol
Compare it to
H_2O + 200 ml HEPA - ethanol extract

Ok, this is really interesting. The idea seems to have worked perfectly.

You have a very broad absorbance peak of the HEPA extract
WITH ETHANOL (denatured alcohol) subtracted out properly.

The peak is @ 232 nm.
The absorbance is also VERY STRONG, even though you only put 200 ul in the UV cuvette.

Will see if we can find what 232 often involves.

Colby has 111 matches @ this level.
We have some functional group analyses to make to see if we can narrow more into Colby.

Lets now work on the Fe^{+3} test.
Also we need NIR - do that next.
We still have blanks available.
NIR & UV alone can allow entry into Colby database.

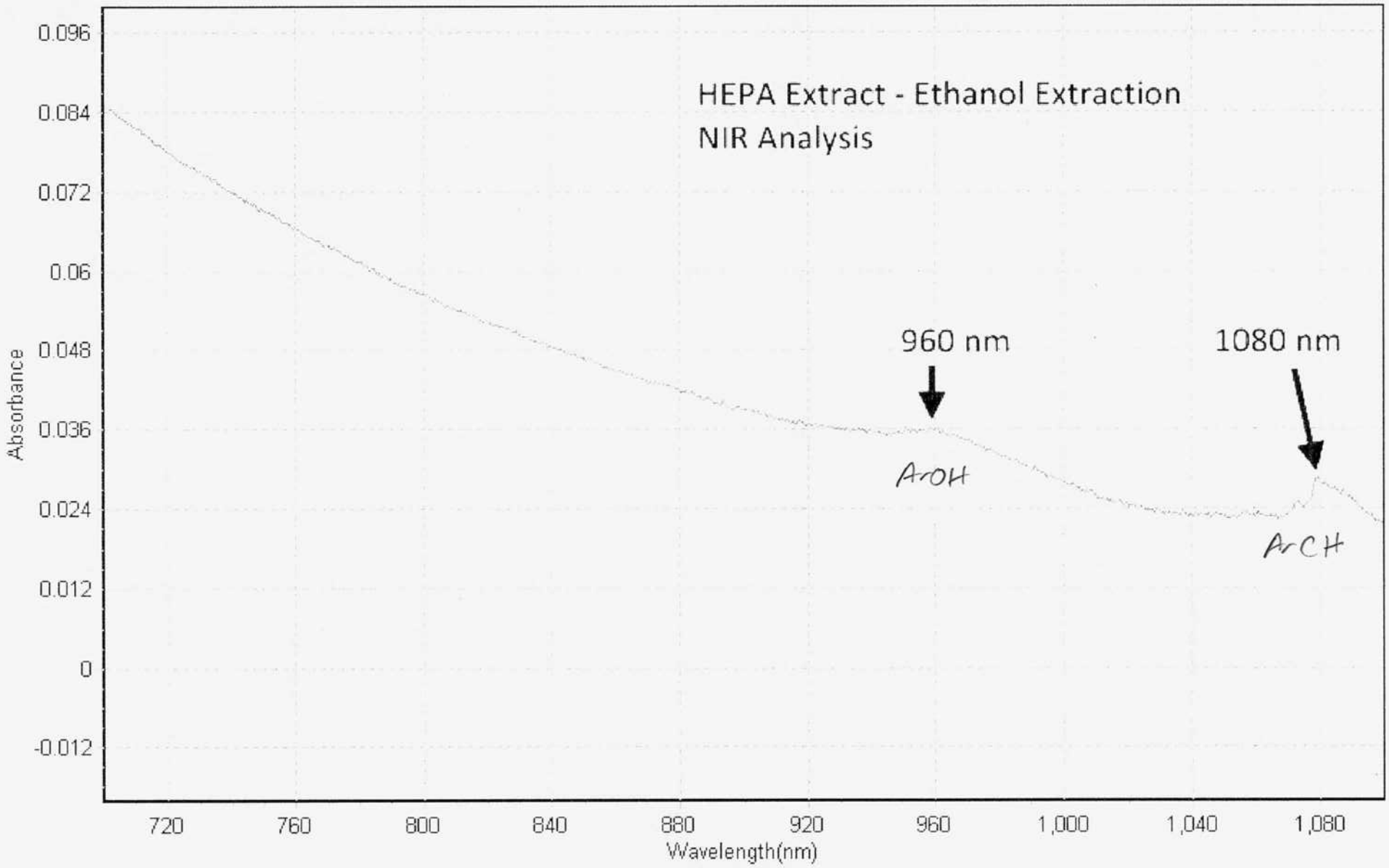
NIR: We do have some absorbance @ 1019 & 960
1019 = Ar CH
960 = Ar OH
] This helps and confirms Cone rain all analyses.

We have picked up 3 peaks between UV & NIR. They can all be used in Colby along w/ functional group analyses.

We have OC and Colby to 94 matches. Candidate matches are Anxline, Arsenophenol
Anxline require nitrogen, which we have no evidence of yet.

HEPA Extract - Proper Ethanol Subtraction

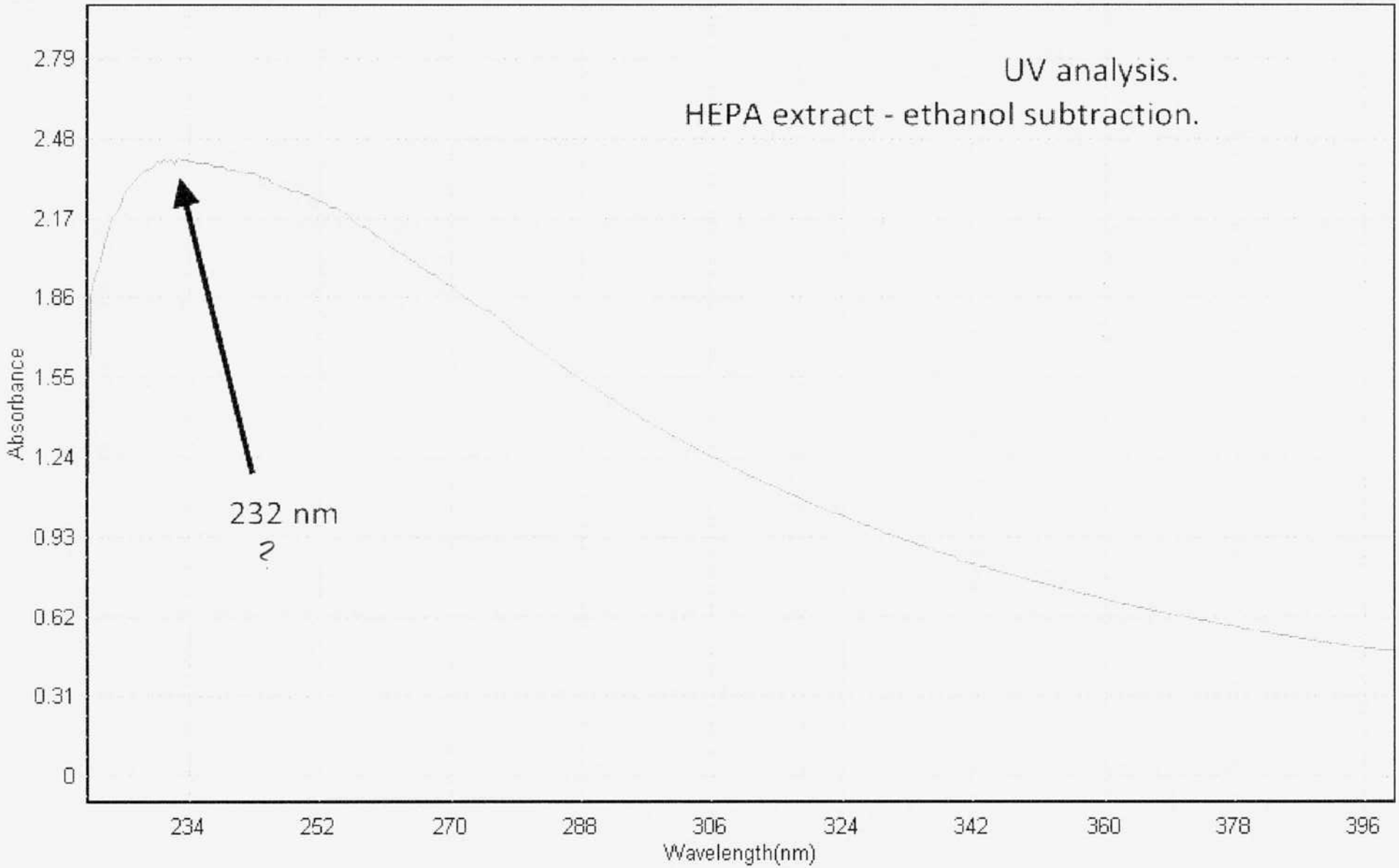
HEPA Extract NIR Analysis Sep 23 2017.jpg

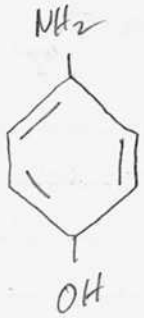


HEPA! Extract UV

Analysis - Proper subtraction of ethanol.

HEPA Extract Dilute with Ethanol Subtraction Sep 23 2017.jpg



Aminophenol is 

Lab use Fe^{+3} test for phenols. Our solutions weak. We may need to increase concentration.
Repeat & Verify NIR results.

We still have IR functional groups. Remember, however, 2 Compounds Comprise. NIR results are Verified.

You do not detect any phenol groups w Fe^{+3} reactor with the HEPA extract. We know that if present, the Concentration w/ 200ml – 400ml is very weak & may not be detectable w/ colorimetric test.

Also, you are tending to get the HEPA extract & rainfall analyses somewhat mixed up.

Take each & keep each analysis separate and then Compare notes at the end.

With HEPA you still have:
1. Functional group analysis
2. Protein test / Pre & Post LC, heating & no heating.
3. LC Separation: you have two components.
4. Remember the sample has been heated & has changed color from green to brown. Not sure how this will affect LC work.

Sep 29 2017

The protein is definitely curtailing germination of plant growth.

The most successful trial is w/ no doubt the mustard seeds. A blackeye pea (bean) trial also shows sprouting. Dill and sesame are not sprouting – we must wonder if the GMO influence is at hand here – most suckenly.

Also the yeast was very strangely affected – it truly looks like a mutation took place here. Doublet & triplet cell formation on a surface layer.

The control yeast culture also has a layer on top. You need to look @ it.
OK, I have now looked. The control culture surface layer DOES have normal cell formation.

It is systematic to make the weeky mustard seed report – today is the 1st day. – Done.

Since we know that the HEPA extract separates into two components (prior to heat treatment - evaporation) we should probably repeat that process. As we want to understand the nature of that separation in more detail.

We have the first compound separated again very easily by GC. H_2O elute pulls it out immediately Signs are:

1. Drip rate increases
2. Index of refraction increases (1.343)
3. Color ↓ (opaque)
4. Odor
5. UV absorption @ 278 & 230 nm
6. NIR definite abs @ 1080 nm → Ar CH is definite.

Test for phenol
protein

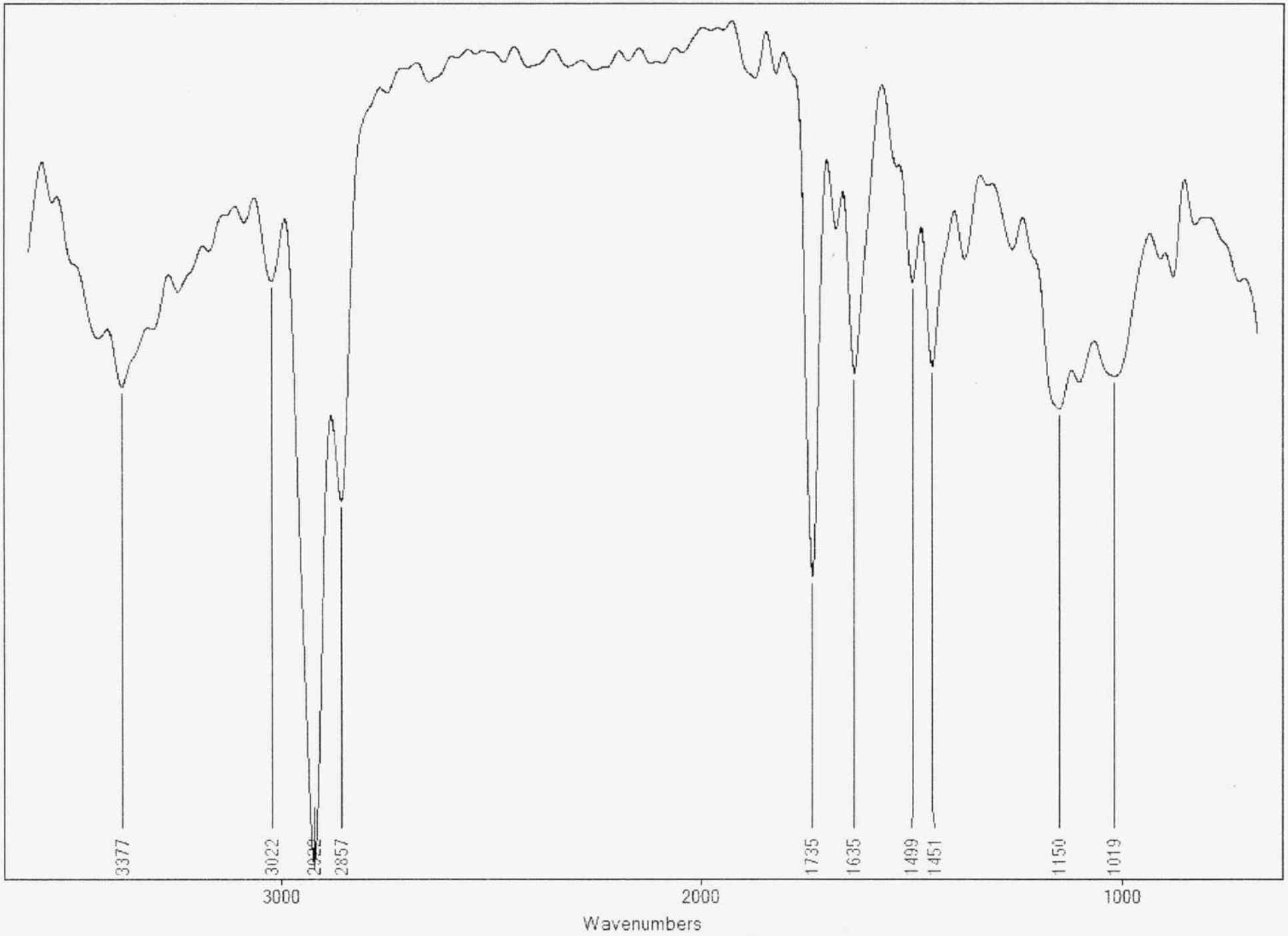
—

We now have second component coming thru. Heat treatment remains brown-yellow in color. Alkalis remove it from column as before.

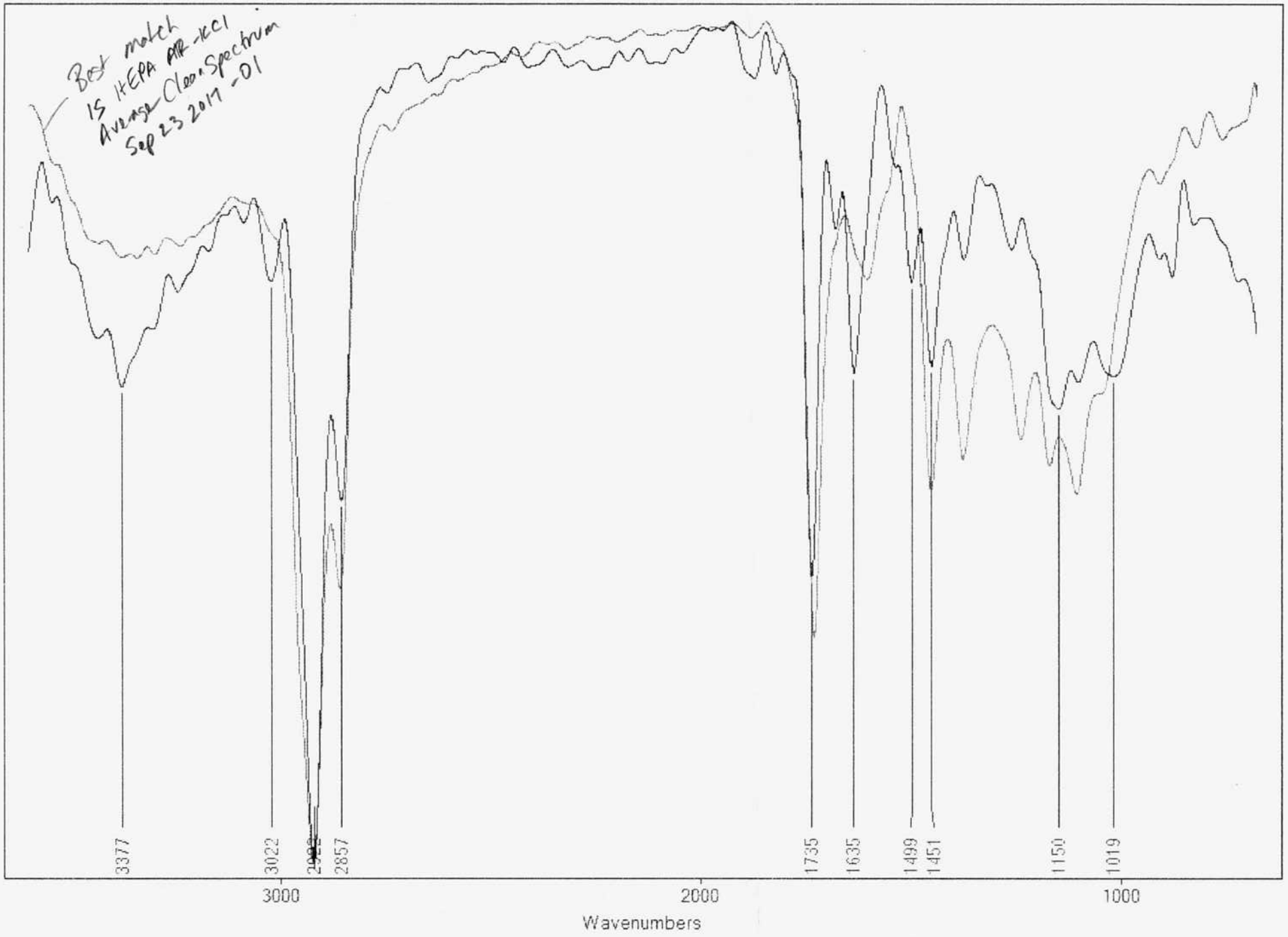
We now have two successfully isolated components. Component 1 is opaque, good spectrum (UV, NIR, IR) obtained, light yellow
Component 2 is clear, yellow, pH is ~10.5 so we would like to neutralize it for ATR.

Ok, we have neutralized it. This turns the solution opaque and it looks close to the nature and color of component #1 but IR, NIR & UV will tell us what is the case.

HEPA Filter LC Component 01 Sep 24 2017 - 01.JPEG



HEPA Filter LC Component 01 Best Match Sep 24 2017 - 02.JPEG



LC Component #2, now neutralized.
Start w/ UV analysis.

There is no distinct aroma so we already know that it is different from Component #1.

We have a singular peak @ 224 nm.
No peak @ 278 so again this speaks to a different Composition than LC Component #1.
UV absorbance increases monotonically from 400 nm to ~~278~~ 224, then a droppy off @ 224.

Now for NIR: We have absorbance @ 1008 RNH_2
879 Ar-CH
& 754 nm ~~CH~~

There is quite different.

Study IR, phenol & protein

NIR does once again give us an indication of potential protein. It actually has quite a lot of oxy donor activity. Let's see if we can get this into IR.

There is a settling out takes place within the collection tube. We also have a second tube that looks viable
neutralized 1-7] both appear useful.
pH High! 1-8]

Concentration now insufficient for NIR. Must concentrate by evaporation. Neutralization of pH causes NaCl crystal to form - all gone here.

It can be seen that the LC component #2 is more viscous. Full concentration is required by careful evaporation to produce this.

Increasingly, a protein nature does seem likely. We clearly have a more viscous material in the sample.

The material is highly IR absorbent and had to be reduced considerably on the KCl disk.
It has a very unusual spectrum on the KCl disk.
Major Thiocyanate existence..

We really have something this time.
It may be hard to detect but the front end peak is @ ~ 3425 3413 is a strong peak.
As the sample is dryer at the front end peak is getty strong but the thiocyanate peak is disappearing. This is unusual. Thiocyanate they are appear to be volatile.

The KCl spectrum seems to be dramatically different from the ATR spectrum. The KCl seem to be closer to one better of the two but it also showed quite a bit of dynamic change as it evaporated.

The closest match is the Rainwater residual R=0.04
The CDB lipids are immediately behind. R=0.04
Thiocyanate shows up in both comparisons.

This is a case where ATR did not work well & KCl worked superbly. Also the dynamic nature w/ evaporation is also new.

We have very good spectra (IR) for both LC Component #1 & LC Component #2 from the WEBA folder.

They appear to be very interesting compounds.

Also, we form the average of Components 1 & 2 to see if we can recover the combined IR plot of September 23 2017.

I do believe we largely have exactly that.

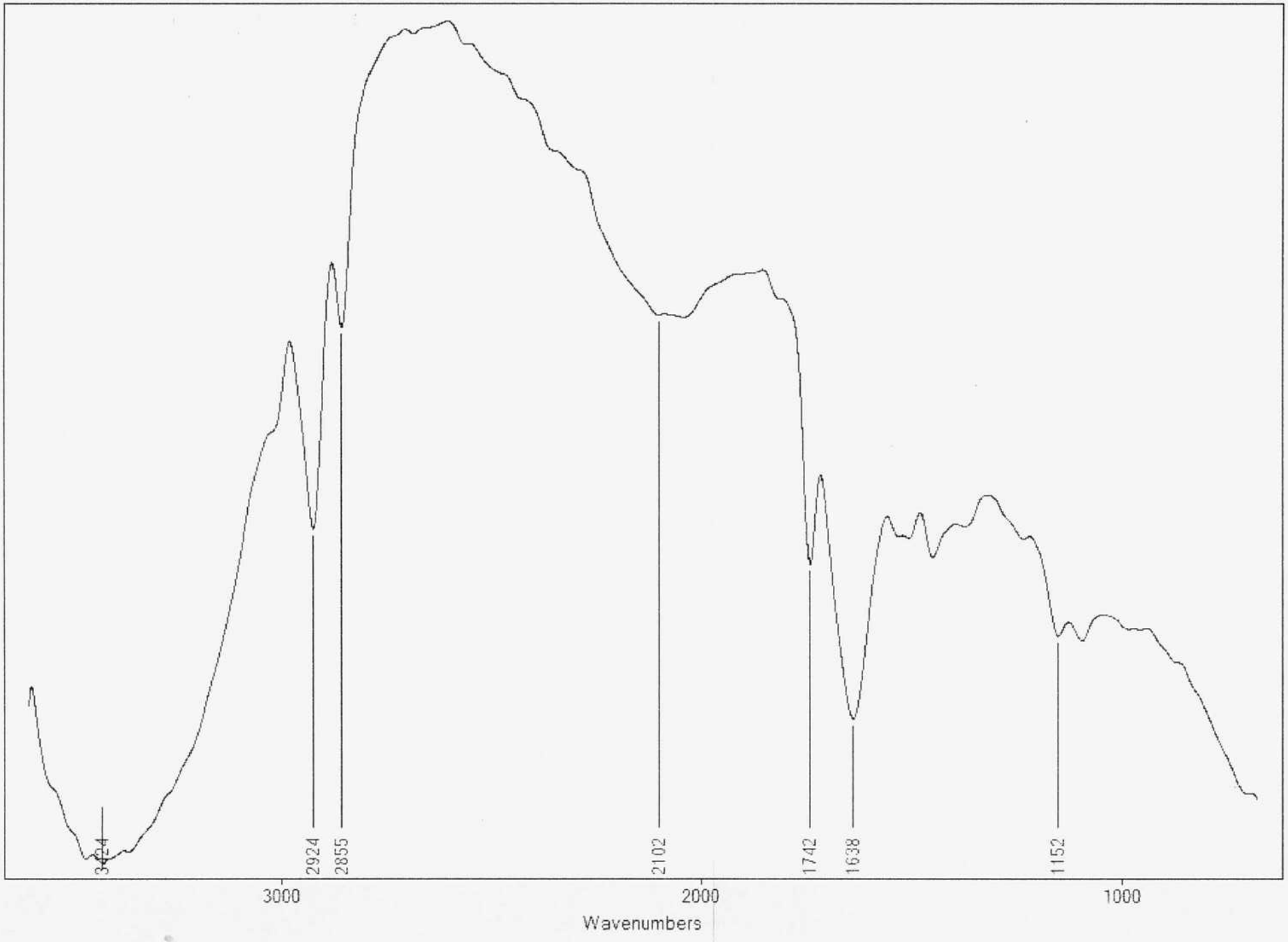
Now additional questions:

1. Phenol?
2. Protein?
3. Thiocyanate?

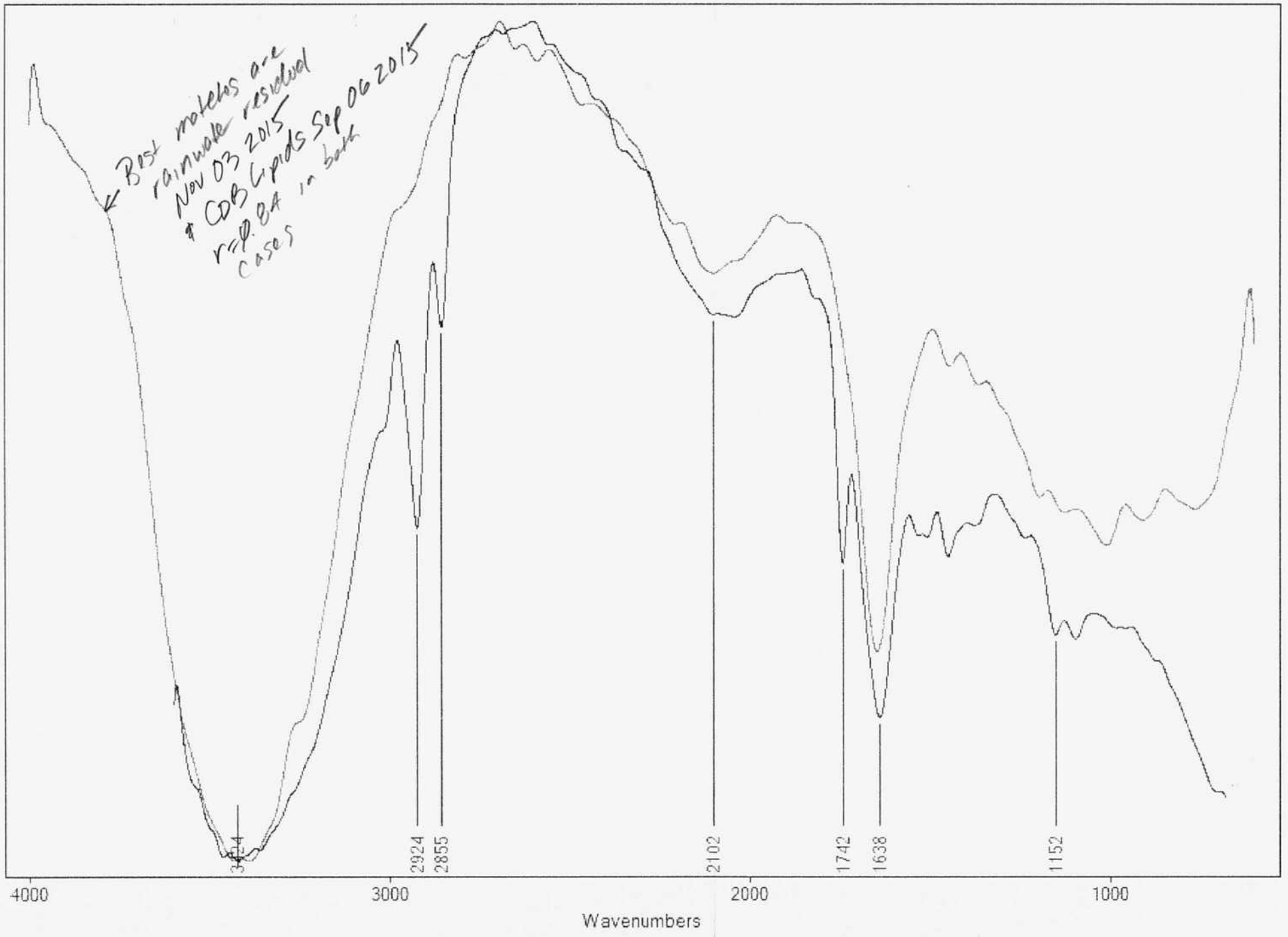
] You need to test Components 1 & 2 for these materials.

There is rather complex work. Full laboratory skills are being used here.

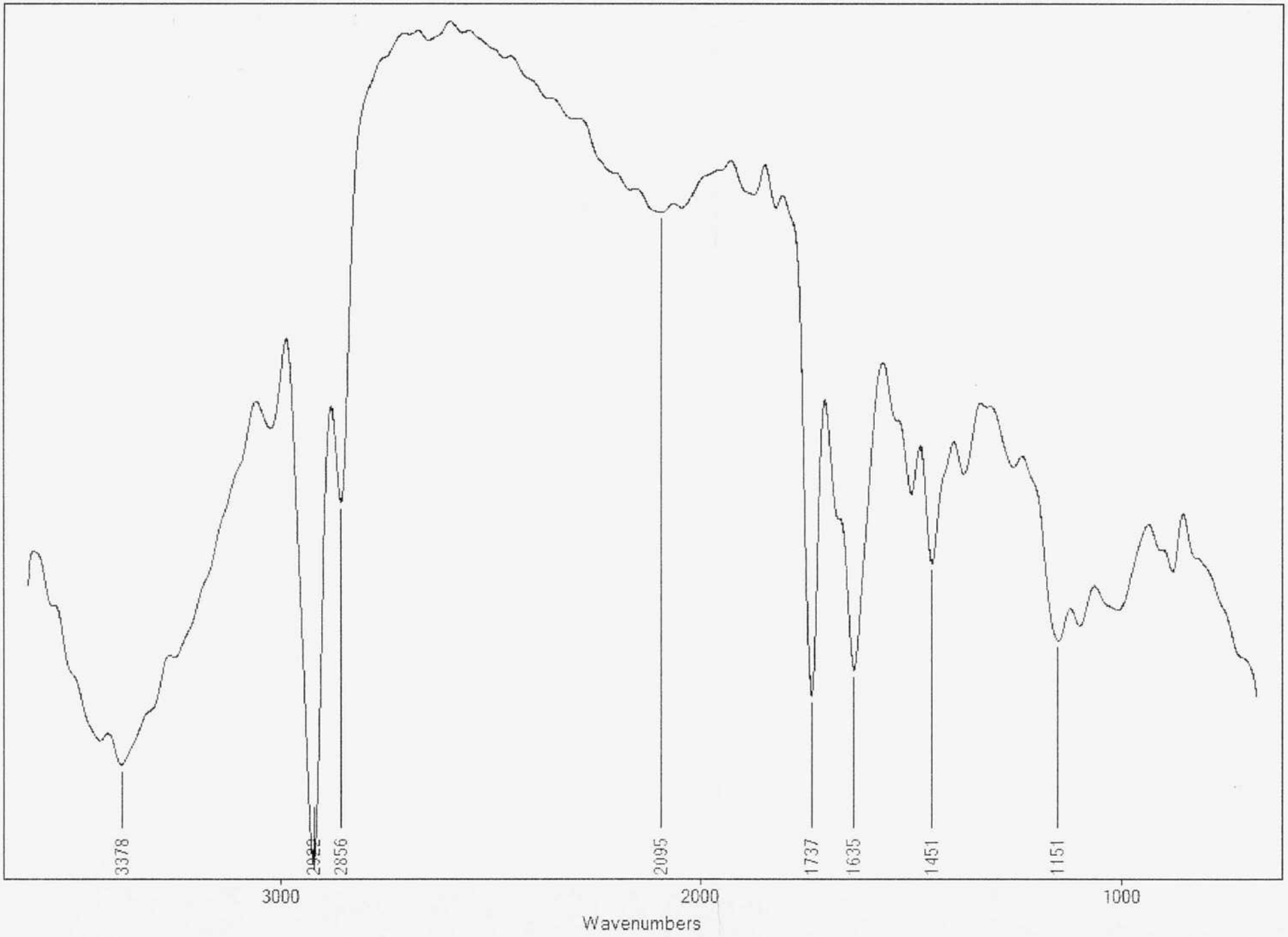
HEPA Filter LC Component 02 KCl Sep 24 2017 - 01.JPEG



HEPA Filter LC Component 02 KCl Comparison to Rainwater Sep 24 2017 - 01.JPEG



HEPA Filter LC AVERAGE OF COMPONENTS 01 AND 02 Sep 24 2017 - 03.JPEG



Sep 25 2017

1. The worms are on their way - they have been shipped.

2. The first protozoa kit has arrived. They need to be cultured. It is in dry form.

3. We have a fair amount of basic information accumulating on the HEPA air filter extract. Methods include:
   1. UV
   2. VIS
   3. NIR
   4. Liquid Chromatography separation & detection

   Need:
   1. Phenol test
   2. Cyanide test
   3. Protein test.

   Detection includes:
   1. pH
   2. ORP
   3. Conductivity
   4. Index of Refraction
   5. Color
   6. Drop Rate
   7. UV analysis

4. We have learned about dilutions of ethanol required for UV. No real difficulty there and discontinuities are now something to be more aware of.

5. Successful pasta on mustard seed & yeast trials.

The phenol test (i.e., $FeCl_3$ added to a test solution) has come out negative for

1. LC #1 diluted solution
2. LC #2 dilute solution
3. LC #2 Concentrated & evaporated solution.

We will need to concentrate additional LC #2 for a proper protein test.

Initial & Dilute Protein Test for LC #1 & LC #2

1. LC #1 definitely fails the Bradford Reagent Protein Test
2. LC #2 might, and that we definitely a might be showing evidence of protein but it's too early & dilute to tell. We must concentrate the elute to determine if protein is present. It's too ambiguous in a dilute form.

We have an ABSOLUTELY POSITIVE protein test result (601 nm vs 632 control) for the Concentrated LC #2 so we definitely have a Protein there, as we have determined in the past. The air, therefore, contains a protein. 400 ul / 3 ml H_2O

The test for phenol (Fe^{+3}) continues as negative, even for the Concentrated LC #2.

Sep 26 2017

The osmometer has arrived, along w/ a manual to see how much works. I think there we are dealing with a $15K instrument that we are trying to get working for less than $1K.

Rough Calibration solutions:

100 mOsm = 0.1 Osm

500 mOsm. = 0.5 Osm

Van Hoff factor = 1.0

Osmole = molality * Van Hoff factor

Molality = $\frac{\text{gms}}{\text{kg}}$ so 1 molality of NaCl = $\frac{58.4 \text{ gms}}{1 \text{ kg of solvent}}$

$\frac{58.4 \text{ gms}}{\text{mole}}$ = $\frac{58.4 \text{ gms}}{\text{liter}}$ (1.0) = $\frac{105.12 \text{ gms}}{\text{liter}}$ is a 1 molal solution of NaCl

Therefore 0.1 Osm = 10.51 gms / liter = 1.05 gms / 100 ml

0.5 Osm = 52.56 gms / liter = 5.26 gms / 100 ml

Everything says that the probe is defective. Replacement part is $600. Installation Cost unknown.

We know where we stand now.

We are running a second pass w/ LC. We pretty much have this routine down.

Water brings out LC#1 - first compound
Strong alkaline brings out LC #2 - second compound

Notice that color of LC#2 is the same as the CDB viscous protein formation. Compare.

Sep 27 2017

The protozoa kevo arrived. I am attempting to develop additional cultures using spring water, cat food & yeast.

I have good imagery for more than 2 hr under scope.

Lets try a solution of

~~5 drop culture medium ~= (0.30 ml)~~
3 ml culture medium
200 ul ~~10 drop~~ 0.5% protein solution

Paramecium range from ~50 & 300 um
Amoeba are 250 - 750 um

Culture contains amoeba, paramecia, Chilomonas, 20-24 um
Stentor, Euglena & Volvox

Movement in the euglena. Euglena are 25-100 um
There is the best match.
Power looks like about 400x.

Photoscope will make a gif.

Sep 29 2017

We are repeating the protozoa test.

Protein solution is 1ml + 200ul 0.5% protein.
= 0.083% ≅ 0.1% solution.

Time elapsed ~ 1 hr. 415 x 360

Motility seriously impaired
Mortality increases

after 1½ hrs all Euglena appear to have expired.

70-80% of paramecium appear seriously impaired or expired.
Motion is erratic and usually circular or back & forth. dysfunctional

Control - 2 hrs no motion impairment

3 hrs - paramecium & euglena still alive.

Control 02 ↔ Protein 05
Control 01 ↔ Protein 04

Sep 30 2017

A couple of trials have been set up with mealworms.

	Control:	Trial:
(1)	Mealworms Moisture (H_2O) Chopped Potato Pine Sawdust Bed Dark Room Conditions	Mealworms 5% CDB Protein Solution Moisture Chopped Potato, squeezed dry & saturated with 5% CDB Protein Solution Pine Sawdust Bed Dark Room Conditions
(2)	Mealworms H_2O Saturated Oats H_2O Moisture Pine Sawdust Bed Dark Room Conditions	Mealworms 5% CDB Protein solution saturated oats (old fashioned) 5% CDB Protein Moisture Pine Sawdust Bed Dark Room Conditions

Yeast, plants (2) and protozoa have now been subjected to a protein solution. Results except for bean plant have been posted - all unfavorable. The results for the bean plant are also unfavorable.

Where are we headed for the last days of the season for the lab?

1. The LC#2 protein needs to be examined in more detail between the different Collection tubes. It also needs to be compared more directly to the CDB secreted viscous protein since the color is so similar. Remember that the solution (heated) was browned going into the columns and comes out green. This mean that we may have a redox process in place or pH influence.

2. Point of Reckoning - Part III is entitled and should be done before breaking down.

3. Thiocyanate comment w/in supplemental discussion paper?

4. Meal worm & bean trials are to be monitored and results posted.

5. Ultrasound trials should be continued.

6. Need to isolate desired books for travel & lab shutdown.

7. I would like to get the osmometer fixed if @ all possible. Money is the issue.

8. Protozoa cultures sustained?

Let's start the protein detection w/ UV analyses.

We have 3 tubes. Two appear to be the same and #1 tube has a precipitate and appears different.

With #2 the precipitate isolated, the solution isolated, and then mixed up.

Sample tube #1: Precipitate combined w/ a clear solution

1. Start w/ clear solution on the top, LC #2
   We have absorbance @ 225 nm
   w/ gradually increasing absorbance ~ 310 nm.

2. Now for LC #2, precipitate dissolved in H_2O.
   The absorbance IS THE SAME, even though there are two separate layers, one precipitate and one a clear solution. This was unexpected
   Notice in both cases there is a barely detectable increase in absorption @ ~ 278 nm

There is no need to mix or stir the solution since they are the same.

Now for sample tube #2 of LC #2:

The general pattern of absorbance is exactly the same except that we do not have an exact peak @ 225 nm. If there is a peak, it is close to 222 nm. But nevertheless there may be a difference here. The general pattern of increasing absorbance < 310 nm is the same as well as the mean absorbance of ~278 nm.

In general, it does look to be the same.

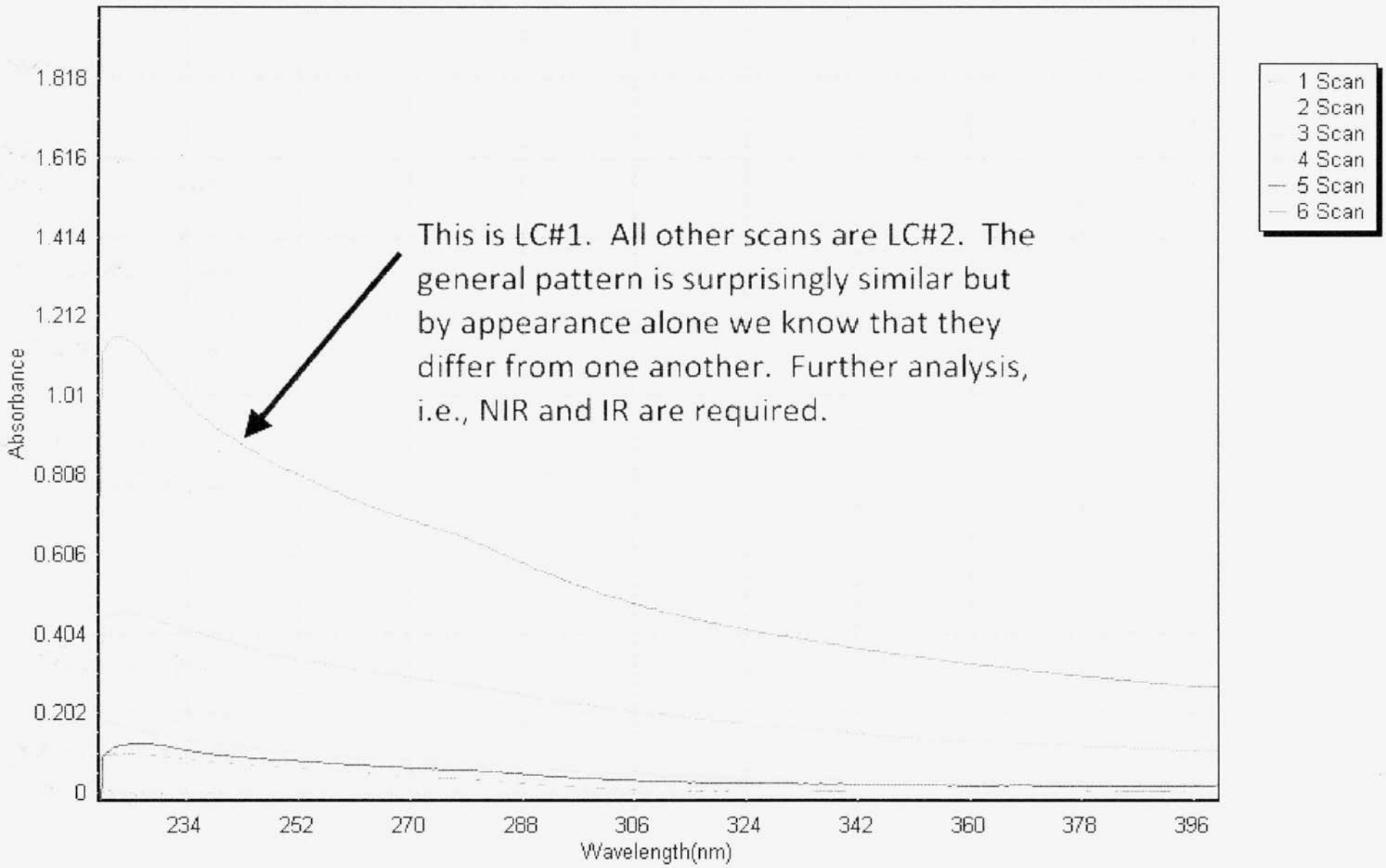
Now for sample tube #3, LC #2
Everything is the same as well for tube #3, except max peak absorbance still shifts to 227 nm.

In essence, the UV absorbance of all 3 sample tubes of LC #2 are essentially the same
They should be essentially equivalent to one another.

Now for LC #1: UV Analysis

We find that the UV spectrum of LC #1 is actually almost identical to LC #2, therefore we do not have adequate distinction @ this point even though by appearance alone we know that they must be different from one another. This was accepted.

LC 1 and LC2 Compared - Suprisingly similiar at this point.jpg



We have no adequate distinction between LC#1 & LC#2 samples @ this time.

Next we go to NIR analyses.

1. LC#1 - Clear Solution portion NIR

There does not appear to be any clear NIR absorption here.

ArCH LC#1 - Precipitate section. Limited material available
RNH2 We do have dynamic absorption @ 1080 nm. ArCH
Also very minor peak @ 1038 nm RNH2.
We also have some absorption @ 858 nm. ArCH and/or RNHR'

OK, now we see that there appear to be a clear distinction between the clear portion & the precipitate section of LC#1.
This makes the case that the precipitate is likely protein in nature. It also has some aroma to it.

2. Now for LC#2 - NIR analyses
Tube#1: We may have a concentration problem here. Possible minor absorption @ 1074 nm but very weak. Try a more concentrated sample.
ArOH (with a greater concentration (but it is still weak) we have some absorption visible @ 962 nm. This is all.

This is ArOH. There appears a difference between LC#1 and LC#2.

This is suggesting that for that the Protein is more likely to be concentrated in the precipitate section of LC#1.

Conflict to be resolved

However look @ the notes of Sep 25.
It states quite emphatically that CONCENTRATED LC#2 POSITIVELY passes a Bradford reagent test for protein

BUT THAT LC#1 fails the Bradford protein test.
Can have to assume the solution was stirred a mixed.

This definately produces a mixed picture.
* This is going to need some serious sorting out.
Very mixed messages here.

LC#2 Tube#2 - NIR
We get exactly the same result. With the original solution (full original concentration) we set the same absorbance @ 966 nm.

ArOH

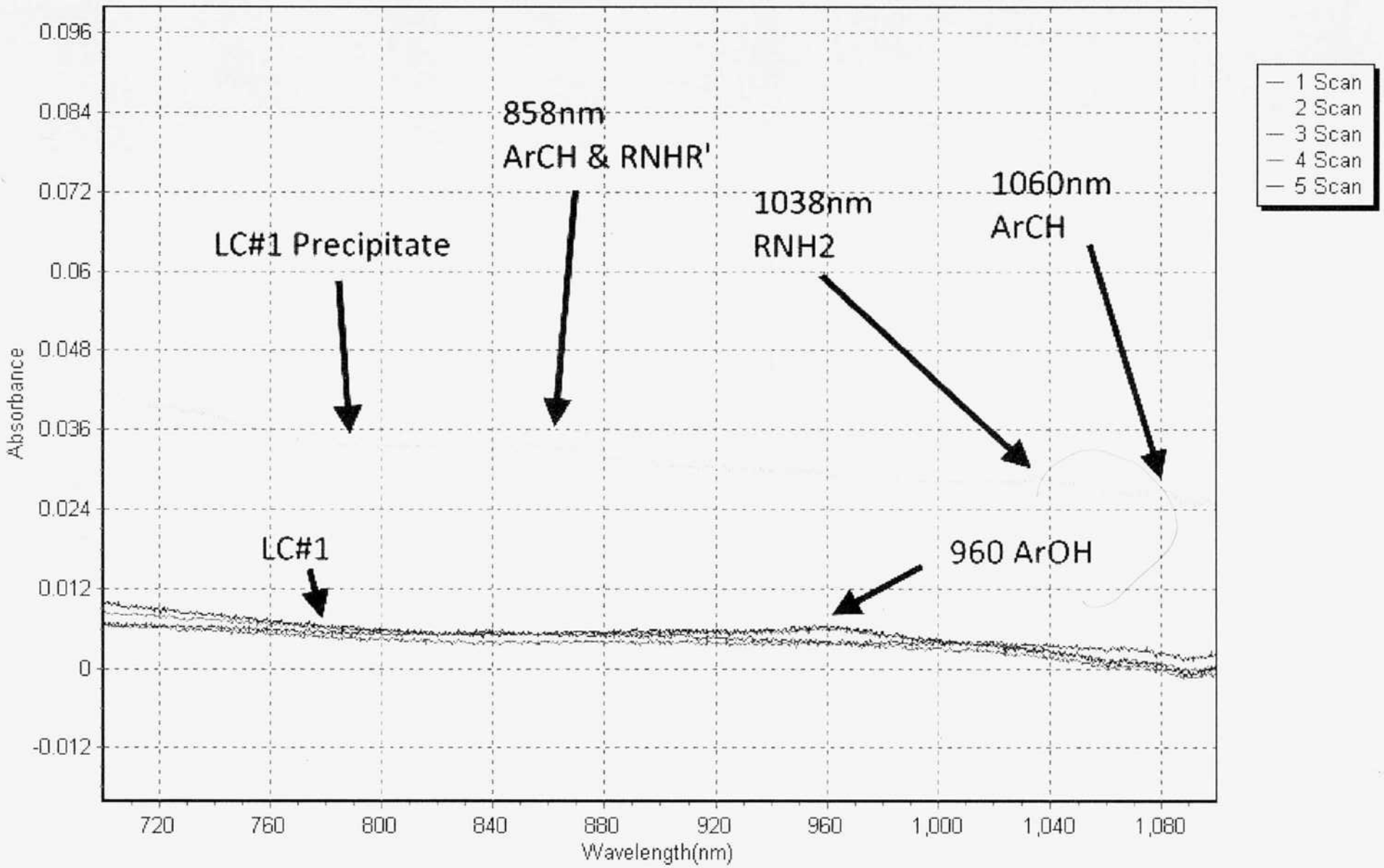
This is ArOH.

How are these results going to compare to analogues of the original CO3 viscous protein?

Also very interested in conductivity of the protein.
Need 3 levels of dilution if possible.

NIR is providing some useful discrimination information between LC#1 (precipitate & clear portions) and LC#2. We do however, have conflict w/ Protein - Bradford reagent tested from Sep 25 2017. Continue to monitor w/ concentration & IR analyses.

LC1 and LC2 NIR Analysis Extended Scale Sep 30 2017.jpg



October 01 2017 Page 61

The meal worm cultures progress report.

The potato-sawdust culture seems to be the have the greatest success so far. I do not think the sawdust alone is a very good base for a culture.

All other cultures seem to be satisfactory thus far.

It does look like the oats-sawdust mix is reasonably successful thus far, roughly a 50-50 mix. Moisture also appears to be key for survival.

The oats appear to be far superior than potato to use. I anticipate the project will need to be replated @ some point but hopefully we can gain some knowledge from the trial.

I think that the oats-sawdust culture will be the primary trial that is made this time.

The bean project is slow and steady. Looks good.

all @ UV-NIR first, later for IR
Now onto the protein situation, which is somewhat complex.

Many issues to resolve here.

done 1. Get the conductivity measurement.

2. Compare LC#2 against the dilute collected CDB viscous protein. UV & NIR

3. The Bradford test has created some complications. We need to sort this out.

4. We also need to sort out the precipitate vs the colored elute from LC #2.

5. We also need to further characterize and identify elute #1, ie LC#1 from the HEPA.

6. We need to look @ the IR plots and continue to sort out the numerous relationships that exist.

Lets try & get a conductivity measurement of the CDB rescue material @ 3 different dilutions but also use as little material as possible.

Weight of weigh boat = ~~3.22 gms.~~ 3.23 gms
Weight w/ toothpick = 3.34 gms.
Weight w/ Protein added = 3.79 gms (High quality protein)
Actual protein weight 0.45

1st Water sample total = 13.62 gms w/ toothpick
Electrical Conductivity: EC = 5.11 EC
13.62 gms − 3.79 gms (weigh boat + pick + protein) = 9.83 gms H_2O
So we have $\frac{0.45 \text{ gms protein}}{9.83 \text{ gms protein} + H_2O} = .046 = 4.6\%$ by total wt.

2nd Water sample total = 20.79 gms w/ toothpick
EC = 3.47 ~~gms~~ EC
20.79 gms − 3.79 gms = 17.00 gms H_2O
So $\frac{0.45 \text{ gms protein}}{17.00 \text{ gms protein} + H_2O} = .026 = 2.6\%$ by wt

3rd Water sample total = 30.18 gms w/ toothpick
EC = 2.45 EC
30.18 gms − 3.79 gms = 26.39 gms
So $\frac{0.45 \text{ gms}}{26.39 \text{ gms}} = .017 = 1.7\%$ by weight

Now let's figure what we have.

These we now know

% Protein by total wgt of Dilute Solution (x)	EC (y)
1.7%	2.45
2.6%	3.47
4.6%	5.11

$EC \cong 0.901(\%\ \text{Protein}) + 1.004 \qquad r^2 = 0.993$

or

$\%\ \text{Protein} \cong 1.102(EC) - 1.086 \qquad r^2 = 0.993$

Now, what we are interested in is extrapolating to the full strength VISCOUS protein.
This would be 100% protein.
This would imply that

$EC \cong 0.901(100\%) + 1.004 = \underline{\underline{91.104}}$

That is significant.
What would it take to produce a salt solution of the level of conductivity?

NaCl solution Comparison:
Wgt of beaker = 127.38 gms.
~~wgt w/ water added = 210.60 gms.~~ 219.48 gms

Therefore mass of H_2O = 83.22 gms = 83.2 ml.

Now add salt.

~~Wgt EC~~
~~211.92~~
—

need an EC of 91

My meter only goes to 19.99 EC so we must max out the meter first and then multiply by the factor.

Mass of beaker + H_2O + salt	EC
219.48 (H_2O alone)	0.00
220.13 gms (w/ salt added)	18.65

We can now equate the two conductivities.

$\frac{\text{EC of } 91.1}{\text{EC of } 18.65} = 4.88$ EC Multiplicative Factor.

Salt used for EC of 18.65 is (220.13 gms − 219.48) = 0.65 gms

And with factor, the mass that we needed to add:

0.65 gms (4.88 EC Factor) = 3.17 gms

And our volume of water is: 219.48 gms − 127.38 gms = 92.10 gms H_2O

Therefore our equivalent NaCl concentration solution is: ~~0.71~~ 3.17 gms NaCl / 92.10 gms H_2O

But by weight, what we actually have is:

$$\frac{3.17 \text{ gms NaCl}}{92.10 \text{ gms } H_2O + 3.17 \text{ gms NaCl}} = .033 = 3.3\%$$

NaCl salt concentration by weight.

Ok, this is interesting.
We can now estimate that the conductivity of the pure CDB viscous protein is approximately equal to a 3.3% NaCl salt solution by weight.

This says that we have dissociation or ionization to a fairly significant degree.

What is the concentration of salt water in the ocean? How about the body?

Well, quite fascinating but the ocean has a concentration of ~ 3.5% by weight of dissolved salts.
So the pure CDB viscous protein is essentially equivalent to the conductivity of salt water in the ocean.

Blood has a concentration of 0.6% dissolved salts

Ok, we have solved the problem and it has been posted. Conductivity is a significant property and gives us a sense of ionization that is likely to be taking place.

As an example of conductivity comparison, does powdered milk in solution conduct electricity?

The conductivity of powdered milk in water is quite less in ~~low~~ comparison.

A very small amount of ~tsp milk powdered in ~ 150 ml of H_2O gave an EC reading of 1.65
vs a very small amount of salt → ~ 2.5 EC
So there is indeed some conductivity to powdered milk in solution but it appears to be less than that of the CDB viscous protein. But it is not to be dismissed, as they are similar in some respects.

Ok, we now go back to UV-NIR analysis of the CDB viscous protein. We have a very pure dilute solution available that is suitable for UV-NIR work.

We also have a dilute collected CDB viscous sample available.

Our interest lies in comparing UV-NIR between
1. LC#2 samples
2. Pure dilute protein sample
3. Collected dilute CDB viscous protein.

As an aside, the ORP (oxidation reduction potential) of the highly dilute protein has been measured @ + 285 mV.
This means that it is of an oxidizing nature.

Powdered milk (also in distilled water) came in with an ORP of +30 mV.

This says that the COB viscous protein is of a stronger oxidizing nature than powdered milk is.

We now have the UV and NIR plots of the purified but dilute COB viscous protein. Good.

Now let's go to the dilute collected COB viscous protein sample and do the same.

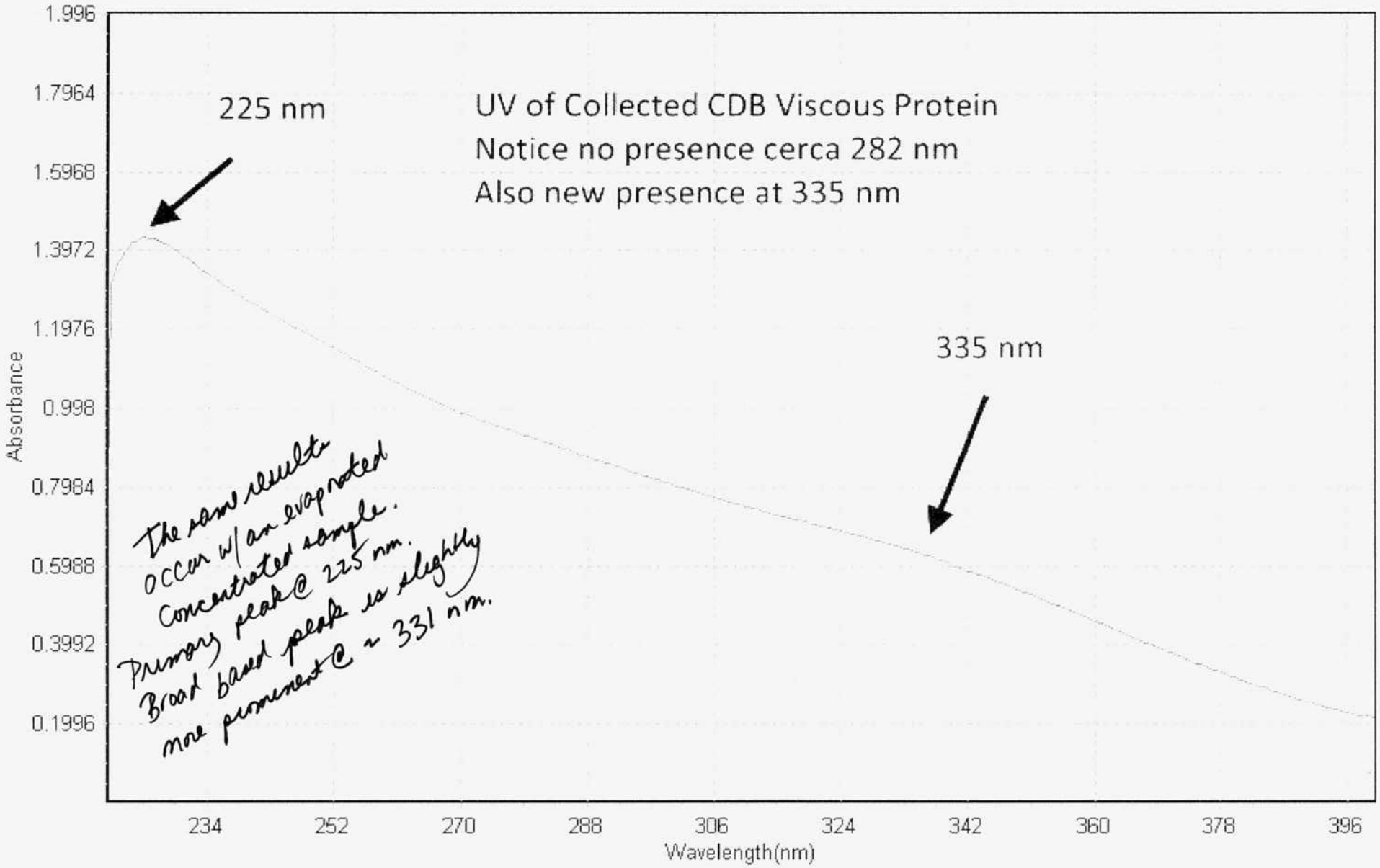
O.K, we now have UV & NIR of:

1. LC #1 & LC #2 (Precipitate & clear portion) and subsequent tube (clear, colored)
2. Collected dilute COB viscous protein
3. Purified but dilute COB viscous protein (most pure & concentrated form).

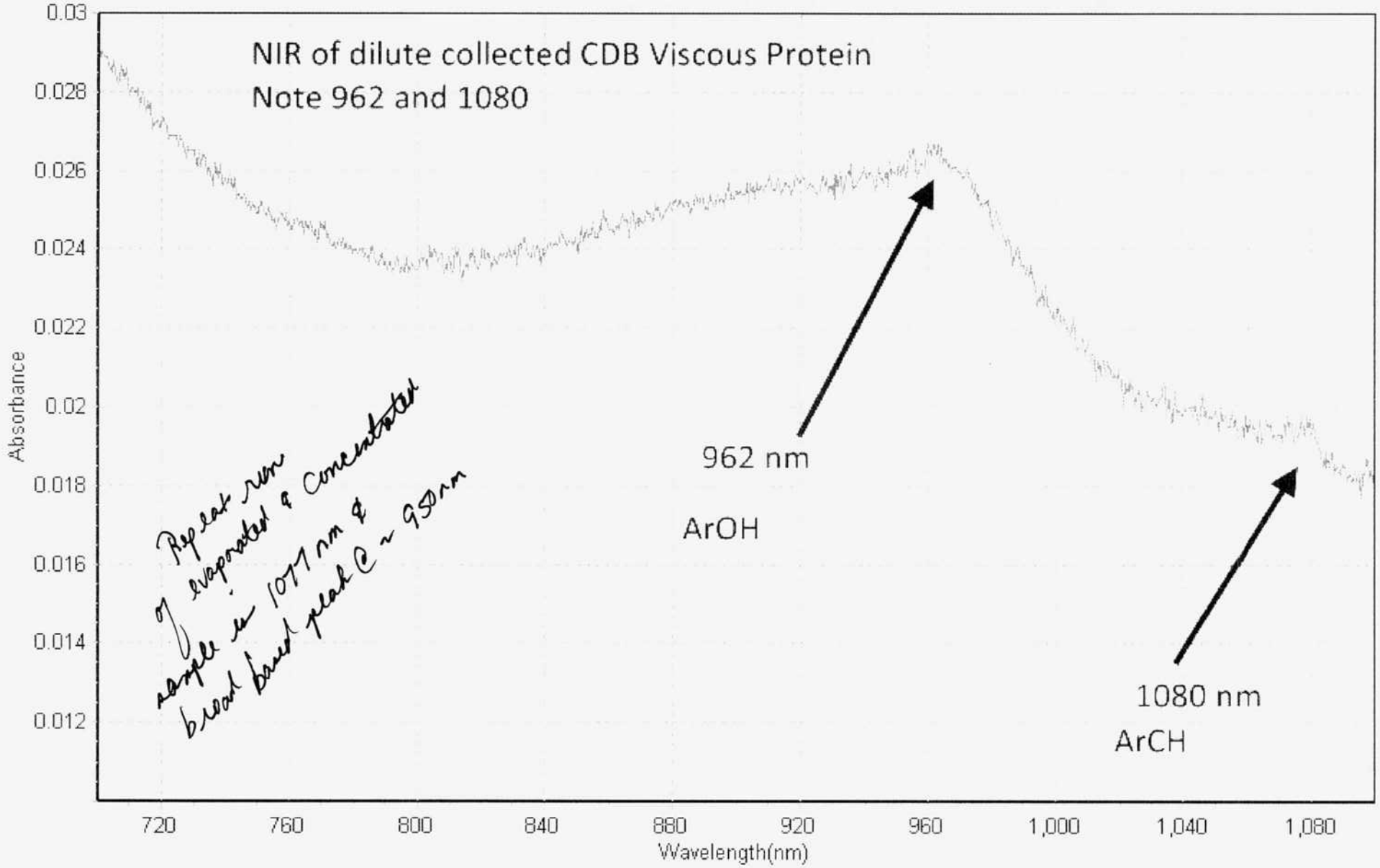
1. Now we need to compare similarities & differences
2. Need to look @ Bradford reaction absorbance & relate to concentration of dilute samples
3. Then we progress to IR analysis

UV & NIR of collected dilute CDB Viscous Protein

UV Collected Dilute CDB Viscous Protein Oct 01 2017.jpg

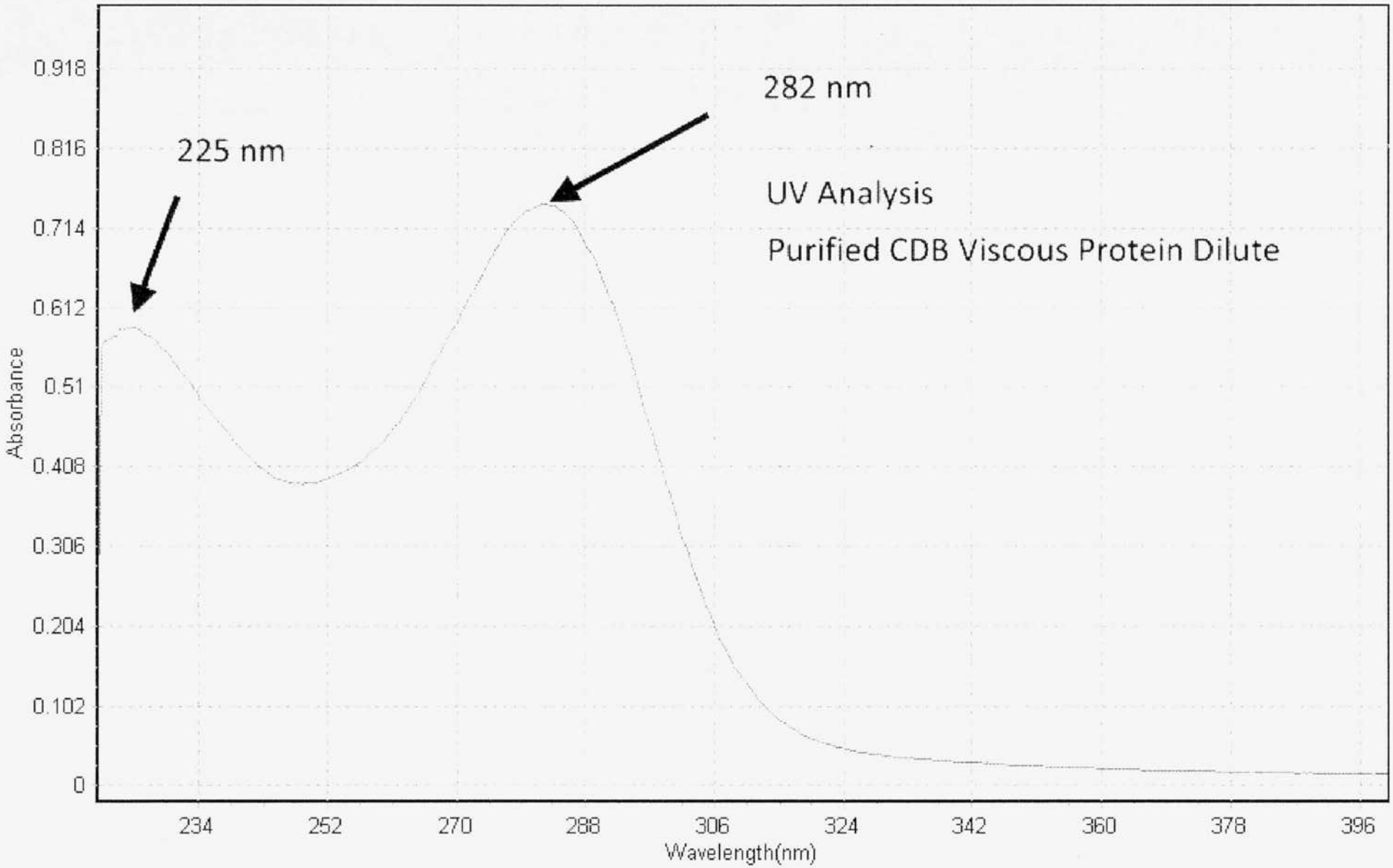


NIR Collected Dilute CDB Viscous Protein Oct 01 2017.jpg

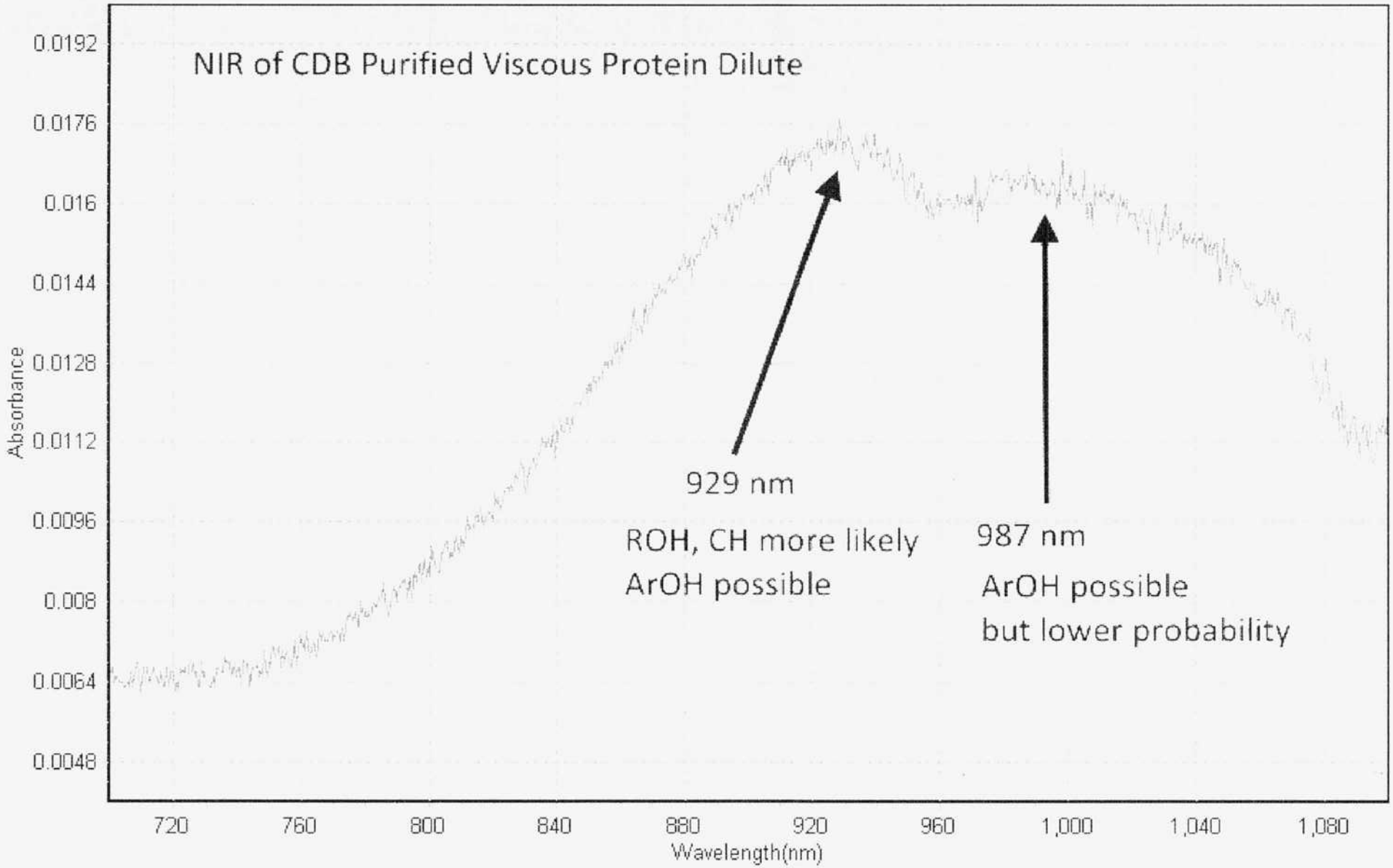


UV & NIR of Purified dilute VISCous CDB Protein

Purified CDB Viscous Protein Dilute Oct 01 2017.jpg



Purified CDB Viscous Protein Dilute NIR Oct 01 2017.jpg



OK, time for regrouping.

(1) We have collected now some good UV-NIR data on three different types of samples.

1. LC separated compounds
2. Dilute Collected CDB protein sample
3. Purified CDB Mucosa protein.

(2) We have the Mean project in place

(3) We have the worm project in place

(4) We would like to get the Point of Reckoning Part III in place but am not sure if this is realistic or not.

Lets look @ the UV-NIR data in more detail now.
Lets start w/ the purified concentrated viscous form first.

(1) Concentrated - Purified CDB Visous Protein

UV		NIR	
225	definite	929	very weak
282	(very strong) Protein Expected	987	extremely weak

(2.) Dilute Collected CDB "Protein"

	(definitive)	ArOH	~950 broad based (repeat run)
225		ArOH	962 noticeable
335	extremely weak	ArCH	1080 noticeable
concentrated 331	broad based peak.		1077 (repeat run)

(3) LC #1 (Turbid - Precipitate) ArCH, RNHR' 858
(aroma) 225 RNH_2 1038
~278 Weak Protein? ArCH 1060

(4). LC #2 (Clear solution) (Tube 1)
225 ArOH 960
Weak Protein? 278 extremely weak

LC #2 (Precipitate) (Tube 1) ArOH 960
225
Weak Protein? 278 extremely weak

LC #2 (Clear & Colored) Tube 2 ArOH 960
225
Weak Protein 278 extremely weak

(1) We now need to characterize any patterns w/in the data.

(2) After that we need to perform Bradford tests

(3) Then we need to go after the IR analyses.

Characterization of UV-NIR data.
UV

Purified, concentrated CDB viscous protein shows a extremely strong absorbance peak @ 278 nm.

It is interesting that the collected CDB dilute "protein did not show any absorbance in the 278 region. Why is this? We need to concentrate the sample to test this - every theory says it should be protein containing.

Also notice that LC#1 & LC#2 in any all forms, precipitate, no precipitate, elute 1, elute 2 all seem to have some level of ~278 absorbance which suggests both LC1 and LC2 may contain protein, not just LC2. Bradford should help to settle this.

We also have important NIR characterizations which still need to be put to use.

Oct 02 2017

The collected COB sample (test tube origen) was evaporated & concentrated. The UV results are identical to the orginal dilute sample.

Bradford test here is done on a fresh test tube sample. The peak is @ ~~~~ ~ 620 nm to 618 nm. This indicates that protein is indeed present.

We also noticed the evaporated condensed concentrated sample once again did appear viscous.

One question is why we do not see a peak prominence in UV @ ~ 270 nm. We do have high absorbance, just not an identifiable peak.

Everything here says a protein w/ ArOH.

Oct 03 2017

The Beam Report is now also posted.
The evidence is stacking.
The evidence from the Beam Report paper is quite compelling.

The question now is if you are in position to produce
A Point of Reckoning – Part III

What exactly is the essence of Part III?

A lot of good work is compiled within Vol XX

(1) The first skin of plate analyses occurs on Aug 02 2017
w/ IR analyses

(2) We start working w/ the environmental filament again on Aug 25 2017. We appear to have an excellent IR plot. Method used:
1. Microwave digestion under strong alkaline
2. Neutralization w/ HCl, produces salt solution
3. IR ATR & KCl synthesis

Environmental filament matches rainfall IR plot on Aug 27 2017 to a high degree. ($r = 0.92$)

Env. Filament digestion repeated on Aug 28 2017

We have the skin plate match on Aug 28 2017 $r = 0.94$

There is a critical tendency. We are also suggesting an

Oct 09 2017

The lab & house are in full stream packing mode. Everything says both are on their way to SE UT in about one week.

The meal worm culture progress is of some interest.

Oat Cultures:

There is a fungus or mold growing in both control & protein cultures. It may be too damp w/ the trial a oats are susceptible.
No real discernible difference in meal worm behavior.

Potato Cultures:

The protein culture is definitely producing a broad based fungus on the bedding (pine sawdust). This is not occurring in the control potato culture.
No discernible difference in behavior.

The potato culture generally seem healthier than the oat cultures.

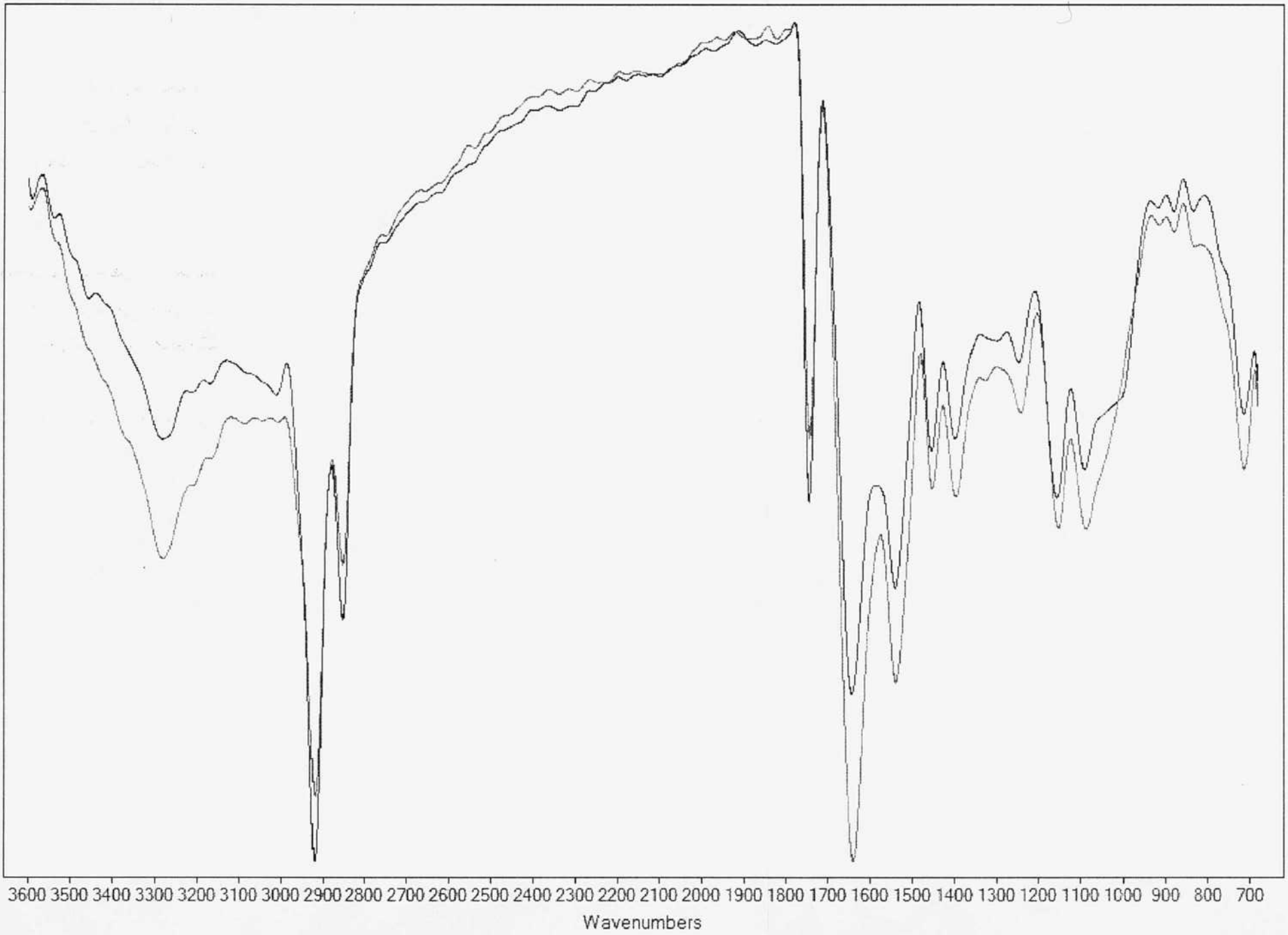
IR spectra on next page suggests at the MOST MARGINAL levels, a potential difference in

1. relative thiocyanate absorption / requires further study @ higher concentrations
2. Difference (again marginal) in alkene presence in control & no alkene presence in protein culture.

Control vs Pro Leg - ATR

1st IR Mealworm Spectra - Marginal if any Difference

Mealworms Control vs Protein Oats Ground Oct 09 2017 - 03.JPEG



This is, to my knowledge, the first time that I have subjected living tissue to IR analysis.

In this case, it is "ground mealworm".

Two different cases & cultures gave very repeatable & similar results. This speaks well for the method. Minor differences might be discernable; this is a topic for further research.

The methacrylate detection proposal is a very interesting one. Resolution may simply require a longer time of exposure, increased concentration, or both.

The alkene (alkene) distinction may also be something important to look for in the future.

There are now many opportunities to consider for live tissue analysis of plants, food, etc. & other life forms.

Oct 30 2017

It is somewhat official today. The laboratory and the bonefront have both been moved now to Monticello Ut-Lab.

There is no question it has been and is quite a production to accomplish this. After a week of unloading, unpacking and setting up it looks to be approximately 40% operative already.

The primary instruments (IR, GC, UV-Vis) appear to have arrived safely. All work is likely dependent upon these instruments nowadays.

The only loss identified thus far is the distillation column. It may yet be found, but an alternate is on order.

It is time to break in the lab gradually again, it is a complex system w/ a fairly small space. Every square inch is pretty much accounted for here.

Oct 31 2017 - Halloween - Monticello UT

We start the lab again w/ a study and exercise in volatility.

The sample material is that of starting fluid. This is a combination of ether and heptane.

It was noticed yesterday w/ an IR trial that the ether signature w/ IR was extremely slight. This was unexpected. The sample came from a bottled sample that is not airtight. And then, of course, it does make sense. The high volatility of ether means that the majority of it has been driven off by evaporation over the last month, even from within a stoppered bottle.

And so now we have a fresh sample for comparison, and the difference in the peak @ 1122 is now quite significant.

And so the lessons are:

1. The sample must be fresh to capture volatiles

2. Separation from the sample, namely the volatiles, can be lost forever and will therefore be missing in the Analysis.

These are important lessons. Not necessarily fatal, but definitely could lead to an incomplete analysis.

IR plots follow.

Lets try & set a conductivity measurement of the CDB viscous material @ 3 different dilutions but also use as little material as possible.

Weight of weigh boat = ~~3.22 gms.~~ 3.23 gms
Weight w/ toothpick = 3.34 gms.
Weight w/ Protein added = 3.79 gms (High quality protein)
Actual protein weight 0.45

1st Water sample total = 13.62 gms w/ toothpick
Electrical Conductivity: EC = 5.11 EC

Weigh boat + pick + protein + water 13.62 gms − 3.79 gms (weigh boat + pick + protein) = 9.83 gms H_2O
So we have $\frac{0.45 \text{ gms protein}}{9.83 \text{ gms protein} + H_2O} = .046 = 4.6\%$ by total wt.

2nd Water sample total = 20.79 gms w/ toothpick
EC = 3.47 ~~gms~~ EC
20.79 gms − 3.79 gms = 17.00 gms H_2O
So $\frac{0.45 \text{ gms protein}}{17.00 \text{ gms protein} + H_2O} = .026 = 2.6\%$ by wt.

3rd Water sample total = 30.18 gms w/ toothpick
EC = 2.45 EC
30.18 gms − 3.79 gms = 26.39 gms
So $\frac{0.45 \text{ gms}}{26.39 \text{ gms}} = .017 = 1.7\%$ by weight

Starter Fluid ATR Hexane - Diethyl Ether Oct 31 2017 - 01.JPEG

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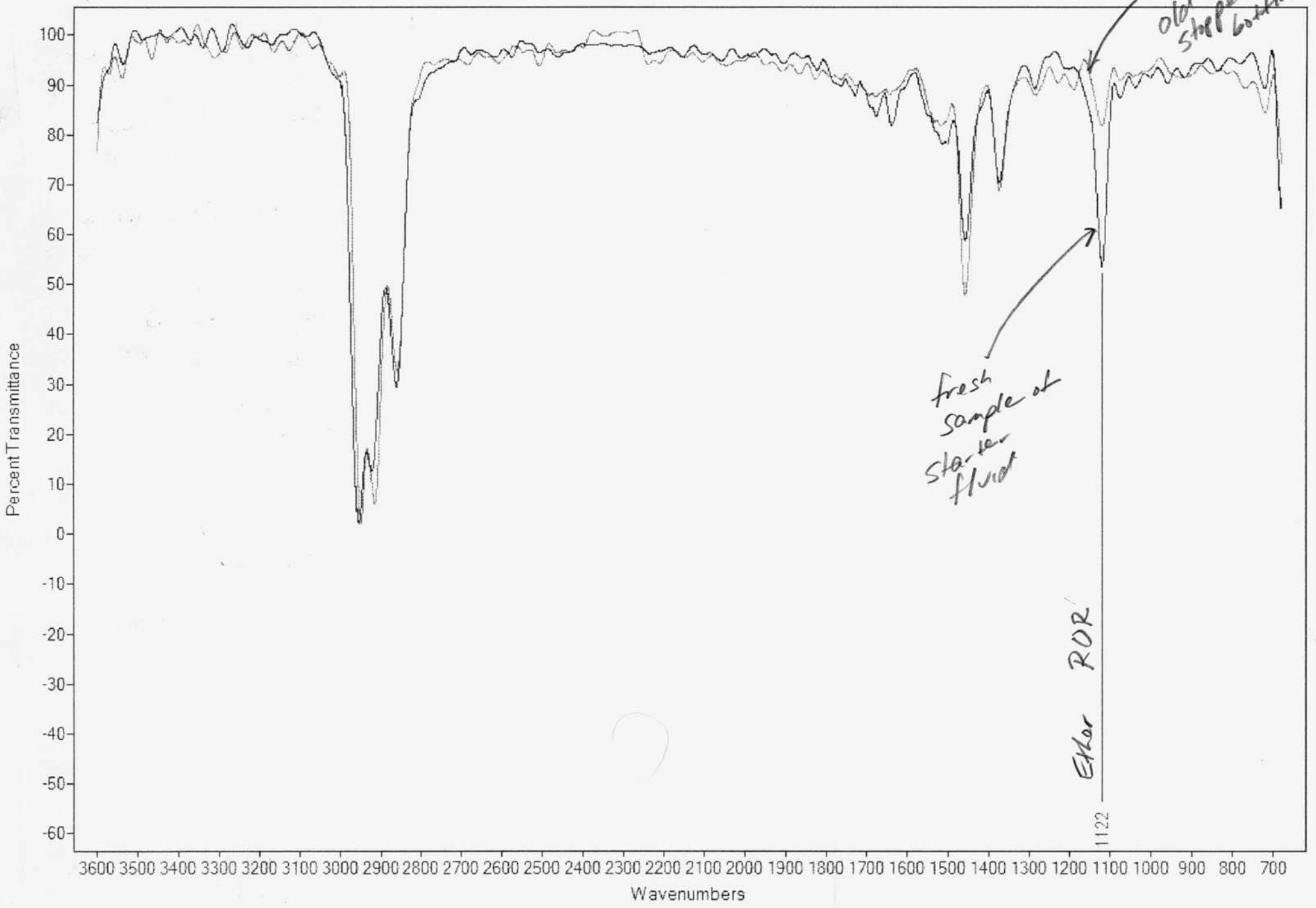
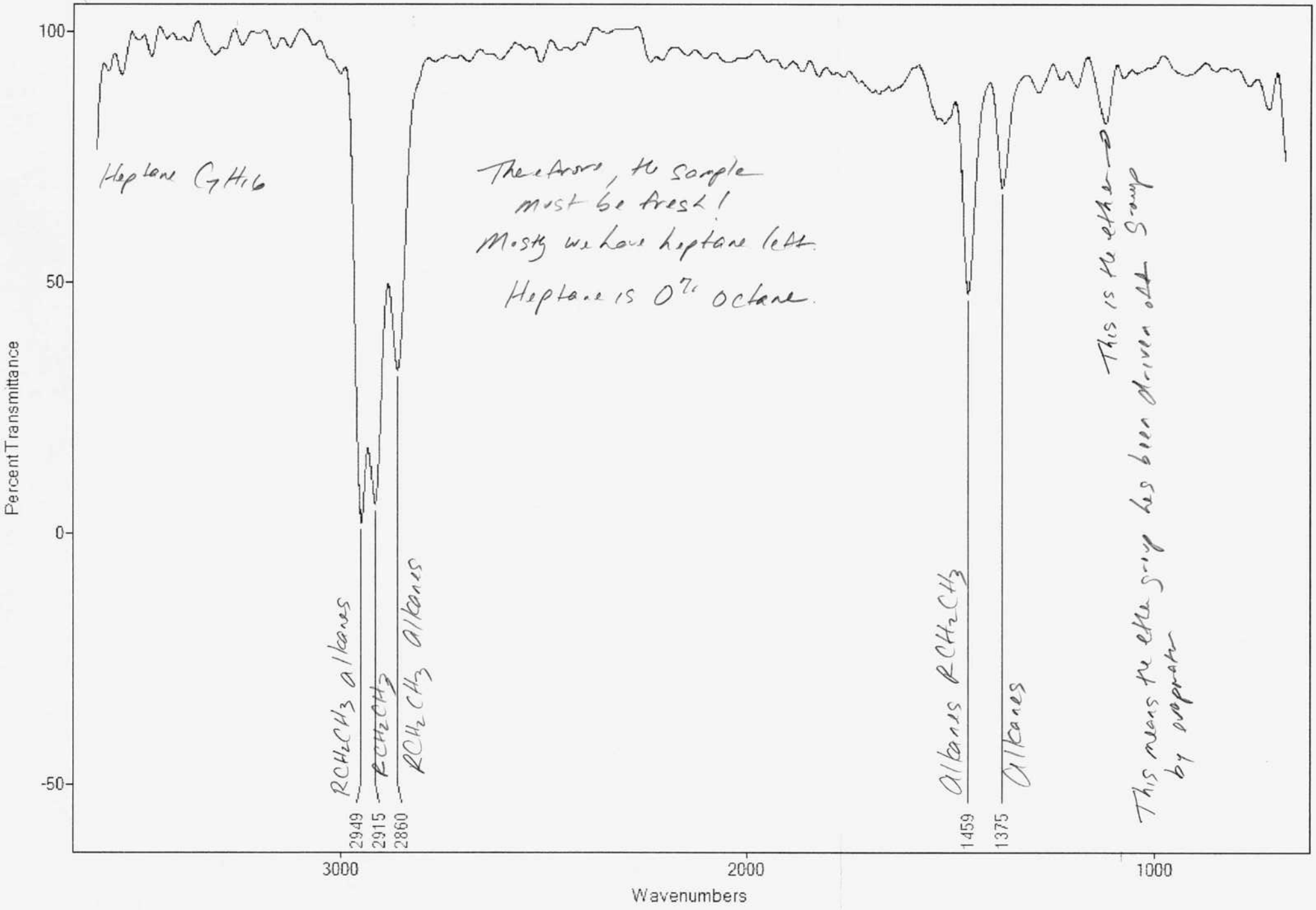


Starter Fluid ATR Hexane - Diethyl Ether Oct 29 2017 - 01.JPEG

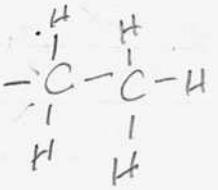


I am looking @ some very basic IR spectra here to
1. Re establish operation & calibration of the IR spectrometer
2. Test the impact of current background spectrum as well as averaging, especially in the CO_2 region. Conclusion is that it does help to be current & to average 3 spectra. We also notice CO_2 levels are varying between 550-900 PPM @ the location with the daily cycles. Wallace ID was seldom over 550 & generally ranged from 450-550 PPM There is an interesting difference that I cannot explain. Also, for example it is reading 860 PPM @ 0000hr so it is not an increase w/ daylight.
3. Conduct a more in depth of functional group presentation, such as Ethers, aldehydes, ketones, & esters for example.

Lets go back to our ether spectra. First, the sample material is auto starting fluid. The means

diethyl ether
heptane
propane
butane
CO_2

ethyl? CH_2? Actually CH_2CH_3

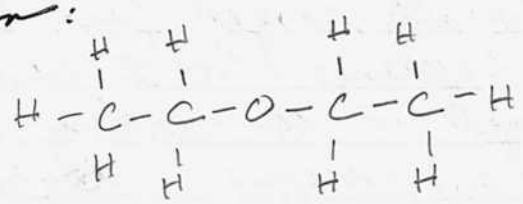


So the ethyl group is actually a modification of ethane (i.e., C_2H_6) to remove one hydrogen to result in C_2H_5.

Now diethyl will have two of these structures.

Next we know that ether is ROR.
What is exactly what we are studying.
This means we anticipate diethyl ether to
evolve.

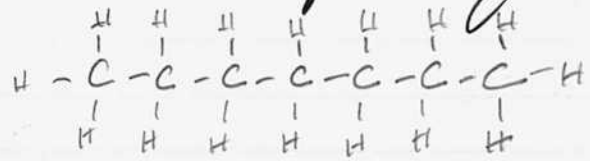
This means that I anticipate a structure of the
form:



Now let's see what it actually is:
It is

$CH_3CH_2OCH_2CH_3$ $\quad C_4H_{10}O$ or $(C_2H_5)_2O$

so we have it right. Good work.
Now for the IR spectrum analysis, allowing disregard
for propane & butane. We will also include
the Unknown heptane of C_7H_{16} (OK).



We expect to see alkane group
CH_2 group
CH_3 group
ether group.

We do see (2949, 2915, 2860, 1459)
RCH_2CH_3 OK
& ROR (1122 cm^{-1})

This is a
perfect match.

You could not get a more textbook example of an IR match than this.
It would obviously be very easy to identify diethyl ether by combination of IR analysis + boiling point determination.

But! it is also highly volatile, so you would easily lose it.
Notice also the intensity of the hydrocarbon peaks vs what is going to happen w/ acetone.

—

With acetone, we have
2992 (but very weak!)

1708 strong
1425 weak
1536 (moderate)
1217 (moderate)

We also know that the acetone is likely not pure.

2992: Alkanes RCH_2CH_3
Carboxylic Acid

1708 RCO-OH Carboxylic Acid
C=O dimer (strong)

1425 S=O sulfate
C-C in ring Aromatic ArC-C

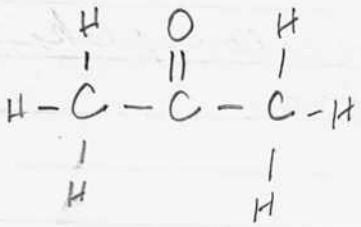
1536 RCONHR', N-O, N=O

1217 N-O, phosphorus, Esters (RCOOR')

Now, this does get interesting. It certainly does not look like the acetone is "pure", as we had already surmised from a MSDS recollection. I also believe manufacturer states that the mixture is proprietary.

Also a case for distillation.

Now let's look @ acetone directly:



Notice we do have $R-CH_2$ & RCH_3 but not exactly RCH_2CH_3

There seems to be more to this acetone than meets the eye. It would appear to be more complicated than the MSDS states.

Notice we do not have a Carboxylic acid but we do have the dimer $C_2=O$.

What exactly is a dimer?

A dimer is two identical molecules linked together

Disregarding proprietary additives, we actually have

RCH_3 where R is $C(=O)$

So we must wonder what the additive actually is. It is clearly not just acetone.

Notice also how weak the hydrocarbon absorption is. Is there an intensity Case

Will the MSDS for Sunnyside 1 gallon acetone says that it is pure acetone so I am not sure exactly how this can be.

Note is a case where GC could be quite useful. How many parts to the whole? Why the MSDS complicate?

Acetone ATR IR - why the strong conflicts w/ MSDS?

Acetone ATR Oct 31 2017 - 01.JPEG

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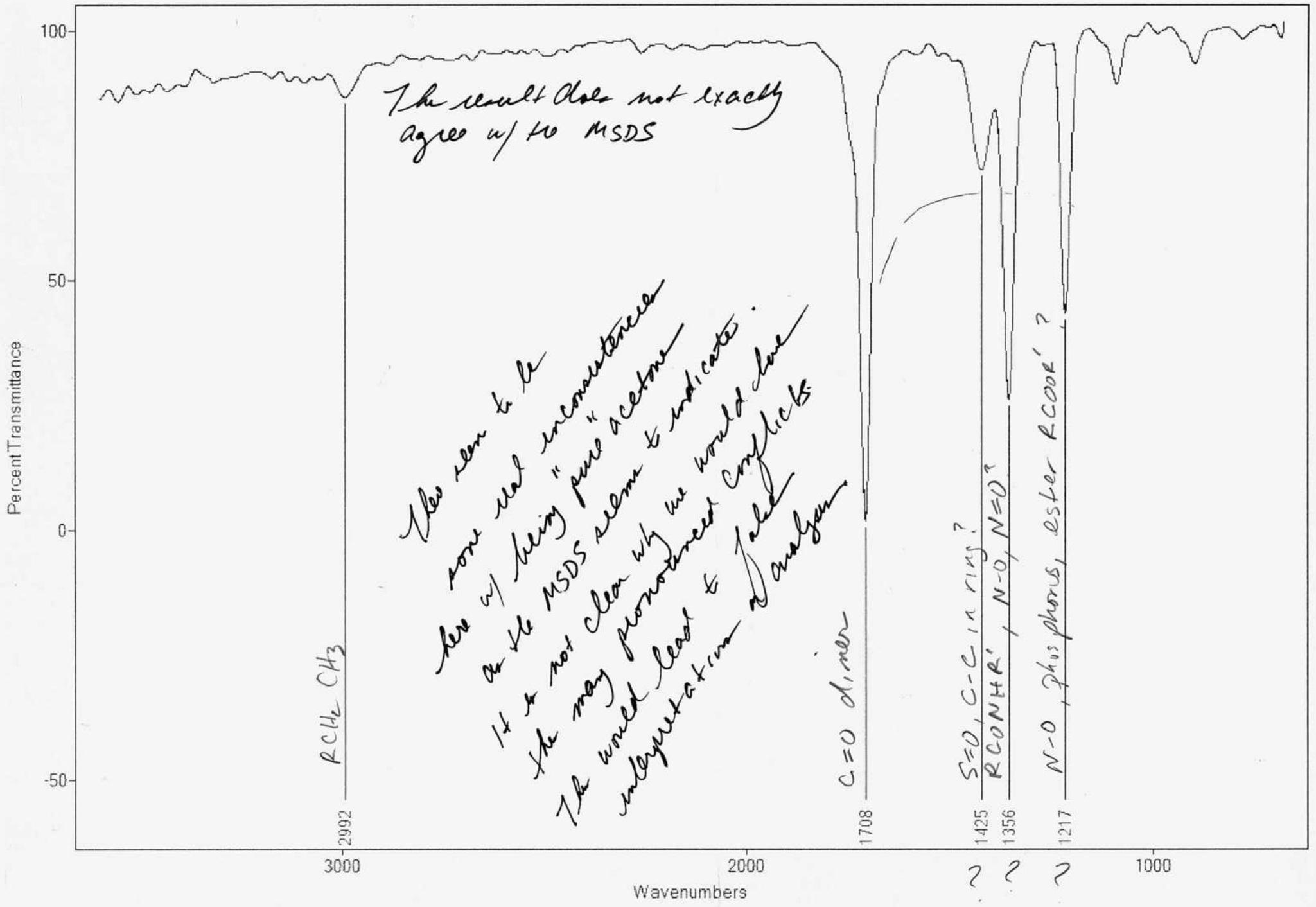


Actually, the plot does thicken, and there is where my memory recall came into play.

On the MSDS sheet, it is stated that it is 100% acetone.

However!

On the product safety sheet it says that it is only 60-100% acetone!

And that the actual composition is a "trade secret" and full composition is not to be disclosed.

So how about that list of additional information?

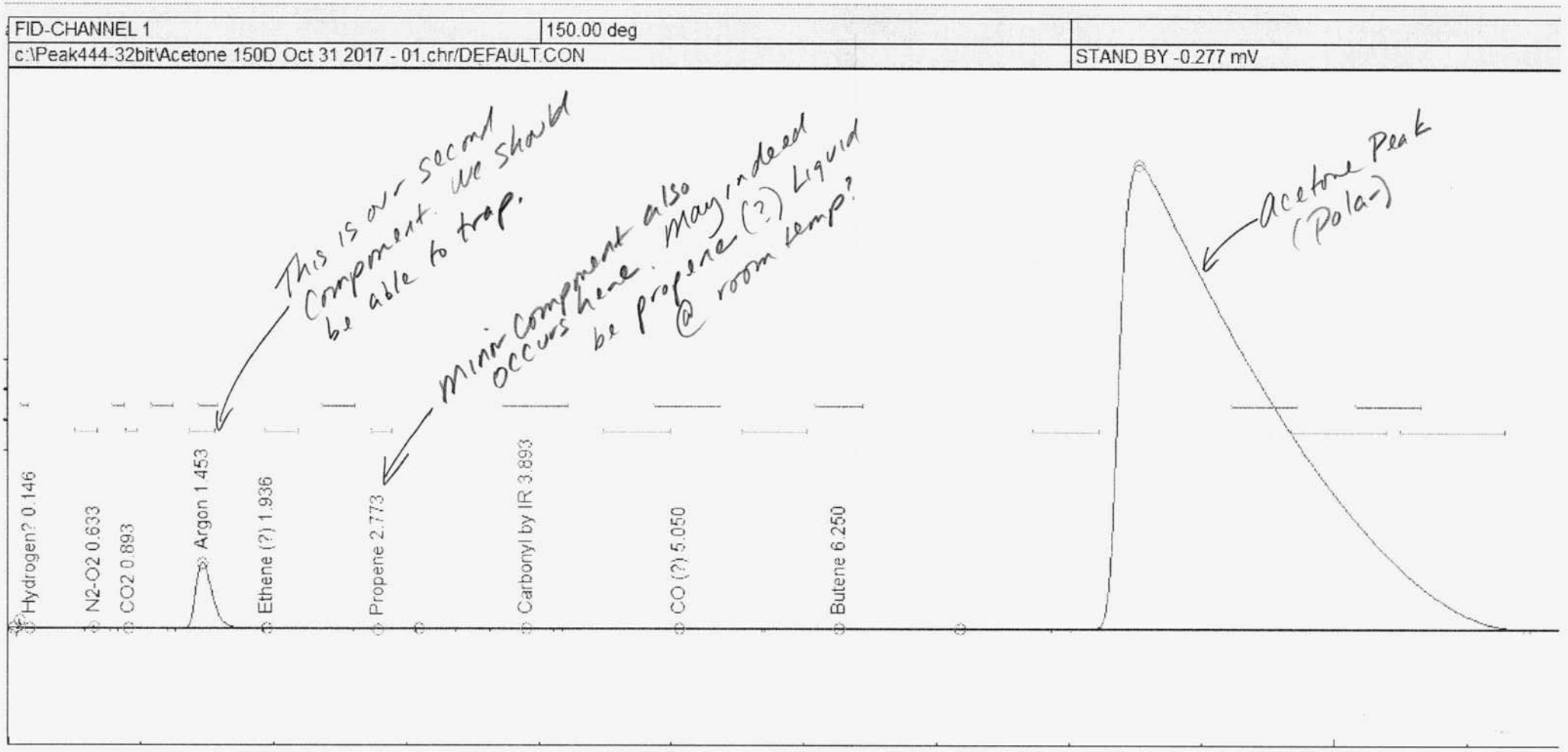
We may therefore very well have additional additives like aromatics in esters (?) but unmyside as a manufacturer is not going to disclose them.

So indeed, it appears that we do indeed not have "pure acetone" but the MSDS continues to contradict themselves.

GC & distillation are probably the next best tools to employ.

Sure enough, GC definitely shows @ least 2 components that is rapid elution, low carbon no, volatile, non polar, then the major component of acetone about 10 min later

Acetone 150D Oct 31 2017



Nov 01 2017

We have an extremely intriguing situation that has developed here. The situation in Ireland involving a mass filament dispersal brings up some very important questions. How do you prove that are not actually spider web filaments as some will claim?

We encountered the same problem w/ the Arizona lab test many years ago. Examples of dispute were
1. size
2 biological w/ w pleurae
3. ribbon appearance of samples

We have conducted an IR spectrum of collected & washed spider webs - Cobwebs.
Our IR spectra presents exactly the same 3 functional groups

3370	dimer OH	Phenols Ar-OH
2074	R-N=C=S	
1633	C=N	Amides, Amines

The suggests we may be dealing w/ truly a synthetic spider web exterior. That is quite feasible technologically & would be a superb cover, as indeed is being used.

We seem to have a deep mystery ahead of us.
The closest match to the spider webs is Carol's hair.
This is actually exactly the most favorable result that can be expected.

Both human hair and spider webs are composed largely of keratin (a keratin form, ref wikipedia on keratin and cell differentiation).

What seems remarkable is that many other items of interest are similar, but just not an exact match, including blood, the environmental filament, and also rainfall concentrate for example.

This indicates the environmental filament may be very similar to spider web, BUT NOT EXACTLY spider web. This is from an IR perspective only. This says nothing about microscopic examination.

* We now require some fine discernment.

1. What exactly is the IR spectrum of the environmental filament?

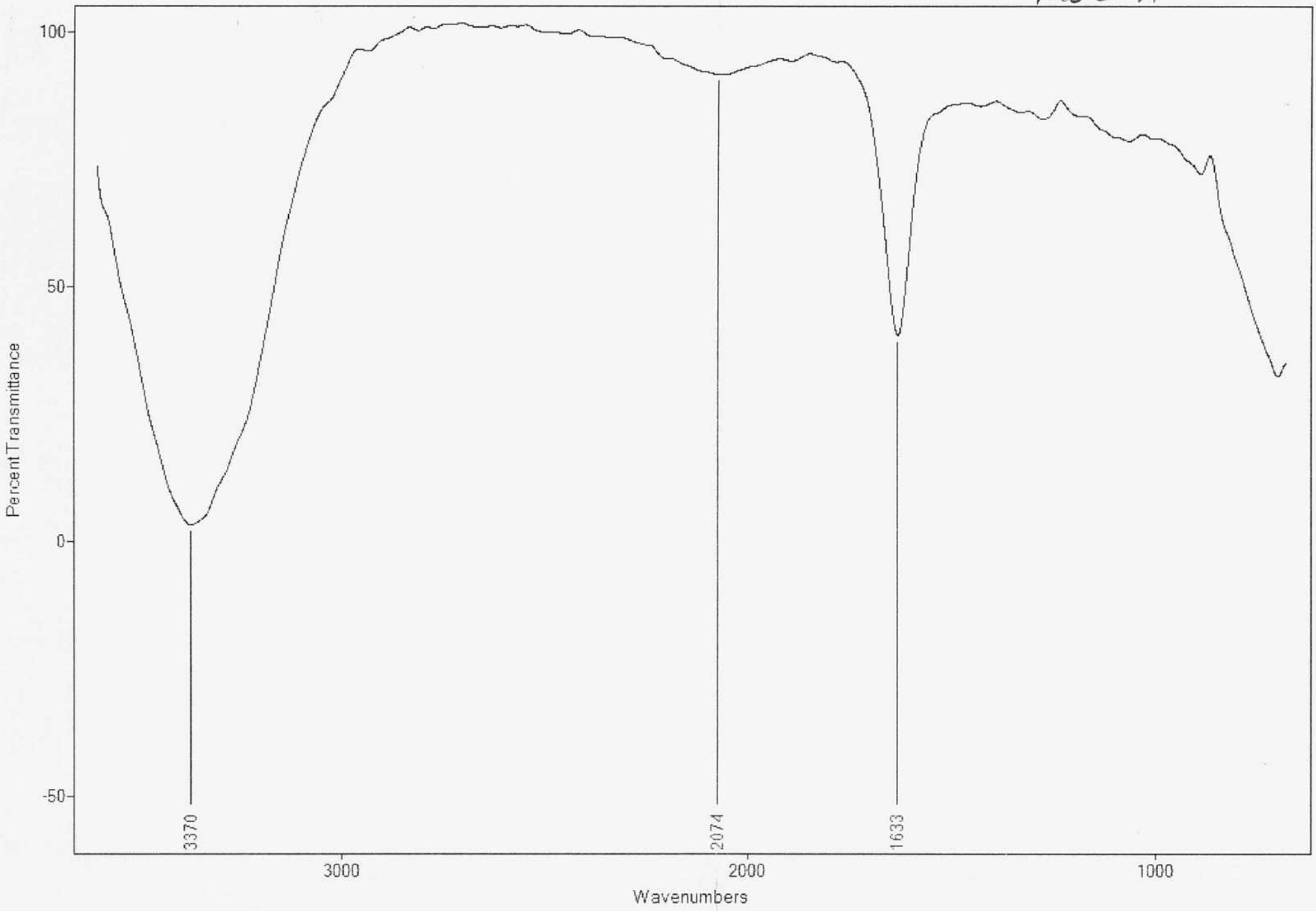
2. What are the visual microscopic differences between the spider web and the environmental filament?

Small differences look to be very important here.

3. What does the spectrum of keratin look like? (IR)

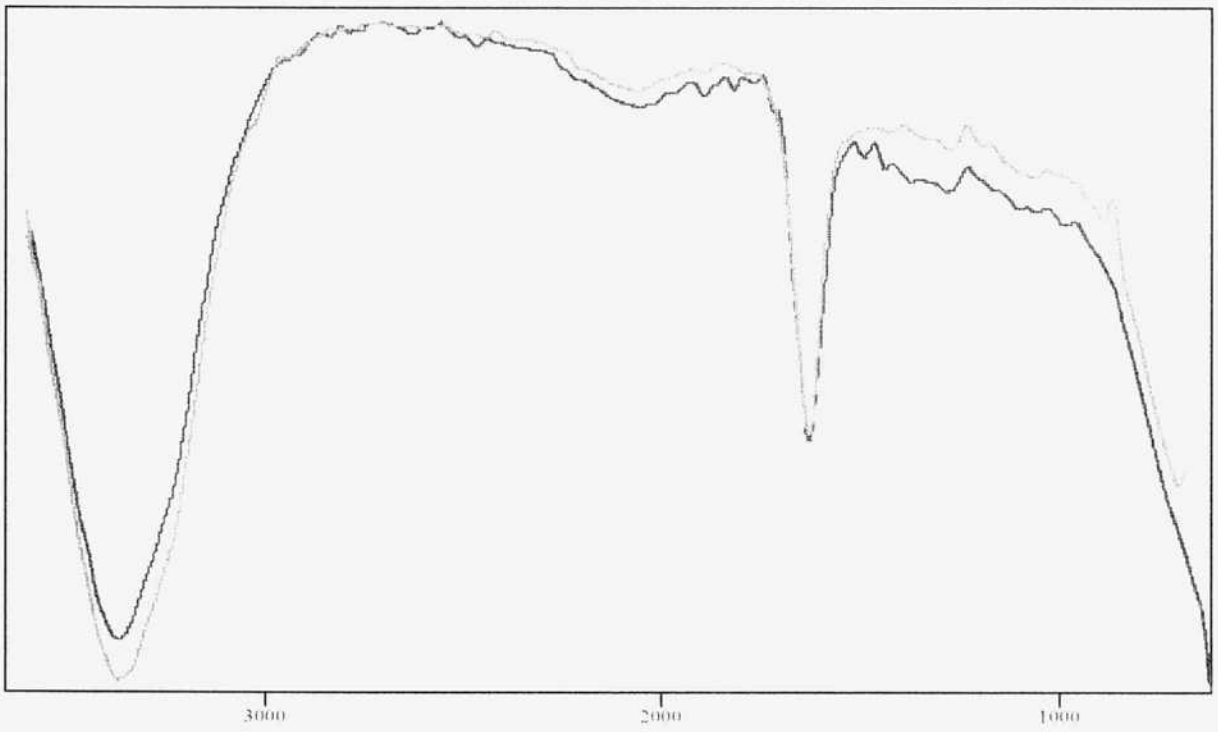
4. What about UV differences?

Spider Webs - Washed Rinsed Cobwebs - NaOH Digestion - Neutral pH Average Nov 01 2017 - 04.JPEG



Spectral Library Search Results

Sample Filename	Spider Webs - Washed Rinsed Cobwebs - NaOH Digestion - Neutral pH Average Nov 01 2017 - 04.spc
File Title	Synthetic spectrum by averaging. See audit trail for details.
Date	Wed Nov 01 21:43:38 2017
Search Algorithm	Correlation Coefficient
Search Regions	Full Spectrum



Spider Webs - Washed Rinsed Cobwebs - NaOH Digestion - Neutral pH Average Nov 01 2017 - 04.spc: Synthetic spectrum by averaging. See audit trail for details.

Hair Microwave Digestion NaOH Carol Aug 30 2017 - 01.spc: Synthetic spectrum by averaging. See audit trail for details.

Metric	Name	Library	Entry
0.966989	Synthetic spectrum by averaging. See audit trail for details.	Master File Set	363: Hair Microwave Digestion NaOH Carol Aug 30 2017 - 01.spc
0.966989	Synthetic spectrum by averaging. See audit trail for details.	Master File Set	364: Hair Microwave Digestion NaOH Carol R0-92 Aug 30 2017 - 01_trn.spc
0.963018	Synthetic spectrum by averaging. See audit trail for details.	Master File Set	564: Skin Foliation NaOH Microwave ATR R0-94 Aug 28 2017 - 01_trn.spc
0.963018	Synthetic spectrum by averaging. See audit	Master File Set	565: Skin Foliation

	trail for details.		NaOH Microwave ATR Aug 28 2017 - 01.spc
0.961737	Synthetic spectrum by averaging. See audit trail for details.	Master File Set	255: Environmental Filament ATR KCl Average NaOH Microwave Digestion Aug 25 2017 - 01_trn.spc
0.953181	Synthetic spectrum by averaging. See audit trail for details.	Master File Set	501: Rainwater Concentrate Average Normalized - ATR Gain 10 Nov 05 2015 - 01.spc
0.952126	Synthetic spectrum by averaging. See audit trail for details.	Master File Set	256: Environmental Filament ATR NaOH Microwave Digestion Aug 25 2017 - 01.spc
0.949219	Synthetic spectrum by averaging. See audit trail for details.	Master File Set	500: Rainwater Concentrate Average Normalized - ATR Gain 10 R0-85 Nov 05 2015 - 02_trn.spc
0.949219	Synthetic spectrum by averaging. See audit trail for details.	Master File Set	502: Rainwater Concentrate Average Normalized - ATR Gain 10 Nov 05 2015 - 02.spc
0.942804	06/27/2016 22:48:59 title	Master File Set	467: Rainfall Acetone Extraction Jun 27 2016 - 02.spc

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- loss of nuclei and organelles, in the final stages of cornification

Metabolism ceases, and the cells are almost completely filled by keratin. During the process of epithelial differentiation, cells become cornified as keratin protein is incorporated into longer keratin intermediate filaments. Eventually the nucleus and cytoplasmic organelles disappear, metabolism ceases and cells undergo a programmed death as they become fully keratinized. In many other cell types, such as cells of the dermis, keratin filaments and other intermediate filaments function as part of the cytoskeleton to mechanically stabilize the cell against physical stress. It does this through connections to desmosomes, cell-cell junctional plaques, and hemidesmosomes, cell-basement membrane adhesive structures.

Cells in the epidermis contain a structural matrix of keratin, which makes this outermost layer of the skin almost waterproof, and along with collagen and elastin, gives skin its strength. Rubbing and pressure cause thickening of the outer, cornified layer of the epidermis and form protective calluses, which is useful for athletes and on the fingertips of musicians who play stringed instruments. Keratinized epidermal cells are constantly shed and replaced.

These hard, integumentary structures are formed by intercellular cementing of fibers formed from the dead, cornified cells generated by specialized beds deep within the skin. Hair grows continuously and feathers moult and regenerate. The constituent proteins may be phylogenetically homologous but differ somewhat in chemical structure and supermolecular organization. The evolutionary relationships are complex and only partially known. Multiple genes have been identified for the β-keratins in feathers, and this is probably characteristic of all keratins.

Silk

The silk fibroins produced by insects and spiders are often classified as keratins, though it is unclear whether they are phylogenetically related to vertebrate keratins.

Silk found in insect pupae, and in spider webs and egg casings, also has twisted β-pleated sheets incorporated into fibers wound into larger supermolecular aggregates. The structure of the spinnerets on spiders' tails, and the contributions of their interior glands, provide remarkable control of fast extrusion. Spider silk is typically about 1 to 2 micrometres (μm) thick, compared with about 60 μm for human hair, and more for some mammals. The biologically and commercially useful properties of silk fibers depend on the organization of multiple adjacent protein chains into hard, crystalline regions of varying size, alternating with flexible, amorphous regions where the chains are randomly coiled.[22] A somewhat analogous situation occurs with synthetic polymers such as nylon, developed as a silk substitute. Silk from the hornet cocoon contains doublets about 10 μm across, with cores and coating, and may be arranged in up to 10 layers, also in plaques of variable shape. Adult hornets also use silk as a glue, as do spiders.

Clinical significance

Some infectious fungi, such as those that cause athlete's foot and ringworm (i.e. the dermatophytes), or *Batrachochytrium dendrobatidis* (Chytrid fungus), feed on keratin.

Diseases caused by mutations in the keratin genes include:

- Epidermolysis bullosa simplex

OK, our first clear distinction occurs under the microscope. Spider webs are generally quite uniform, appear to be on the order of 1-2 microns in thickness, are smooth in general, no significant internal structure, and they are smoothly.

We have them @ 500x, 1250x & 5000x.

—

Now lets go to microwave digested NaOH neutralized pH, UV analysis.

OK, we now have the spider web microwave digestion UV plot.
We have noticeable peaks @ 229 & 278 nm.

We can compare this to hair and the environmental filament.

Spide Web

Spider web vs Env. Filament vs Hair

We will have

Env Filament

done 1. observation (microscopic & macroscopic)
done 2. IR analysis
done 3. UV analysis.
done 4. NIR analysis done
5. Metals analysis ICP-MS

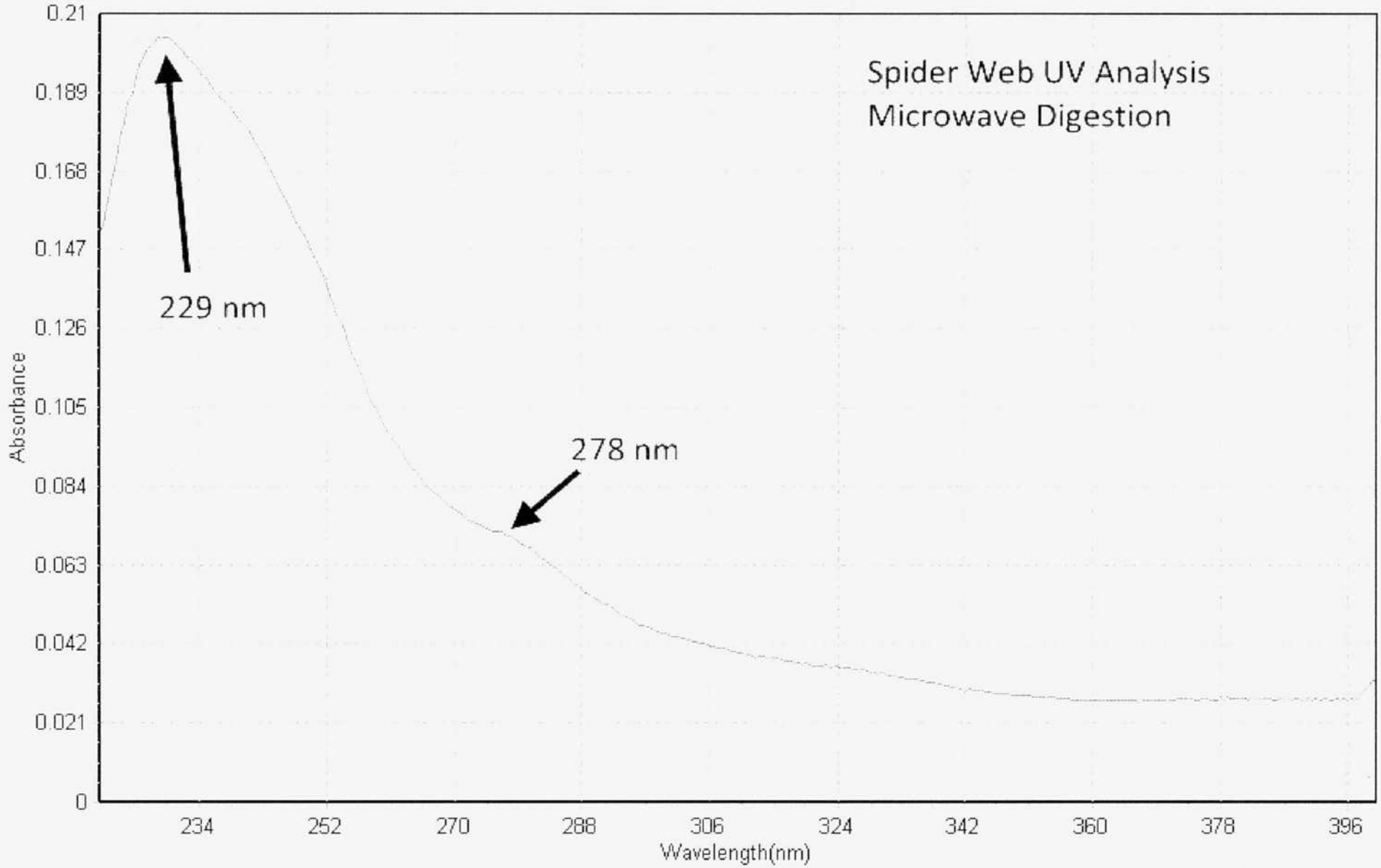
We also have a good photograph of the sample spider web.

6. Electrochemical?

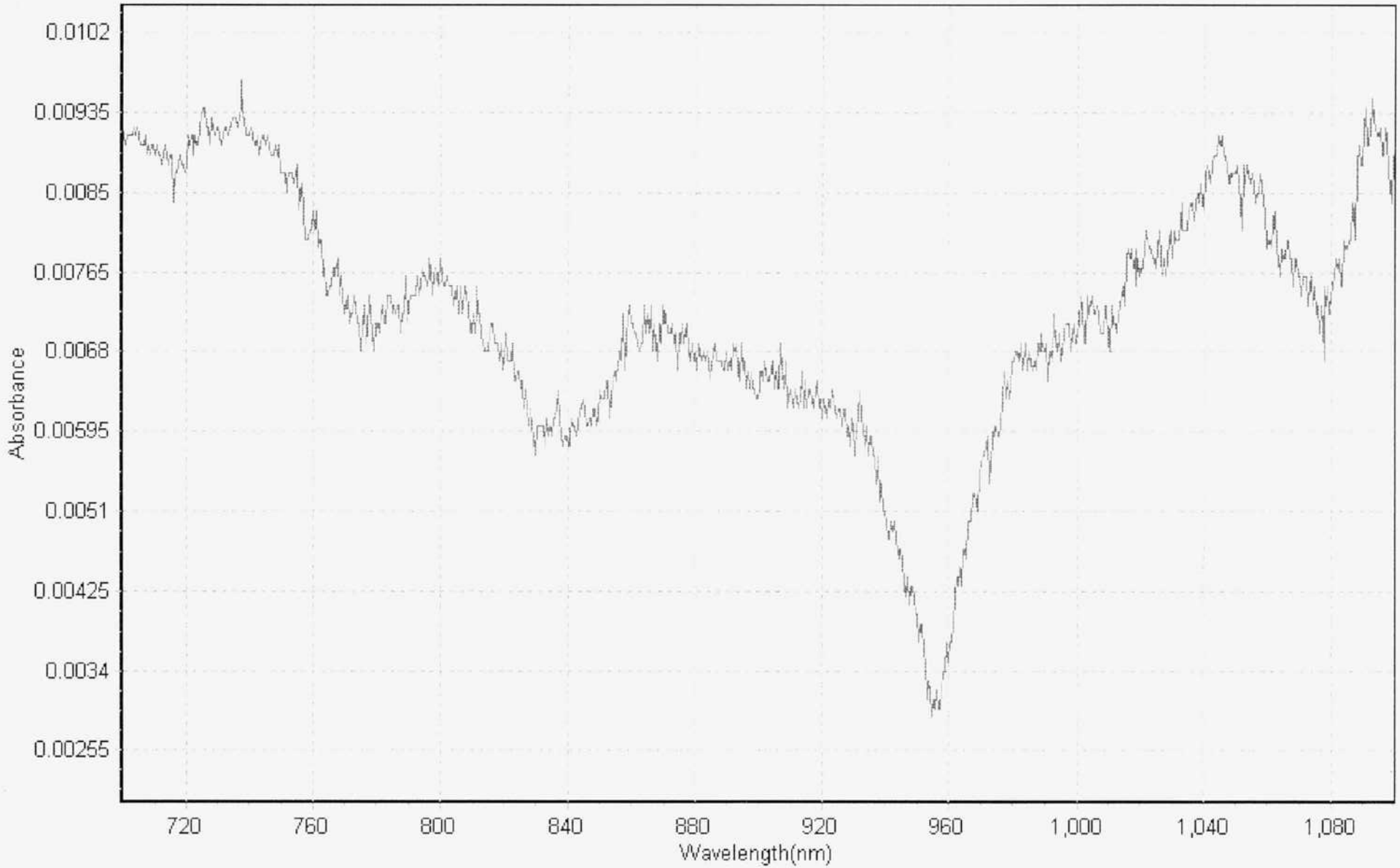
NIR analysis of spider web is quite interesting w/ many peaks (magnitude is low)

1093	ArCH	There is a significant amount of structural information.
1045	RNH_2	
863	ArCH	Amines, aromatic hydrocarbons & hydrocarbons.
791	RNH_2	
736	CH_2	There is likely to show considerable unsaturation

UV Spider Web Microwave Digestion Nov 01 2017 - 01.jpg



NIR Spider Web Analysis Microwave Digestion Nov 01 2017 - 01.BMP



Nov 02 2017

We learned yesterday that the infrared instrument suffered some harm during the move. The sensitivity of the detector has been reduced by about 1/2, but seems.

You will have to plan for a $1000 upgrade at an unknown future date.
For now, you must increase the gain by a factor of 10 to compensate. You have also adjusted for maximum sensitivity, but it is still weak.

I am curious if digestion of keratin (eg hair) varies with use of NaOH or combined NaOH & KOH.

We are headed toward electrochemical analysis as well.

Our first stage of comparison however, is simply visual, comparing spider silks against the environmental sample.

It is quite clear, from direct observation under the microscope @ 500x, 1250x & 5000x that the spiderweb and the "environmental filament" are entirely different from one another.

Spectroscopic methods will further confirm this difference, but they are not necessary to prove that the difference exists.

Now, the question is, in anticipation of a paper to the letter based on an event that took place in Ireland, do I or should I conduct the additional test methods?

There is, of course, much to be learned in the process. The methods are:

1. IR analyses
2. UV analysis
3. NIR analyses
4. Electrochemical methods

One complication affecting the decision is that sample environmental pollutant material is required to conduct these tests, and the material is in very short supply.

If you had sufficient material, you should be able to perform all tests w/ only one sample microwave digestion.

Before deciding, determine if there is a difference between NaOH vs NaOH + KOH

NaOH: 40 gms/mole $(50\text{ml}/1000\text{ml}) = .05 \Rightarrow .05(40.0) = 2.0\text{ gms}$
therefore 1M = 20 gms/50 ml.

KOH: 56.1 gms/mole
$(.05)(56.1) = 2.805$ gms/mole
therefore 1M = 28 gms/50 ml.

Therefore to make 1 50ml NaOH
means 20 gms / 50 ml

But to make 10 M NaOH + KOH
we need 20gms / 50ml + 28 gms / 50ml = 100ml
NaOH KOH

so we actually need:

40 gms / 100 ml of NaOH
28 gms / 50 ml of KOH

and then mix 50ml 10M NaOH w/ 50 ml KOH = 100 ml

✓ setup 0.36 gms CEC hair into 10 ml 10M NaOH

Filler Results

0.39 gms CEC hair in 10M (NaOH + KOH)

0.36 gms CEC hair in Commercial NaOH + KOH

The next thing we learn is that hair dissolved very well in 10M NaOH Microwave Digestion 30 min @ 10% Power.

Spider web material did not do this. It took much longer.

I am filtering the results. - TWICE

Well, a little mistake here. NaOH + KOH trial has failed, or at least been obviated. The aluminum funnel reacted w/ the strong NaOH + KOH concentrate and contaminated the filtrate. It is unusable.

If you ever need dissolved aluminum this is definitely a way to do it.

Your NaOH filtrate looks beautiful. Very clear amber - olive colored filtrate.

Lesson: only use a plastic or glass funnel w/ the strong alkaline. The 30 minute microwave digestion for 30 min @ 10% power is working exceptionally well.

—

We have neutralized the pH of the NaOH solution.

—

Now test IR on NaOH + KOH commercial alkaline. The produces a darker filtrate than NaOH alone.

—

We learned many important things from these recent IR digestion-calibration trials.

1. KOH + NaOH is superior @ microwave digestion than NaOH alone. This is why this combination is used commercially. It picked up several important peaks, albeit small, that aid considerably in the keratin - hair - spider web analyses.

2. Run a background spectrum for each session. IR is sensitive enough to CO2 levels in the room that it can make a big difference in the plot result.

3. Do not use gain yet on the IR. Even though the detector has weakened, adding gain will introduce clipping artifacts. Do not use gain if any clipping results. The detector is still sensitive enough to work well.

4. We have worked up the hair IR spectrum from created w/ multiple calibration digestions & IR background analyses. The closest match to hair, as in the reverse case, is indeed spider webs. They are both keratin. Now we just need to examine the environmental filament again.

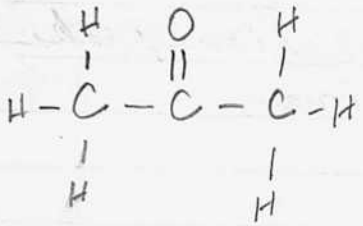
5. We are going after UV & NIR on the two hair digestions to compare to the spider web results.
   1. NaOH
   2. NaOH + KOH

Now, this does get interesting. It certainly does not look like the acetone is "pure", as we had already surmised from a MSDS recollection. I also believe manufacturer states that the mixture is proprietary.

Also a case for distillation.

Here is a case where GC could be quite useful. How many parts to the whole? Why the MSDS complication?

Now lets look @ acetone directly:



There seems to be more to this acetone than meets the eye. It would appear to be more complicated than the MSDS states.

Notice we do have $R-CH_2$ & RCH_3 but not exactly RCH_2CH_3

Notice we do not have a Carboxylic acid but we do have the dimer $C_2=O$.

What exactly is a dimer?

A dimer is two identical molecules linked together

So disregarding proprietary additives, we actually have

RCH_3 where R is $C(=O)$

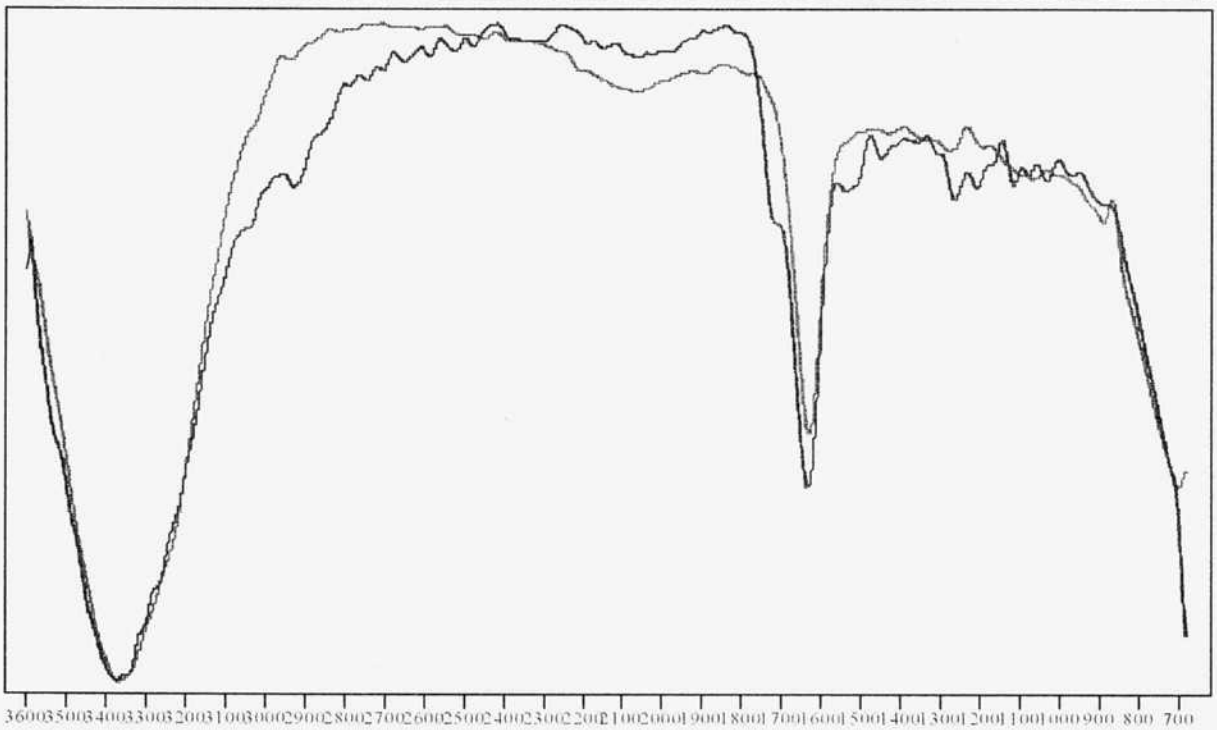
So we must wonder what the additive actually is. It is clearly not just acetone.

Notice also how weak the hydrocarbon absorption is. It is an entirely Case.

Will the MSDS for Sunnyside 1 gallon acetone say that it is pure acetone so I am not sure exactly how this can be.

Spectral Library Search Results

Sample Filename	Hair CEC NaOH and KOH Commercial Microwave Digestion Nov 02 2017 - 02.spc
File Title	Synthetic spectrum by averaging. See audit trail for details.
Date	Fri Nov 03 01:54:04 2017
Search Algorithm	Correlation Coefficient
Search Regions	Full Spectrum



Hair CEC NaOH and KOH Commercial Microwave Digestion Nov 02 2017 - 02.spc: Synthetic spectrum by averaging. See audit trail for details.

Spider Webs - Washed Rinsed Cobwebs - NaOH Digestion - Neutral pH Average Nov 01 2017 - 04.spc: Synthetic spectrum by averaging. See audit trail for details.

Metric	Name	Library	Entry
0.987506	Synthetic spectrum by averaging. See audit trail for details.	Master File Set	574: Spider Webs - Washed Rinsed Cobwebs - NaOH Digestion - Neutral pH Average Nov 01 2017 - 04.spc
0.986774	Synthetic spectrum by averaging. See audit trail for details.	Master File Set	577: Spider Webs - Washed Rinsed Cobwebs - NaOH Digestion - Neutral pH Nov 01 2017 - 03.spc
0.980556	Synthetic spectrum by averaging. See audit trail for details.	Master File Set	576: Spider Webs - Washed Rinsed

Hair CEC NaOH and KOH Commercial Microwave Digestion Nov 02 2017 - 02.JPEG

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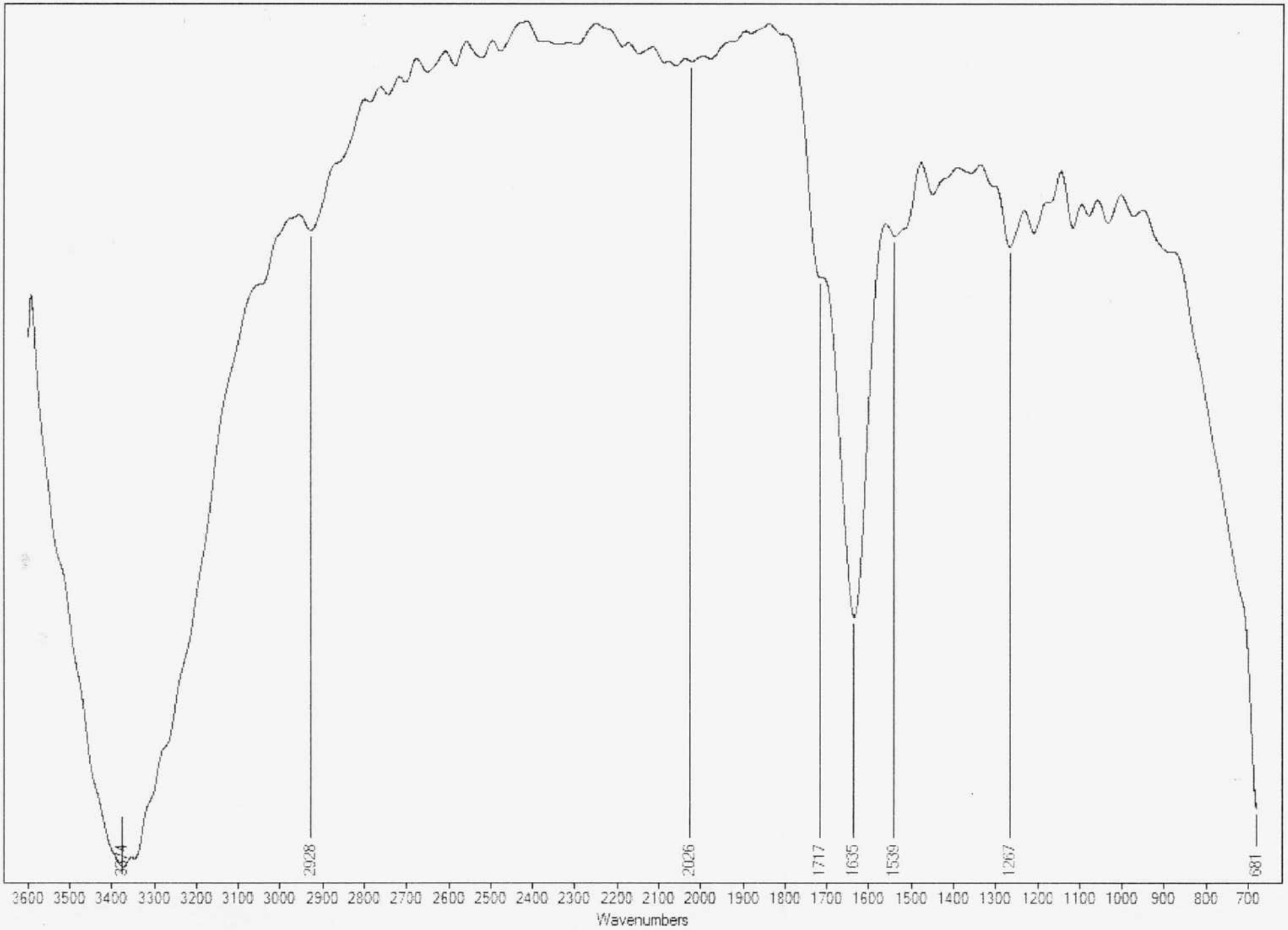


UV analysis of Human Hair. NaOH

We had peaks @ 275 nm & 222 nm.

NIR analysis gives a simple result:

~ 1095 ArCH
963 ArOH
and that is all.

UV Analysis of Human Hair NaOH + KOH

275 nm & 232 nm.
(vs 278 for spider webs) (vs 229 for spider webs)

NIR Analysis: NaOH + KOH

1080 ArCH
962 ArOH (same result)

KOH + NaOH gives exact match w/ human hair to spider webs. Another method of showing that they are both keratin.

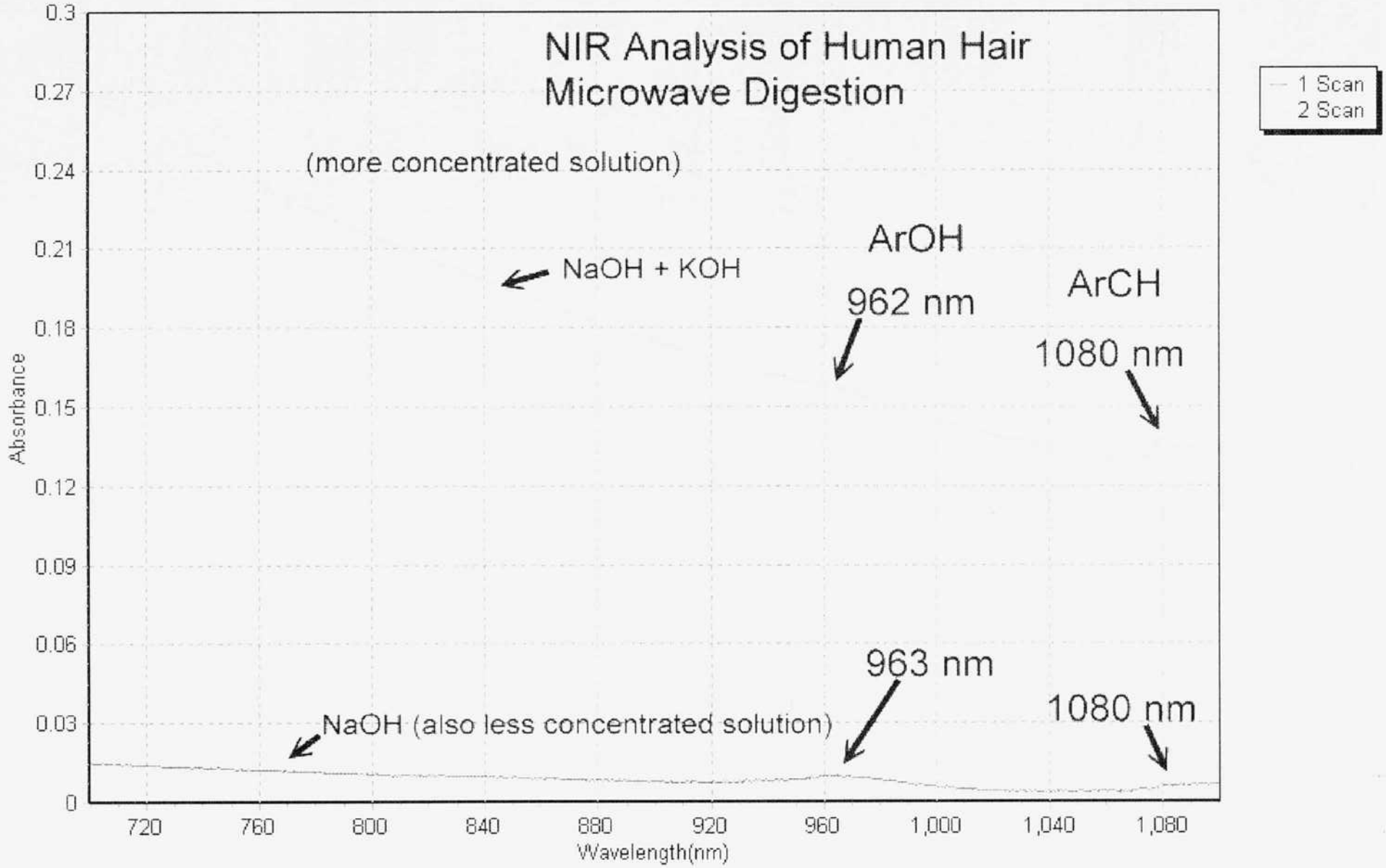
.BMP plots are on USB drive if you want to use this

We only need the env. file next again.

Nov 03 2017 UV Hair Analysis: NaOH + KOH
Microwave Digestion

NIR Hair Analyses : NaOH + KOH
Microwave Digestion

NIR Human Hair NaOH and KOH Microwave Digestion Nov 02 2017.jpg



Spider webs & human hair have now both been compared

Spider Webs — Human Hair

Keratin Nature — IR Spectrum Essentially Identical — Keratin Nature

Closest IR Match

NIR done
UV done
Microscope done
Microwave digestion seems more difficult

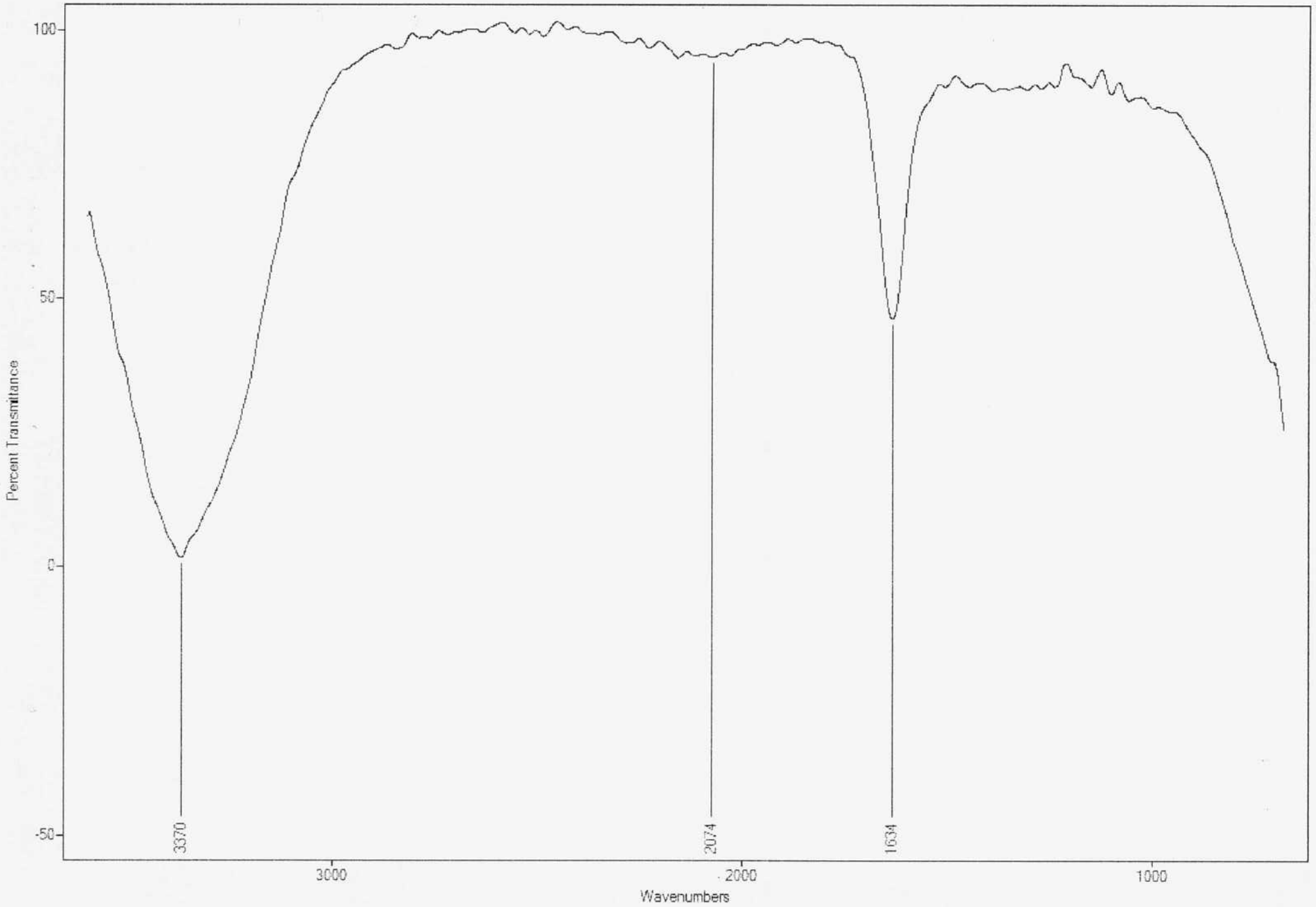
NIR done
UV done
Microscope Available
Microwave Digestion not difficult

We also observe that microwave digestion of the env. filament absorbs more energy. This was also noticed in earlier trials. Metal analyses of the env. fil. supports the observation. The env. filament is ABSORBING MICROWAVE ENERGY.
This is likely a very important observation.
Spark, energized sound.

Ok, now for the first time in quite some time we are studying the "Environmental Filament."
We have human hair and spider webs as a reference point and yet we know w/ certainty they are all quite different from each other.

Environmental Filament: NaOH & KOH IR Analysis
Microwave Digestion

Environmental Filament NOH - KOH Microwave Digestion Nov 03 2017 - 02.JPEG



Env Filament IR peaks:

	Hair	Spider Web
3370	3374	3310
2074 (+ or -)	2026	2074
1634	1635	1633

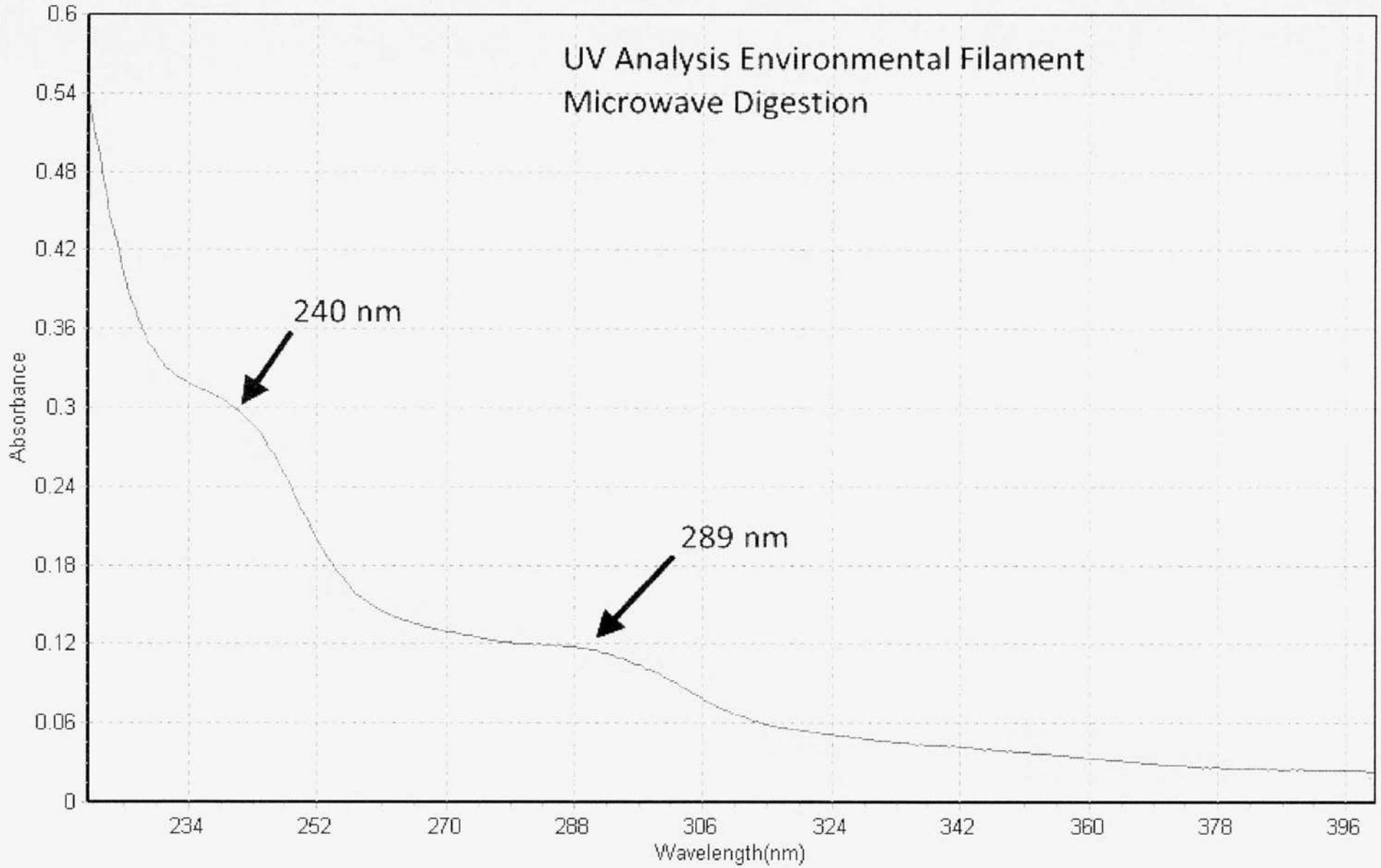
+ additional peaks

Therefore the coating of the env. filament is identical to spider web IR signature & yet we positively know that it is unique from spider webs by microscopic examination. This is profound!

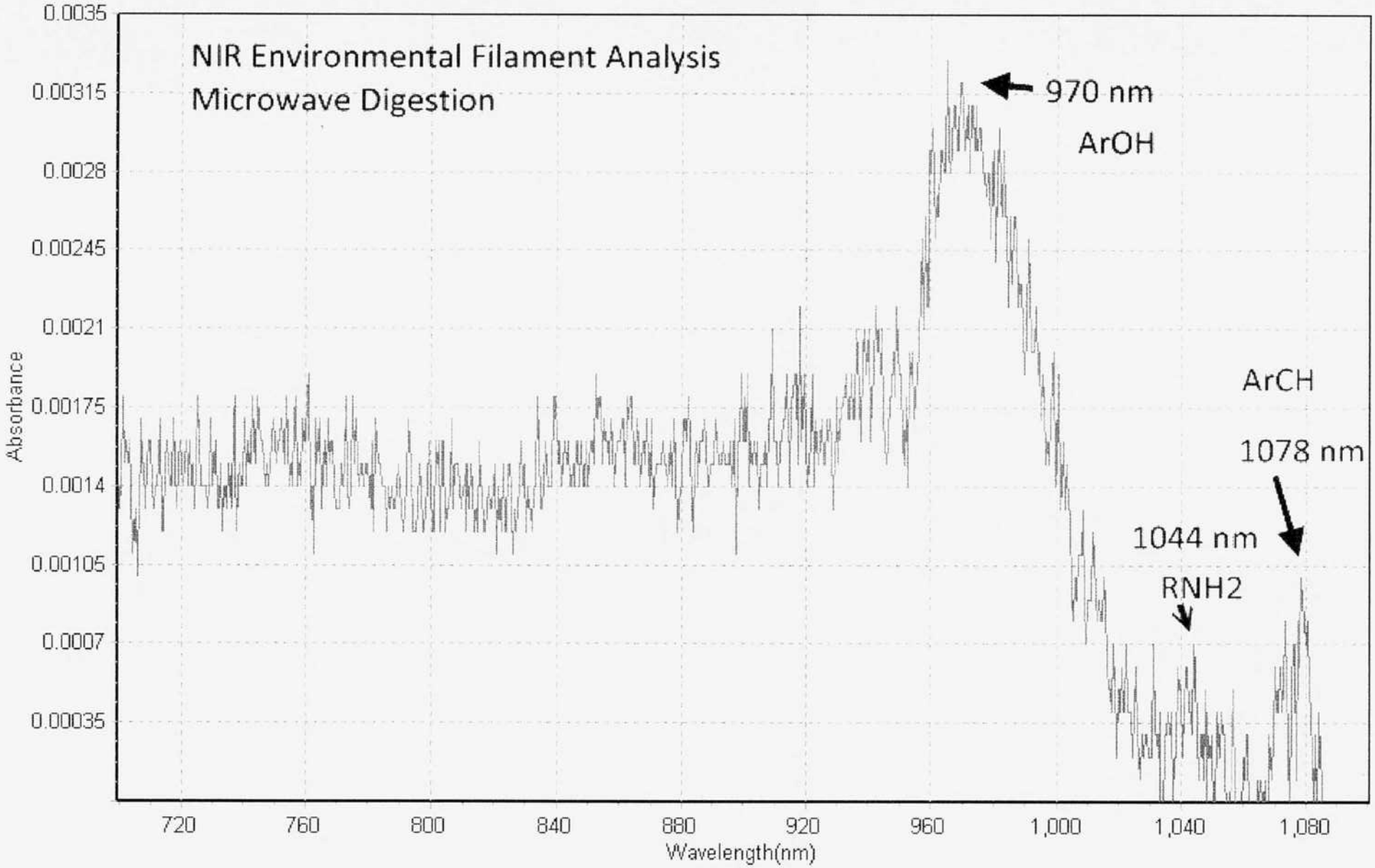
Notice the potential shift from 2026 of hair; this may or may not be real.

The above stat finding is most amazing.

UV Environmental Filament NaOH - KOH Microwave Digestion Nov 03 2017.jpg



NIR Environmental Filament NaOH - KOH Microwave Digestion Nov 03 2017.jpg



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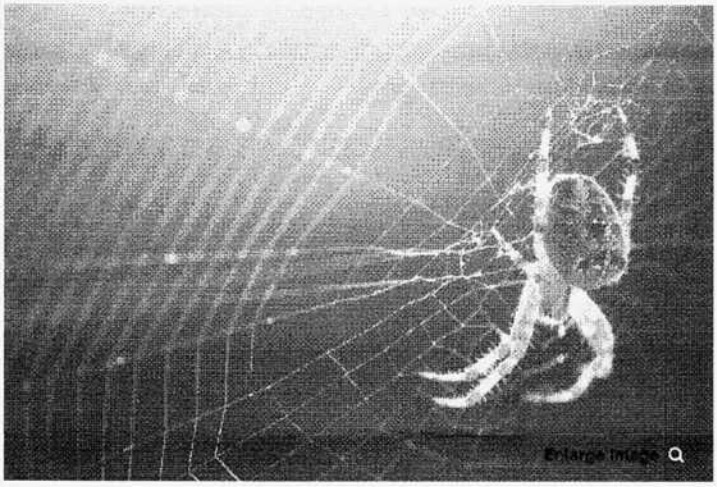
SCI-TECH

Researchers finally figure out how to make artificial spider web

Humans: 1, spiders: 0.

BY DANIEL VAN BOOM / JANUARY 11, 2017 11:14 PM PST



Getty Images/EyeEm

Spiders, as we all know, are terrifying little jerks who won't tell us the secret to their super strong webs.

Well the joke is on you, spiders, because a group of researchers from the Swedish University of Agricultural Sciences and the Karolinska Institutet has figured out how to make artificial spider silk.

The researchers discovered that there's a certain acidity in a spider's silk gland, where webs are stored as a protein before being converted into a fiber. They developed a way to replicate the silk gland, leading to them being able to make a kilometers-worth of artificial spider's web.

You can read their full scientific explanation here.

Apart from being ridiculously strong -- more durable than steel, even -- spider silk has impressive medical utility. It can help regenerate skin following a burn or cut, for instance, and some researchers think it can even replace ligaments.

Despite its usefulness, spider silk has been difficult to farm, the University said, because the critters are hard to keep captive and don't actually produce that much web.

"This is the first successful example of biomimetic spider silk spinning," claimed

Tech Today 00:00 / 00:30

Nov 06 2017

I have finally worked up a fume hood in the lab. Most of this is due to the work of [redacted] a couple years ago. He has done great work & I have succeeded in installing it. It is working quite well.

The fume hood would have saved me the neck CDB Pirtlin reaction on my skin. The was fairly severe and took two weeks to heal & never want to encounter that again.

First trial for organic content of a pine cone.

Weigh boat mass: 11.05gms

Wgt w/ Cone: 14.78gms

Wgt of Cone 3.73 gms H_2O: $\frac{3.73-.27}{3.73} = 92.8\%$

Mass after combustion ~~11.32~~ 11.32 + Organic (CO_2)

Mass of ash: 0.27gms In Organic = $\frac{0.27}{3.73} = 7.2\%$

$92.8 + 7.2 = 100\%$

Nov 07 2017

Working on the spider web question.

One estimate is that a strand around the earth weighs 500 gms.

$$\frac{500 \text{ gms}}{24000 \text{ miles}} = .02083 \frac{\text{gms}}{\text{mile}} \cong 20 \text{ mg/mile}$$

$$\frac{20 \text{ mg}}{1609.3 \text{ meters}} = .02083 \text{ gms} = \frac{x}{1} \qquad x = .000013 \text{ gms per meter.}$$

how large is a typical spider web and how many strands

It is common for a web to be about 20 times the size of the spider building it.

Most ballooning spider are ~ 1 mg.
Therefore a spider can make approx $20(1 \text{ mg}) = 20 \text{ mg}$ of material
$= .020$ gms of material

$$\frac{.020 \text{ gms}}{.000013 \text{ gms/meter}} = 1.5 \text{ million meters of web.}$$

no way.

Nov 10 2017 Page 115

Another paper has been developed - it is about 80% completed and the main point is made. The paper proves the point that the "environmental filament material is not spider web. The case is clear. The paper is entitled

Global Validation: (the spider web problem...)

—

Two important emails to take care of but we are entering more profoundly into EIS, cyclic voltammetry and electrical species identification. Redox titration also.

To start with, we study QUCS, the circuit simulator along w/ some video tutorials on EIS

The desire is to simulate EIS work / circuits on a spice simulator, in this case QUCs.

We have progress of a working model of Randles circuit on QUCs now. The wgsbot.

Next steps are to work towards inputting the component values found in the preliminary EIS of hair, spider web, and the environmental filament.

1. Explore the behavior of those circuits.
2. Work toward extracting impedance information from the circuits and the Randle circuit in general
3. Look @ variation w/ respect to placement of the external resistor.

---

1. Add the UV segment to the Global Validation paper.
2. Study organic Chemistry Course

I have made good progress w/ SPICE (QUCs) simulation. I have workable AC circuit diagrams of both the spider web and the environmental filament EIS steady. We have current flow as a function of frequency.

Page 117

Now I have all three: Spider Web, Env. Filament, & Human

Spider Web & Env. Filament - EIS Simulations via SPICE (QUCs)
Next we need human hair.

Spider Web - Nov 11 2017 - 01 200Hz.sch

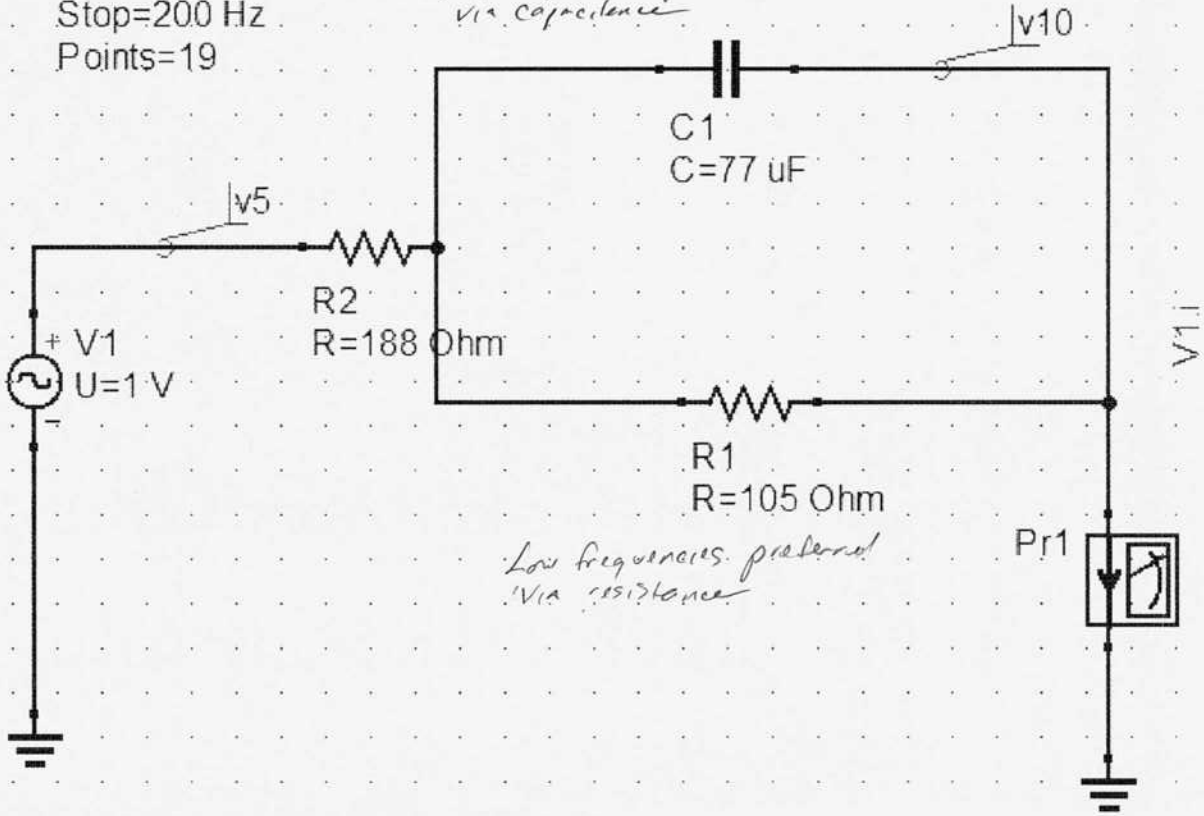
Spider Web - Resistor Pre Bridge Nov 11 2017 1400 - 01.dpl

ac simulation

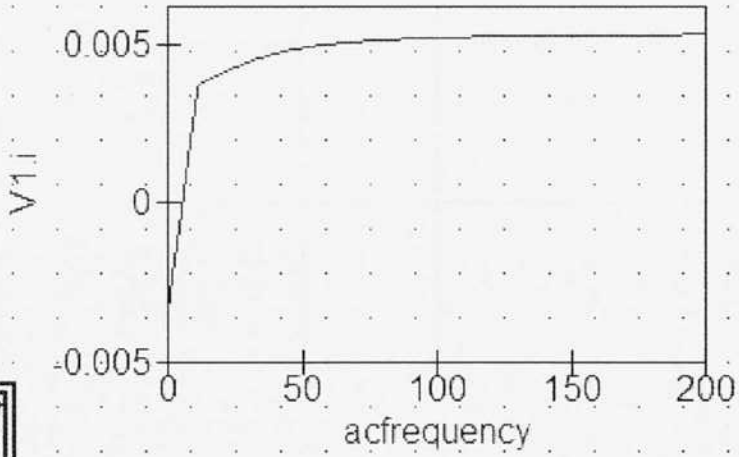
AC1
Type=lin
Start=0
Stop=200 Hz
Points=19

acfrequency	Pr1.i	V1.i	v10.v	v5.v
0	0.00341	-0.00341	0	1
11.1	0.00368 / 9.53°	0.00368 / -170°	0	1
22.2	0.00417 / 12.5°	0.00417 / -167°	0	1

High frequencies preferred here via capacitance



Low frequencies preferred via resistance



Env Filament - Nov 11 2017 - 06 200Hz.jpg

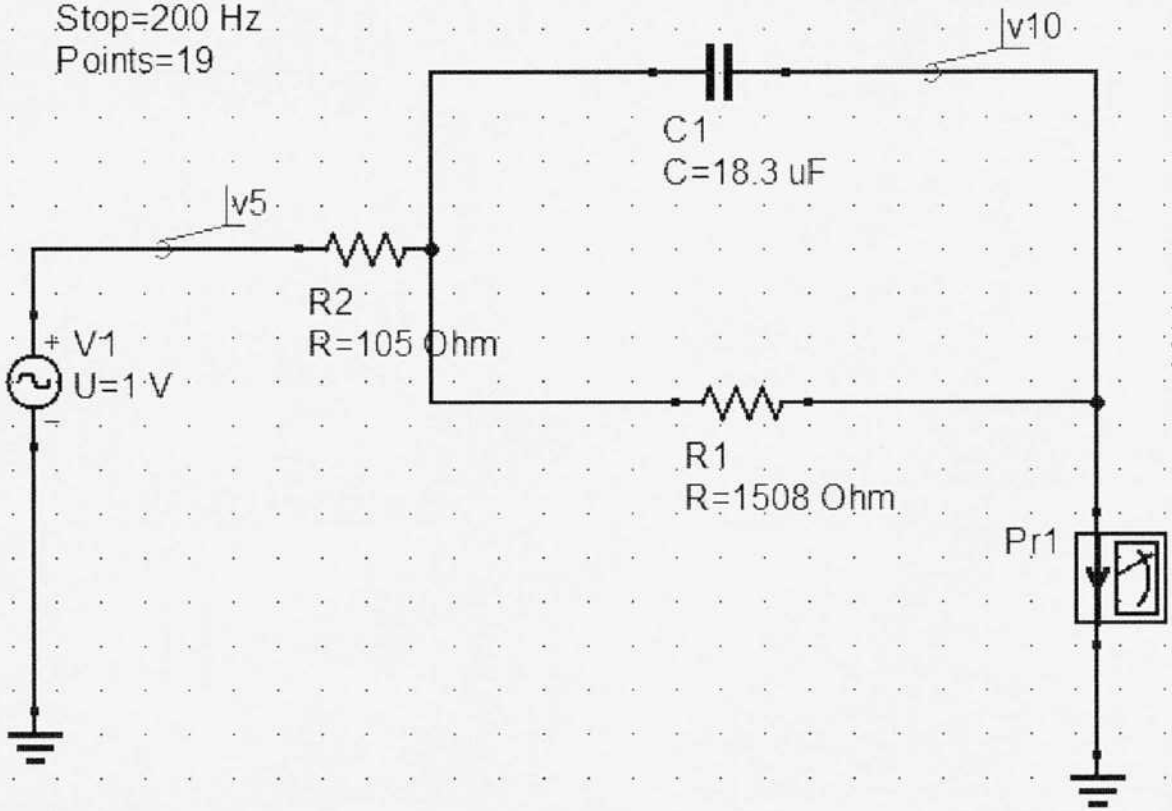
Env Filament - Nov 11 2017 - 06 200Hz.sch

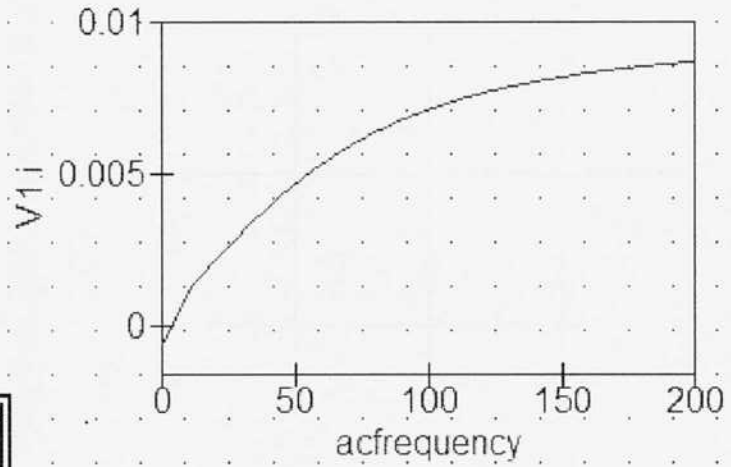
Environmental Filament - Resistor Pre Bridge Nov 11 2017 1330 - 06.dpl

ac simulation

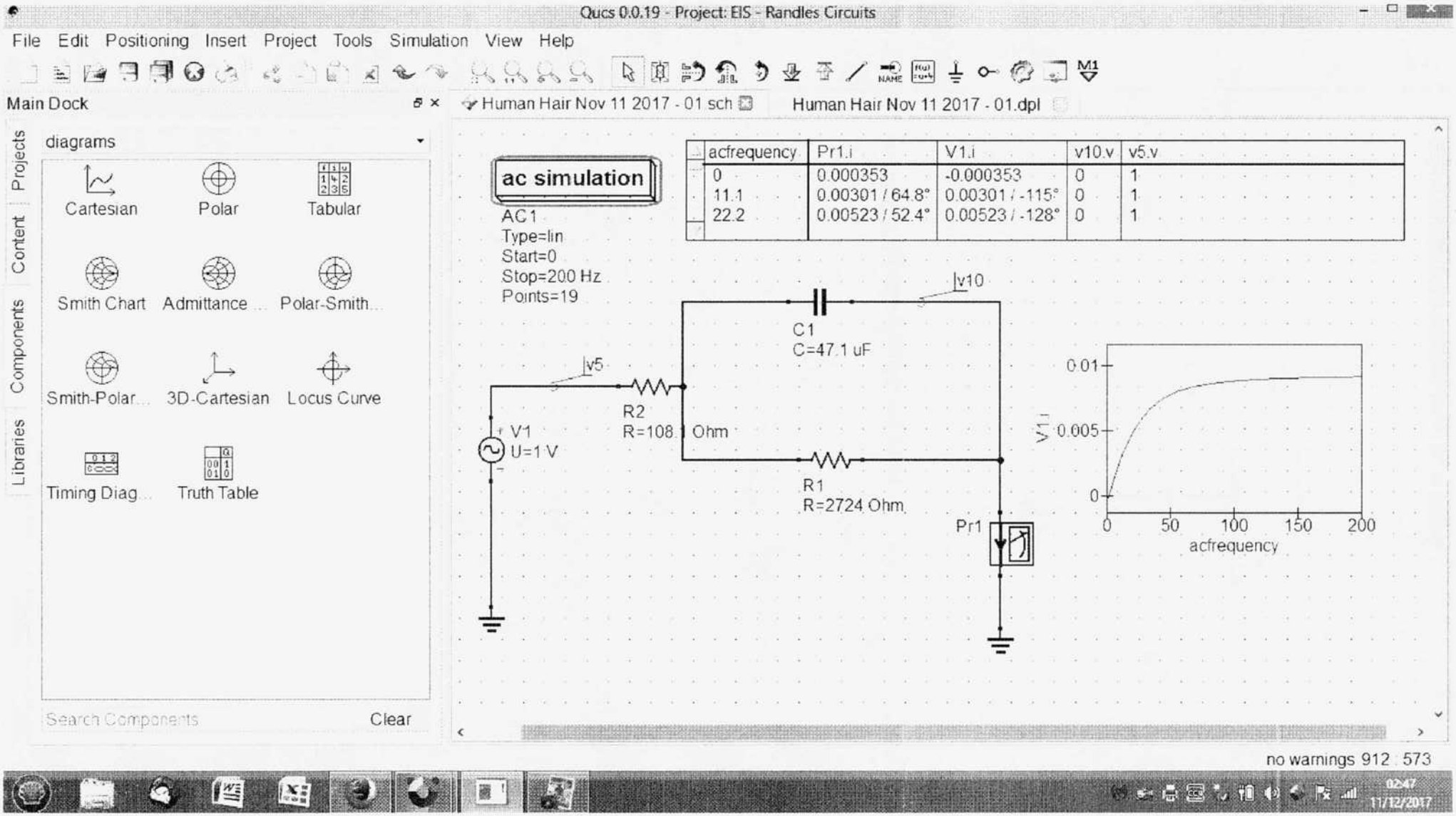
AC1
Type=lin
Start=0
Stop=200 Hz
Points=19

acfrequency	Pr1.i	V1.i	v10.v	v5.v
0	0.00062	-0.00062	0	1
11.1	0.00134 / 55.4°	0.00134 / -125°	0	1
22.2	0.00239 / 61.4°	0.00239 / -119°	0	1





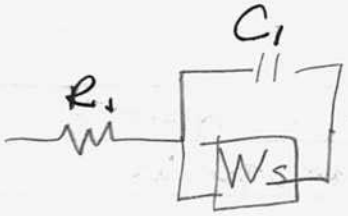
Human Hair Nov 11 2017 - 01 200Hz.jpg



We are finally that insertion of a Warburg short element is giving a smaller better fit to the data.

σ is the Warburg coefficient.

Our best model, @ least for the last sample is:



You set big n/y variable results here.

			Error
R1	849 Ω		0.8
C1	3.28 uF		12.2
01	7615 σ	(coefficient)	2.2
01	1.218 √s		5.06
01	1.285 φ		0.6

Ws

the really does seem to be the best fit.

Warburg impedance is diffusion impedance. Warburg impedance is better @ low frequencies.

One source says that Warburg elements can be modelled in SPICE w/ successive RC circuits.

A warburg element is not a good fit added to R2.

The circuit above is a good fit but the solution does not always converge.

A R-RC model gives

		Error
R_1 =	108.1	8.41%
C_1 =	41.1	9.72%
R_2 =	2424	18.0%

Actually a straight Warburg element can work

	Error %
$R_1 = 95.1\ \Omega$	3.72
$C_1 = 16.33\ uF$	8.57
$R_2 = 4.1E-4$	Very high 2.06E6
$\sigma = 3593$	5.107

Warburg element by itself works:

	Error	
$R_1 = 95.1$	3.64%	The is actually the
$C_1 = 16.33$	8.2%	best model.
$\sigma = 3593$	5.01%	

But lets try the R-RC circuit in QUCS.

Nov 12 2017

Studying diffusion as a mechanism in electrochemistry.
D. Wipf has a video segment.
He estimates diffusion rate @ about 1 micron per millisecond.
So 15 secs of an experiment = 15000 milliseconds
≅ 15000 microns = 0.015 meters = 1.5 cm,
just about the width of my test tube.

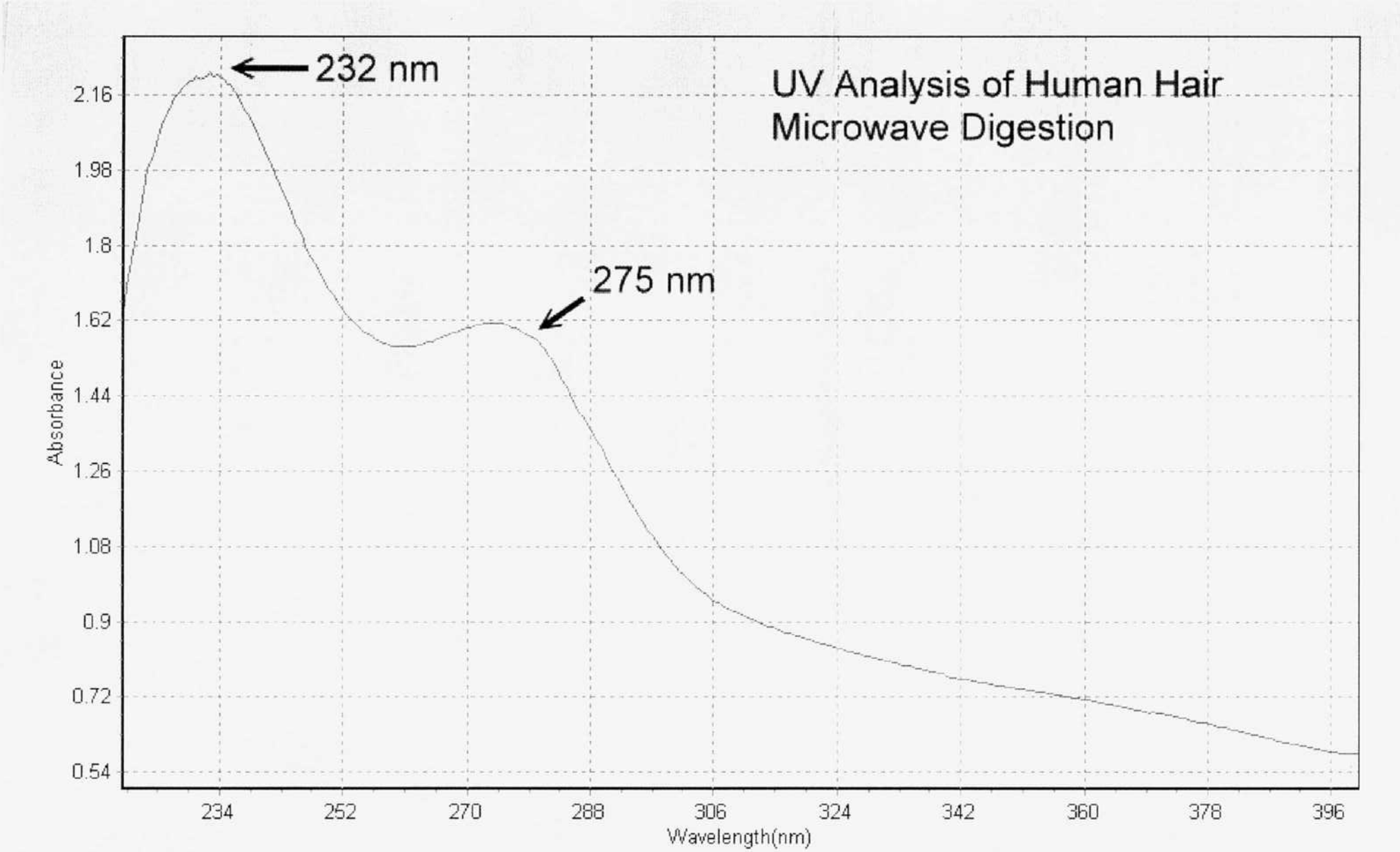
"Random walk" model applied
No driving force is required (this would mean potential, I would suppose)
Fick's Law 1 – Depends upon:
1. Concentration gradient
2. Diffusion Coefficient

I would like to look @ EIS of the three sample types across a broader frequency range.

Hair: We have good looking plots @:
E_{ac} = 0.1 V
n = 20
Max f = 1000 Hz
Min f = 0.1 Hz

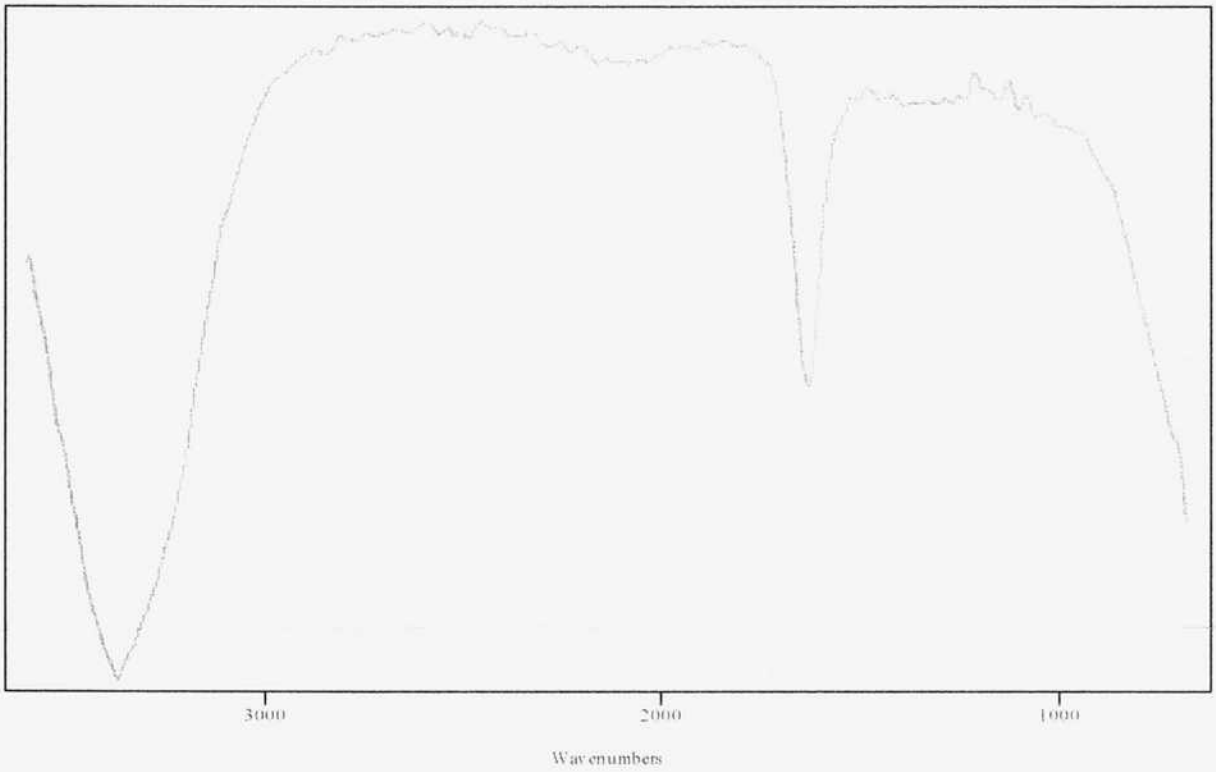
OK we have a very interesting finding here.
The impedance is normally not a straight line.
I found a reference suggesting using the OCP (open circuit potential).

Human Hair NaOH and KOH Digestion Nov 02 2017 - 01.jpg



Spectral Library Search Results

Sample Filename	Environmental Filament NOH - KOH Microwave Digestion Nov 03 2017 - 02.spc
File Title	Synthetic spectrum by averaging. See audit trail for details.
Date	Fri Nov 03 16:16:57 2017
Search Algorithm	Correlation Coefficient
Search Regions	Full Spectrum



Environmental Filament NOH - KOH Microwave Digestion Nov 03 2017 - 02.spc: Synthetic spectrum by averaging. See audit trail for details.

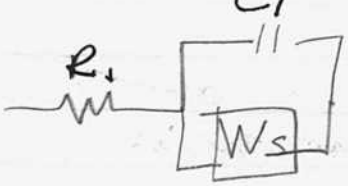
Environmental Filament NOH - KOH Microwave Digestion Nov 03 2017 - 02.spc: Synthetic spectrum by averaging. See audit trail for details.

Metric	Name	Library	Entry
1.000000	Synthetic spectrum by averaging. See audit trail for details.	Master File Set	283: Environmental Filament NOH - KOH Microwave Digestion Nov 03 2017 - 02.spc
0.996204	Synthetic spectrum by averaging. See audit trail for details.	Master File Set	580: Spider Webs - Washed Rinsed Cobwebs - NaOH Digestion - Neutral pH Nov 01 2017 - 03.spc
0.993995	Synthetic spectrum by averaging. See audit trail for details.	Master File Set	577: Spider Webs - Washed Rinsed Cobwebs - NaOH Digestion - Neutral

We are finally that insertion of a Warburg short element is giving a small better fit to the data.

σ is the Warburg coefficient.

Our best model, @ least for the last sample is:



You set this only variable here.

			Error %
R1	849 Ω		0.80
C1	3.28 uF		12.2
W1	7615 σ	(coefficient)	2.2
W1	1.218 $\sqrt{s}$		5.06
W1	1.285 φ		0.67

the really does seem to be the best fit.

Warburg impedance is diffusion impedance.
Warburg impedance is higher @ low frequencies.

One source says that Warburg elements can be modelled in SPICE w/ successive RC circuits.

A warburg element is not a good fit added to R2.

The circuit above is a good fit but the solution does not always converge.

A R-RC model gives

		Error
R_1	= 108.1	8.41%
C_1	= 41.1	9.72%
R_2	= 2424	18.0%

I am now getting a drastically different result.
It is definitely not a straight line.

My settings are

Edc = 1.0V
Eac = 0.1V
n = 20 (0.1 - 1000 Hz) n = 50 (0.1 - 10000 Hz)
☑ Measure vs OCP
☐ Edc versus OCP
t max OCP = 1.0 sec
Stability Criterion .001 sec

Now I get a full half circle! It looks quite traditional.
All that I did was change f from 0.1 to 10,000
and n to 50!

This is amazing. It looks like I have a real plot now.
What the heck is OCP about?
You also had no undercurrents or overloads.

OK I have succeeded in getting a very proper EIS fit
to Herron hair

		Error	
R1	76.6 Ω	1.06%	superb!
C1	209.4 uF	2.59%	
R2	355.5 Ω	2.03%	

Equilibrate 4 sec
Edc = 0.4 V
Eac 0.1 V
n = 50
f = 0.1 to 8000 Hz
✓ measure OCP
✓ Edc vs OCP

Ok, this is a great accomplishment.
I love my first successful + reasonable EIS plot and model!

The model conforms to the conventional Randles' circuit very well. No Warburg element is even required now.

The catch seems to be the inclusion of the OCP parameters and setting the DC voltage properly. Definitely some trial and error work coupled w/ the OCP reference that I found in relation to mentioning by straight line EIS empedance result (this is not correct).

We can see for a Randle circuit that current leads a slope voltage to create the semicircle on the Nyquist Plot. Straight line impedance does not create this effect.

We now have our first EIS model that makes sense, this time applies to microwave digested human hair.

Now lets go on to the spider web sample.

EIS Improvment Human Hair Nov 12 2017 - 01.jpg

EIS Improvment Human Hair Nov 12 2017 - 02.psmethod

Measurement

Technique: Impedance Spectroscopy ?

Notes: Connect CE, RE and WE to WE test at side panel

Select current range(s):

100 pA | 1 nA | 10 nA | 100 nA | 1 uA | 10 uA | 100 uA | 1 mA | 10 mA

▷ Pretreatment Settings

◢ Impedance Spectroscopy Settings

t equilibration	4	s
Scan type	FixedPotential	
E dc	0.4	V
E ac	0.1	V
Frequency type	Scan	
n frequencies	50 = 10 / dec.	list
Max. frequency	8000.0	Hz
Min. frequency	0.1	Hz

☑ ◢ Measure vs OCP

☑ E dc versus OCP

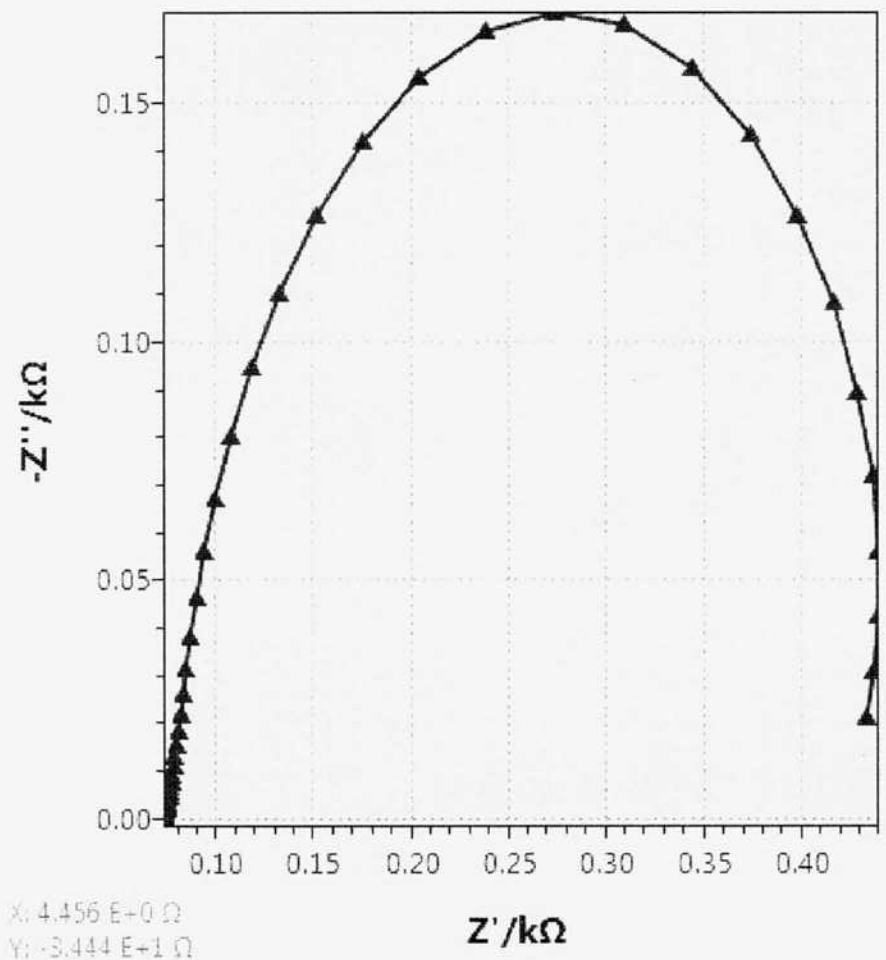
t Max. OCP	1.0	s
Stability criterion	0.001	mV/s

Expected duration: 00:01:42s
50 datapoints

Edc(V): 1.253 f(Hz): 0.100 Z(Ω): 433.917 Z'(Ω): 43

Idc: 0.157 *1 mA Iac: 0.244 *1 mA -Phase(°): 2.82 Z''(Ω): 21

Plot: -Z'' / Z' Equal axes scaling Circuit Fitting



X: 4.456 E+0 Ω
Y: -3.444 E+1 Ω

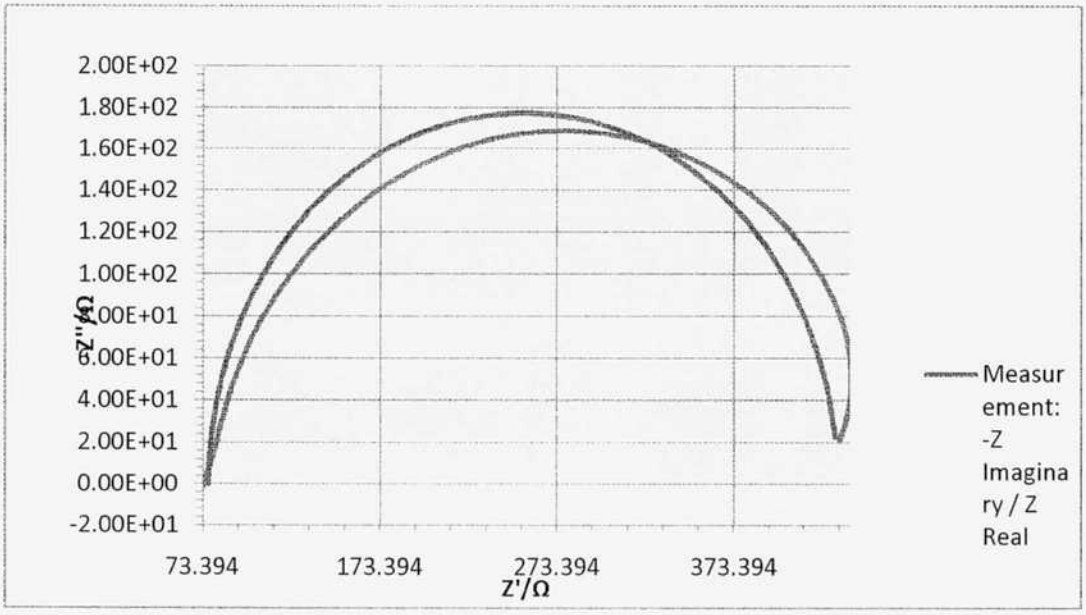
Human Hair Improvement Nov 12 2017

Fixed Element	Fitted Value	Min Value	Max Value	Unit	Error%
False R 1	76.56	1.00E-6	1.00E+12	Ω	1.062
False C 1	289.4	1.00E-6	1.00E+3	μF	2.593
False R 2	355.5	1.00E-6	1.00E+12	Ω	2.032
Chi-Squared:	0.0034	Iterations:	16		

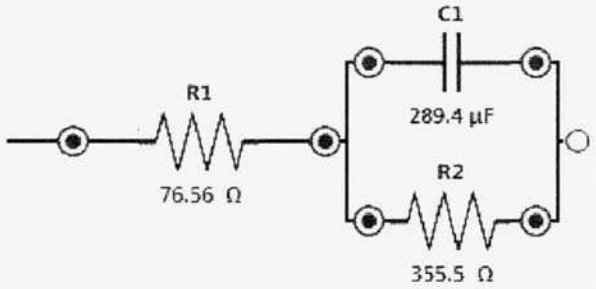
Terms are:

Measurement: -Z Imaginary / Z Real		Fit: -Z Imaginary / Z Real	
Ω	Ω	Ω	Ω
74.0303797	-1.570486153	76.55909697	0.068743914
73.57847573	-1.208661269	76.55910454	0.086136123
73.42315315	-0.553578925	76.55911702	0.108890347
73.39376813	-0.262633852	76.55913636	0.13686912
73.49951024	0.31435215	76.55916708	0.17220347
73.52816844	0.299664357	76.55921556	0.216543251
73.54763318	0.721246248	76.55929412	0.273531882
73.63848724	1.035769926	76.55941438	0.342894337
73.7621261	1.228626985	76.55960956	0.432398592
73.91334507	1.617326633	76.55992228	0.546031666
74.08404714	1.985916109	76.56040516	0.685443068
74.30091118	2.429207415	76.56119389	0.866168183
74.51196129	2.88054891	76.56239848	1.085593835
74.76628928	3.444117378	76.56439611	1.374307845
75.05092168	4.050697133	76.5674242	1.721994772
75.38142473	4.784271477	76.57232491	2.169683409
75.75772091	5.644460206	76.58008028	2.732134273
76.21058901	6.576867055	76.59261942	3.452818635
76.67786296	7.729525475	76.61176289	4.327407071
77.25710165	9.12695911	76.64270287	5.451828681
77.94847681	10.80805998	76.69159755	6.862621059
78.66174903	12.78769651	76.76914893	8.63950888
79.43684438	15.19472074	76.892622	10.88452156
80.32406578	18.12393221	77.08827196	13.70635401
81.36050372	21.74810307	77.40053501	17.27588276
82.62629009	26.0432821	77.88489685	21.67056211
84.25960135	31.53116721	78.66642689	27.29084612
86.4963279	38.17568116	79.88771494	34.23974656
89.49135957	46.18587183	81.80366401	42.8616209
93.64829505	55.76780574	84.79975091	53.49696188
99.51410599	67.04698471	89.45907355	66.48284909
107.5199586	80.02394055	96.58707401	81.97256703
118.393944	94.55688515	107.3063798	99.93146943
133.0686174	110.2596122	122.9699635	119.7775198
152.0672444	126.3800316	144.9551409	140.1394703
175.7539858	142.1734119	173.9845449	158.5758425
204.5282892	155.5319602	209.7048448	172.0755329
238.1865447	165.2961939	249.668316	177.7057412
273.926631	169.058013	290.1342845	174.122885
310.0817145	166.4285	327.0809181	162.196557
344.0577382	157.4734196	357.7423847	144.5892228
373.8482274	143.6708225	381.2705088	124.4430657
398.1677764	126.5896281	398.2514974	104.3380611
416.4370539	107.9631466	409.9693443	85.88437374
429.0221266	89.42649516	417.8093023	69.81535504

436.6612826	71.82927461	422.9476434	56.28244617
439.4634266	56.1020898	426.2688595	45.12897546
439.4747061	42.39683047	428.3967915	36.06009256
436.8901867	30.8799767	429.7523585	28.75000997
433.393118	21.31518582	430.6128498	22.88922551


2.00E+02
1.80E+02
1.60E+02
1.40E+02
1.20E+02
1.00E+02
8.00E+01
6.00E+01
4.00E+01
2.00E+01
0.00E+00
-2.00E+01
-Z''/Ω
73.394
173.394
273.394
373.394
Z'/Ω
Measurement: -Z Imaginary / Z Real

EIS Improvement with OCP Human Hair Nov 12 201.jpg



Now we work w the spider web. We see indeed that Edc is controlling the curvature of the impedance graph.

Hair was set @ 0.4V Edc. Hair set to 0.4V
Spider web is requiring 0.65 V Edc

We are now looking @ repeatability.
It looks like ya want to form a semicircle and end up @ Ø when ya ~~finish~~.

Ok, yes, you want to set Edc to form a symmetrical semicircle that ends ~ Ø.

We have accomplished this for the spider web.
The circuit is substantially different.

	Error	
$R_1 = 53.63\ \Omega$	0.86%	$E_{dc} = 0.6V$
$C_1 = 337.8\ uF$	4.75%	
$R_2 = 52.81\ \Omega$	2.13%	

Ok, we see now that you really want to force these your graph to be truly symmetric.

Small changes in Edc make a big difference.

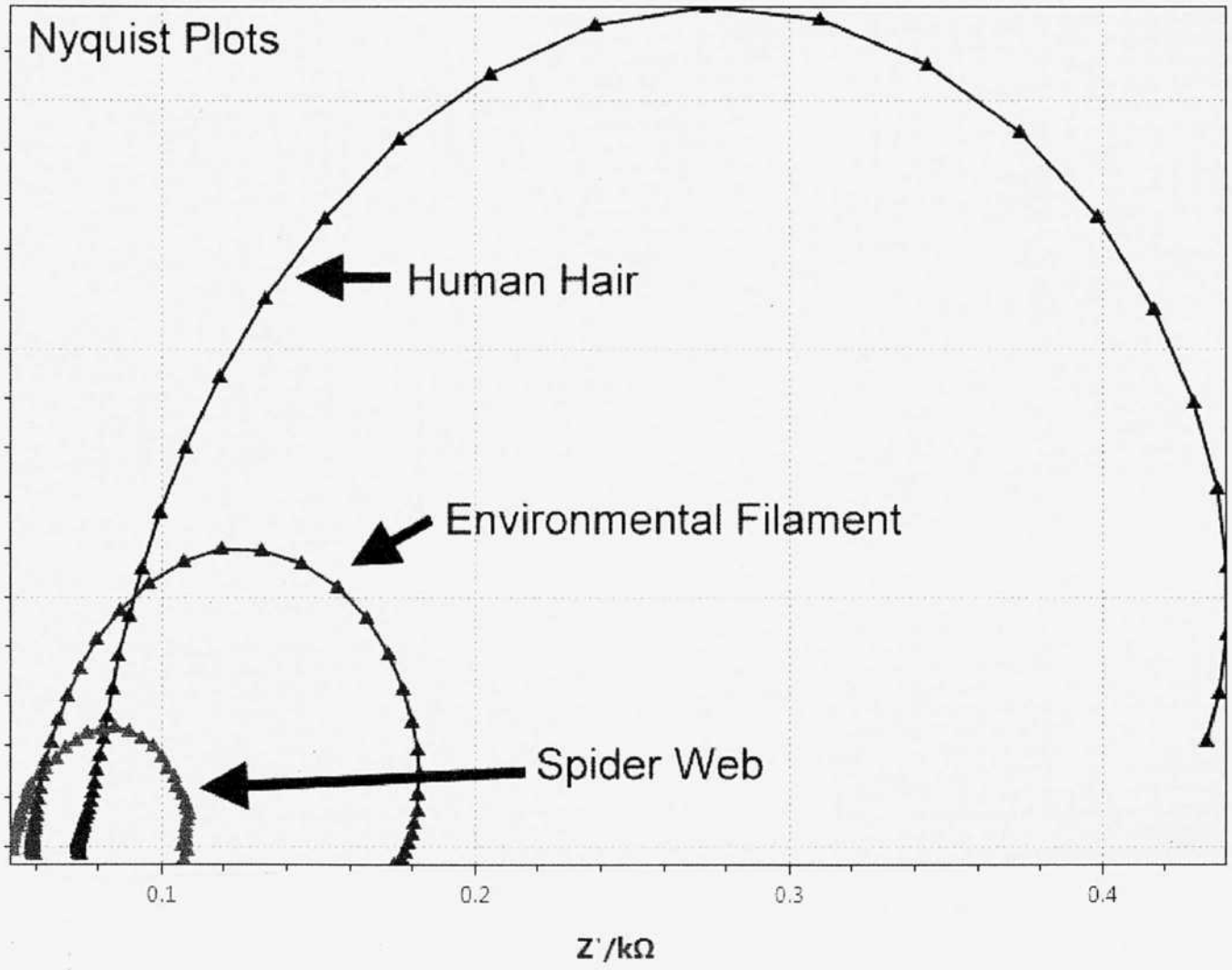
The Environmental Filament Produces the Following Circuit:

		Error:
R_1	59.5 Ω	0.51%
C_1	250.9 UF	1.76%
R_2	121.1 Ω	0.96%

Attention to achieving symmetry produces a better Circuit Result

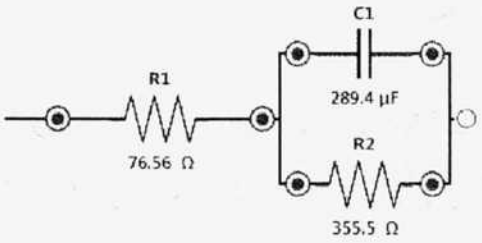
EIS Nyquist Plot Hair - Spider - Env Filament Nov 12 2017.jpg

ectrical Impedance Spectroscopy : Hair, Spider Web, Env. Filament



EIS work : Hair, Spider Web, Env Filament

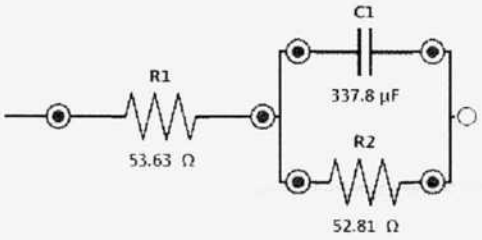
Electrical Impedance Spectroscopy (EIS) Equivalent Circuits



Human Hair

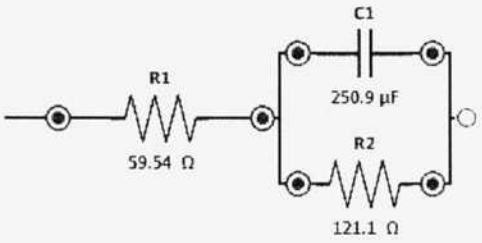
Regression Error:

R1 1.06%
C1 2.59%
R2 2.03%



Spider Web

R1 0.86%
C1 4.75%
R2 2.13%



Environmental Filament

R1 0.51%
C1 1.76%
R2 0.96%

This says to me that:

1. Human hair is ~~less~~ the least conductive of the three over 0-1000 Hz.

2. Spider web is the most conductive

3. The env. filament is midway

based upon the materials that can dissolve with the process of microwave digestion.

I do not know how to get impedance data out of QUCS, but we have it in both tabular and graphical form from the Palmsens instrument and software.

There really is no need to simulate the circuit with QUCS, it just seems like a good thing to be able to do.

I would say that we have made tremendous progress w/ EIS over the last 2-3 days.

This work makes the case that all three samples are electrically quite different from one another.

Next we will repeat the work to see if we can reproduce it.

Nov 13 2017

What we are doing now is examining the reproducibility of the EIS solution.

There seems to be some level of convergence after several iterations but just also how a level of variability involved that I can not explain either for.

Symmetry of the plot by controlling Edc does still seem to be a most important factor.

R_1 & C_1 seem quite stable. R_2 seems to have the most variability. 6-10 runs seems to stabilize the solution

Human Hair Reproducibility.

					$\bar{X}$	1st Solution	% Error
R_1	80.0	80.6	80.3	81.2	80.5	76.6	4.8
C_1	218.4	220.3	223.4	227.5	222.4	289.4	30
R_2	350	423	332	269	343.5	355.5	3.5% from 1st run.

These are saved results

This is actually quite close to our proposed solution accepted w/ a single run before we observed the pattern of convergence.

The largest error was in C_1. Convergence and a series of consistent runs will be used w/ an average. The hair circuit is definitely different from the melon & the env. plasmid, even with the error range above future runs should be considerably lower now.

Of the circuit sets for a while, it seems to be less stable until it converges again with several runs.

When you set a symmetric run, then you take your measurement. An average of symmetric runs should produce the ~~best~~ best result.

Another goal of fitting seems to be to try and match the ~~best~~ estimated time in the run. The also means little symmetry.

Next I am exploring Conditioning of the electrode The does not seem to be ~~help~~ very matters @ all.

Next we explore t Max OCP.
Increasing from 1 to 5 secs.
This seems to be helpful. We can see the electrode is active now.

You do have a good fit, but the values are different.

E_{dc} = Ø.58	Error	E_{dc} = Ø.56		
R_1 = 67.6	.37%	77.8	Ø.82%	} We are right back in range of original solution
C_1 = 291.0	1.92%	2814	1.89%	
R_2 = 72.5	Ø.89%	410.4	1.58%	

↑ Very good initial value

Max OCP t = 5 secs does seem to help.

Nov 16 2017

I now have a very effective combustion chamber that has been constructed w/in a fume hood.
It is working flawlessly. It depends upon an aquarium pump for a source of forced oxygen into the combustion chamber.
The combustion chamber is approximately a 2 oz stainless steel container w/ a canning lid cover w/ an inserted basal oxygen tube from the aquarium pump.

Ashing is now quite efficient.

I now need to start working again w/ identification of redox species by voltammetry.

We have introduced ourselves to
1. Linear voltammetry
2. Cyclic voltammetry
3. Electrical impedance spectroscopy.
(OCP & fine tune of EDC is critical to the success of EIS results).

Let's start w/ solutions of

1. Iron
2. Copper
3. Aluminum

We are going to try & recall normal pulse voltammetry.

$FeSO_4$

(3, 30, 10) [-3.2, 3.2] low noise .005, .008, .03

You can see Fe forming on the electrode! (black electrode)

Have same resolution results

(-3, 30, 10) [-3.2, 3.2] low noise.

((3, 30, 10) [3.2, -3.2] High noise level here

Have same resolution results

(-3, 30, 10) [3.2, -3.2]

It appear conditioning did not have that much influence.

We appear to have peaks @

-1.96
+1.09
-.75
+1.20

1.96	1.9 FeO_4, Fe^{3+}	
1.20	1.22 (O_2, H_2O), (H_2O, OH^-)	We want
.75	.77 Fe^{3+}, Fe^{2+}	.77, 1.12 & 1.9
1.09	1.12 SO_3^{2-}, SO_4	

So you actually do have a model.

Now we head towards conditioning since we could not replicate

1. We condition electrode.
2. We use AC voltammetry
3. We replicate results

-2.26 $H_2 + 2e^- \rightarrow 2H^-$ (-2.25)

-1.06 SO_3, SO_4 (1.12)

-.71 Fe^{3+}, Fe^{2+} (.71)

+2.1 $HFeO_4$, Fe^{3+} (2.07) S_2O_8 $H+$, HSO_4

O_3, $H+$, O_2 H_2O (2.07) S_2O_8, SO_4

Highly reproducible work

Mins
Max's
Zero Crossings] May all be important.

Slope Breaks seem to be very important (2nd derivative)

Slope breaks — It looks like we have something here

.77
.69
2.05
2.1, 2.1

You are going [-3,3]

You should also be able to go [3,-3]

Repeat trials and giving better results.
eg 5 iterations.

1. For initial function, you will look @ slope breaks and min maxs.

2. For derivative, you will look for zero crossings and large changes (ie, peaks).

I am getting fantastic resolution and truly unique & repeatable spectra. Now / w CuSO4.
Convergence w/ repetitions also does seem to take place, 5 secs
If you condition @ the starting voltage (eg -30V) you may get a steadier curve.

Nov 17 2017

We now have AC voltammograms of $FeSO_4$, $CuSO_4$ & $AlCl_3$.

Next we compile an AC voltammogram of a solution mixture.

Our goal is to see how much we can identify of Fe, Cu, Al, SO_4, & Cl_3

We look for slope breaks and min/maxs in the AC plot.

We look for zero crossings & peaks in AC'.

AC

Slope Breaks [-3,3]	Slope Breaks [3,-3]	Min/Max
~~-2.40~~		
~~-1.64~~	~~-1.34~~	
~~-.91~~	~~-.76~~	
~~-.62~~	~~-.62~~	
~~-.35~~		
~~-.14~~	~~-.12~~	
~~+1.02~~	~~+1.27~~	
~~+1.96~~	~~+1.89~~	
~~+2.24~~		
~~+2.56~~	~~+2.43~~	
~~+2.68~~	~~+2.61~~	
	~~+2.84~~	

AC'

Min/Max [-3,3]	Min/Max [3,-3]	Zero Crossings [-3,3]	Zero Crossings [3,-3]
~~-1.85~~	~~-2.16~~	~~-2.42~~	
~~+1.53~~	~~+1.53~~	~~-.89~~	~~-.89~~
~~2.40~~	~~+2.64~~	~~-.59~~	~~-.59~~
	~~+2.82~~	~~-.39~~	~~-.39~~
	~~+2.59~~	~~-1.03~~	~~-1.03~~
	~~2.93~~	.003	.003
		~~2.22~~	

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Fe, Cu, Al, SO_4, Cl_3

2.40, 2.43, 2.40, 2.42
1.64
.97 (.56 $FeOH_3$) (.62 ClO_3^-)
.62, .62, .59, .59 (.33 Cu)
.35, .39, .39
.14, .12
1.02, 1.03, 1.03 (2.25 H_2)
1.96 (1.48 Cl^-)
2.24, 2.22 (.81 Cl^-) (.77, Fe^{3+})
2.56, 2.59, 2.64, 2.61, 2.68
2.84, 2.82, 2.93 (1.9 Fe^{3+})
1.53, 1.53 (.12 CuCl) (.08 $CuOH_2$)
.89, .89 (2.12 HSO_4^+)
.76
1.34 (1.64 $HClO_2$) 1.61 Cl_2) (1.66 Al^{3+})
1.85, 1.89 (.95 ClO_2) (.93 SO_4)
2.16 (2.00 SO_4) (1.90 Fe^{3+})
.003 (.77 Fe^{3+})

$\bar{X}$	n			
~~2.41~~	~~4~~	?	2.62	5
1.64	1	?	2.41	4
.97	1		.60	4
~~.60~~	~~4~~		.38	3
~~.38~~	~~3~~	?	1.03	3
~~.13~~	~~2~~	?	2.86	3
~~1.03~~	~~3~~		2.23	2
1.96	1		1.53	2
~~2.23~~	~~2~~		.82	2
~~2.62~~	~~5~~	?	1.34	2
~~2.86~~	~~3~~		1.87	2
~~1.53~~	~~2~~		.13	2
~~.89~~	~~2~~		2.16	1
.76	1		.003	1
~~1.34~~	~~2~~		1.64	1
~~1.87~~	~~2~~		.97	1
2.16	1		1.96	1
.003	1		.76	1

The analysis does show a retrieval of Fe, Cu, Al, SO_4 & Cl_3
(5) (3) (1) (3) (5)

You would now also screen by commonality.

We have such a list of common elements now. We take as a reference point.

Next, we ran a trial w/ an ashed pine cone (spruce). The solution has been acidified w/ HCl.

The solution appears weak so we increase concentration from 30 ml to 130 ml. The looks much better.

Sequence was to
1. Thoroughly ash the pine cone
2. Dissolve ash in minimal water & acidify w/ 10M HCl 6 drops.
3. Double filter.

We have about 3 ml of solution now.
We now have ascending & descending AC Voltammetry curves on the ashed spruce cone.

AC•

slope break a peak:

[-3, 3]	[3, -3]
~~-2.13~~	~~-2.63~~
~~-1.45~~	~~-1.80~~
~~-.72~~	~~-1.41~~
~~-.26~~	~~+.14~~
~~+.71~~	~~+1.89~~
~~+2.41~~	~~+2.43~~
~~+2.59~~	~~+2.59~~
~~+2.43~~	
~~+2.79~~	~~+2.88~~

AC'

zero crossy a peak

[-3, 3]		[3, -3]	
~~-1.80~~	~~+2.74~~	~~-2.70~~	~~+2.5~~
~~-1.61~~	~~+2.79~~	~~-1.82~~	~~+2.~~
~~-1.47~~	~~+2.84~~	~~-1.52~~	~~+2.~~
~~-.99~~	~~+2.90~~	~~+0.11~~	~~+2.~~
~~-.73~~		~~+1.45~~	~~+2.~~
~~-.58~~		~~+2.45~~	~~+2.~~
~~-.05~~		~~+2.91~~	
+1.01		~~+2.98~~	
~~+2.42~~		~~+1.10~~	
~~+2.50~~		~~+.14~~	
+2.53		~~+.26~~	
~~+2.59~~		+.54	
~~+2.60~~		+2.41	
+2.68			

Here ??? Prob ≈ $\frac{3.01}{1+44.36e^{-.047 \cdot h}}$ (8.93)

Score = $\frac{\text{No. of Mentions} \cdot \text{Rank}}{(\text{Abundance Rank}/5)}$ Highest Freqency

n	$\bar{x}$	Now sort.	Est Prob		Ranking: Model Prob	Ln	Score
1	2.13	2.13	95%	Fe		2.42	11.2
5	1.46	1.45, 1.41, 1.47, 1.52, 1.45	90%	Cl		2.27	9.7
3	.72	.72, .71, .73,	83%	S		1.19	3.3
6	2.45	2.47, 2.43, 2.42, 2.50, 2.45, 2.41	80%	Cr	64%	1.10	3.0
2	.26	.26, .26	75%	Co		1.03	2.8
7	2.59	2.59, 2.63, 2.59, 2.59, 2.60, 2.61, 2.53	70%	Na		.99	2.7
6	2.72	2.73, 2.68, 2.74, 2.79, 2.70, 2.70	65%	Ca		.92	2.5
3	2.83	2.79, 2.84, 2.85	60%	K	↑	.87	2.4
3	1.81	1.80, 1.80, 1.82	50%	Mn		.74	2.1
3	.13	.14, .14, .11	40%	Al		.69	2
1	1.89	1.89	20%	Sr		.10	1.1
4	2.90	2.88, 2.91, 2.90, 2.90	6%	Ba		-.22	Ø.8
1	1.61	-1.61	5%	Cu		-.36	Ø.7
2	.61	-.58, .65					
2	2.96	2.98, 2.95					
1	1.70	1.70					

Use 2.25 cut-off point ≈ 55% w/ model Cut-off pt

Our model is: % ≈ $\frac{130.6 \cdot \text{Score}}{(\text{Score} + 3.08)}$ r = 0.90

	SORTED:	Candidates:		Score
7	2.59	?		
6	2.72	Na (2.71)		Na 2.7
6	2.45	?		
5	1.46	Cl (1.48, 1.47, 1.45)	(7(5))/(18/5)	Cl 9.7
4	2.90	K (2.92) Ba (2.9) Sr (2.89)	K=2.4	Ba=Ø.8
3	.72	Cl (.76) Fe (.77) .69 (O_2, H_2O_2), Co (.73) Cr (.74)	Fe=11.2	Co=
3	2.83	Ca (2.87) Sr (2.88, 2.89)	Ca=2.5	Sr=1.1
3	1.81	Co (1.82) 1.77 (H_2O_2, H^+)		
3	.13	S (.14) .12 (Cu, Cl) Cr (.13)	S=3.3	Cu=Ø.7
2	.26	Co (.28)		
2	.61	Cl (.62) Mn (.60)	Mn = 2.1	
2	2.96	K (2.92)		
1	1.70	Mn (1.69) Al (1.66)	Al=2	
1	1.61	Cl (1.61) Mn (1.56) Al (1.66)		
1	1.89	Fe (1.90) N_2 (1.87)		
	2.13	SO_4 (2.12) Fe (2.07)		

We now have a Elemental Probability Model for use w/ AC Voltammetry.

The model is in 2 parts:

1) Element Score:

$$\text{Element Score} = \frac{\text{No. of element mentions} \cdot \text{Highest Freq Rank from Redox Search Plot}}{(\text{Element Abundance Rank} / 5)}$$

2) $\text{Prob}\% \cong \frac{130.6 \cdot \text{Element Score}}{(\text{Score} + 3.08)}$ $\quad r \cong 0.90$

Applied to the Pine Cove Analysis:

Score	Prob	
2.25	$\approx 55\%$	← This is a good cut off point.
1.9	$\approx 50\%$	
2.6	$\approx 60\%$	
3.5	$\approx 70\%$	
4.9	$\approx 80\%$	
6.9	$\approx 90\%$	

These are our analyses indicate that, within the Spruce cone, are likely some the elements or ions:

POS! Fe
POS! Cl
POS! S
POS! (low) Cr
Positive trace Co
Moderate Normal Pulse V. and AC Voltammetry. Na
POS 2000PPM Ca
POS ~150PPM K

These are all very reasonable assessments.

Iron, sulfur, chromium, cobalt, sodium, calcium & potassium are the strongest candidates. (skip Chlorine)

2 independent methods.

All of this was determined by ashing a Pine Cone and applying AC voltammetry to the problem.

Secondary tests could then be developed.

This is indeed superb superb work. I have indeed now confirmed the presence of both Fe+2 and Fe+3 w/ Colorimetric tests and the use of VIS spectrometry. This was done w/ the use of 100ul of our solution added to ~ 2 ml of H_2O. The is quite weak and yet the VIS spectrometer easily picked it up.

Your methods are working. There is iron in a spruce pine cone! Do we have a test for sulfur? (Sulfates)

Barium Chloride forms barium sulfate.

We absolutely also got a positive white precipitate results when testing for sulfates w/ the use of barium chloride. Our solution is already acidified. Also used 100 ul in ~ 2 ml of solution. A very sensitive test.

Nov 18

I have now confirmed colorimetrically / visual spectroscopy that Chlorine is also present in the spruce cone. This is done w/ a colorimetric chlorine/bromine test kit. The cola is not strong enough to pick up by eye w/ ~ 100 ul in 2 ml distilled water sample.

But it can be and is detected w/ visual spectrometry. The signal is weak but definitive w/ a max absorbance @ ~ 437 nm.

Another perfect result.

→ Our ashing experiement is working quite well.

What tests might exist for Chromium + Cobalt?

Chromium Chemistry is very rich in color formation

	Oxidation State is:	Color is:
$Cr_2O_7^{2-}$ (OH^- ↓ ↑ H^+)	+6	Orange
CrO_4^{2-}	+6	yellow
Cr^{2+}	+3	Green
Cr^{2+}	+2	Blue - (unstable)

(orange) $Cr_2O_7^{2-}$ + Zinc + H^+ (acid) → Cr^{3+} (green) This is reduction.

Cr^{3+} + H_2O_2 (peroxide) + OH^- (base) ⇌ CrO_4^{2-} This is oxidation

Cr^{3+} + Zn + No air → Cr^{2+} (very difficult to do & it wants to go back to +3)

(+3 oxidation state) (+2 oxidation state)

Nov 13 2017

What we are doing now is examining the reproducibility of the EIS solution.

There seems to be some level of convergence after several titrations but just how at level of variability involved that I can not explain either for.

Symmetry of the plot by Controlling Edc does still seem to be a most important factor.

R_1 & C_1 seem quite stable. R_2 seems to have the most variability. 6-10 runs seems to stabilize the solution

Human Hair Reproducibility.

					$\bar{X}$	1st Solution	% Error
R_1	80.0	80.6	80.3	81.2	80.5	76.6	4.8
C_1	218.4	220.3	223.4	227.5	222.4	289.4	30%
R_2	350	423	332	269	343.5	355.5	3.5% from 1st run.

These are good results

The $\bar{X}$ is actually quite close to our proposed solution accepted w/ a single run before we observed the pattern of convergence.

The largest error was in C_1. Convergence and a series of consistent runs will be used w/ an average. The hair circuit is defenitely different from the melanoma & the env. plasmid, even within the error range above. Future runs should be considerably shorter now.

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??? Here

Prob ≈ 3.01 / (1 + 44.36e^(-.047·ln(Score)))

Highest Fraying

(8.93)

(Score) = No. of Mentions · Rank / (Abundance Rank / 5)

n	x̄	Now sort.	Est Prob		Ranking: Model Prob	Score
1	2.13	2.13	95%	Fe	2.42	11.2
5	1.46	1.45, 1.41, 1.47, 1.52, 1.45	90%	Cl	2.27	9.7
3	.72	.72, .71, .73,	83%	S	1.19	3.3
6	2.45	2.47, 2.43, 2.42, 2.50, 2.45, 2.41	80%	Cr	1.10	3.0
2	.26	.26, .26	75%	Co	1.03	2.8
7	2.59	2.59, 2.63, 2.59, 2.59, 2.60, 2.61, 2.53	70%	Na	.99	2.7
6	2.72	2.73, 2.68, 2.74, 2.79, 2.70, 2.70	65%	Ca	.92	2.5
3	2.83	2.79, 2.84, 2.85	60%	K	.87	2.4
3	1.81	1.80, 1.80, 1.82	50%	Mn	.74	2.1
3	.13	.14, .14, .11	40%	Al	.69	2
1	1.89	1.89	20%	Sr	.10	1.1
4	2.90	2.88, 2.91, 2.90, 2.90	6%	Ba	-.22	Ø.8
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2	.61	-.58, .65				
2	2.96	2.98, 2.95				
1	1.70	1.70				

Use 2.25 cut-off point ≈ 55% w/ model

one model is: % ≈ 130.6 · Score / (Score + 3.08) r = Ø.90

SORTED Candidates:

				Score
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1	1.89	Fe (1.90) N2 (1.87)		
1	2.13	SO4 (2.12) Fe (2.07)		

Unfortunately, we do not seem to have a source of Chromium in the lab.

I have found a source on Ebay for a reasonable cost and it has been ordered.

In the meantime,
the acidified ash absorbs @ the yellow end of the spectrum.

but the alkaline version starts to absorb more highly in the orange sector of the spectrum.

so the acidified is supposed to be more orange. (Scan 2)
alkaline ~~acid~~ is supposed to be more yellow.

We have some interesting things going on today with the pursuit of identification of Chromium.

The VIS spectroscopic analysis of acidified or alkaline ashed spruce cone is suggestive but not definitive.

The spectrum of the acidified ash is shifted toward 400 nm. relative to the alkaline. This says that the acidified is absorbing toward the violet end. This means the visual appearance should be slightly more yellow than the alkaline ash.

But w/ the Chromate - dichromate ion we see that the Chromate ion (yellow) occurs in a more alkaline solution. So the observation does not match the expected color reaction of the Chromate ion

But there is another interpretation of the spectra that also can be made.

The magnitude of absorbance of the alkaline ash is greater @ ~~485 nm (orange visible appearance)~~

435–480 nm (yellow visible appearance) than the acidified ash sample is. There is one way of interpreting that the chromate & dichromate ions do exist in the solution and that they are following expected color change reactions.

But since the remains ambiguous, I can fortunately report that I do have a qualitative Chromium colorimetric test reagent available! It comes from the water testing kit for pollution that I have. I am most fortunate to have the opportunity available.

Let's see...

(Incidentally, the fact that I am getting any absorbance @ all in the yellow-orange region

and I am

suggests that we may indeed have Chromium in the ashed sample.)

Continuing w/ the color test, using 100 µl ~ 2.5 ml H_2O.
Result is not visible by eye.
Let's try VIS spectrometry.

The Co(II) test is supposed to produce reddish-purple (pink or red) but it must be slightly acidic.

I have added 1 drop 1M HCl.

What we had initially was broad band absorption increasing as we went towards the yellow (ie visible color) portion of the spectrum w/ no distinctive or identifiable peak.

We have a ever so slight absorbance increase @ ~~480~~ ~ 498 nm.

This is right on the boundary of red - red purple.
I would say we have it!

I have now:
1. Doubled the concentration of the ash sample.
2. Doubled the reagent Concentration.

We have a slight absorbance increase @ 500 nm.
This is, once again red to red-purple.

We do have Chromium detection @ a very low Concentration level.

The peak is barely discernible but it occurred 3 times.

I may have already developed a colorimetric test for cobalt.

This is acidified!

Control	Sample
3 ml H_2O	3 ml H_2O
30 ul RH emerald dye (Green)	30 ul RH emerald dye
1 drop 1M NaOH (10M?)	1 drop 1M NaOH
pinch tartaric acid	pinch tartaric acid
	small amt of cobalt chloride
turns blue green	turns purple

both solutions are clear

The looks promising.

No, I think the pH is causing the difference. The sample is highly acidified.

Cobalt Colorimetric - UV Detection Test Developed.

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If you develop any colorimetric test, as a part of installing controls, you must include pH as one of the factors of control.

Dyes frequently change color as a function of pH. Make sure that all tests are done @ the same pH.

I have now run a color - pH test on each of the four RIT dyes

Dye Name	Acid	Neutral	Base
Lemon yellow	Light yellow	Yellow	Rich Yellow
Emerald	Light Purple	Light Blue	Light Blue - Green
Purple	~~Light~~ Purple	Dark Purple	Dark Purple
Cherry Red	Rich Red	Rich Red	Very Dark Purple - Red

We now know that the Emerald dye is most subject to color change w.r.t. pH. This is not a disadvantage; it is important to know.

Next biggest change is w/ Cherry Red w/ base.

Purple + yellow dyes are the least variable.

COBALT TEST DEVELOPED

Attempting to develop a Color test for Cobalt.
Variables under consideration:

Yellow dye w/ NaOH — Cobalt or no Cobalt.
$FeSO_4$, $CuSO_4$?
Tartaric acid

Centrifuge shows no reaction.

Great news. I have produced a Color shift w/ the presence of Cobalt Chloride.

Control	Sample
~ 2.5 ml H_2O	~ 2.5 ml H_2O
30 ul RIT Emerald Dye	30 ul RIT Emerald Dye
1 drop 10M NaOH	1 drop 10M NaOH
1 Syringe drop .5M $CuSO_4$	1 Syringe drop .5M $CuSO_4$
Shake & Sit	Pinch Tartaric acid (4th & 3rd)
Centrifuge	Shake & Sit
also Pinch Tartaric acid (4th)	Centrifuge

Max & Primary Absorption @ 615 nm

Similar absorption @ 615 nm
Additional significant Absorbance @ 400 nm.

Let Sit 10 min to develop the Color.

Repeat the test.
Increase absorption from 700 to 220 nm.

Centrifuging will probably not be required w/ trace testing.

COBALT TEST DEVELOPED

This is a UV Test as much or more than a colorimetric test.

We have changed the absorbance range from 700 to 220 nm since there is strong UV absorption taking place with a peak @ ~297 nm in addition to the VIS peak @ ~617 nm.

Well there is certainly interesting. The absorbance peaks for both treats are actually essentially the same. The shape of the curves, however, is different, especially in the range of ~330 nm to ~450 nm.

The sample w/ Cobalt has a much higher level of absorbance in this region, which appears in the yellow to yellow green portion of the spectrum.

Visually, the Cobalt treated sample appears to be much more blue green than the more blue Control sample.
Let's repeat this test.

There is a case where the derivative of the curves would be more telling. The Control curve is much more flattened from ~380 to 480 nm. (orange + yellow absorption visible).

Let's repeat this test. The stakes are high for it.

Let the result set 10 min to develop the color change
You have used a very small sample of Cobalt Chloride this time.
No centrifuge may be required w/ addition of tartaric acid?

After 10 min of setting, there appears to be a very slight difference in color. We will centrifuge. Yes, I can see the difference again.

COBALT TEST DEVELOPED

I have centrifuged both samples. I can once again see the difference between the control & the Cobalt sample. Control is blue; Cobalt sample is blue green.

Both seem appear visually identical; Cobalt presence is detected as a Color change & spectral change under the reagent conditions established.

A difference plot from the reference (control reagent) will be the most practical way of recording the impact of the Cobalt.

We have had a discontinuity appear when validating the reference plot. It occurred @ ~350 nm.

Regardless of the discontinuity, we can see that the max difference absorbance between the Cobalt sample and the Control-reference sample also occurs @ ~350nm. They actually appear to be coincidental. The regular plots will reveal the same situation.

So the maximum difference occurs in the UV portion of the spectrum.

Let's run a plot from 300-400 nm.

The discontinuity is because of a gain issue. Let's dilute the samples

COBALT UV Detection Test Developed.

The gain change in the instrument is what is causing the discontinuity. I have deleted the solution.

Recall that the UV absorbance was very high. If the gain goes to 8 it introduces a discontinuity. I have shifted the discontinuity to 308 nm which is sufficient.

Let's now scan from 310 to 410 nm wanted to remove it. OK, now you have managed the discontinuity properly. It is gone.

We cut it just a little too close. The maximum absorption difference appears to be @ almost exactly ~ 310 nm. Dilute it further.

The lesson here is that our test is actually taking place in the UV portion of the spectrum and the reference solution is too ~~strong~~.

The is not exactly true. The max difference between the two vials (reference & Cobalt) actually occurred near 350 nm, not 300. This says that dilution is affecting the results disproportionately.

I am using backup trial set & using only 200 ul of each solution in 3 ml distilled H_2O from 300 to 500 nm. Difference plot being developed

COBALT UV Detection Test Developed.

The low concentration of 200 ul of each solution is more than enough to create a suitable difference plot. Lets see if we can get it on the instrument 250-500nm.

Another lesson: the reference reagent and the sample must be prepared @ exactly the same concentrations and in the same way.

We made it to 260 nm nm. Try that.
Using a reagent reference instead of water is definitely causing some complications in the process but we are getting there. A jump to a gain of 8 causes a problem.

This is the first time that we have had to work so intently within the VIS-UV boundary & overlap.

Another thing we see over & over is that this UV-VIS-NIR spectrometer is a VERY sensitive instrument. Only very low concentrations are required to detect differences.

OK. For the first time we have a successful difference plot @ the same concentration level.

The max difference is occurring @ ~ 314 nm.

Definately a UV absorption Cobalt test!

Cobalt UV-Vis Detection Method

We also see that there is continually increasing absorption difference in the Cobalt solution from 500 to 400 nm in the visible range as well. This is the orange - yellow - yellow green visible portion of the VIS spectrum and this is causing the color shift that you can detect by eye.

There is, however, even greater absorption difference taking place in the UV portion of the spectrum w/ one significant absorption that takes place @ ~ 314 nm.

There undoubtedly is a Complex being formed therefore. Is Cobalt or its complexes known to absorb significantly in the UV portion of the spectrum?

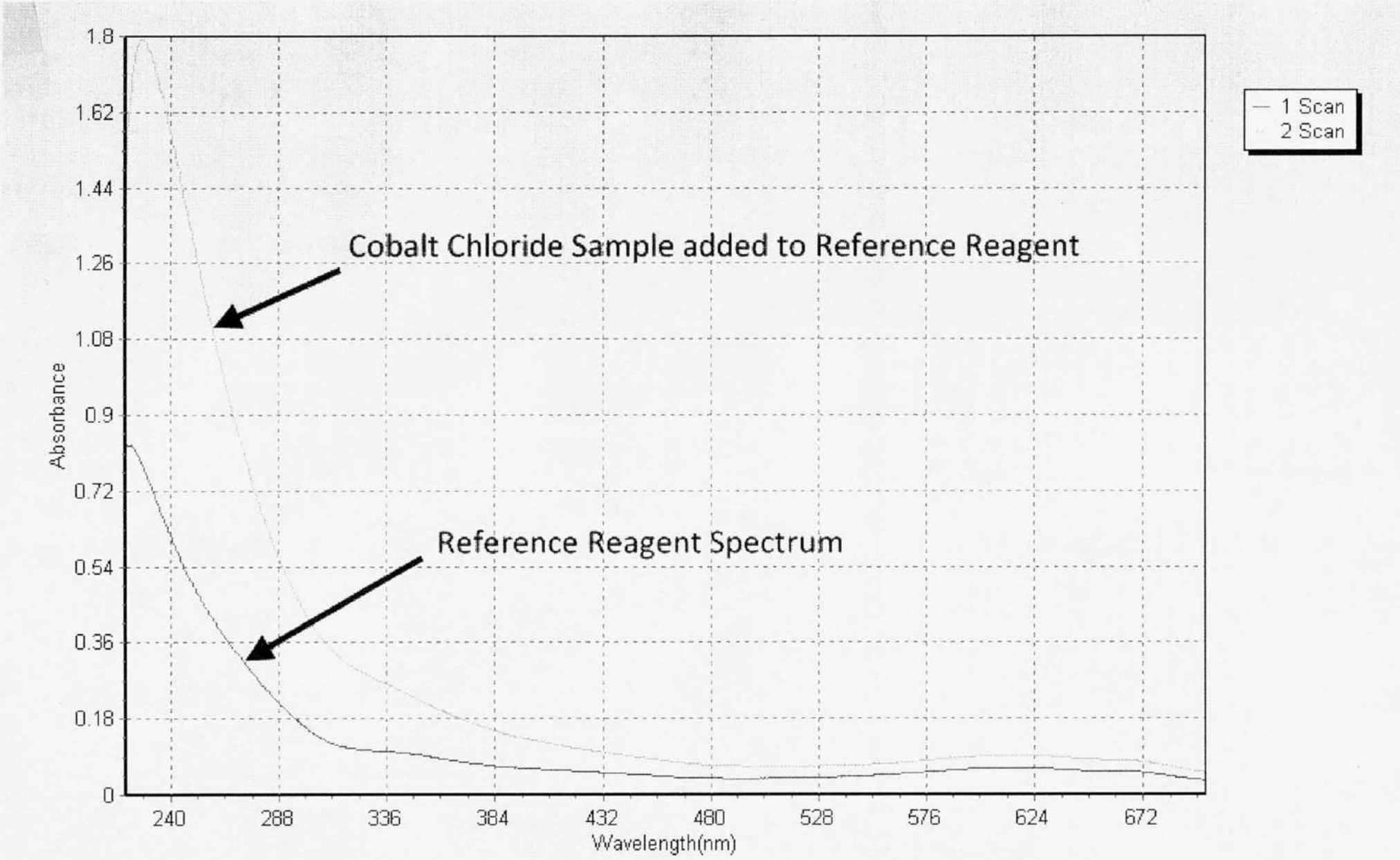
Ok, the answer is a resounding yes! Both copper and Cobalt have significant UV absorption exactly in the 300 - 500 nm range as I have zeroed in upon.

I would say that we have established and created a UV - VIS detection method for Cobalt in an ingenious and affordable manner.

This is worthy of presentation in the ourreight.

UV-VIS Cobalt Detection Method Spectrum

Cobalt Testing Plot AshedCone -Direct Spectrum Nov 19 2017 - 05.jpg



Now we apply the test to the asked spruce cone.

Cobalt $^{+2}$ is pink
Cobalt $^{+3}$ is orange/yellow

What do we have w/ cobalt chloride? $CoCl_2$ means Co^{+2}

Interesting that we seem to be picking up yellow-orange on our spectrum but since that is a complex could it not be a Co^{+3} complex just as easily?

—

Our result when testing the asked sample is that we get a strongly negative absorption in the UV spectrum in the range of interest. We can see that the color is much more purple than it is green as w/ $CoCl_2$. There a complex is definitely being formed but it may well not be a cobalt complex. We pretty much have a mirror negative spectrum of the cobalt spectrum and a different color which is toward the 500-600 nm portion not 300-500. So we have something being formed but we do not know what it is.

Cobalt therefore remains as undecided. Further testing is going to be required, electrochemically as a starting point, to investigate the Cobalt Candidacy further.

We do see after setting for an hour a more that the known cobalt sample turned a rich green color.

What are we w/ the UV - VIS spectral analyses so far:

1. The UV spectrum in the VIS range is very similar between the reference & the asked sample even though we can see a color difference by eye.

The reference reagent has a peak @ 604 nm
The asked sample has a peak @ 599.5

There is indeed a small red shift in the spectrum. This indicate the presence of a red-orange-yellow component, which is what is causing it to look more purple visually. So this makes sense, even w/ cobalt.

Next, in the UV spectrum we see that the magnitude of UV absorption is dramatically less than with our forced & some simulated cobalt trials.

But if you look very closely you see a small absorbance peak @ ~ 329 nm.

This is not really that far off from 315.

It may be that we are indeed picking up a slight trace of the cobalt ion with respect to both of the above findings. We do expect a red shift component and we do expect some UV increased absorption near ~ 315 nm.

We actually have both.

Notice w/ the reference Cobalt reagent we actually have a blue shift. This is what caused it to turn green.

This is therefore in an opposing direction to what we found w/ the asked Cone Total.

But the UV absorption still has some similarity.
329 nm vs 315 nm.

An interesting observation.

Let's review the red shift - blue shift question again.

With the reagent development, we see now that the Cobalt solution shows a red shift in ABSORBANCE relative to the control reagent.
~615 for reference reagent
~618 for Cobalt solution

Since 615 is in the visible appearance as greenish blue (which is accurate) the shift of the sample is actually towards purple. (The shift is still so slight that it still appears greenish blue).

Now, when we look at the asked Cone we have
~618 for the reference
~615 for the asked Cone.] This is exactly the reverse.

I am running every thing over from scratch.
Some things we learn are:

1. It is difficult to use a reference material that absorbs more strongly than water, such as a Copper solution in the UV range.

2. The Cobalt absorbs considerably more (or at least the Complex that is formed) than Copper does by itself.

3. From ~400 to 480nm the Cobalt absorbs more strongly than the reference Copper solution. This is in the Orange - Yellow - yellow green (appearing) section.

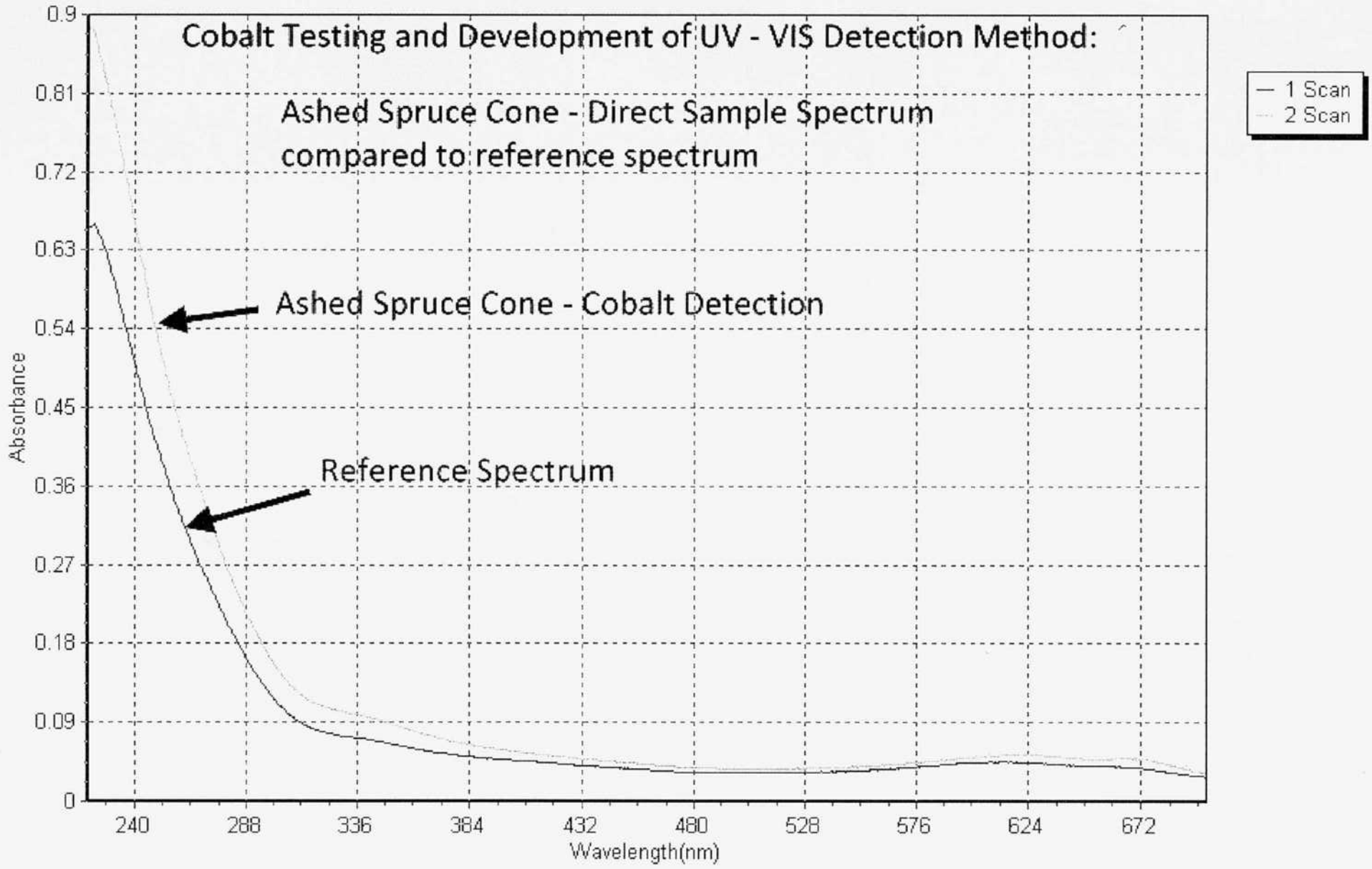
4. Only 200 ul added to 3 ml was required to produce a substantial plot w/ absorbance up to ~2.0. Cobalt is therefore highly UV absorbing.

5. A direct spectra readout is now included on this slate. The differences between the reference reagent and the Cobalt sample can be observed directly.

---

* Upon a very close inspection, the spectra of the ash sample shows all the signs of containing trace elements of Cobalt. This became evident in the increased absorbance in the visible range circa 600 - 700 nm and especially the shape of the curve and increased absorbance in the UV range as well. The purple tone did not arise — although the alkalinity of the sample was maintained w/ additional NaOH in spite of the acidity of the ash sample.

Cobalt Testing Plot AshedCone -Direct Sample Spectrum Nov 19 2017 - 06.jpg



Calcium:

Next we have tested for Calcium. The test is highly positive.

We add 200 ul to 5 ml H_2O.
We get a reagent result of 80 PPM.

Therefore the original concentration is:

$\frac{5000\ ul}{200\ ul} = 25$ factor

4 drops turns color from pink to purple = 80 PPM

$80(25) = 2000$ PPM is the original ash solution concentration.

Potassium Reagent Test is also Positive.
We estimate ~150 PPM in original ash solution

Na^+ is a more difficult ion to establish chemical identity of.

Flame test is not exactly practical w/ ½ ml of liquid sample remaining w/in a mixed & complex composition.

Ion selective electrodes are one method. A potable meter from the UK will cost ~$500.
Apparently no colorimetric test exists.

My suggestion @ this time is to attempt a 2nd electrochemical method.

Microscopic analysis fails to detect cubic crystals @

I have reopened Normal Pulse Voltammetry investigations. The method does appear to be very useful as found before. It is picking up Na^+ @ 2.7 immediately.

We have what seems to be four important electrochemical methods in place:

1. Conditioning of electrodes
2. Normal Pulse Voltammetry (NP, NP')
3. AC Voltammetry (ACV & ACV')
4. EIS & circuit development.

5. Concentrations w/ ACV?

Note:
Cyclic
Cyclic

Now, if you want a great test, you will compare the results of Normal Pulse Voltammetry & AC Voltammetry and see how they compare, without looking @ both of them together.

It does, however, look like Na^{+1} is immediately rejected.

Let's go:

I want to run a cyclic NPV run after the electrodes are conditioned and then compare to previous run. We notice the electrical noise here seems to be quite low.

1. 1600
2. 1300
3. 275
4. 1100
5. 400

The results between conditioning and non conditioning appear almost identical.

Conditioning appears to help in one case, & possibly hurt in the other. We have created an average; now collect data:

[-3.1, 3.1]

~~-2.91~~
~~-1.98~~
~~-1.14~~
~~-1.15~~
~~-.82~~
~~-.35~~
~~+.34~~
~~+.79~~
~~+2.31~~

[3.1, -3.1]

~~-2.92~~
~~-2.13~~
~~-1.04~~
~~±.66~~
+2.73

n	$\bar{x}$		$\bar{x}$	
2	2.94	-2.97, -2.92	2.94	K (2.92)
1	1.98	-1.98		SO_4 (2.00)
1	1.77	-1.77		H_2O, H_2O_2 (1.77)
1	1.15	-1.15		Cl(1.15) Cu(1.12) Mn(1.15) SO_4(1.12)
2	.805	-.82, .79	.805	Cl (.81) Fe(.77)
2	.345	-.35, .34	.345	Cu (.33) Co(.28) Cu(.35)
1	2.31	2.31		Al (2.31) Al(2.33) Mg(2.37)
1	2.13	2.13		O_2 (2.07)
1	1.04	1.04		?
1	.66	.66		Cl(.66) Cl(.62) Co(.73)
1	2.73	2.73		Na (2.71) Mg(2.68)

Variable in model should include:

1. No. of redox entries
2. ~~No. of entries for each element or ion~~ Redox Rank #
3. ~~Deviations~~ Avg deviation of element from avg redox value
4. Rank of commonality of element or ion

Still need this.

Our model is currently in two parts:

(1) An intermediate score

(2) Probability Estimate in %

$$\text{Intermediate Score} = \frac{\text{No Mentions}_{(\#1)} \cdot \text{Highest Freq}_{\#2} \; \text{Redox Rank}}{(\text{Element Abundance Rank}_{\#4}/5)}$$

$$\text{Prob\%} = \frac{130.6 \cdot \text{Int. Score}}{\text{Score} + 3.08} \quad r = .9$$

n	$\bar{x}$		$\bar{x}$	
2	2.94	-2.91, -2.92	2.94	K (2.92)
1	1.98	-1.98		SO_4 (2.00)
1	1.77	-1.77		H_2O, H_2O_2 (1.77)
1	1.15	-1.15		Cl(1.15) Cu(1.12) Mn(1.15) SO_4(1.
2	.805	-.82, .79	.805	Cl (.81) Fe(.77)
2	.345	-.35, .34	.345	Cu (.33) Co(.28) Cu(.35)
1	2.31	2.31		Al (2.31) Al(2.33) Mg(2.37)
1	2.13	2.13		O_2 (2.07)
1	1.04	1.04		?
1	.66	.66		Cl(.66) Cl(.62) Co(.73)
1	2.73	2.73		Na (2.71) Mg(2.68)

Variable in model should include

1. No. of redox entries
2. ~~No. of entries for each element or ion~~ Redox Rank #
3. ~~Deviations~~ Avg deviation of element from avg redox value
4. Rank of commonality of element or ion

Still need this.

Our model is currently in two parts:

(1) An intermediate score

$$\text{Intermediate Score} = \frac{(\#1)\ \text{No Mentions} \cdot \#2\ \text{Redox Highest Freq Rank}}{(\text{Element Abundance Rank}/5)}$$

#4

(2) Probability Estimate in %

$$\text{Prob\%} = \frac{130.6 \cdot \text{Int. Score}}{\text{Score} + 3.08}$$

Now what your model should do is combine both Cyclic AC Voltammetry and Cyclic Normal Pulse Voltammetry results.

Revises to

ACV	n	Eo			n	Eo	
	7	2.59					
	~~6~~	~~2.12~~	7	2.12	8	2.92	K(2.92)
	~~6~~	~~2.45~~	6	2.45	(7 2.59) ?		Sc(2.6) ???? Big Mys
	5	1.46			7	2.72	Na(2.71)
	~~4~~	~~2.90~~	8	2.92	(6 2.45) ?		Big Mystery
	3	.72			5	1.46	Cl(1.48, 1.47, 1.45)
	3	2.83			3	.72	Co(.73), Cl(.76), Fe(.77) O_2(.
	3	1.81			3	2.83	Ca(2.87) Sr(2.88, 2
	3	.13			3	1.81	Co(1.82) 1.77(H+)
	2	.26			3	Ø.13	S(1.14) Cu(.12) Cl(.12) C
	~~2~~	~~.61~~	3	Ø.63	3	Ø.63	Cl(.62)
	~~2~~	~~2.96~~			2	Ø.26	Co(.28)
	~~1~~	~~1.90~~			2	2.13	SO_4(2.12) Fe(2.07)
	~~1~~	~~1.61~~			2	.805	Cl(.81)
	~~1~~	~~1.89~~			2	.345	Cu(.33)
	~~1~~	~~2.13~~	2	2.13			
NPV	~~2~~	~~2.94~~					
	~~2~~	~~.805~~	2	.805			
	2	.345					
	~~1~~	~~1.98~~					
	~~1~~	~~1.77~~					
	~~1~~	~~1.15~~					
	~~1~~	~~2.31~~	~~1~~	~~2.31~~			
	~~1~~	~~2.13~~					
	~~1~~	~~1.04~~					
	~~1~~	~~.66~~					
	~~1~~	~~2.13~~					

Next assume we disregard all entries of ≤ 1. This leaves us with:

Element	Incidence	Rank n	abundance/5	Sc
K	1	8	3.4	2.
Na	1	7	2.2	3.
Cl	7	5	3.6	9.
Co	3	3	3.2	2.
Fe	3	3	Ø.8	11
Ca	1	3	1.2	2.
Sr	2	3	6.0	1.
S	2	3	1.8	3.3
Cu	2	3	4.2	1.
Cr	1	3	2	1.5

Next, we compute Prob % = $\frac{130.6 \cdot \text{Score}}{\text{Score} + 3.08}$

These scores indicate that you have just cause to be looking to verify all elements & ions w/ a score > 40%.

If something is doubtful, it can of course still be checked. ! ! !

I have completely succeeded w/ the project. I have successfully identified 7 different metals and two ions by two independent methods in each case as applied to a random sample (ie, a spruce cone).

The opens up inorganic analysis to anything which can be dissolved into solution, especially aqueous.

n (7) 2.59 & n (6) 2.45 & Two Mystery Components

Prob.	Element/Ion		Ranked:	%	Verif.
57%	K		Fe	102	POS
67	Na	(verified 2 methods)	Cl	99	POS
99	Cl		S(SO_4)	68	POS
62	Co		Na	67	POS(li
102	Fe		Co	62	POS (t
59	Ca		Ca	59	POS(20
32%	Sr		K	57	POS(15
68	S (SO_4)		Cr	43	POS (tra
41%	Cu		Cu	41	POS
43%	Cr		Sr	32	(trace

Not adequate basis $\leq .25$ PPM

Nov 21 2017

I have now started applying the electrochemical methods to concentrated rainfall.

I start w/ cyclic AC voltammetry (CACV) and I get a definite result but the elements seem to be less certain than w/ the spruce cone ash.

Aluminum is going to be a question. There are some indications that it may be involved but it is lacking the qualitative ion test w/ the use of sodium hydroxide.

The plot is much smoother (less complex) than the ashed spruce cone but there nevertheless is definite redox activity of some sort.

Before we get too involved, lets compare distilled water, tap water, and filtered water.

But while we have the setup in place, lets continue with Cyclic normal pulse voltammetry (CNPV)

(We also have the yeast cultures that look to be of interest for the microscope).

When working w/ Chronopotentiometry (i.e., conditioning the electrode) we notice the original curve starts @ 0.0V and then eventually converges in the process towards ~ 0.62V.

Then is exactly the DC voltage level that was found to cause EIS to give suitable results, along w/ the discovery (from a forum post) that the open circuit potential (OCP) incorporation into the EIS measurement was what changed everything and produced the first successful Nyquist Plot.

How is that for a run on sentence?
It means that OCP is a critical factor w/ our graphite electrode system and that you must learn about this.

I now have the results for CACV & CNPV for rainwater. Data remains to be extracted.

It would now be of interest to compare w/ distilled, tap & filtered water.

I have Cyclic ACV Voltammetry plots available now. Cyclic Normal Pulse voltammetry can be used as a backup.

Lets look @ distilled water a bit:

For 'NPV plot we want slope breaks and minimums/max
for NPV' we want peaks and zero crossings

~~NPV~~ ACV

Could copper pipes be used in the distillation process? This does seem possible.

~~NPV~~ ACV'

Trace Signals:
-1.22 O_2, H_2O
.14 Cl, O_2, Fe
-.13 Cu, Cl
.37 Cu, Cu

Nitrogen appears to dominate -

Now for rainwater
~~NPT~~ ACV

ACV'

-1.71 peak
+1.48 peak

? -1.04 peak NO (1.04)
1.41 zero crossing NH (1.42)
-.89 peak H_2O (.88)
Mn(-1.56) 1.52 peak NO(1.59) Mn(1.5)
N_2(1.77) -1.73 zero crossing H_2O(1.77)
1.71(N_2O) -1.81 possible small peak

The increased probability of N indicates the possibility of an organic constituent.
UV, NIR, IR all come to the fore @ this point, proteins far more so than metals do.

From the methods now in place, it becomes immediately clear that we are dealing w/ significant organic constituents w/in the rainwater concentrate sample.

1. Cyclic AC voltammetry repeatedly indicates the presence of nitrogen based compounds in the solution. As we see above, the organic aspect dominates the redox character of the solution, much more so than the metallic signature.

2. UV Analysis shows a very strong organic signature. It includes a peak near 270 nm which is indicative of protein content

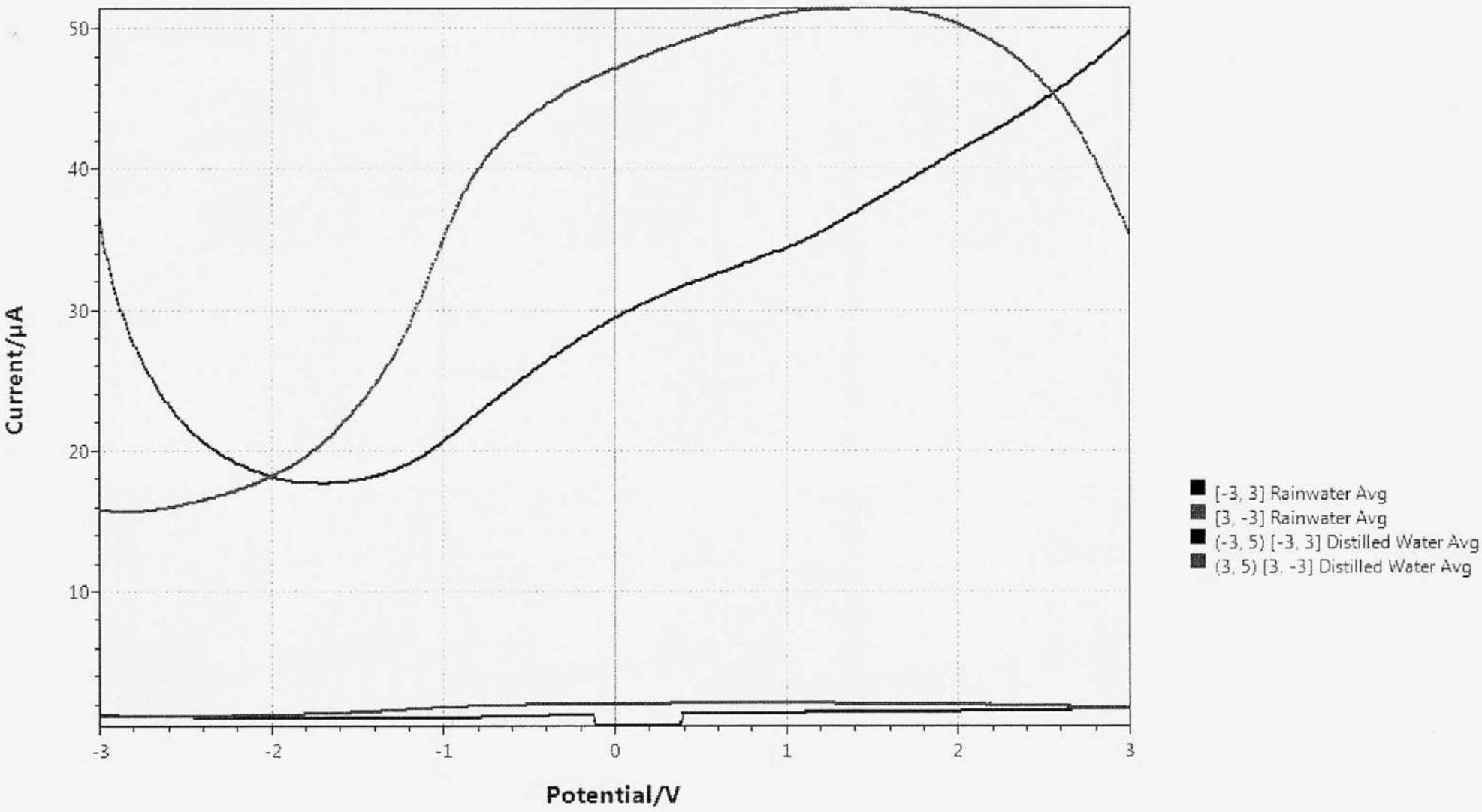
3. NIR further substantiates an important organic signature. Functional groups of Ar OH & Ar CH are indicated w/in the spectrum, namely an aromatic aspect.

4. Bradford for protein is an obvious next test, along w/ IR Analysis.

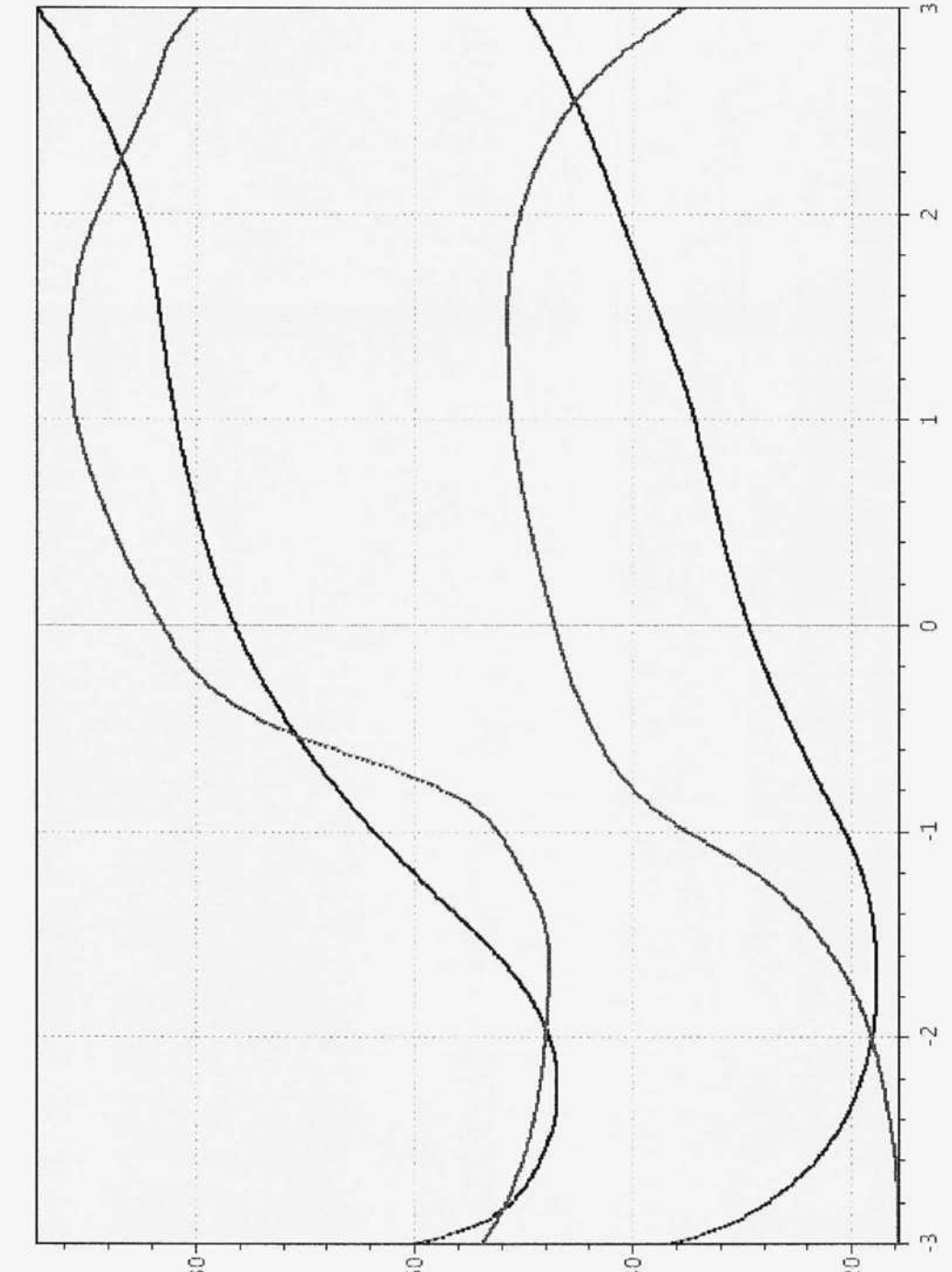
Cyclic AC Voltammetry Plots

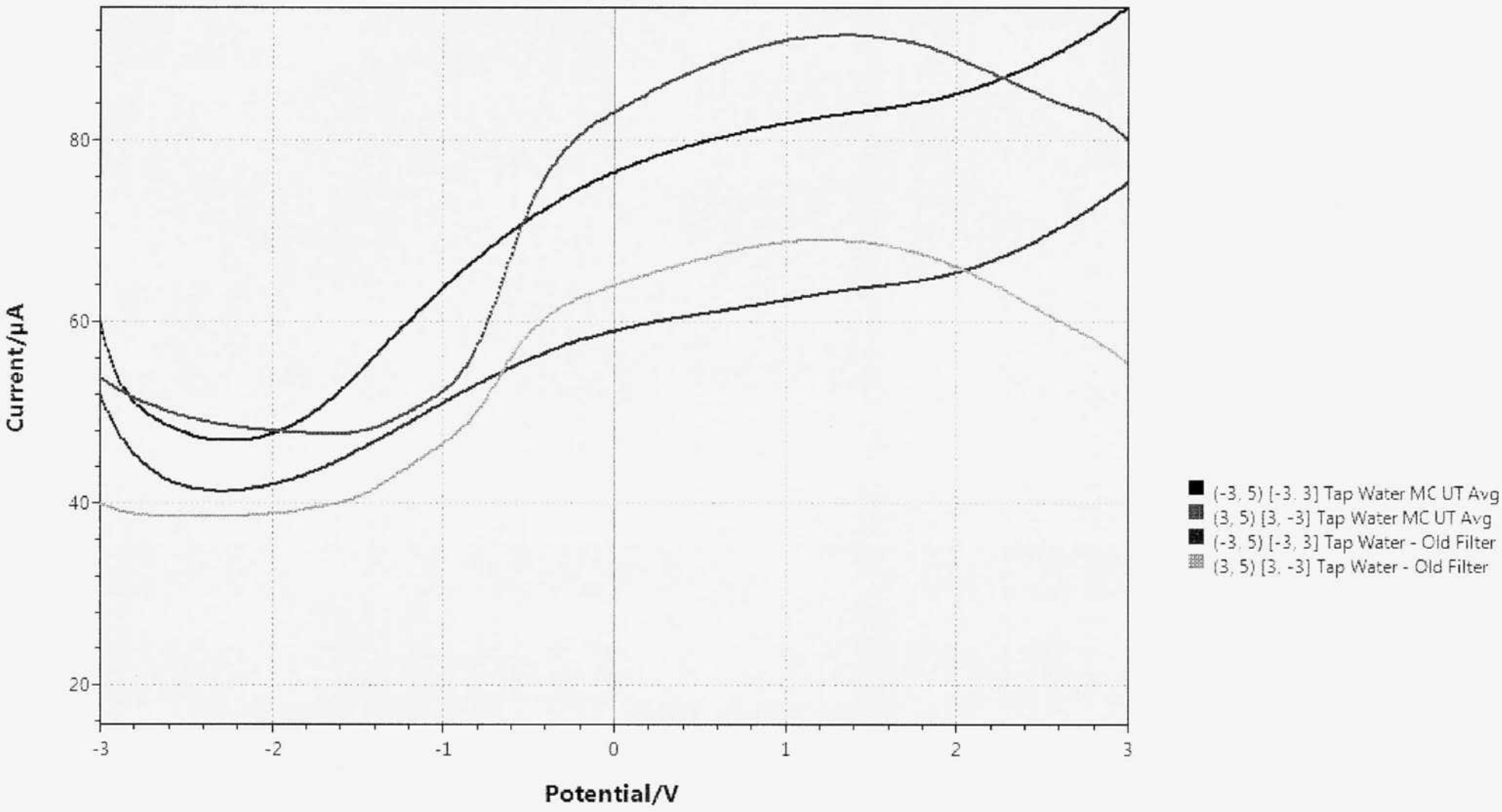
Conc. Rain, Top Water, Filtered Water (Old Filter, New Filter)

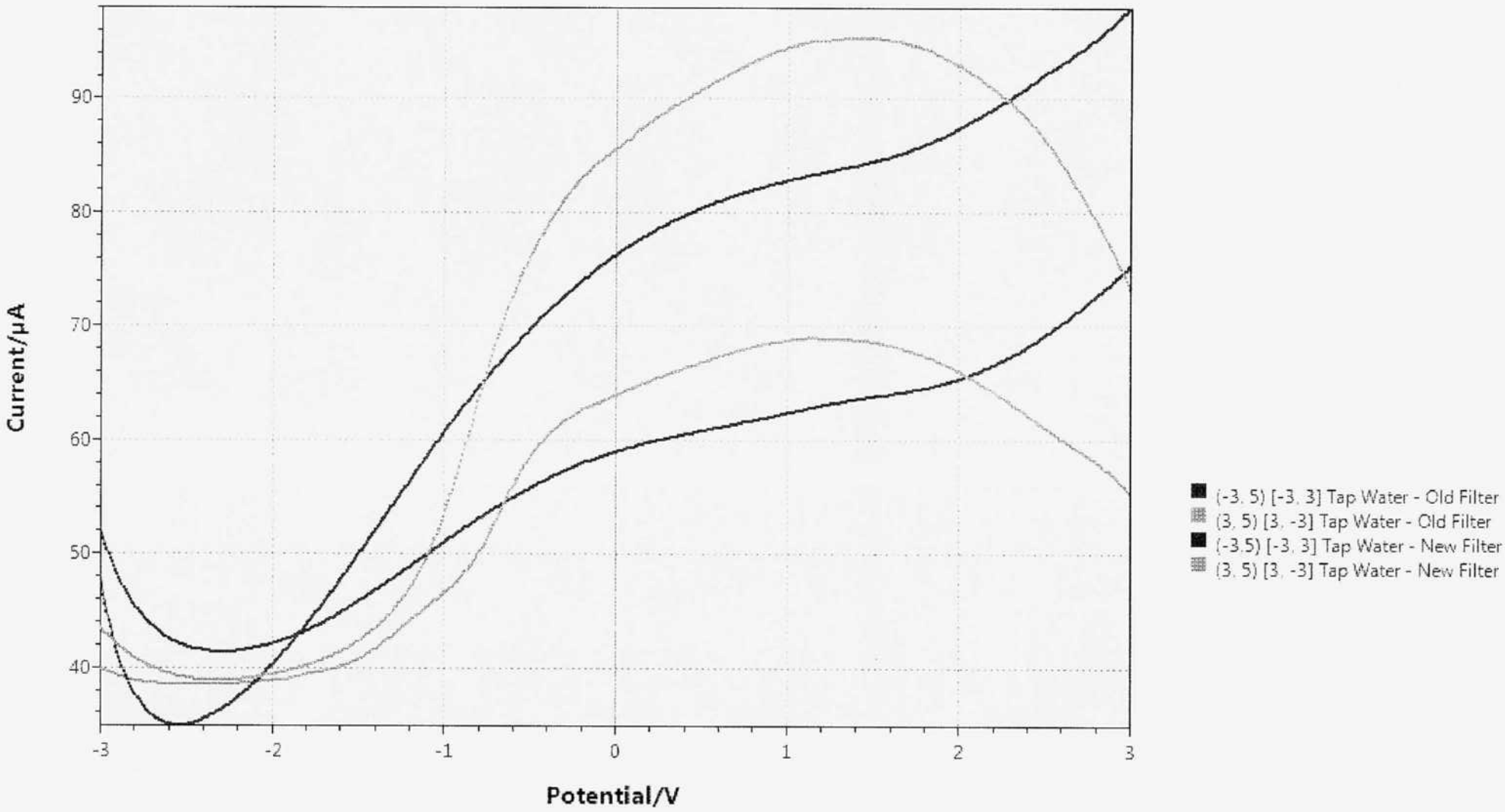
Rainwater vs Distilled.jpg



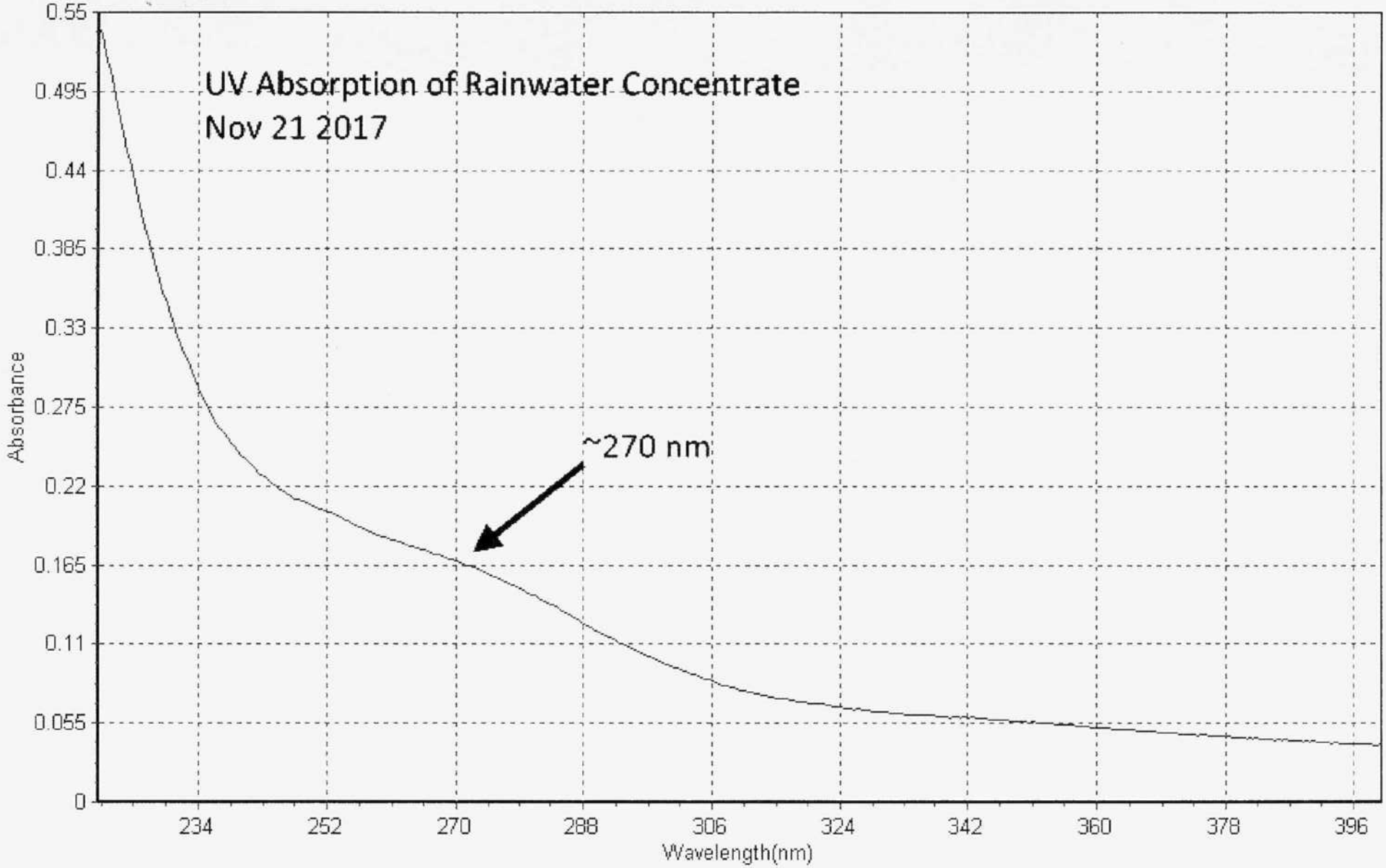
Page 167B


Current/μA
80
60
40
20
-3
-2
-1
0
1
2
3
Potential/V
[-3, 3] Rainwater Avg
[3, -3] Rainwater Avg
(-3, 5) [-3, 3] Tap Water MC UT Avg
(3, 5) [3, -3] Tap Water MC UT Avg

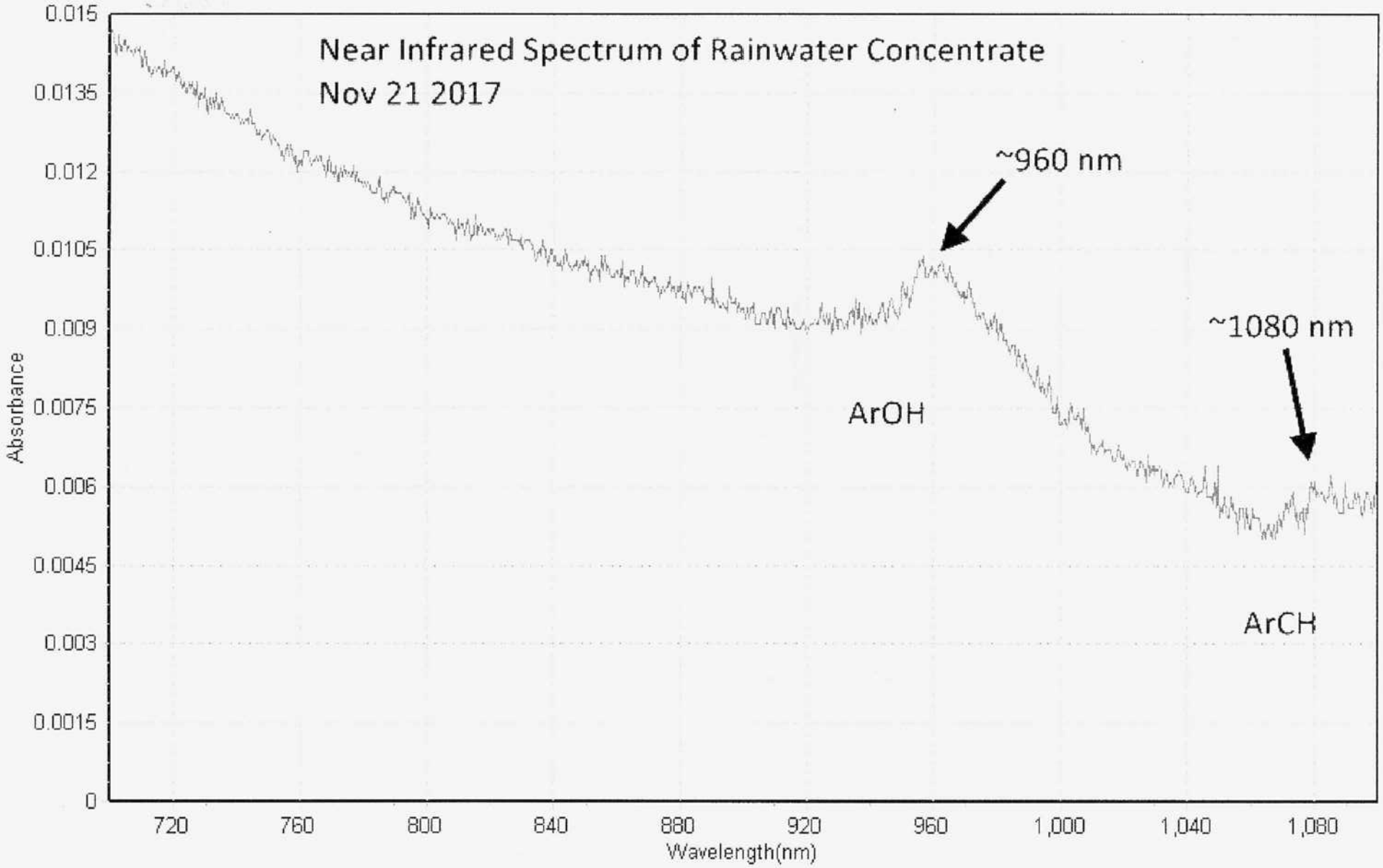

Current/µA
80
60
40
20
-3
-2
-1
0
1
2
3
Potential/V
(-3, 5) [-3, 3] Tap Water MC UT Avg
(3, 5) [3, -3] Tap Water MC UT Avg
(-3, 5) [-3, 3] Tap Water - Old Filter
(3, 5) [3, -3] Tap Water - Old Filter


Current/µA
90
80
70
60
50
40
-3
-2
-1
0
1
2
3
Potential/V
(-3, 5) [-3, 3] Tap Water - Old Filter
(3, 5) [3, -3] Tap Water - Old Filter
(-3,5) [-3, 3] Tap Water - New Filter
(3, 5) [3, -3] Tap Water - New Filter

UV Analyses of Rainwater Concentrate


UV Absorption of Rainwater Concentrate
Nov 21 2017
~270 nm
Absorbance
0.55
0.495
0.44
0.385
0.33
0.275
0.22
0.165
0.11
0.055
0
234
252
270
288
306
324
342
360
378
396
Wavelength(nm)

Near-Infrared Analysis of Rainwater Concentrate (NIR)


Near Infrared Spectrum of Rainwater Concentrate
Nov 21 2017
~960 nm
~1080 nm
ArOH
ArCH
Absorbance
0.015
0.0135
0.012
0.0105
0.009
0.0075
0.006
0.0045
0.003
0.0015
0
720
760
800
840
880
920
960
1,000
1,040
1,080
Wavelength(nm)

Bradford Test Rainwater - No additional Concentration of Sample

No substantial result.

It does not appear the Concentration in this sample rainwater is sufficient to justify Protein existence via the Bradford Test.

We need to work other problem or more than one angle and open the problem up to organic detection VS Protein detection.

--

Nov 22 2017

A very exploratory observation is taking place. We are working w/ an extraction method, this time using xylene.

If you mix xylene & distilled water, xylene is on the top layer when shaking. Ok, this will remain the case.

I hour set up

1. ~ 3 ml distilled H_2O + 30 drops xylene
2. ~ 3 ml conc. rainfall + 30 drops xylene
   Shake vigorously.

First observation: Rain + distilled adheres to test tube wall more than rain + xylene

Nov 23 2011

What we have accomplished is a non polar extraction of the rainwater into xylene. The method is to

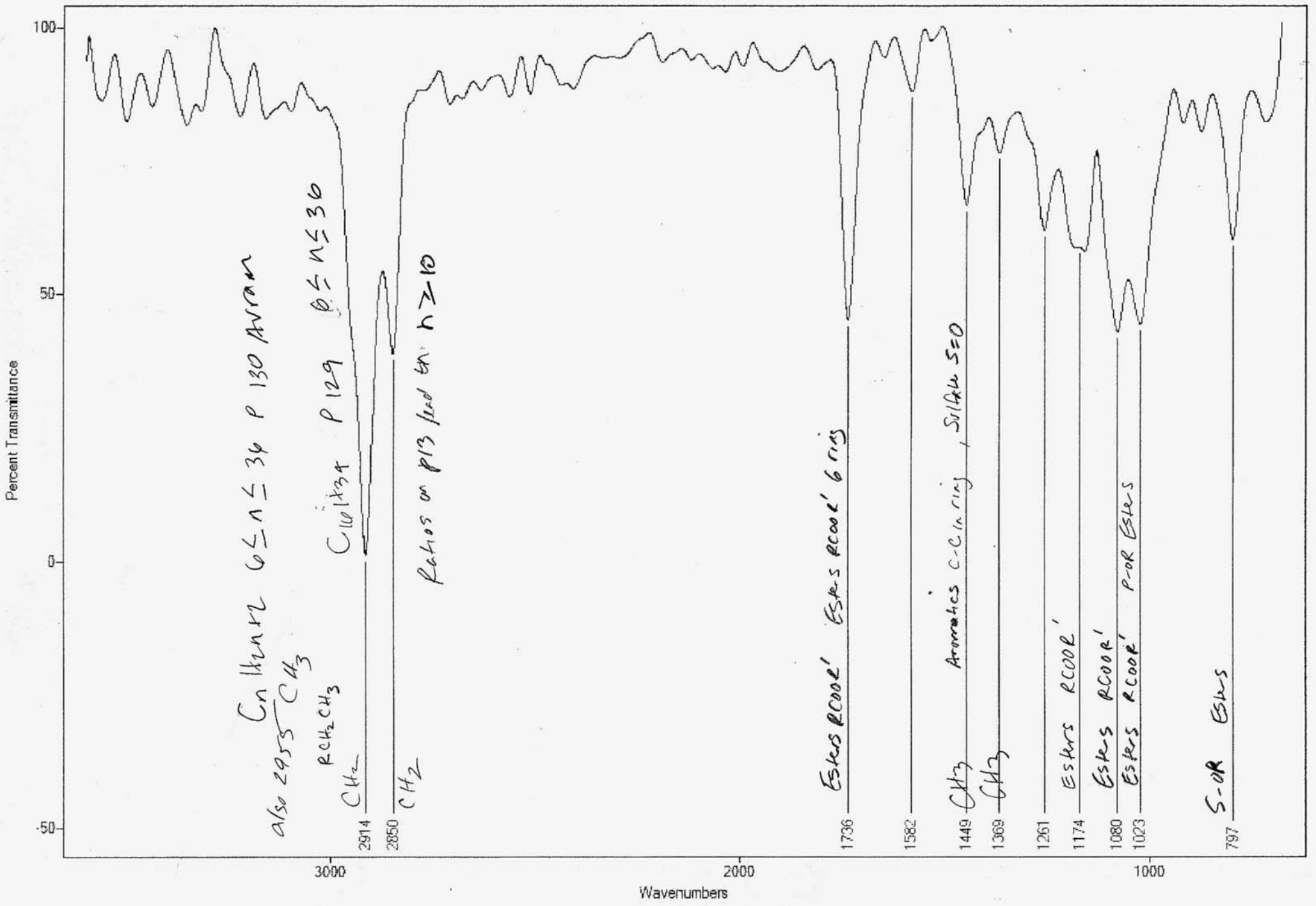
1. Start w/ Conc. rainwater - Conc. factor ~ 12.5
2. Concentrate a smaller sample by approximately a factor of 4 w/ moderate heat.
3. The give an end concentration of ~ 50X.
4. Use ~ 4 parts rain to 1 part xylene, mix & shake
5. Extraction layer will be on top, ~1 ml.
6. Pipette carefully the top layer to IR ATR.
7. Producing the film layer is difficult but possible w/ care and patience & careful use of the small handlens.
8. It is difficult to capture an adequate IR signal but we do have it as shown on next page

9. The interpretation seems quite straightforward here. AVERAGE IR look is crucial w/ nuances here. We find
   1. small peak @ 2955 (lost in smoothing) corresponds to C_nH_{2n+2} w/ $6 \leq n \leq 36$ or 2914 - check
   2. We also have a statement of ~ 2914 cm^{-1} leading to 2850

   leading to $n \geq 10$.

So one way or another we have a fairly significant Carbon-Chain going on.

Rainfall Xylene Extraction Evaporated Clean Average Nov 22 2017 - 05.JPEG



Remember this discussion only applies to the non polar extraction. We will examine a polar extraction into alcohol later.

Concerning. Entry of this data @ the level of

Peaks: 2914 2850 1736 1080 1023 ± 10 cm^{-1}
w/ Transmittance ≤ 60%
and C between 10 & 36
shows that the top 10 entries found in the SDBS IR search
All have a benzene ring within them.

So this would increase the chance of this occurring.

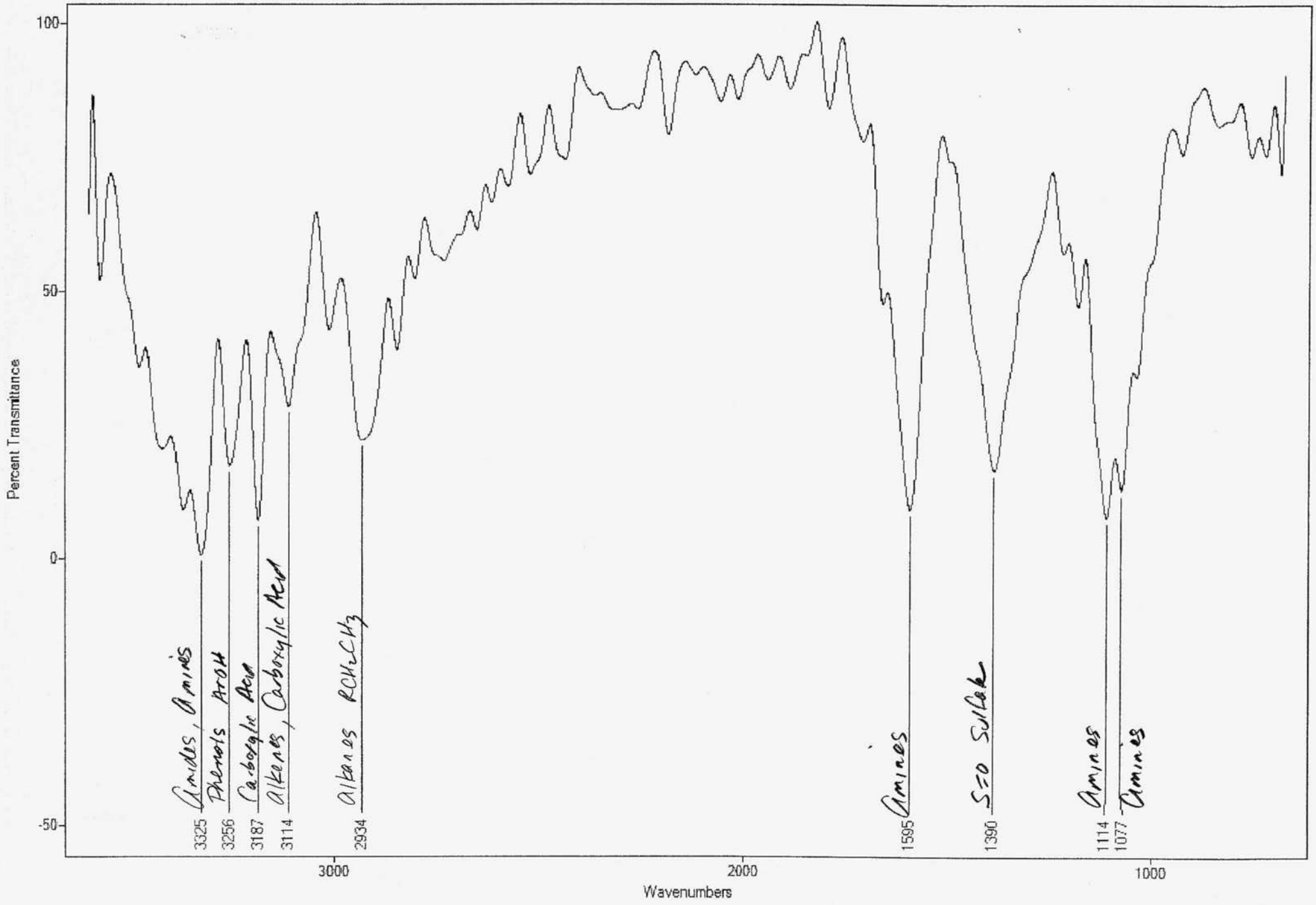
We also recall that the NIR plots of the original Concentrated rain samples (IR, no extraction) show Ar OH & Ar CH activity.

We are therefore seeing indications of a:
1. benzene ring
2 Aliphatic Chain?
3. Ester

Phthalates may be a topic here.

I have the polar extraction into ethanol. It is equally difficult but I have strong resolution of answer.

Rainwater Extraction into Alcohol Evaporated Avg Nov 23 2017 - 03.JPEG



Nov 25 2017

I am doing some very interesting decomposition work using combined techniques of:

1. ashing
2. microwave digestion

1. Ashing is taking place in the fume hood. Excess O_2 is delivered to the combustion chamber. A good ash product is resulting in ~ 30 to 45 min.

2. We then follow w/ microwave digestion for 30 min under low power (10%) in a double sealed container. I have no bursting of containers taking place under these conditions & the breakdown products are quite good. Solvent is 10M NaOH.

3. The digestion product is then filtered into a test tube. This is a highly alkaline solution.

4. In fact, it is even more alkaline than expected It required ~3-4 times as much 10M HCl to neutralize the solution (pH). With the spruce cone I also have some settling white precipitate that forms upon neutralization.

5. The techniques are being applied to spruce cone & mealworms (no leaf). I hope to post process w/ both electrochemistry and infrared.

Nov 26 2017

6. I then evaporate a portion of the neutralized sample under moderate heat. The acid-base combination / neutralization produces a high NaCl solution, perfect for final drying w/ ATR on IR.

7. What I am looking @ while waiting for evaporation is the precipitate that is settled in the neutralized solution. It is white. The sample is the spruce pine cone, ashed & digested & neutralized. Method is IR.
Notice no hydrocarbon are immediately visible but some functional groups are.

8. I seem to have a very strong phosphorus component to this settled precipitate material. Does ash have high phosphorus content?

Wood ash is < 2% phosphorus. Has high Calcium.
Wood ash:

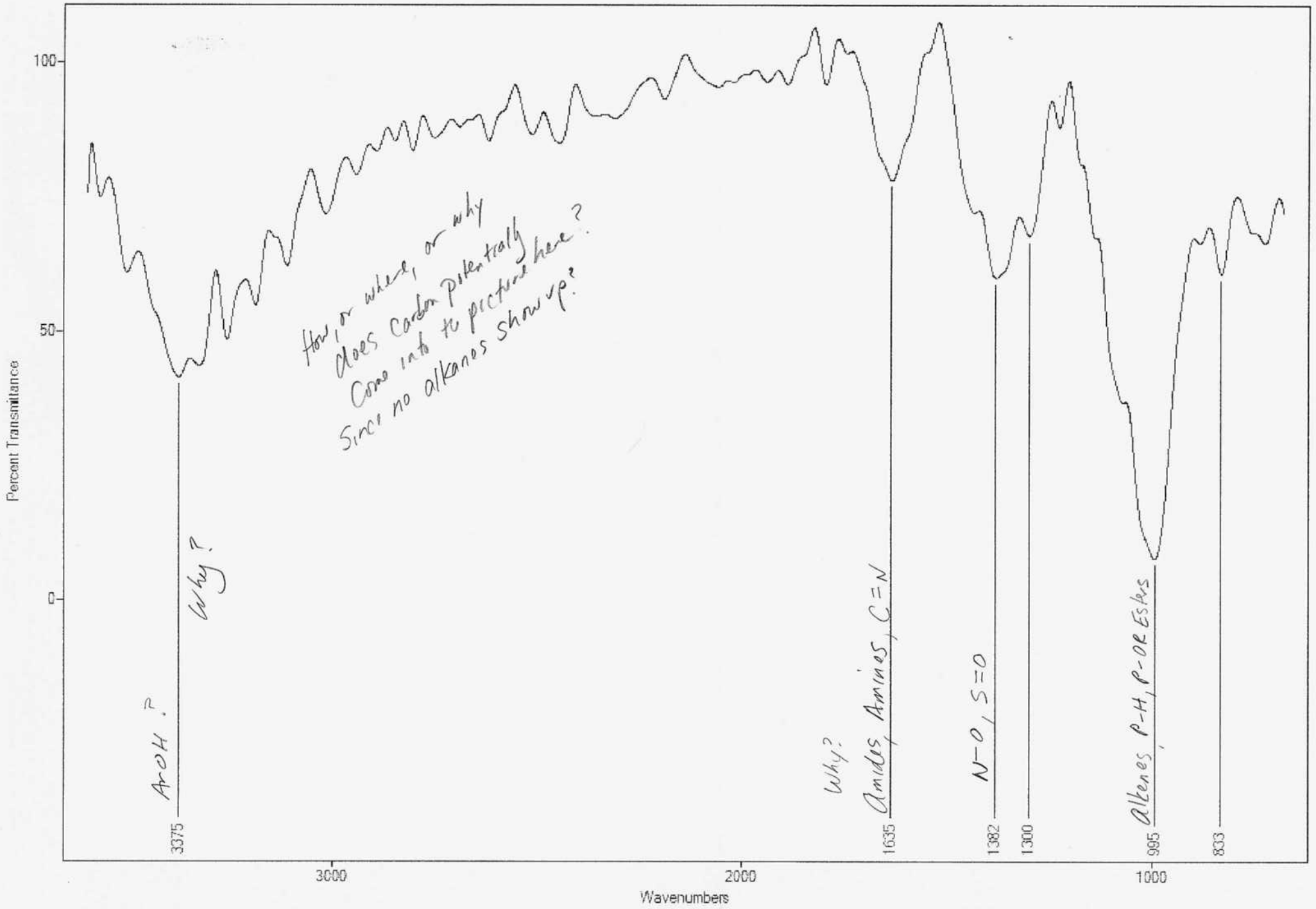
Calcium ~20%
Potassium 4%
<2% Phosphorus
Magnesium
Aluminum
Sodium

Wood ash is a low grade fertilizer.

Trace elements:
Boron
Copper
Molybdenum
Sulfur
Zinc

Spruce Cone - Ashed & Microwave Digested

Spruce Cone Ashed and Digested Nov 25 2017 - 02.JPEG



"Muka" in Russia

What an incredibly fascinating topic has come up here. The path of insight and discovery begins by learning about what is known as Moka Muka in Russia. It is a supplemental cattle feed of modest but important protein content made by heating pine needles @ 210°C and then milling. (Up to about 8% protein developed by the method.

Basically, I believe that I have produced a "muka" type product from the treatment of a spruce pine cone.

1. It has been subjected to the proper level of heat.

2. The exposure to 10M NaOH a microwave digestion only further aids in breaking down lignin & cellulose structures. Look @ hair and keratin.

Subsequently, I believe the rigors of protein extraction within my ashed & digested spruce cone are quite real. This is quite phenomenal as a means of accessing protein in material that is normally quite inaccessible to such consideration.

This opens up many avenues of very interesting research in both health, nutrition & botany, etc. I only anticipated inorganic results — this does not appear to be the case, and it is quite well known in Russia w/ the production of Muka.

How does my sample fare w/ Bradford & UV analysis?

"Muka" in Russia

Page 180A

Description

Click on the "Nutritional aspects" tab for recommendations for ruminants, pigs, poultry, rabbits, horses, fish and crustaceans

Common names

Wood, wood by-products, untreated wood, treated wood

Feed categories

- Other plant by-products
- Plant products and by-products

Related feed(s)

- Wood pulping by-products
- Wood sugar and wood molasses

Processes

The most promising treatments for making wood more digestible are the following:

Ball milling

This method reduces the wood to microparticles. As particle size decreases, more cellulose is exposed. Carbohydrate digestibility in ball-milled wood approaches that of feed grain (70-80%).

Chemical treatments

These include treatment in aqueous solutions of alkali and vapour-phase treatment with sulphur dioxide. Red oak has been made 55% digestible by chemical treatment. After steeping in a solution of 15% sodium hydroxide, a poplar species with 5% dry matter digestibility in vitro had a digestibility of 50%. Alkali-treated aspen and birch sawdust have constituted up to 30% of ruminant rations with good results.

Steam

In some cases steaming has been a very effective method of increasing digestibility. Steamed aspen wood with a dry matter digestibility of 48% was used successfully as a substitute for hay in sheep rations.

Muka

The needles of conifers and the leaves of deciduous trees can be made suitable for animal feeds with little processing. Basically all that is required is heating at 210 C for a few minutes to drive off moisture and unpalatable essential oils, followed by milling. The major development of this process has taken place in the USSR, where about 100,000 tons of the product, called Muka, are fed to animals each year. Muka is somewhat similar to lucerne, being rich in cellulose, carotene and minerals and containing one half to two thirds as much protein. It is fed as a supplement to poultry, cattle, milking cows and pigs at 5-8% levels. The major impetus to the development of

Page 180 B

Nutritional aspects

Nutritional attributes

Wood contains a high percentage of potentially digestible carbohydrates, but when fed in the form of untreated sawdust or chips it is largely indigestible, even by ruminants. The structural components of wood - lignin, cellulose and hemicellulose - form a close physical and chemical complex called lignocellulose. Lignin gives plants strength and rigidity. The content of lignin varies from about 2% in immature forages to about 15% in mature forages, whereas in wood the percentage is somewhat higher. It is completely indigestible and also lowers the digestibility of cellulose and hemicellulose by acting as a physical barrier to cellulose-splitting enzymes. Hemicellulose consists of digestible polysaccharides constructed mainly or 5-carbon sugars. The sugar xylose is the commonest component of hemicellulose in forages. The digestibility of hemicellulose varies from 45-90%, depending on the sugars it is composed of. Cellulose is usually the most abundant polysaccharide of the lignocellulose complex and consists of 6-carbon sugar glucose. Pure cellulose is fully digestible by ruminants. The lignocellulose complex accounts for most of the gross energy in common forages and wood. The mechanism by which lignin affects digestibility is complex. Rye straw has nearly the same lignin content as birchwood, but rye straw is far more digestible. Hence the lignin content in itself is not a reliable yardstick for measuring digestibility. Wood species differ widely in lignin content, but as a rule the wood of conifers contains more lignin than deciduous or broad-leaf trees.

Numerous feeding trials and laboratory experiments have shown that the nutrients in untreated wood are essentially unavailable to farm animals with the exception of a few less lignified hardwood species. The new concept of feeding cattle on high-grain rations has increased the possibility of using wood residues like sawdust and chips as the roughage component. Experiments have shown that sawdust is an effective roughage substitute when it constitutes up to 15% of the total ration. Cattle compensate for the lower energy of sawdust-diluted feed with higher intake. Some sawdusts - poplar is an outstanding example - are partly digestible by cattle. The in vitro digestibility of spruce sawdust is nil, of oak sawdust 5% and of poplar sawdust 30%. Comparatively mild treatments can markedly increase the digestibility of wood from certain species by exposing the cellulose from the protecting lignin to render it more accessible to attack by cellulose-splitting enzymes. In some woods the cellulose is partly exposed by openings in the lignin which can be widened by swelling. In other woods - for instance, white oak - these openings are plugged and chemical treatments are of little value.

Now what your model should do is combine both Cyclic AC Voltammetry and Cyclic Normal Pulse Voltammetry results.

Revises to

ACV	n	Eo		n	Eo	
	7.	2.59				
	~~6~~	~~2.12~~	7 2.12	8	2.92	K(2.92)
	~~6~~	~~2.45~~	6 2.45	7	2.59 ?	Sc(2.6) ???? Big Mystery
	5	1.46		7	2.72	Na(2.71)
	~~4~~	~~2.90~~	8 2.92	6	2.45 ?	Big Mystery
	3	.72		5	1.46	Cl(1.48, 1.47, 1.45)
	3	2.83		3	.72	Co(.13), Cl(.76), Fe(.77) O_2(.69)
	3	1.81		3	2.83	Ca(2.87) Sr(2.88, 2.89)
	3	.13		3	1.81	Co(1.82) 1.77(H+)
	2	.26		3	Ø.13	S(1.14) Cu(.12) Cl(.12) Cr(.13)
	~~2~~	~~.61~~	3 Ø.63	3	Ø.63	Cl(.62)
	~~2~~	~~2.96~~		2	Ø.26	Co(.28)
	~~1~~	~~1.70~~		2	2.13	SO_4(2.12) Fe(2.07)
	~~1~~	~~1.61~~		2	.805	Cl(.81)
	~~1~~	~~1.09~~		2	.345	Cu(.33)
	~~1~~	~~2.13~~	2 2.13			
NRV	~~2~~	~~2.94~~				
	~~2~~	~~.805~~	2 .805			
	2	.345				
	~~1~~	~~1.98~~				
	~~1~~	~~1.44~~				
	~~1~~	~~1.15~~				
	~~1~~	~~2.31~~	~~1 2.31~~			
	~~1~~	~~2.13~~				
	~~1~~	~~1.04~~				
	~~1~~	~~.66~~				
	1	2.13				

Next assume we disregard all entries of ≤ 1. This leaves us with:

Element	Incidence	n	abundance/5	Rank Score
K	1	8	3.4	2.4
Na	1	7	2.2	3.2
Cl	7	5	3.6	9.7
Co	3	3	3.2	2.8
Fe	3	3	Ø.8	11.2
Ca	1	3	1.2	2.5
Sr	2	3	6.0	1.0
S	2	3	1.8	3.3
Cu	2	3	4.2	1.4
Cr	1	3	2	1.5

Next, we compute Prob % = $\frac{130.6 \cdot \text{Score}}{\text{Score} + 3.08}$

These scores indicate that you have just cause to be looking to verify all elements & ions w/ a score > 40%.

If something is doubtful, it can of course still be checked.

!!!!

I have completely succeeded w/ the project. I have successfully identified 7 different metals and two ions by two independent methods in each case as applied to a random sample (ie, a spruce cone).

The opens up inorganic analysis to anything which can be dissolved into solution, especially aqueous.

(7) & (6)

2.59 & 2.45

& Two Mystery Components!

Prob.	Element/Ion		Ranked:	%	Verified
57%	K		Fe	102	POS
67	Na (verified 2 methods)		Cl	99	POS
99	Cl		$S(SO_4)$	68	POS
62	Co		Na	67	POS (2 ways)
102	Fe		Co	62	POS (trace)
59	Ca		Ca	59	POS (2000 ppm)
32%	Sr		K	57	POS (150 ppm)
68	$S(SO_4)$		Cr	43	POS (weak positive)
41%	Cu		Cu	≤.25 ppm 41	POS (trace)
43%	Cr	Not adequate basis	Sr	32	

I have positively identified and isolated a protein from the spruce cone. It admirably passes the Bradford test as shown on ~~VIS~~ VIS plot on next page.

I never expected to achieve this result. I expected only inorganics to result from the relatively high temp ashing process & subsequent 10M NaOH microwave digestion.

NOT TRUE. You therefore can produce highly significant organic & inorganic materials for further analysis by the method.

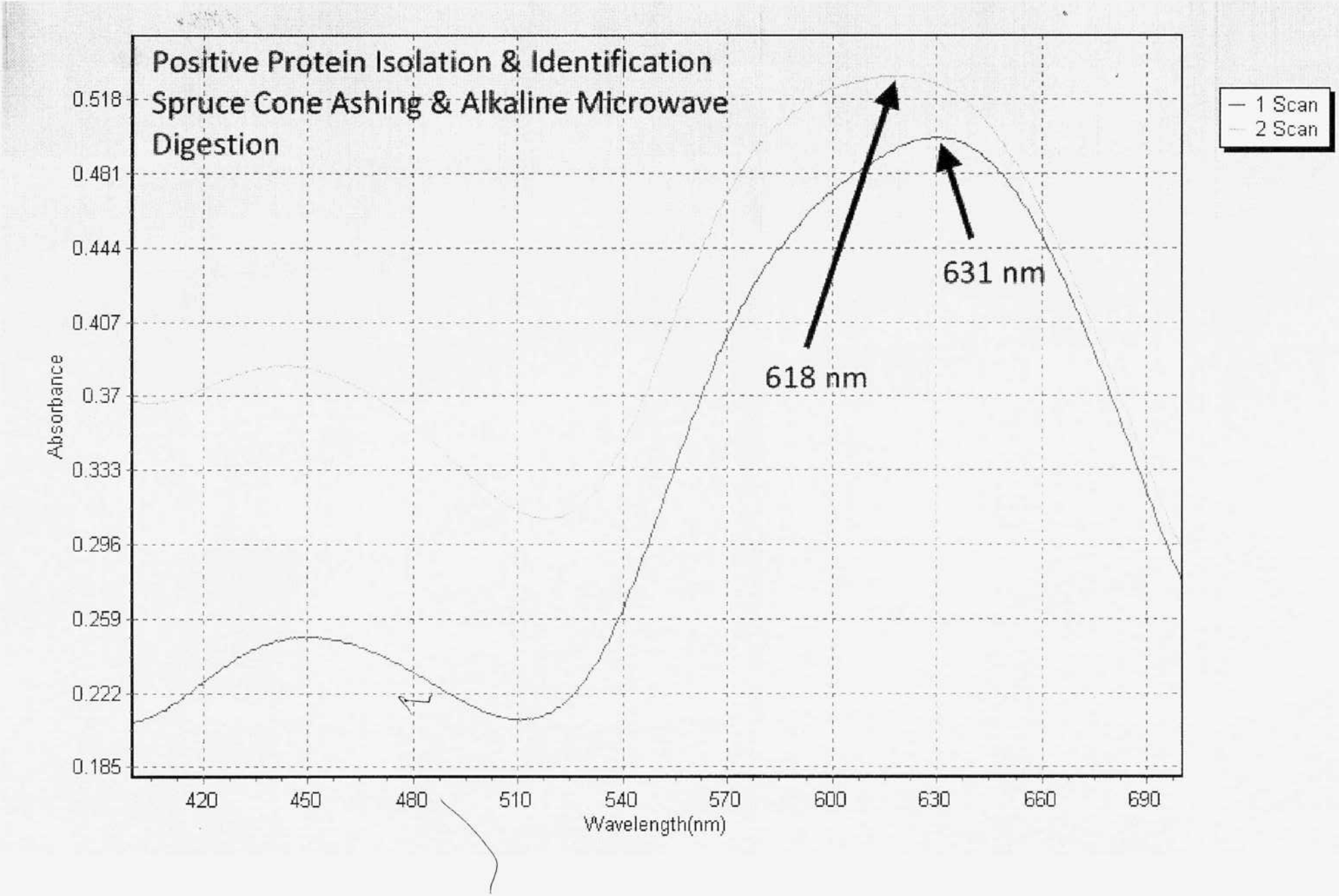
The tipoff was provided by the protein signature w/ enzyme. This was not expected.

As when you recall, you have now identified
1. numerous metals by AC voltammetry & normal pulse voltammetry
2. additional elements such as Sulfur & Chlorine electrochemically
3. Proteins & phosphorus by IR
4. Proteins confirmed by Bradford VIS
5. Qualitative Chemical Verification of electrochemical results.

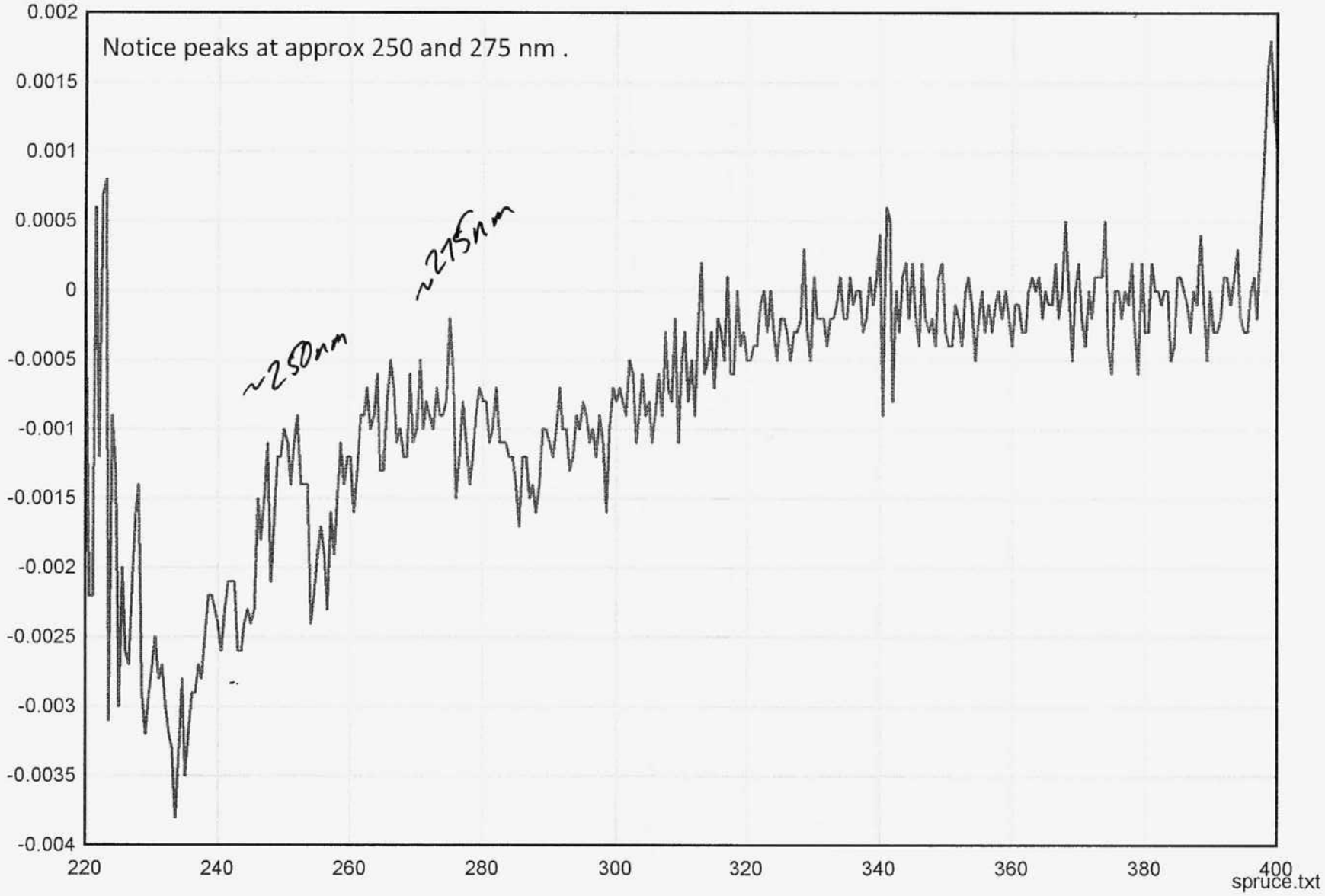
And have created an entirely new method for decomposition and analysis

all from investigating such prospects as ashing & microwave digestion using a spruce cone as a random & arbitrary sample material.

VIS Plot - Spruce Cone - Protein Isolation Identification


Positive Protein Isolation & Identification
Spruce Cone Ashing & Alkaline Microwave Digestion
1 Scan
2 Scan
631 nm
618 nm
Absorbance
0.518
0.481
0.444
0.407
0.37
0.333
0.296
0.259
0.222
0.185
420
450
480
510
540
570
600
630
660
690
Wavelength(nm)

Spruce Cone UV Differential Analysis



We also have high magnitude UV absorption w/ the ashed-digested spruce core.

We see a peak (hump) near 275 nm which further substantiates proteins & amino acids.

What we really need w/ the UV plots is differential analysis. This will require export of the data and then analysis in DPlot. The software is not sophisticated enough.

UV software will export to Excel easy enough.

In the future, DPlot will require a spacing of 1 nm in the data collection in order to smooth the data.

Thus far now, we have sufficient peak identification @ ~250 & 275 nm / see next page.

Now lets try NIR.

Marketing methods now include

Portable
1. Infrared
2. VIS if possible
3. UV
4. NIR
Portable
5. Electrochemistry
6. Gas Chromatography – (& Pyrolysis)

Quite the powerhouse now.

960 nm – Prominent Peak – Ar OH is Confirmed!

We also have high magnitude UV absorption w/ the ashed-digested spruce cone.

We see a peak (hump) near 275 nm which further substantiates proteins or amino acids.

What we really need w/ the UV plots is differential analysis. This will require export of the data and then analysis in DPlot. The software is not sophisticated enough.

UV software will export to excel easy enough.

In the future, DPlot will require a spacing of 1 nm in the data collection in order to smooth the data.

For now, we have sufficient peak identification @ ~ 250 & 275 nm / see next page.

Now lets try NIR.

Marastay methods now include

1. Infrared
2. VIS if possible
3. UV
4. NIR
5. Electrochemistry
6. Gas Chromatography – (& Pyrolysis)

Portable

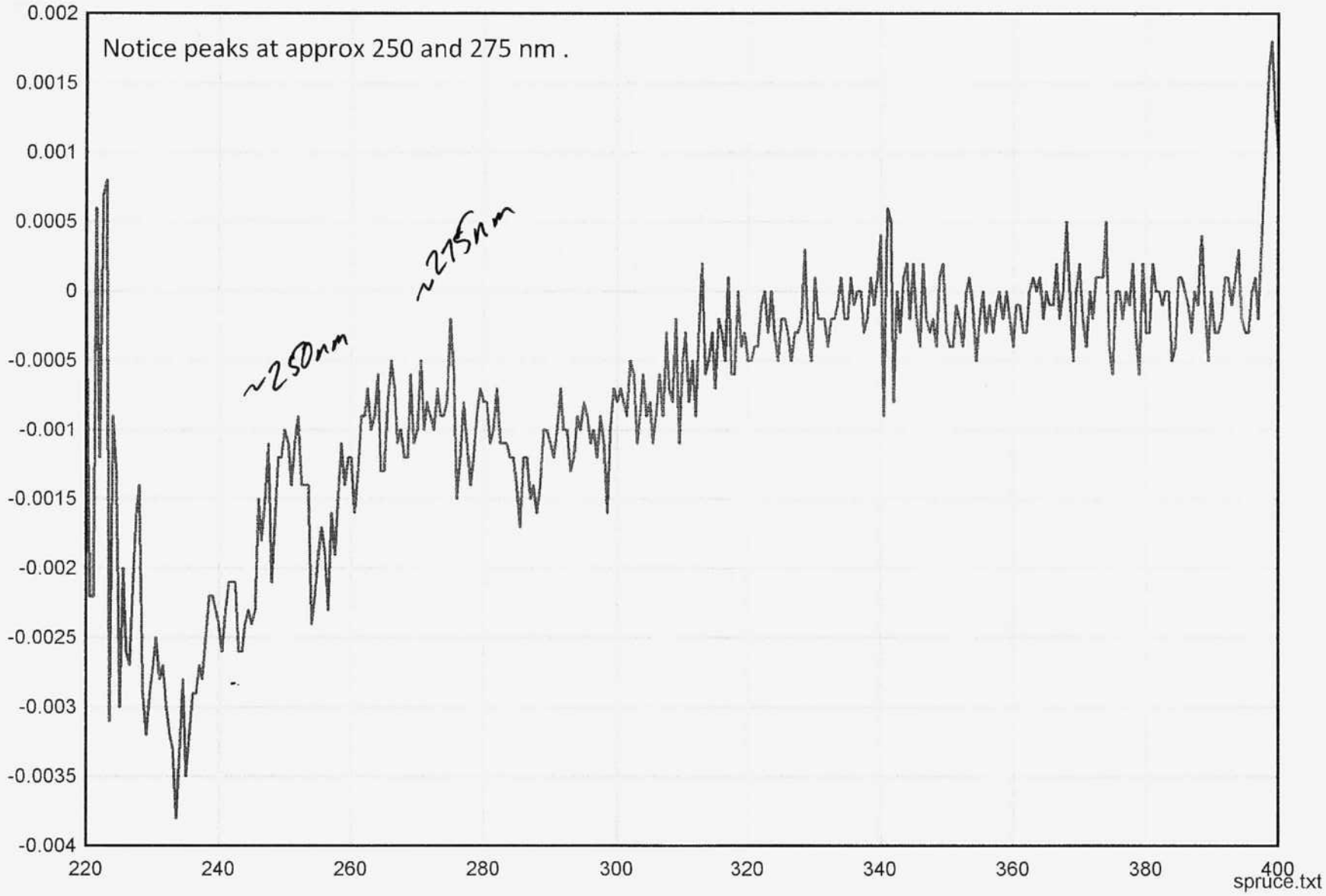
Portable

Quite the powerhouse now.

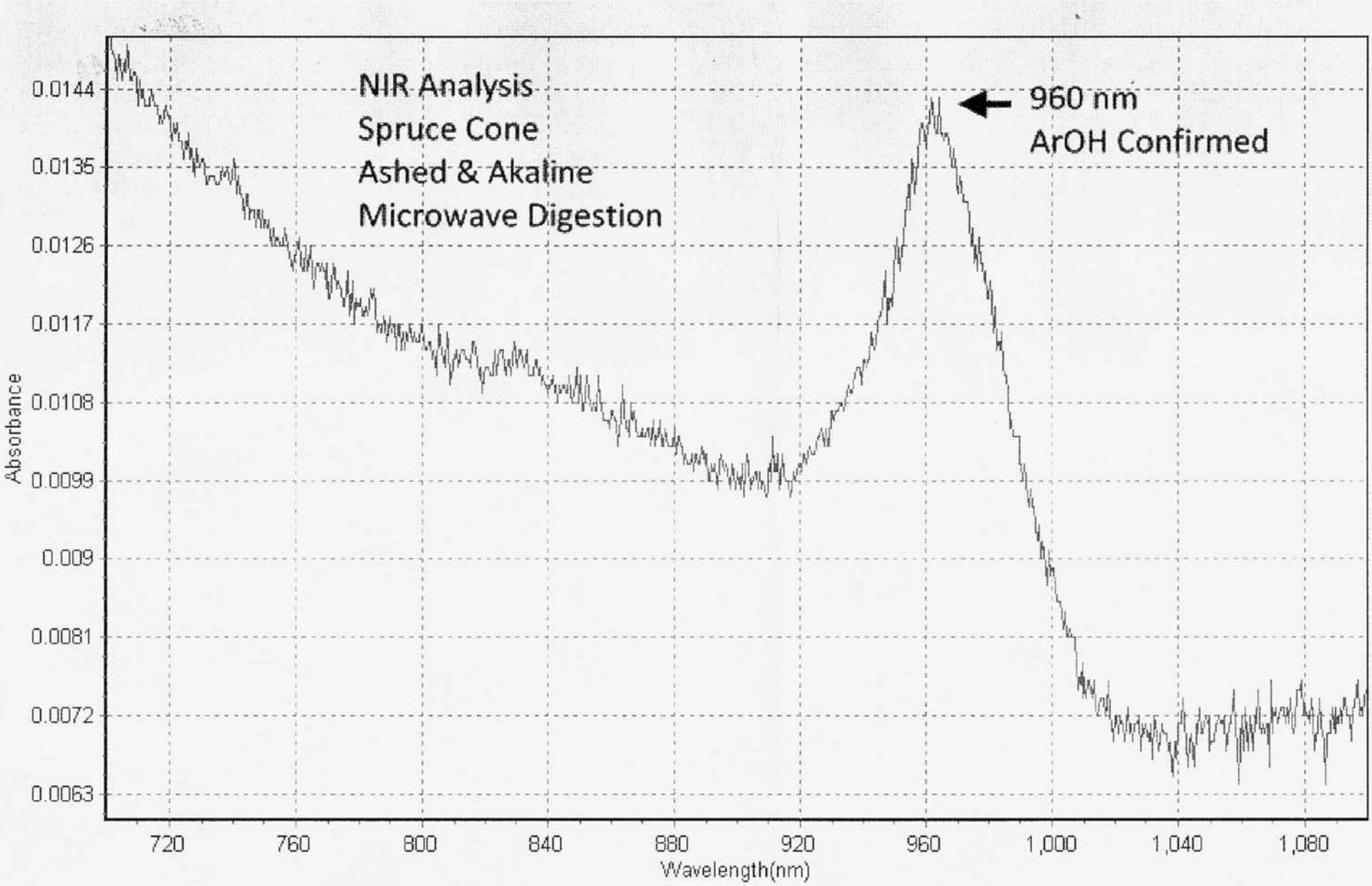
960 nm – Prominent Peak – Ar OH is Confirmed!

UV Analysis (differential) of Spruce Cone

Spruce Cone UV Differential Analysis



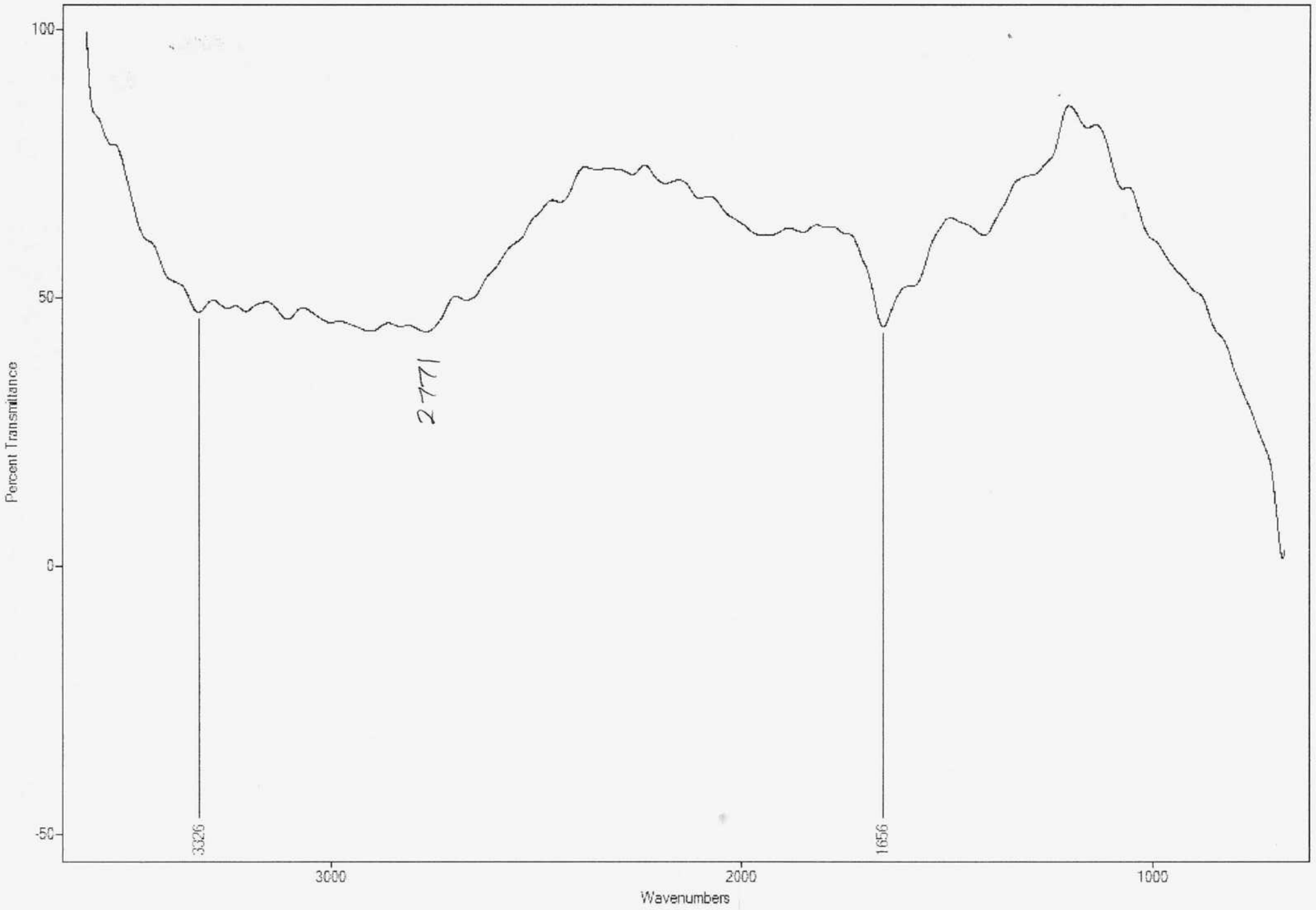
NIR Analysis - Spruce Cone - ArOH Confirmed


NIR Analysis
Spruce Cone
Ashed & Akaline
Microwave Digestion
960 nm
ArOH Confirmed
Absorbance
0.0144
0.0135
0.0126
0.0117
0.0108
0.0099
0.009
0.0081
0.0072
0.0063
720
760
800
840
880
920
960
1,000
1,040
1,080
Wavelength(nm)

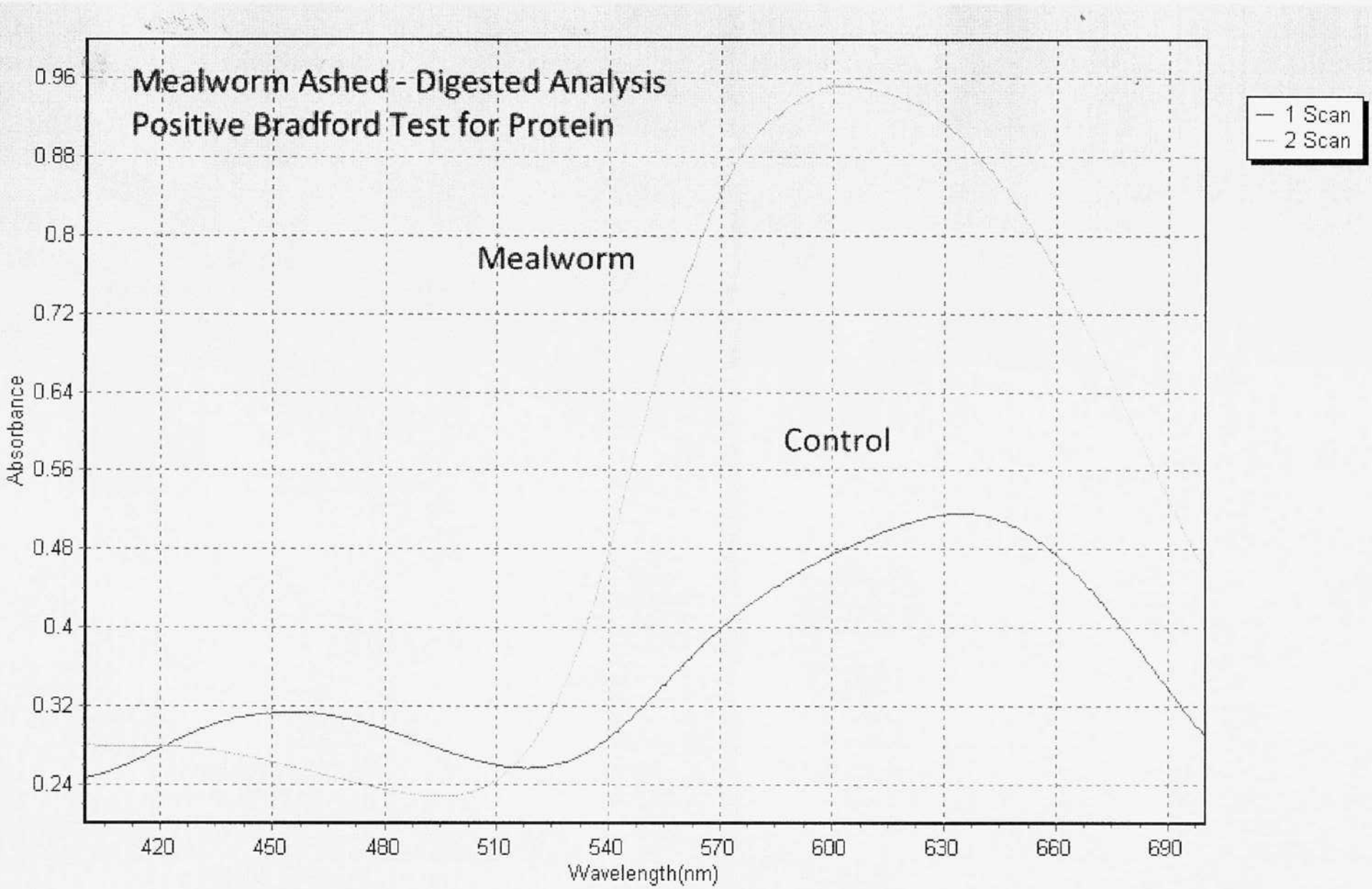
Mealworm Analysis * Ashed & Digested

Our methods have succeeded admirably. IR plot of ashed-digested mealworms (& beetles) suggest protein once again. Bradford test is highly positive. Protein concentration appears to be extremely high.

Mealworms Ashed Digested Nov 26 2017 - 01.JPEG



Malawarra VIS Analysis – High Protein Concentration


Mealworm Ashed - Digested Analysis
Positive Bradford Test for Protein
Mealworm
Control
1 Scan
2 Scan
Absorbance
0.96
0.88
0.8
0.72
0.64
0.56
0.48
0.4
0.32
0.24
420
450
480
510
540
570
600
630
660
690
Wavelength(nm)

Nov 27 2017

There has been some good work done with combined methods of ashing along w/ alkaline microwave digestion.

Important organics can show up here, especially proteins have been quite the surprise.

This has been worked over with a spruce cone and meal worms as non-specific samples.

Electrochemistry seems quite effective @ inorganics but also surprisingly effective w/ organics, especially the nitrogen compounds.

We also have good work w/ rainwater separation into both polar & non polar components. The deeper analysis of these results is certain to be helpful & important.

The electrochemical work on this latest rainwater sample did not indicate a strong inorganic signature. This will also need to be reviewed and especially w/ the other samples available.

I will go ahead now with the preliminary electrochemical examination of the CDB viscous (secreted) protein We have Cyclic AC voltammetry data already collected.

Look @ both ACV & ACV'

Intermediate Score = $\frac{\text{No. Redox Entries} \cdot \text{Highest Redox Rank}}{(\text{Abundance}/5)}$

Prob% = 30.6 · Int Score / Score + 3.08

ACV: We want the slope breaks and the peaks.
ACV': Peaks & Zero Crossings

ACV		ACV'	
Slope Breaks	Peaks	Peaks	Zero Crossings
-1.45 ✓	-2.30 ✓	-1.88 ✓	-2.33 ✓
	.32 ✓	-2.84 ✓	-.08 ✓
	-.46 ✓	-.54 ✓	+.23 ✓
	+.29 ✓	-2.07 ✓	
		-1.87 ✓	Ranked:
		-1.46 ✓	4 .26 Co(.23) SO_4(
		-1.14 ✓	4 .505 S(.50, .48)
		-1.42 ✓	3 1.44 Cl(1.45)
		-.81 ✓	3 .81 Cl(.81) NO_3^-(.80,
		+.03 ✓ O_2(.01)	3 .06 NO_3(.01) Fe(.0
		-.20 ✓	2 2.315 H_2(2.25) Al
		+.08 ✓	2 1.815 Fe(1.90) N_2
		+.96 ✓	1 2.84 Ca(2.87)
		+.85 ✓	1 2.07 SO_4(2.00)
		.76 ✓	1 1.14 Cl(1.15) SO_4(1
		+.57 ✓ SO_4(.93)	1 .96 NO_3^-(.96, .94) C
		+1.54	1 1.54 NO(1.59)
		+.57 ✓	

$\bar{X}$	n	
✓ 1.44	3	-1.45, -1.46, -1.42
✓ -2.315	2	-2.30, -2.33
✓ 0.26	4	.32, .29, .23, -.20
✓ .505	4	.46, .51-.54, .51
1.875	2	-1.88, -1.87
2.84	1	-2.84
2.07	1	-2.07
1.14	1	-1.14
✓ .81	3	-.81, .76, .85
✓ .06	3	.03, .08, -.08
.96	1	.96
1.54	1	1.54

Element	Int Score	Prob%
Co	1.25	38%
S	8.9	91%
Fe	15.0	108%
Cl	2.5	58%
N	3.75	72%
Ca	.83	28%

Therefore, we conclude that viscous proteins (CDB) to contain

- Iron
- Sulfur
- Nitrogen
- & Chlorine

> important

Electrochemical analysis of the COB Problem. :.

The method of establishing a probability for each element seem quite sound.

The results make perfect sense. It also suggests that tap water provided a source of chlorine.

Iron & sulfur would be important constituents of the protein.

Nitrates also appear to show up repeatedly when as they're expected to be in w/ the observed nitrile production.

The work to be good solid work.

Now analyses by IR & NIR should continue to be helpful to identify some of the properties of the protein.

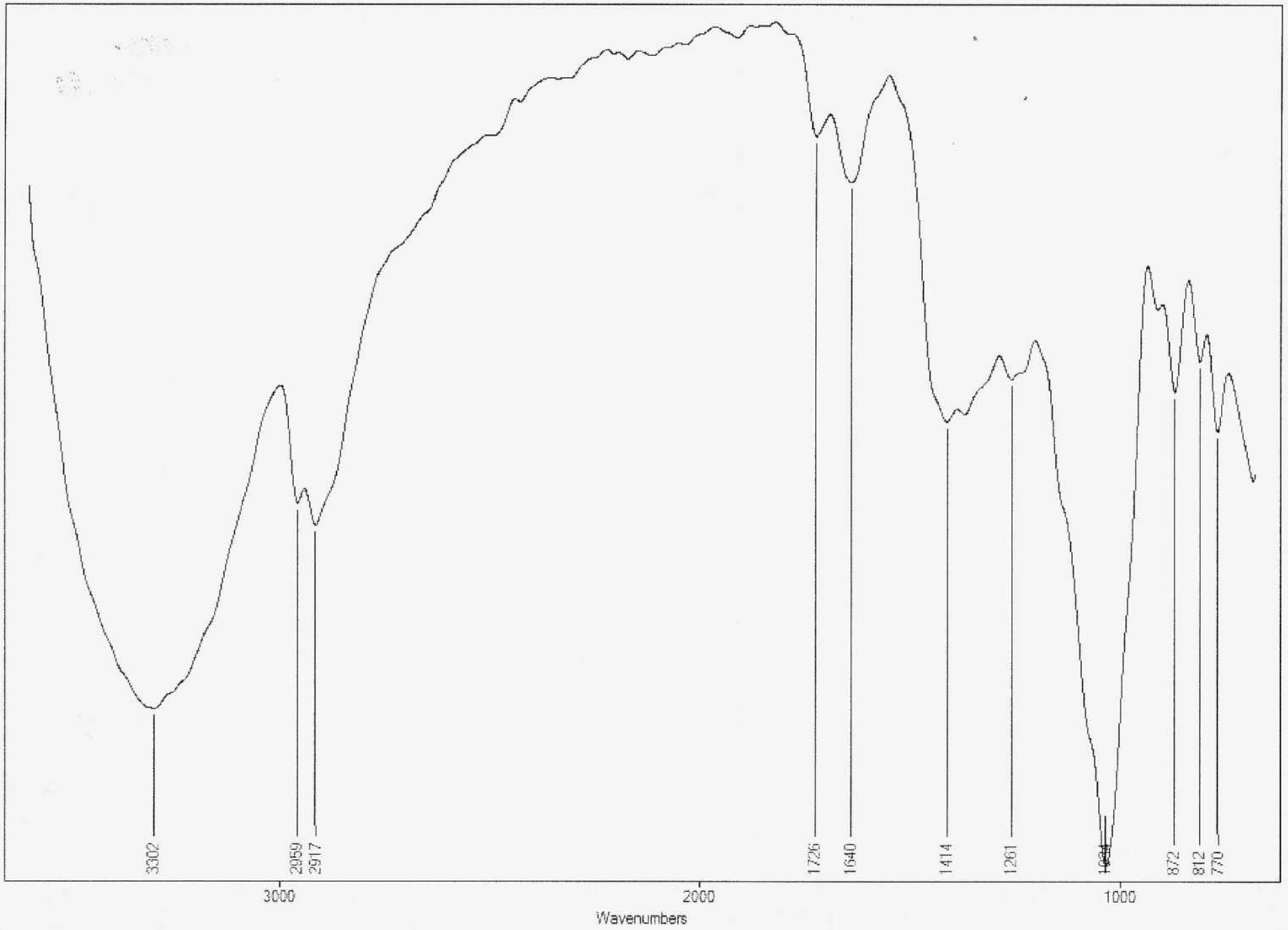
Iron
Sulfur
Nitrogen (esp Nitrates)
Chlorine

We have an extremely solid IR plot of the protein on the next page. The sample was dissolved in ethanol & applied cleanly to both ATR & KCl. It correlates well w/ previous sample runs. The will now be regarded as the reference.

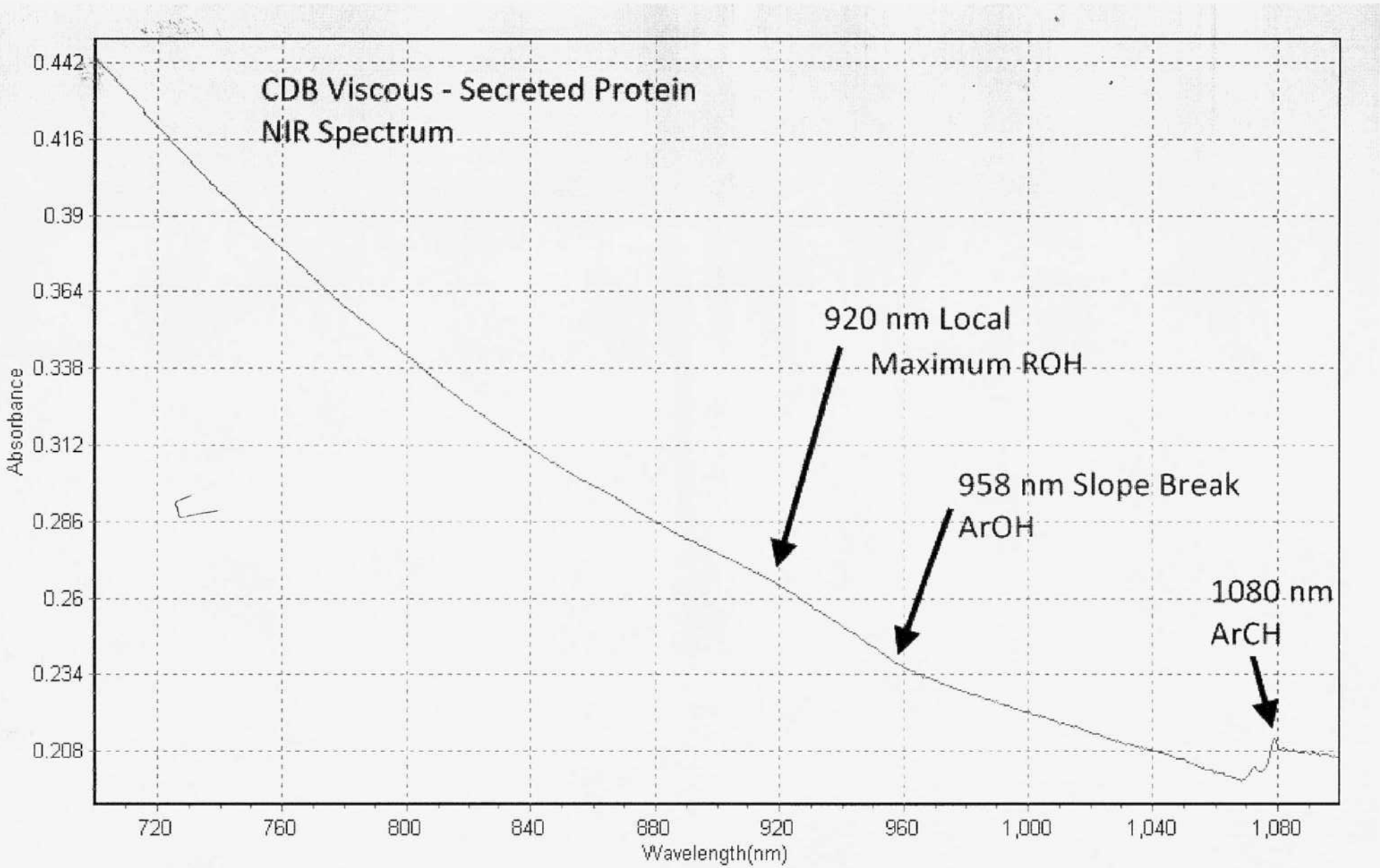
This is to be combined w/ elements known above.

High Quality IR Plot of the CDB Viscous Secreted Protein.

CDB Protein Secreted Viscous Primary Sample KCL ATR Avg Nov 27 2017 - 03.JPEG



CDB NIR Spectra - Viscous - Secreted Protein


CDB Viscous - Secreted Protein
NIR Spectrum
920 nm Local
Maximum ROH
958 nm Slope Break
ArOH
1080 nm
ArCH
Absorbance
0.442
0.416
0.39
0.364
0.338
0.312
0.286
0.26
0.234
0.208
720
760
800
840
880
920
960
1,000
1,040
1,080
Wavelength(nm)

Nov 29 2017

Open Circuit Potential (OCP) is another interesting method. Probably the simplest of all. Useful for titrations.
Apparently most in common w/ and Oxidation Reduction Potential (ORP) meter. Easily demonstrates the effects of oxidation and reduction in a measurable way.
First investigations involve the use of
Iron Sulfate (Fe^{+2})
Hydrogen Peroxide H_2O_2
Bleach
Vitamin C

Iron Fe^{+2} and Vit C are reducers
H_2O_2 & Bleach are oxidizers

Next investigation would likely involve
dichromate, Fe^{+2}, peroxide, bleach & vit C.

Iodine & permanganate are oxidizers as well.
Vits A, D, & E are also reducers, but fat soluble

MW of $Na_2Cr_2O_7 \cdot 2H_2O$ Sodium Dichromate Dihydrate is 262.1 gms/mole

$$\frac{262.1 \text{ gms}}{1000 \text{ ml}} = \frac{X}{60 \text{ ml}} \qquad X = \frac{16.93 \text{ gms}}{60 \text{ ml}} = 1M \text{ solution}$$

Solubility is 73 gms/100 ml. This is extremely high.
Lets make a 0.1 M solution =

$$\frac{1.69 \text{ gms}}{60 \text{ ml } H_2O}.$$

ORP can also be used as a general identification method to determine the relative oxidizing or reducing capability of a species in solution, at least relative to water.

The Palmsens seems to give a more reliable reading and can be logged and stored as data as opposed to the aquarium ORP meter.

10 min data collection periods seem to be sufficient to establish stability in the readings.

You can also label each curve in palmsens.

Remember H_2O as the reference here

1. We started with filtered H_2O : −27 mV
2. added 1 drop sodium dichromate (0.1 M) : −16 mV
3. Next we add 1 drop $FeSO_4$: ~~+1 mV~~ to 0 mV

We can see here visually that the dichromate has oxidized or is oxidizing the Fe^{+2}. It turns the reddy turbid color as w/ peroxide. Dichromate is a clear orange color @ other pH.

4. Now we add some Vit C. Expect reduction. Small amount added. No real change here, in fact, range is 0 to +5 mV.

5. Added more Vit C. Contrary to expectations, the potential has risen to +17 mV. A higher oxidized state. Not sure why this is yet but maybe dichromate is an extremely effective oxidizer?

6. Now add 1 drop NaOH. My recollection is that NaOH reduces dichromate to chromate. Yes, reduction is occurring. Reduces to −18 mV. Significant reaction here.

So Vit C does not always reduce, depending upon the circumstances.

The method (OCP) is a great way to see and learn about redox reactions, and combinations of reactions, in general.

We notice the solution is not starting to clear as soon as reduction is taking place.
So far, our combination includes:

1. Water
2. $Na_2C_2O_4$
3. $FeSO_4$
4. Vit C
5. NaOH

7. Add Vit C again, and it again increases, this time the jump is from the −18 mV to +2 mV. The solution, however, continues to clear up.

8. Now we added additional $FeSO_4$. Solution has become almost clear now. We are at +6 mV now, close to where Fe^{+2} was before.

We noticed the solution turned almost perfectly clear even though it had now become a fairly complex solution.

9. Now we increase NaOH concentration. The term NaOH finally increased the ORP to +9 mV. It now longer no longer has the effect of reducing dichromate, most likely because it has now in the chromate form.

10. We added more dichromate. It has reduced slightly now to +4 mV but it is still high.

11. Now we have added HCl. It certainly seems, as somewhat anticipated, that it is much easier to create an oxidized state vs a reduced state. It seems very difficult to escape the oxidized state. Acid has definitely introduced some instability into the situation. It caused a delayed spike to ~ 15 mV.

Acid continues to introduce instability. Range is running from +12 mV to -3 mV. May be stabilizing near +6 mV.

A very interesting topic is to see how many of these elements can be recovered by cyclic AC Voltammetry. That would be one very useful exercise. The solution is perfectly clear now.

HCl appears to be stabilizing now after ~ 30 min.

Not exactly true. HCl is much more variable w/ its impact but appears to be stabilizing @ ~ +19 mV.

The solution is now entirely clear and looks no different than water. This would be a very good opportunity to apply Cyclic AC voltammetry to see which species can be recovered from a combined (known) solution.

OK, ORP Mix data has been collected by Cyclic AC Voltammetry

(Peaks) ACV & ACV' Zero Crossing

-1.36	+2.04 -2.32	-1.58	-.16	-2.30	+.37
-.58	+1.39	-1.39	-.10	-1.74	+.58
-.42	+.78	-1.17	+.02	-1.61	+1.54
-.14	+.42	-1.07	+.08	-1.01	+1.84
+.02	+.24	-1.02	+.16	-.58	+2.36
+.17	+.02	-.78	+.31	-.39	+2.55
+1.88	-.11	-.50	+.99	-.13	+2.68
+.83	-.19	-.58	+1.98	-.04	+2.79
+2.88	-.52	-.25	+2.38	+.15	

$\bar{x}$		~ $\bar{x}$		
1.38	-1.36, 1.39, -1.39 Co(1.33) Fe(1.56)	4 1.02	-1.07, -1.02, .99, -1.01 S(1.04)	
.56	-.58, -.52, -.50, -.58, -.58, +.58 C-(1.4)	3 .38	.37, -.39, +.37 Fe(.36) C+(.4)	
.40	-.42, +.42, +.37 Fe(.36) (-.44) S(.4, .45, .45)	1 1.98	1.98	S(2.00)
.16	-.14, +.17, -.16 +.16, .15 S(.17, .17, .20)	1 1.74	1.74	?
.03	+.02, +.02, -.04 Fe(.04)	1 2.55	2.55	?
1.86	1.88, +1.84 Fe(1.90) Co(1.82)	1 2.68	2.68	Na(2.71)
.80	+.83, +.78, -.78 Cl(.81) Fe(.77) N(.8, .8)			
2.84	2.88, 2.79 ?			
2.04	2.04 Fe(2.07) O_2(2.01) Cl(2.10)			
.21	.24, -.19 Co(.17) S(.20)			
0.105	-.10, -.11, -.13, +.08 Co(.10) S(.09) O_2(.07)			
2.34	2.32, 2.38, -2.30, 2.36 Al(2.33, 2.31)			
1.58	-1.58, -1.61, +1.54 N(1.59) Al(1.66)			
1.17	1.17 Cl(1.15, 1.19)			
.42	.42, .37, .39, .37			

I need to reestablish the Scoring & Probability Model Protocol:

The Two Part Model is:

$$\text{Int Score} = \frac{\text{No. Redox Entries} \times \text{Highest Redox Rank}}{\text{Abundance}/5} \quad \& \text{ Prob\%} = \text{Score} \cdot 130.6 + \text{Score} + 3.08$$

The first part is to rank the no. of redox ~~scor~~ entries @ a particular Eo. Then assign the various elements to those ranked mean Eo's. We have actually already done this:

n	Eo	Element	Score	Prob
6	.56	Fe (.56)	Fe: 6(5)/(4/5) = 37.5	121
5	.16	S(.17, .17, .20)	S: 5(8)/(9/5) = 22.2	115
4	.105	Co(.10) S(.09) O_2(.07)	Co: 4(3)/(6/5) = 3.8	72
4	2.34	Al (2.33, 2.31)	Al: 4(3)/(5/5) = 12	104
4	1.02	S(1.04)	Cr: 3(2)/(10/5) = 3	64%
3	1.38	Cr (1.33)	Cl: 3(1)/(8/5) = .8	27%
4	Ø.39	Cr (.40) Fe(.36, .44) S(.40, .45, .45)		
3	0.03	Fe(.04)	N: 3(1)/(2/5) .75	26%
3	Ø.80	Cl(.81) Fe(.77) N(.8, .8)		
3	1.58	N(1.59), Al(1.66)		

Weak

2	1.86	Fe(1.90) Co(1.82)
2	2.04	?
2	Ø.21	Co(.17) S(.20)

Very Weak — Not good

1	2.04
1	1.17
1	1.98
1	1.74
1	2.55
1	2.68

We can see, therefore, that the method and system is far from infallible.

We pick up some that are real, miss some that should be there, and pick up some that have no basis.

Using the criteria of $\geq 40\%$

Real:

Fe
S
Cr

The work is excellent

Missed:

Na
Cl

This set is tenuous

No Basis:

Co
Al

This has no understanding @ this point!

$FeSO_4$
$Na_2Cr_2O_7$

NaOH
HCl

No basis ??

One candidate elements ranked are:

Fe 121% } Good work
S 115% }

Al 104% }
Co 72% } These 2 have no basis (???)

Cr 64% } Good work

Look for a separation factor: Magnitude / peaks

Nov 30 2017

I am seeking to examine the Co & Al signals to assess their standing w/in the Cyclic AC Voltammetry Plot.
Are these signals prominent? How do they compare with the identifications of Fe, S & Cr that are accurate?

The numbers of interest are:

Co .10, ~~.11~~, 1.82

Al ~~2.33~~, ~~2.31~~, 1.66

Now notice if you sacrificed n=2 as well as n=1 you would have lost Co in the dust.
The score would drop to $\frac{4(1)}{(16/5)} = 1.25 = \text{Prob}\% = 38\%$
38% < 40% or 50% so it would have fallen out of error.

Aluminum would show up no matter what because of its abundance and signals found.
Let's look @ the aluminum signals. There is no chance for anticipatory contamination @ this point.

-2.31 or -2.33 on [3, -3] is not a peak, thus is not to be accepted
-2.31 or -2.33 on [-3, 3] is not a peak, not to be accepted
+2.31 or +2.33 on [3, -3] is not to be accepted.
+2.31 or +2.33 on [-3, 3] is also not to be accepted.

This means that neither of these were actual signals.

Intermediate Score = No. Redox Entries · Highest Redox Rank / (Abundance / 5)

Prob% = 130.6 · Int Score / Score + 3.08

ACV: We want the slope breaks and the peaks.
ACV': Peaks & Zero Crossings

ACV

Slope Breaks	Peaks
-1.48 ✓	-2.30 ✓
	.32 ✓
	-.46 ✓
	+.29 ✓

ACV'

Peaks	Zero Crossings
-1.88 ✓	-2.33 ✓
-2.84 ✓	-.08 ✓
-.54 ✓	+.23 ✓
-2.07 ✓	
-1.87 ✓	
-1.46 ✓	
-1.14 ✓	
-1.42 ✓	
-.81 ✓	
+.03 ✓	
-.20 ✓	
+.08 ✓	
+.96 ✓	
+.85 ✓	
.76 ✓	
+.57 ✓	
+1.54	
+.57 ✓	

We can see that ACV' is far more effective @ indicating redox points.

-1.45, -1.46, -1.42
-2.30, -2.33
.32, .29, .23, -.20
.46, .51 -.54, .51
-1.88, -1.87
-2.84
-2.07
-1.14
-.81, .76, .85
.03, .08, -.08
.96
1.54

Ranked:

4	.26	Co(.28)	SO_4(.2)
4	.505	S(.50, .48)	Fe(.56)
3	1.44	Cl(1.45)	
3	.81	Cl(.81)	NO_3^-(.80, .80)
3	.06	NO_3(.01)	Fe(.04)
2	2.315	H_2(2.25)	Al(2.33)
2	1.815	Fe(1.90)	N_2(1.87)
1	2.84	Ca(2.87)	
1	2.07	SO_4(2.00)	
1	1.14	Cl(1.15)	SO_4(1.12)
1	.96	NO_3^-(.96, .94)	Cl(.95)
1	1.54	No(1.59)	

Element	Int Score	Prob%
Co	1.25	38%
S	8.9	91%
Fe	15.0	108%
Cl	2.5	58%
N	3.75	72%
Ca	.83	28%

Therefore, we conclude that viscous proteins (CDB) to contain

- Iron
- Sulfur
- Nitrogen
- & Chlorine

> important

The solution is now entirely clear and looks no different than water. This would be a very good opportunity to apply Cyclic AC voltammetry to see which species can be recovered from a combined (known) solution.

OK, ORP Mix data has been collected by Cyclic AC Voltammetry

(Peaks) ACV	ACV'	Zero Crossing			
-1.36 ✓	+2.04	-2.32 ✓	-1.58 ✓	-.16 ✓	-2.30 ✓	+.37 ✓
-.58 ✓	+1.39 ✓		-1.39 ✓	-.10 ✓	-1.74 ✓	+.58 ✓
-.42 ✓	+.78 ✓		-1.17 ✓	+.02 ✓	-1.61 ✓	+1.54 ✓
-.14 ✓	+.42 ✓		-1.07 ✓	+.08 ✓	-1.01 ✓	+1.84 ✓
+.02 ✓	+.24 ✓		-1.02 ✓	+.16 ✓	-.58 ✓	+2.36 ✓
+.17 ✓	+.02 ✓		-.78 ✓	+.31 ✓	-.39 ✓	+2.55 ✓
+1.88 ✓	-.11 ✓		-.50 ✓	+.99 ✓	-.13 ✓	+2.68
+.83 ✓	-.19 ✓		-.58 ✓	+1.98 ✓	-.04 ✓	+2.79 ✓
+2.88 ✓	-.52 ✓		-.25	+2.38 ✓	+.15 ✓	

n	$\bar{x}$		n	$\bar{x}$	
3 ✓	1.38	-1.36, 1.39, -1.39 Cr(1.33)	4 ✓	1.02	-1.01, -1.02, .99, -1.01 S
6 ✓	Ø.56	-.58, -.52, -.50, -.58, -.58 Fe(.56) +.58	3	.38	.37, -.39, +.37 Fe(.36) Cr
3 ✓	Ø.40	-.42, +.42, +.37 Fe(.36) (-.44) (-.14) S(.4, .45, .45)	1	1.98	1.98 S(2.00)
5 ✓	Ø.16	-.14, +.17, -.16 +.16, .15 S(.17, .17, .20)	1	1.74	1.74 ?
3 ✓	Ø.03	+.02, +.02 ✓, -.04 Fe(.04)	1	2.55	2.55 ?
2	1.86	1.88, +1.84 Fe(1.90) Co(1.82)	1	2.68	2.68 Na (2.71)
3 ✓	.80	+.83, +.78, -.78 Cl(.81) Fe(.77) N(.8, .8)			
2	2.84	2.80, 2.79 ?			
1	2.04	2.04 Fe(2.07) O_2(2.01) Cl(2.10)			
2	Ø.21	.24, -.19 Co(.17) S(.20)			
4 ✓	0.105	-.10, -.11, -.13, +.08 Co(.10) S(.09) O_2(.07)			
4 ✓	2.34	2.32, 2.38, -2.30, 2.36 Al(2.33, 2.31)			
3	1.58	-1.58, -1.61, +1.54 N(1.59) Al(1.66)			
1	1.17	1.17 Cl(1.15, 1.19)			
	.42	.42, .37, .39, .37			

I need to reestablish the Scoring & Probability Model Protocol:

The Two Part Model is:

$$\text{Int Score} = \frac{\text{No. Redox Entries} \times \text{Highest Redox Rank}}{\text{Abundance}/5} \quad \& \text{ Prob\%} = \text{Score} \cdot 130.6 \,/\, \text{Score} + 3.08$$

The first part is to rank the no. of redox ~~scores~~ entries @ a particular Eo. Then assign the various elements to those ranked mean Eo's. We have actually already done this:

n	Eo	Element	Score	Prob
6	.56	Fe (.56)	Fe: 6(5)/(4/5) = 37.5	121%
5	.16	S(.17, .17, .20)	S: 5(8)/(9/5) = 22.2	115%
4	.105	Co(.10) S(.09) ~~O2 (.07)~~	Co: 4(3)/(6/5) = 3.8	72%
4	2.34	Al (2.33, 2.31)	Al: 4(3)/(5/5) = 12	104%
4	1.02	S(1.04)	Cr: 3(2)/(10/5) = 3	64%
3	1.38	Cr (1.33)	Cl: 3(1)/(18/5) = .8	27%
4	Ø.39	Cr (.40) Fe(.36, .44) S(.40, .45, .45)		
3	0.03	Fe(.04)	N: 3(1)/(20/5) .75	26%
3	Ø.80	Cl(.81) Fe(.77) N(.8, .8)		
3	1.58	N(1.59), Al(1.66)		
2	1.86	Fe(1.90) Co(1.82)		
2	2.84	?		
2	Ø.21	Co(.17) S(.20)		
1	2.04			
1	1.17			
1	1.98			
1	1.74			
1	2.55			
1	2.68			

Not good

I see a signal for $+0.23$. on $[3,-3]$ but never a signal for $2.31-2.33$.

. Our actual signal is $+0.24$ on $[3,-3]$
Thus is the only signal I actually see and that eliminates 2 out of 3 AI signals.

Too many minor peaks may have been recorded.
We also see that 0.24 is much closer to S (@ 0.20) than it is to Co @ (.17), thus also eliminates Co since it is actually out of the preferred range ($\leq .05\ \Delta$)

Thus are the scores @ the point drop to
AI = 4(1)/(5/5) = 4 vs 12 $\Rightarrow$ Prob = 74%
Co = 4(2)/(16/5) = 2.5 $\Rightarrow$ Prob = 59%
so they still are in the running but considerably reduced.

Another thing we see is that when you have a tie, the most common element is key for the more likely choice. Eg sulfur is more common than Co @ $\bar{E}_0 = .105$
There is therefore a case for giving S preferential treatment here.
You also see AI is actually out of range @ 1.58 ($\Delta = .06$ which is greater than 0.05) so AI is actually out of the running in all ways.

In summary, we see that
1. Small n's are vulnerable, ie $n \leq 2$
2. Signals $\Delta \geq 0.05$ are vulnerable
Then you would have had 1 Co $\Rightarrow$ 1(3)/(16/5) $\Rightarrow$ Prob = 30%
3. Define a prominent peaks as zero crossings to be accepted (0.94)
and no AI exists. And your solution is fundamentally correct w/ Fe, S, & Cr being identified w/ Scores of 121%, 115% & 64% all of which are $\geq 50\%$.

There are some lessons here in this recovery simulation that have arisen. They act to instill some reasonable constraints and precautions in the method Criteria for acceptance of data.

1. If the solution has a complex voltammogram, it is advisable to use extreme caution with $n \leq 2$ on redox entries.

2. If $\Delta \bar{x} \geq 0.05$ the expectation is that you are outside of the error range.

3. Restrict yourself by at all possible to fundamental, central constituents w/ a high score (eg $\geq 50\%$)

4. The candidates established are not infallible but they are a superb starting point. It is best to confirm, under all occasion possible, with an independent colorimetric, spectrometric, or redox or qualitative chemical test. This is sometimes difficult but by very hard to do so.

5. If in doubt, you use a 2nd electrochemical method, namely Cyclic Normal Pulse Voltammetry!

This is how you verified Na^+.

Cobalt testing can take place by UV spectroscopy
Chromium by Water Pollution Kit
Ca^{+2}, K^{+1}

For Inorganic Analyses:
We see that the first thing to do to confirm the results is to run two independent electrochemical tests, ie
Cyclic AC Voltammetry
Cyclic Normal Pulse Voltammetry

Then switch to:
1. Colorimetry (if possible)
2. Spectrometry (if possible)
3. Qualitative Chemistry (if possible)

Infrared Study Tonight:

You have learned some things that are quite important. The salt plate background from the microwave digestion process has a reasonably significant IR signature on its own right. It appears to be accounting for some significant absorption in the 3360 Cm^{-1} region of its own accord. I can not say exactly what is coming from or the sample appears to be dry. Or, I am seeing the problem. The salt plate will need to be subtracted from any samples that are using the same process to prepare for IR.

The is why the
1. Plastic Bottle Top
2. Env. Filament
3. Hair
4. Spider Web

Have such a remarkably similar spectrum. It means that little to no material actually went into solution. You are going to need to repeat some very important work here.

Working through w/ the bottle cap example has taught us that there is no significant organic material remaining after the combustion process take place. You can only be getting inorganics in the microwave @ other points.

Now, this is not always the case. The spruce cone and the meal worms do naturally produced proteins (organics, & therefore of it signature).

But your work w/ hair, spider web & the ext. skeleton will all need to be reworked w.r.t. the organic claims. Hair is the obvious sample to start with.

There is also a difference between

Combustion + Microwave Digestion
vs
Only Microwave Digestion.

Our primary interest lies in potential complications in the

Combustion + Microwave Digestion process.

This is where you may have received false positive absorptions from the produced NaCl ATR film layers.

Let's try hair first.

The has nothing to do w/ the inorganic alkali analysis w/ electrochemistry.
It also raises some questions about UV & NIR work also.

We definitely do have a signal w/ digested hair (no combustion involved). The is good & great.

A lesson here is that to keep everything on a level playing field, make sure that you neutralize the NaOH microwave digestion product to produce a NaCl matrix, and then make sure you use a NaCl matrix as the background for ATR analysis.

Your existing work is generally fine, but you will have some important refinements to make for I will work on the hair extraction part.

Your first pass w/ an existing digested hair sample (devitalization state is uncertain, but it most likely is neutralized) does produce a nice spectrum in IR.

---

50 ul is plenty for a suitable IR ATR film w/ the pipette. Hair extract turns muddy color @ a neutral pH. Digestion was quite easy @ 10% power 10M NaOH for 30 min.

Ok, we have a good spectrum IR for hair now with the NACl ATR salt plate removed as reference. First time.

Our spectrum is fine & matches previous work quite well.

Dec 01 2017

We now have a very clean spectrum of human hair that take into account the effects from a NaCl ATR crystal developed from neutralization of the digestion product.

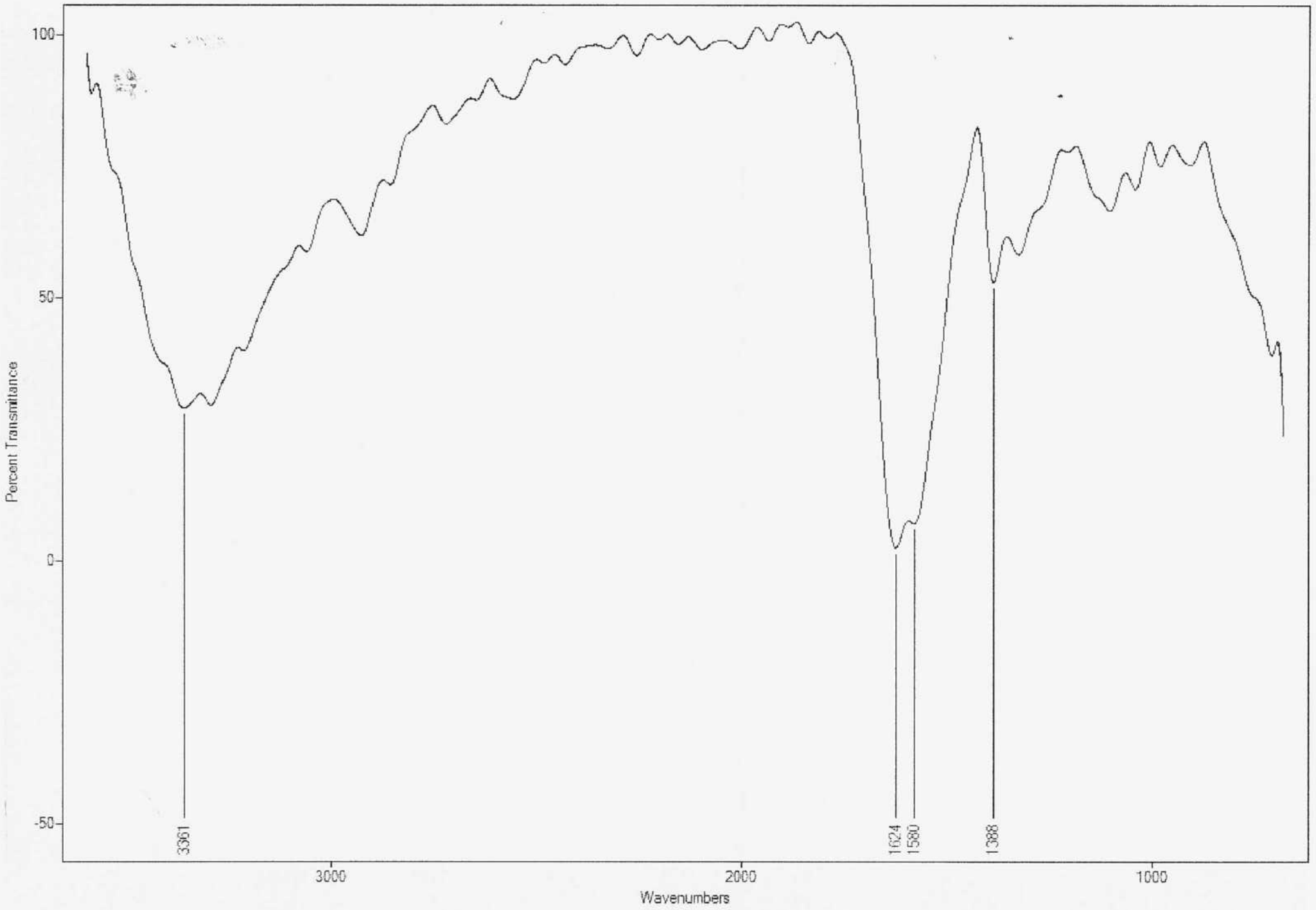
The spectrum is not different in any significant fashion from those obtained earlier. The influence from the ATR salt crystal appears negligible, therefore.

I will, nevertheless, pursue any difference that can be determined w/ a spider web sample or an environmental filament sample, if sufficient material for analysis remains.

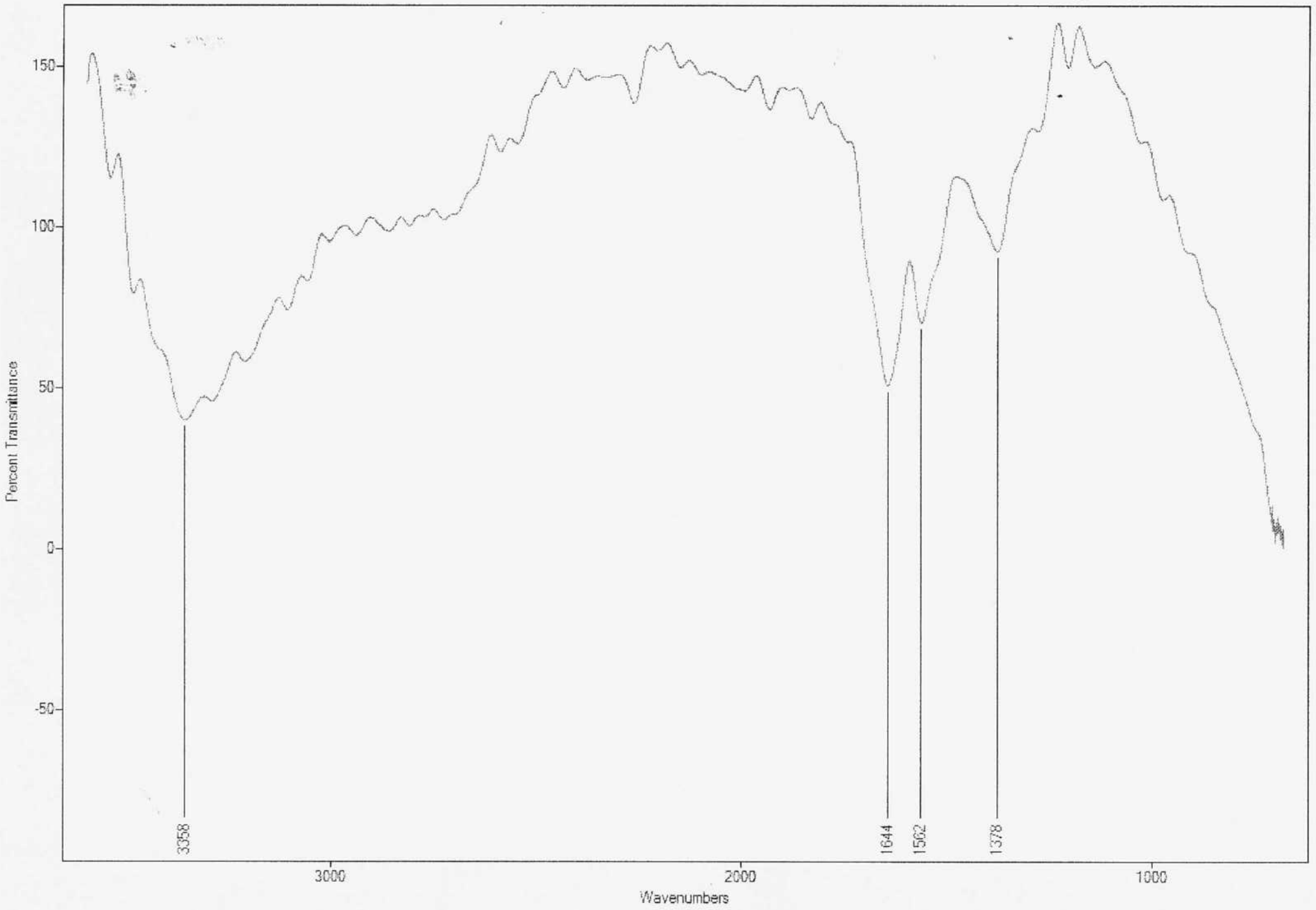
An interesting observation is that the hair digestion product still under high pH (10M NaOH) has congealed somewhat overnight. I would like to repeat this. We may have denatured protein in abundance w/ in that sample.

A very clean spectrum of digested ~~[illegible]~~ hair removing a reference ATR salt crystal ($NaOH + HCl \rightarrow H_2O + NaCl$)

Hair Digestion - NaCl Reference Removal ATR Nov 30 2017 - 02.JPEG

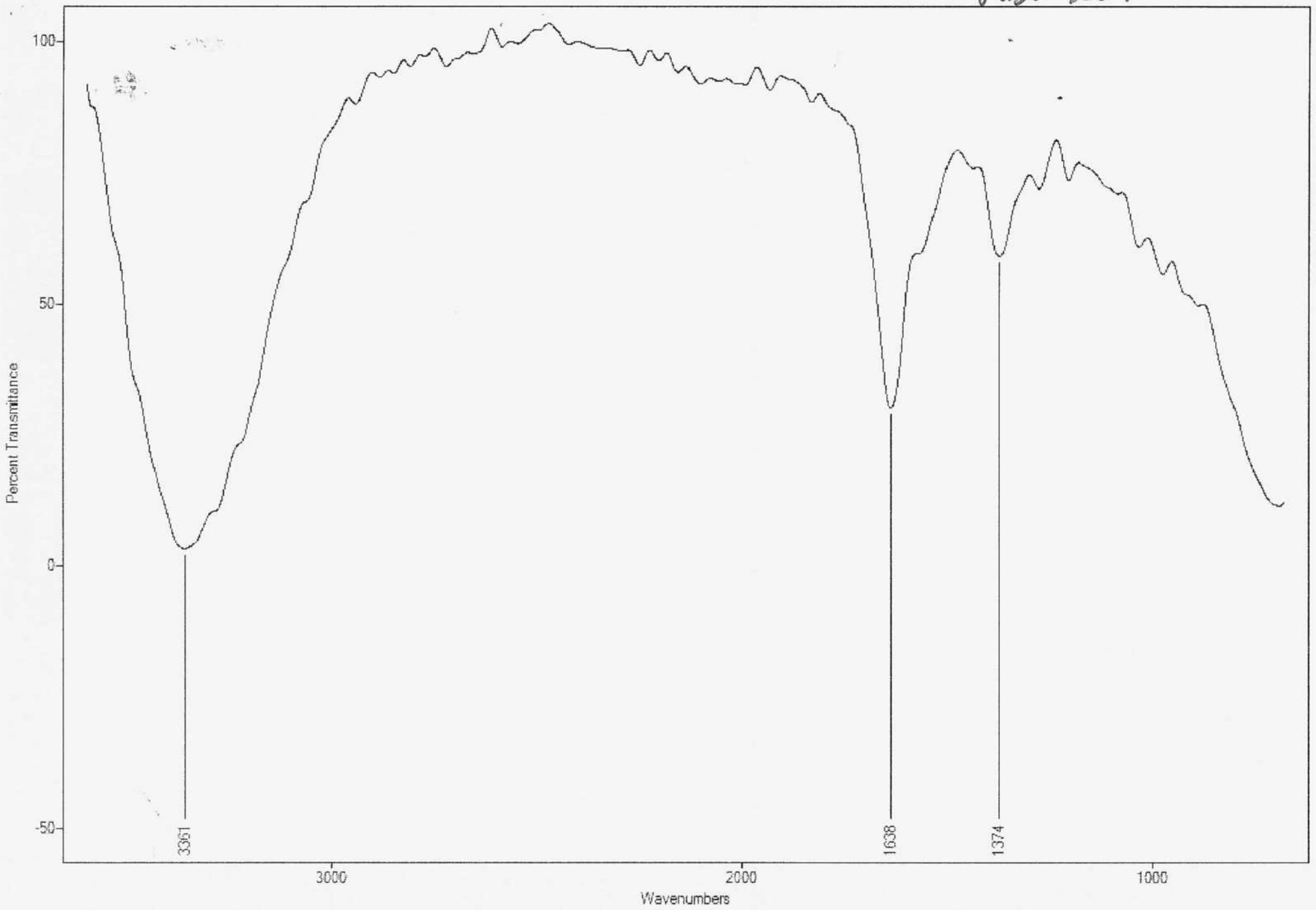


Spider Web Digested Neutralized NaCl Reference Average ATR Dec 01 2017 - 02.JPEG



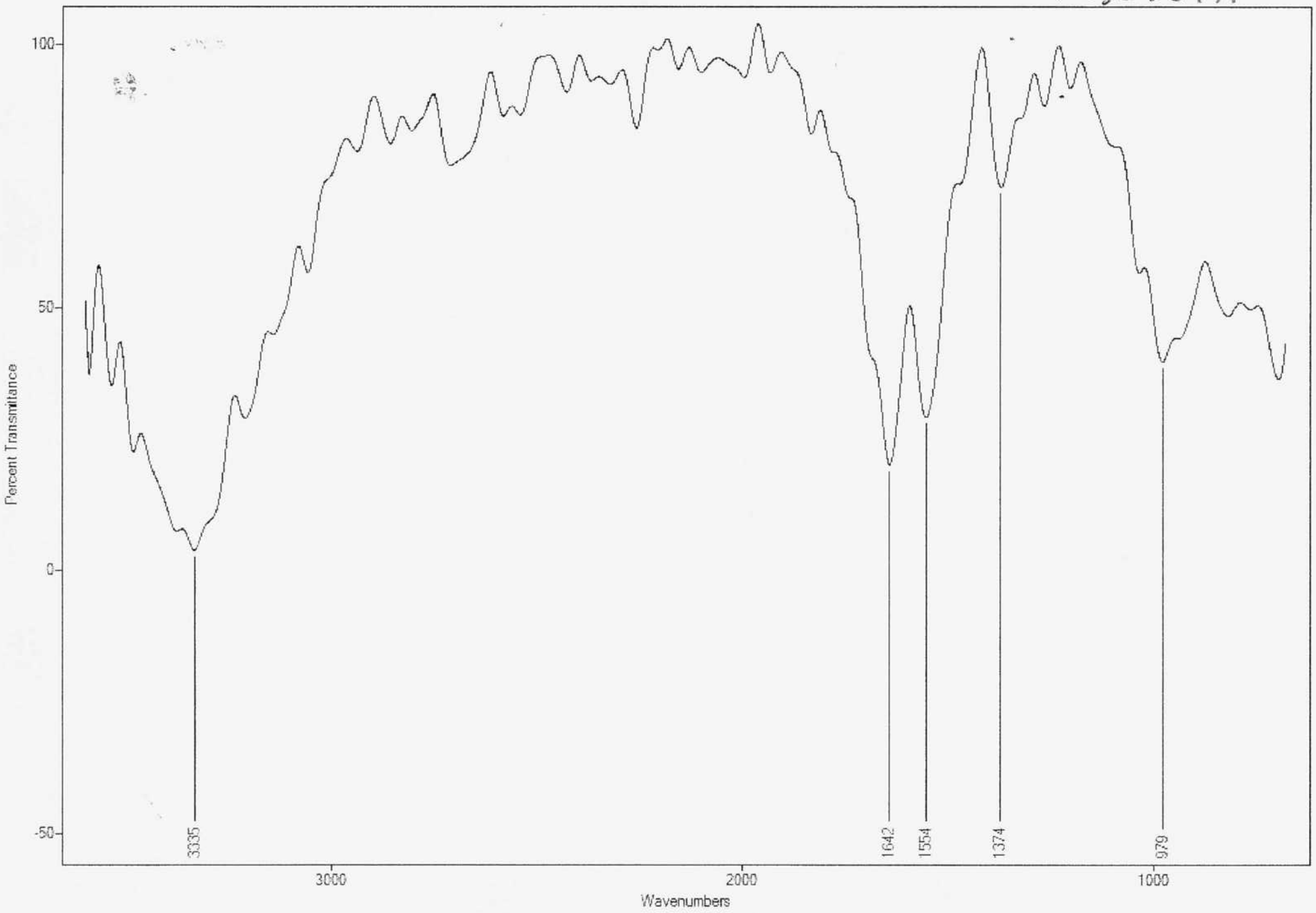
Orisinal 1999 EPA Sample

Environmental Filament 1999 Sample Digested Neutralized Salt Plate Reference ATR Dec 01 2017 - 02.JPEG



Weak signal - subject to greate error - a more Contemporary Sample

Environmental Filament Digested Neutralized Salt Plate Reference ATR Dec 01 2017 - 01.JPEG



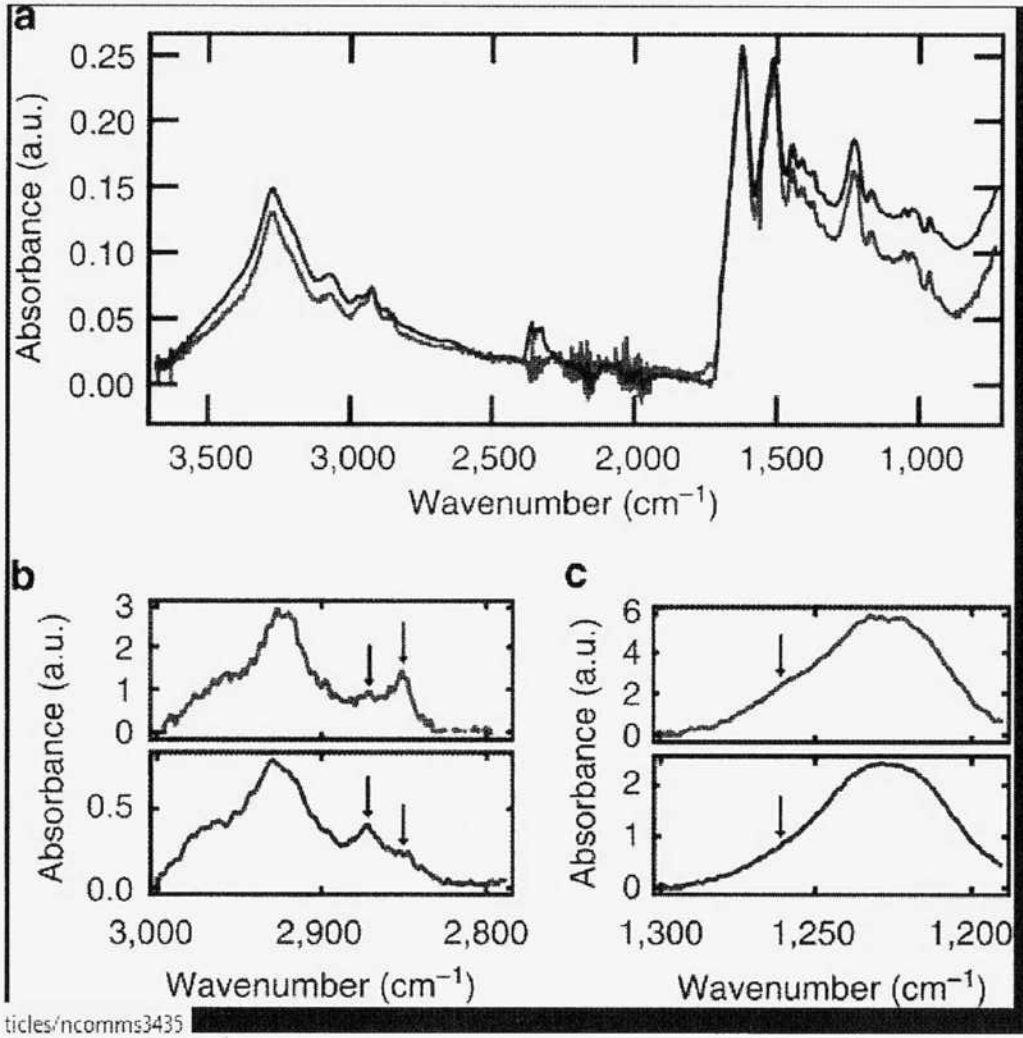
Term "Acid hydroxyl" is used, presumably towards the ~3300 peak

Amide bands modification of spider silk

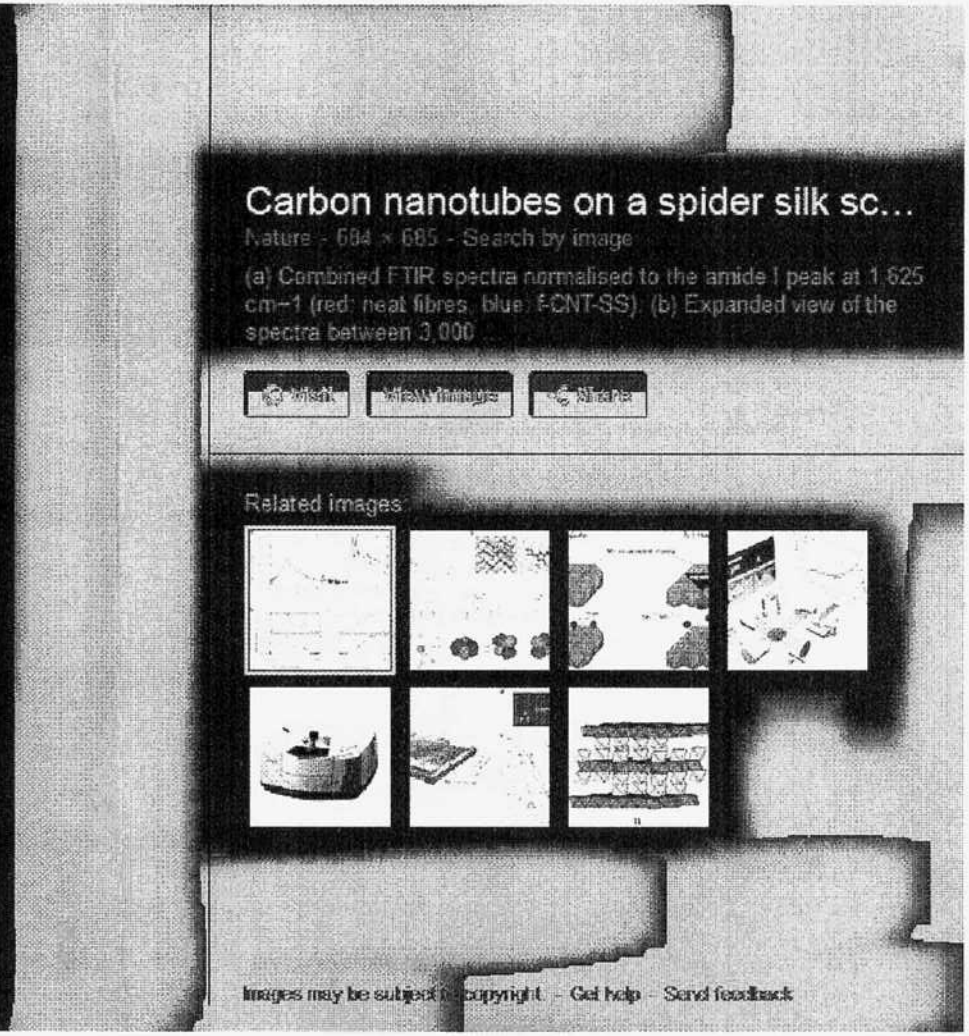
We do have it.

Spider Silk FFT Study

Page 209 B



ticles/ncomms3435



We now have a pretty good handle on the IR spectra comparisons between human hair, spider webs and the environmental filaments. The current user has established the reference spectrum as a NaCl plate on the ATR plate ($NaOH + HCl \rightarrow NaCl + H_2O$)

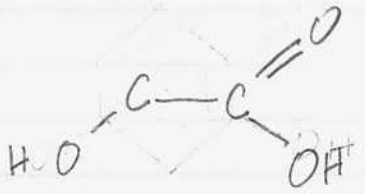
They are amazingly similar as was determined previously. This is because they each share a keratin chemical nature. Spider webs are more interesting than is realized by many with the keratin connection.

Only one IR spectra of a spider web was located, and it involve a study that modified the amide bands.

Each spectra is dominated by the peaks of
~ 3360 cm^{-1} "acid hydroxyl" terminology?
~ 1640 cm^{-1} amides $RCONH_2$ or $RCONHR'$ bring
~ 1380 ~~amides~~ Sulfate S=O

Arramph 45

The 3360 strong absorbance is a little confusing. IR Pal shows both ArOH & Carboxylic acids
Acid hydroxyl seems to imply the type of combination.



Alpha Hydroxyl Acid

"Alpha Hydroxyl Acids have a profound effect on keratinization".

"AHAs w/ greater bioavailability appear to have deeper dermal effects".

Sound relevant to the Morgellons issue, does it?

All three materials seem to be sharing the chemical nature.

Hydroxy acids are indeed very relevant to human hair, spider webs and the "environmental filament"

Page 211

From the point, you can as and so on to compare

UV
NIR
Electrochemistry

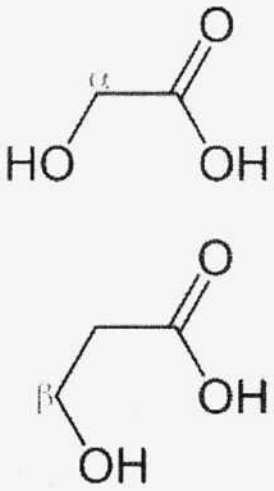
Lactic acid is an AHA Alpha Hydroxy Acid

Page 211 A

WIKIPEDIA

Alpha hydroxy acid

α-Hydroxy acids, or **alpha hydroxy acids** (**AHAs**), are a class of chemical compounds that consist of a carboxylic acid substituted with a hydroxyl group on the adjacent carbon. They may be either naturally occurring or synthetic. AHAs are well known for their use in the cosmetics industry. They are often found in products that aid in the reduction of wrinkles as well as to soften strong, defining lines and improve the overall look and feel of the skin. They are also used as chemical peels available in a dermatologist's office, beauty and health spas and home kits, which usually contain a lower concentration of around 4%. Effective results through continuous treatment have resulted in AHAs being a successful & developmental method of curbing harsh ageing effects in the skin & cosmeceutical industry.[1][2]



α-, β- and γ-hydroxy acids

Contents

1 **Cosmetic applications**
- 1.1 Epidermal effect
- 1.2 Dermal effects

2 **Other applications**
- 2.1 Organic synthesis

3 **Alpha hydroxy acids at different concentrations**

4 **Chemical acidity**

5 **Safety**

6 **See also**

7 **References**

8 **External links**

Cosmetic applications

Understanding skin structure and cutaneous aging is helpful to a discussion of the topical action of AHAs. Human skin has two principal components, the avascular epidermis and the underlying vascular dermis. Cutaneous aging, while having epidermal concomitants, seems to involve primarily the dermis and is caused by intrinsic and extrinsic aging factors.

AHAs are a group of organic carboxylic compounds. AHAs most commonly used in cosmetic applications are typically derived from food products including glycolic acid (from sugar cane), lactic acid (from sour milk), malic acid (from apples), citric acid (from citrus fruits) and tartaric acid (from grape wine). For any topical

compound to be effective, including AHA, it must penetrate into the skin where it can act on living cells. Bioavailability (influenced primarily by small molecular size) is an important factor in a compound's ability to penetrate the top layer of the skin. Glycolic acid, having the smallest molecular size, is the AHA with greatest bioavailability and penetrates the skin most easily; this largely accounts for the popularity of this product in cosmetic applications.

Epidermal effect

AHAs have a profound effect on keratinization; which is clinically detectable by the formation of a new stratum corneum. It appears that AHAs modulate this formation through diminished cellular cohesion between corneocytes at the lowest levels of the stratum corneum.

Dermal effects

AHAs with greater bioavailability appear to have deeper dermal effects. Glycolic acid, lactic acid, and citric acid, on topical application to photodamaged skin, have been shown to produce increased amounts of mucopolysaccharides and collagen and increased skin thickness without detectable inflammation, as monitored by skin biopsies.[3]

Other applications

Organic synthesis

α-Hydroxy acids are useful building blocks in organic synthesis. For example, α-hydroxy acids are generally useful as precursors in the preparation aldehydes via oxidative cleavage.[4][5] Compounds of this class are used on the industrial-scale and include glycolic acid, lactic acid, citric acid, and mandelic acid.[6][7]

Alpha hydroxy acids at different concentrations

At low concentrations (5-10%), as found in many over-the-counter products, glycolic acid (GA) reduces cell adhesion in the epidermis and promotes exfoliation. Low concentration makes possible daily application as a monotherapy or as part of a broader skin care management for such conditions as acne, photo-damage, wrinkling as well as melasma.[8][9] Care should be taken to avoid irritation to avoid the worsening of melasma or other pigmentary problems. Newer formulations combine glycolic acid with an amino acid such as arginine and time-release formulations that reduces the risk of irritation without affecting glycolic acid efficacy.[10] Supplemental use of an anti-irritant such as allantoin may also be helpful in reducing irritation.

At higher concentrations (10-50%) the effects of GA are more pronounced, but application must be limited. Such application may be used to prepare the skin for stronger glycolic acid concentrations (50 - 70%) or to prime the skin for stronger chemical applications (e.g. trichloroacetic acid).

At highest concentrations (50-70%) applied for 3 to 8 minutes under the supervision of a physician, glycolic acid promotes slitting between the cells and can be used to treat acne or photo-damaged skin (e.g. due to mottled dyspigmentation, melasma). The benefit of such short-contact application (chemical peels) depends on

the pH of the solution (the more acidic the product, or the lower the pH, the more pronounced the results), the concentration of GA (higher concentrations produce more vigorous response), the length of application and prior skin conditioning such as prior use of topical vitamin A products. Although single application of 50-70% GA will produce beneficial results, multiple treatments every 2 to 4 weeks are required for optimal results. It is important to understand that glycolic acid peels are chemical peels with similar risks and side effects as other peels. Some of the side effects of AHAs chemical peeling can include hyper-pigmentation, persistent redness, scarring, as well as flare up of facial herpes infections ("cold sores").

Chemical acidity

Although these compounds are related to the ordinary carboxylic acids, and therefore are weak acids, their chemical structure allows for the formation of an internal hydrogen bond between the hydrogen at the hydroxyl group and one of the oxygen atoms of the carboxylic group. Two effects emerge from this situation:

- Due to the "occupation" of electrons of the carboxylic oxygens in the hydrogen bonding, the acidic proton is held less strongly, as the same electrons are used in bonding that hydrogen too. So the pK_a of 2-hydroxypropanoic acid (lactic acid) is a full unit lower compared to that of propionic acid itself (3.86[11] versus 4.87[12])
- The internal bridging hydrogen is locked in its place on the NMR timescale: in mandelic acid (2-hydroxy-2-phenylacetic acid) this proton couples to the one on carbon in the same way and magnitude as hydrogens on geminal carbon atoms.

Safety

AHAs are generally safe when used on the skin as a cosmetic agent using the recommended dosage. The most common side-effects are mild skin irritations, redness and flaking. The severity usually depends on the pH and the concentration of the acid used. Chemical peels tend to have more severe side-effects including blistering, burning and skin discoloration, although they are usually mild and go away a day or two after treatment.

The FDA has also warned consumers that care should be taken when using AHAs after an industry-sponsored study found that they can increase photosensitivity to the sun.[13] Other sources suggest that Glycolic acid, in particular, may confer a photoprotective effect.[14]

See also

- Beta hydroxy acid
- Omega hydroxy acid
- Salicylic acid, a β-hydroxy acid

References

1. Kempers, S; Katz, HI; Wildnauer, R; Green, B (June 1998). "An evaluation of the effect of an alpha hydroxy acid-blend skin cream in the cosmetic improvement of symptoms of moderate to severe xerosis, epidermolytic hyperkeratosis, and ichthyosis". *Cutis*. **61** (6): 347–350. PMID 9640557 (//www.ncbi.nlm.nih.gov/pubmed/9640557).

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Lactic Acid in Hair

by FRED DECKER | Last Updated: Oct 03, 2017

PEOPLE ARE READING

1 Skin Products That Contain AHA W Lactic or Glycolic ...

2 Which Fruits Contain Alpha Hydro: Acid?

3 Alpha & Beta Hydroxy Acids

4 What Are the Side Effects of Alpha Hydroxy Acid and 12 ...

5 Over the Counter Cleansers & Moisturizers With Glycolic...

Lactic acid is one of a group of organic compounds collectively referred to as alpha hydroxy acids, or AHAs. They're widely used in cosmetic products, primarily as gentle exfoliants for skin care. Lactic acid is also frequently incorporated into hair-care products. Although it is largely associated with dairy products, the acid is also produced by the human body.

Lactic Acid

Lactic acid was first isolated and described in 1780 by Swedish scientist Carl Wilhelm Scheele. It was originally found in soured milk, hence the term "lactic," or "milk-derived" acid. A century later, French researchers discovered that lactic acid was also produced by fermentation, which allowed commercial production of lactic acid. It's widely used in a number of industrial processes as well as food production. It's an ingredient in biodegradable plastics, detergents and other household cleaners. Pharmaceutical companies use lactic acid to adjust the pH of medications. It's also found in personal-care and hair-care products.

Hair Care

Page 211 I

Lactic acid is an ingredient in many shampoos and other hair-care products. It serves a number of purposes, depending on how the product is formulated. With hair that has become dry and scaly, lactic acid and other mild acids relax and smooth the hair, making it more manageable. One major shampoo manufacturer touts lactic acid on its website as an ingredient that helps restore strength and elasticity to hair by altering the ionic charge of its molecules. In slightly higher concentrations, lactic acid also strips away part of the hair's protective keratin coating. This improves hair's ability to absorb other restorative ingredients.

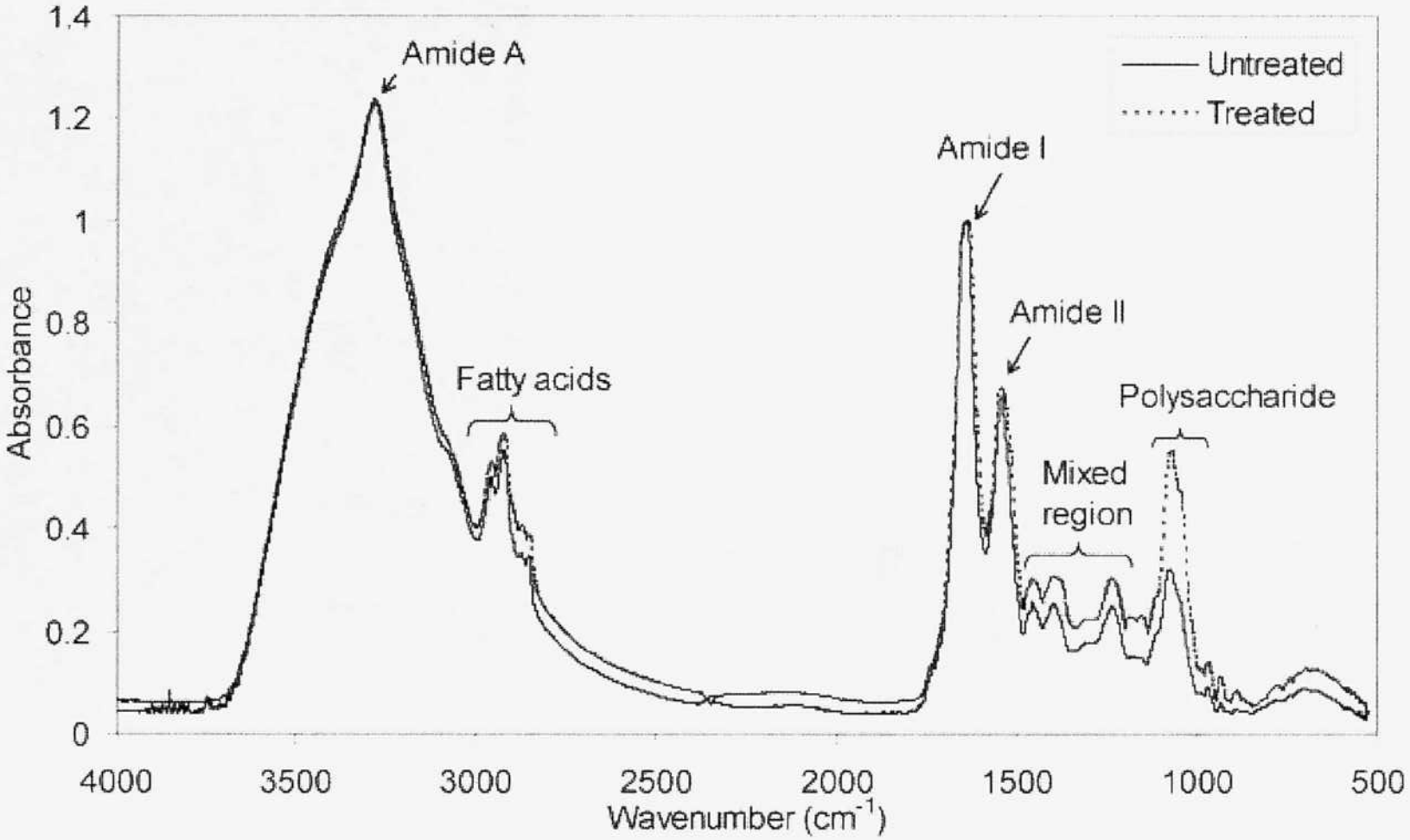
Hair Removal

Lactic acid's ability to strip away your hair's protective keratin coating is also harnessed in a number of hair-removal products. These depilatories remove unwanted hair by using lactic acid to remove the protective keratin layer, then weakening the hair itself with more potent chemicals such as acetylsalicylic acid, better known as aspirin. The hair is weakened by the acidic depilatory and can be rinsed away in the shower. Unwanted hair loss sometimes occurs in people with medical conditions that cause excessive perspiration. Sweat naturally contains lactic acid, and in excessive amounts, it can cause hair loss.

Sanitation

Oddly, there is a common connection joining lactic acid, hair and pickles. When foods are pickled, the yeasts that begin the fermentation are aided by a number of beneficial bacteria. Some of those bacteria generate lactic acid, which lowers the pH of the food to the point that dangerous bacteria can't live in it any longer. A similar thing occurs in your hair follicles, which naturally manufacture lactic acid. This modest quantity of lactic acid acts as an antibacterial agent, protecting your scalp from bacterial and fungal infections.

We are dealing w/ amide bands w/ all peaks in general, at least Amide A + Amide I
Could be in combination w/ hydroxy acids.
The 3360 band is apparently the Amide A band.
If any? What's relationship (in IR) between Amide A & hydroxy acids?


Amide A
Amide I
Amide II
Fatty acids
Mixed region
Polysaccharide
Untreated
Treated
Absorbance
Wavenumber (cm-1)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
4000
3500
3000
2500
2000
1500
1000
500

There is indeed a close relationship between Carboxylic acids & amides. See Chapter 8 Avram.

IR analyses of proteins

Fourier-Transform Midinfrared Spectroscopy for Analysis and Screening of Liquid Protein Formulations, Part 1

Understanding Infrared Spectroscopy of Proteins

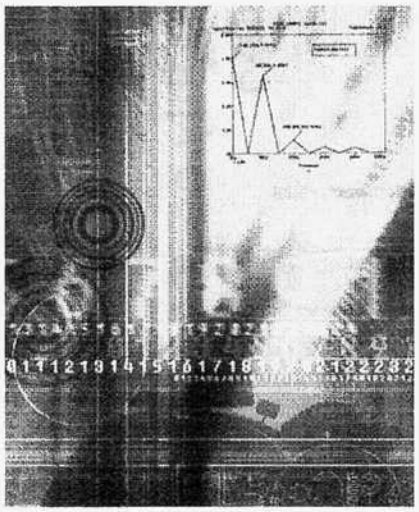
Patrick Garidel and Heidrun Schott

Sir William Herschel discovered infrared light in 1800, over two centuries ago. The first spectra of the molecular vibrations of organic liquids were registered in 1881 by Abney and Festing. Most applications at that point were of academic interest. In the early 1930s, researchers at BASF (Badische Anilin und Soda Fabrik) in Ludwigshafen, Germany, realized the importance of infrared spectroscopy for industrial purposes. In 1937, the first infrared (IR) instrument with a modulated beam was built by Lehrer (1). Five years later, the first nondispersive infrared analyzer was presented (2).

By 1950, Elliot and Ambrose showed that IR spectroscopy provides information on the secondary structure of proteins (2, 3). But the utility of this technique for structural analysis of proteins was not widespread. That changed with the rediscovery of Fourier transform analysis — as described by Jean-Baptiste Joseph de Fourier (4) in 1822. It led to the development of Fourier-transform infrared (FTIR) spectroscopy in the early 1980s (5).

The transition from IR to FTIR spectroscopy allowed strong improvements in spectral quality. The "Advantages" box summarizes major benefits of FTIR. Additionally, outstanding developments in analytical technology (e.g., mathematical tools for data processing and handling and new possibilities in computer science) enabled significant progress for data analysis, allowing extraction of information on protein conformations from IR spectra (1, 2).

Only since the past decade has FTIR spectroscopy become an accepted and powerful technique for development of protein formulations. The relation between protein structure (Figure 1) and bioactivity was long ago recognized. Changes in bioactivity were believed to result from alteration in protein structure and organization. Several methods were developed — and now are commonly used — for determination of secondary and tertiary structure of proteins. For obtaining structural information at high resolution, two methods are mainly used: X-ray crystallography and multidimensional nuclear magnetic resonance (NMR) spectroscopy. However, high-resolution studies of proteins are not always feasible (6).



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X-ray crystallography requires well-ordered single crystals of high-quality. For many proteins, that is not possible. And it is unclear whether the relatively "static" structure of a single crystal adequately represents protein conformation in a complex and dynamic liquid environment. Multidimensional NMR spectroscopy presents an alternative to X-ray crystallography, offering somewhat better flexibility in studying the structures of proteins in solution (their natural environment). However, data evaluation and interpretation of NMR spectra are very complex and

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Advantages of FT Technical Improvements

Time-Saving: All spectral elements are measured at the same time (Fellgett or multiplex advantage).

Better Signal-to-Noise Ratio: Method provides high optical throughput (Jacquinot advantage).

High Accuracy and Reproducibility: HeNe laser serves as an internal frequency standard (Connes advantage).

No Spectral Artifacts: Fourier transform method strictly excludes other frequencies (e.g., stray light).

Figure 1: Chemical structure of pectine lyase with representations of different secondary structure elements

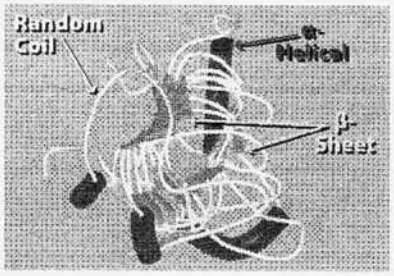
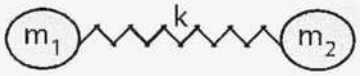


Figure 2: Mechanism of adsorption and the Hooke's law



$$\partial \mu_{el} / \partial r \neq 0$$

$$f = \frac{1}{2\pi} \sqrt{\frac{k}{m_{red}}}$$

$$m_{red} = \frac{m_1 \cdot m_2}{m_1 + m_2}$$

$$C=O \cdots\cdots H-O$$

extremely time consuming, especially for molecules that are larger than ~30 kDa. So NMR applications are limited to small proteins (~15–30 kDa).

Those restrictions have led to development of alternative methods including circular dichroism (CD) and vibrational (infrared and Raman) spectroscopy (1, 7). They are often referred as "low-resolution" techniques because they provide only global insight into the overall secondary structure of proteins. They offer no way to establish the precise three-dimensional location of individual structural elements. However, there are a number of advantages to using Fourier-transform infrared (FTIR) spectroscopy.

FTIR measurements can be made using extremely small protein samples (~1 mg/mL) in a number of environments (e.g., solid, semisolid, liquid, or adsorbed to a surface). Here we focus mainly on liquid sample analysis. A major benefit of FTIR is the ease and rapidity of acquiring high-quality spectra (an IR spectrum registers in just a few seconds). This allows a rapid screening of various protein formulations to determine the best conditions for optimal protein stability. Using IR spectroscopy eliminates problems associated with background fluorescence, for example, as detected by Raman or light scattering.

All these facts have made FTIR spectroscopy practical for studying biological systems. By contrast with, for example, electron spin resonance spectroscopy, it does not require additional probe molecules. FTIR is a noninvasive technique. Water absorption, omnipresent especially in the spectral range between 4000 and 400 cm^{-1}, can be subtracted mathematically, which makes study of hydrated (H_2O) biomolecular samples almost custom (8–11). Another approach is using D_2O. Mathematical methods are now available and routinely used to separate subcomponent bands that overlap in the spectra of proteins.

The infrared region is subdivided into three regions: near infrared (NIR: 12,500–4000 cm^{-1}), midinfrared (MIR: 4000–400 cm^{-1}), and far infrared (FIR: 400–10 cm^{-1}). The useful unit is the wave number, which is expressed in cm^{-1}. Herein we focus on information derived from MIR spectroscopy.

The Basis of Infrared Spectroscopy

The atoms within a molecule constantly oscillate around an equilibrium position, r. Consequently, bond lengths and angles change. In the classical model, two atoms are fixed by a spring, with a spring/force constant, k (Figure 2). The frequency of such motion is within the infrared region, between 1 and 100 µm, and IR radiation of such energy can excite vibrational motions. Thus, infrared spectroscopy is a vibrational spectroscopic method (8) that measures the wavelength and intensity of infrared light absorbed by a sample (dry, wet, or gaseous).

The frequency f of the vibration between two atoms of mass m_1 and m_2 depends on k and the reduced mass m_{red} (Figure 2 shows the exact relation). Changes in k or m_{red} induce a change in f. The equation in Figure 2 describes the frequency of vibrations for a diatomic molecule. Usually the frequency f of a vibration is given in Hz (s^{-1}). However, in vibrational spectroscopy it is common to express frequencies in wave number units (waves per unit length), which is the reciprocal of wavelength (12).

A linear molecule has $3n - 5$ degrees of vibrational freedom (n = the number of atoms present); a nonlinear molecule has $3n - 6$. For proteins, that can be several thousands, if not more, possible vibrational motions. These motions may not only be excited to the first energy level, but also to higher levels.

However, for three main reasons, not all such excitations are observed individually in a spectrum. As mentioned above and shown in Figure 2, IR radiation is absorbed by valence electrons (bonds) (1, 2).

- For infrared absorption, the transition dipole moment (μ_e) must change: $\partial\mu_{el} \div \partial r \neq 0$ (Figure 2). So a molecule such as nitrogen N_2 is not IR active because during its vibrational motion, there is no change in transition dipole moments. By contrast, carbon dioxide (CO_2) is IR active, and an IR spectrum is obtained.
- Transition probability dictates the intensity of an observed IR absorption. If that probability is too small, a transition is too weak to be observed. The transition probability of a vibrational excitation is proportional to the change in dipole moment

during the vibration.

• When vibrations have similar excitation frequencies, they cannot be resolved or identified.

Most IR spectroscopic investigations of proteins use the MIR spectral region. Within this region, first-order excitations of the vibrations are detected for small molecule parts or groups. Those are called *functional group vibrations*, and they include methyl and methylene stretching vibrations as well as stretching of carbonyl bonds. The carbonyl group is a functional part of the amide groups in proteins and peptides. They give rise to well-defined vibrations (well-defined IR bands) and thus are observed in characteristic regions and frequency positions of a spectrum. Figure 3 shows an infrared spectrum of a protein molecule with its absorptions in the spectral region between 4000 and 750 cm^{-1}. Table 1 summarizes the most prominent infrared bands observed for biomolecules such as proteins and lipids (6, 13–15). The most important bands for analyzing protein formulations are the amide bands, especially amide I and II.

IR Spectroscopic Experiments

An infrared spectrometer consists of a Michelson interferometer, a light source, a sample chamber, and a detector. The continuum source producing light over a broad range of infrared wavelengths is usually based on silicon carbide (e.g., Nernst Globar). Such IR sources provide high-energy beams at a maximum intensity of 1500–1800 cm^{-1}.

The IR light is split into two beam paths using a half-silvered mirror, then reflected from two mirrors back onto a beam splitter, where both light beams are recombined (a Michelson interferometer). One mirror is fixed, and the second is movable. A laser is used to determine the exact position of the movable mirror. If the distance from the beam splitter to the fixed mirror is not exactly the same as that from the beam splitter to the second mirror, then when the two beams are recombined, there will be a small difference in the phase of their light. Because of the "superposition principle," constructive and destructive interference exists for different wavelengths depending on the relative distances of those two mirrors from the beam splitter. Additionally, the laser used for detecting the mirror position provides a trigger signal for data acquisition.

A data point is collected at each zero crossing — e.g., of the laser interferogram, which corresponds to the quantity measured by the detector (intensity of the combined IR beams as a function of the moving mirror displacement). If the intensity of light is measured and plotted as a function of the movable mirror's position, the resulting graph can be shown to be the Fourier transform of the intensity of light as a function of wave number. In FTIR spectroscopy, the light leaving an interferometer is directed onto a sample, and its intensity is measured using an infrared detector. The intensity of light striking the detector is measured as a function of mirror position. Next, the results are Fourier-transformed to produce a plot of intensity as a function of the wave number.

IR experiments can be performed in transmission or reflection mode (Figures 4 and 5), for which corresponding sample cells are available (15). Each sample cell must be a closed chamber that can be rapidly purged with nitrogen or dry air. Both water and CO_2 have strong absorption bands and so must be removed. Water vapor bands especially appear in the spectral region between 1700 and 1600 cm^{-1}, which overlaps with the amide I vibration. In most commercially available IR spectrometers, the sample chamber can hold only one sample. Therefore, background standards and sample spectra are measured sequentially rather in parallel.

Two main types of infrared detectors are used in MIR: mercury cadmium telluride (MCT) and deuterated triglycine sulfate (DTGS) detectors. The quality of a detector depends on its noise level, detectivity (defined as the reciprocal of its noise equivalent power, which describes the incident radiant power for a signal-to-noise ratio of 1 within a given bandwidth of 1 Hz at a given

Table 1: Band assignments of the main infrared active vibrations of biomolecules

Frequency Range (cm^{-1})	Assignment
3490 and 3280	Asymmetric and symmetric H–O–H stretching
3250–3300	Amide A (N–H stretch in resonance with amide II overtone)
3080	Amide B
3010	=C–H stretching of alkenes
2957	Asymmetric CH_3 stretching
2920	Asymmetric CH_2 stretching
2872	Symmetric CH_3 stretching
2851	Symmetric CH_2 stretching
1738	C=O stretch
1600–1700	Amide I (mainly C=O strech)
1645	H–O–H bending
1480–1575	Amide II (N–H bend in plane and C–N stretch)
1468	CH_2 scissoring
~1395	C=O stretch of COO
1378	CH_3 symmetric bend
1343	CH_2 wagging
1230–1330	Amide III (N-H bend in plane and C-N stretch)
1240	Asymmetric PO_2^- stretch
1170	Ester C–O asymmetric stretch
1080	Symmetric PO_2^- stretch
1047	C–OP stretch
980	Choline asymmetric stretch
625–770	Amide IV (mainly O=C-N deformation)
720	CH_2 rocking

wavelength), and the spectral detection cut-off (1, 2). Another important issue is detector response time, which determines how fast the mirror of a Michaelson interferometer can be moved.

The most common MIR detectors are DTGS detectors, which are very stable but have the drawback of relatively slow response times. MCT detectors offer higher sensitivities. Their response times are about 10 times faster than for DTGS detectors, so spectra can be acquired much faster. The linear detector range is higher for DTGS than for MCT, and DTGS detectors come "ready-to-use," whereas MCT detectors must be cooled with liquid nitrogen to work properly.

Figure 4 shows schematically a sample set-up in transmission mode. The intensity of the beam passing a sample follows the Lambert–Beer law. The transmission T is defined as the ratio of the beam I passing the sample and the initial beam I_0. T spectra can easily be transformed to absorption (A) spectra by the relation shown. This enables IR spectroscopy to be used for determining protein concentration. Attenuated, transmitted IR radiation can be displayed as a function of wavelength, which provides an IR spectrum.

Transmission cells used in MIR spectroscopy typically consist of NaCl or KBr windows for nonaqueous media and CaF_2 or BaF_2 windows for aqueous solutions. Table 2 characterizes the most widely used IR windows, and their properties must be considered in preparing an IR experiment. Analysis of aqueous solutions by MIR spectroscopy is possible using thin sample cells with an optical path length below ~25 µm. This is because increasing the path length renders opaque the region around 1640 cm^{-1} (the bending vibration of water), where the amide I vibration is detected. An alternative is switching from H_2O to D_2O. Commercially available systems for transmission experiments require only very small volume of ~50 µL, with a path length of ~7 µm. Such sample cells come with a temperature control system that enables registration of IR spectra as a function of temperature.

Spectra also can be registered in reflection mode using horizontal attenuated total reflection (hATR) techniques. In ATR, a sample comes into contact with the surface of an internal reflection element (IRE) that has a high refractive index n_1 (Figure 5). Radiation is totally reflected at the boundary between two media of higher (n_1) and lower (n_2) refractive indices (e.g., when a sample is placed on top of the crystal) if it hits this boundary at an incident angle greater than the critical angle

$$\theta_c = \text{arc sin } (n_2/n_1)$$

Reflected radiation energy penetrates the boundary as a so-called *evanescent wave*. Penetration depth d_p is the thickness within which intensity decreases to 1/exp of the intensity at the boundary. As Figure 5 shows, d_p is a function of the refractive indices n_1 and n_2, the incident angle θ, and the wavelength λ. Absorption is a consequence of the IR beam's penetration into a sample. Such hATR set-ups are available for one or multiple path cells, which enhance sensitivity.

Many commercially available hATR systems come with a mounted Peltier element for temperature control. Sample volumes can be as small as 10–20 µL. That is important especially in the preformulation phase of drug development because of a "chronic" lack of material. With hATR techniques, formulation screening for protein stability can be performed at temperature intervals from 0 to 95 °C. Protein concentration can vary between one and at least 100 mg/mL. For investigations of protein spectra, a buffer spectrum must be registered and subtracted from the protein spectrum. ATR set-ups offer several important advantages:

- easy-to-clean ATR crystals (and cells)
- no sample preparation
- consistent path lengths
- useful in investigating both soft powder and liquid samples.

Figure 3: FTIR spectrum of a protein in the spectral region between 4000 and 750 cm^{-1}, showing various regions in which vibrational motions of the amide backbone occur (spectrum taken in the reflection mode)

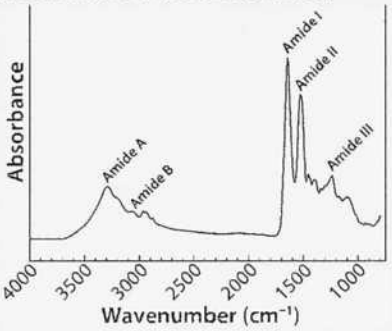


Figure 4: IR experiment in the transmission mode (E = electric field; I = intensity; f = frequency; d = optical thickness; μ_e = dipole moment; T = transmission; A = absorption)

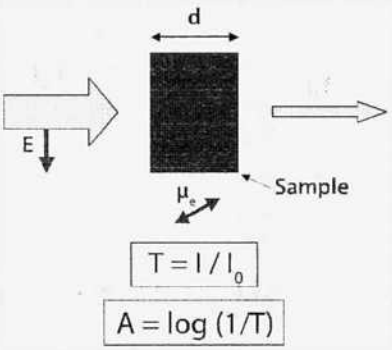
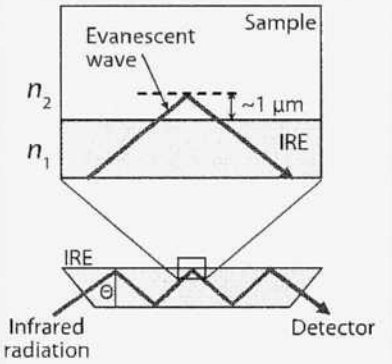


Figure 5: Schematic representation of the ATR set-up in infrared spectroscopy (IR = infrared beam; n_1 = respective index; n_2 = refractive index; IRE = internal reflection element, Θ = angle of incidence; µ = wavelength; dp = penetration depth)



$$d_p = \frac{\lambda}{2 \pi n_1 \sqrt{\sin^2 \Theta - n^2_{21}}}$$

$$n_{21} = n_2 / n_1$$

$$n_1 > n_2$$

Table 2: The most widely used infrared window material: some physicochemical properties

Material	TR	*n*	RL	H	Remarks
NaCl	28,000–700	1.52	~4.5 %	15	Hygroscopic, slightly soluble in alcohol and NH_3
KBr	33,000–400	1.54	~4.5 %	7	Soluble in water, alcohol and glycerine, hygroscopic
AgCl	23,000–400	2.00	~11 %	10	Insoluble in water, soluble in NH_4OH, sensitive to light
CaF_2	66,000–1200	1.40	~2.8 %	158	Insoluble in water, resists most acids and bases, soluble in NH_4 salts
BaF_2	50,000–900	1.45	~3.3 %	82	Low water solubility, soluble in acid and NH_4Cl
Ge	5000–600	4.01	~36 %	550	Insoluble in water, soluble in hot H_2SO_4
ZnSe	20,000–500	2.43	~17 %	150	Soluble in strong acids, dissolves in HNO_3

TR = Transmission range in cm^{-1}
n = Refractive index: at 2000 cm^{-1}
RL = Reflectance loss per surface
H = Hardness according to Knopp

Looking for More Detail?

Midrange FTIR spectroscopy is showing promise for use in formulation stability studies, especially in the early preformulation phase, because it enables fast screening of a number of formulations using only small amounts of protein. The protein concentrations used in FTIR range from 1 mg/mL to at least 100 mg/mL.

Next month, Part 2 concludes this article by dealing with the interpretation of protein infrared spectra and presenting various applications and results derived from FTIR screening of protein formulations.

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Fourier-Transform Midinfrared Spectroscopy for Analysis and Screening of Liquid Protein Formulations

Part 2: Detailed Analysis and Applications

Patrick Garidel and Heidrun Schott

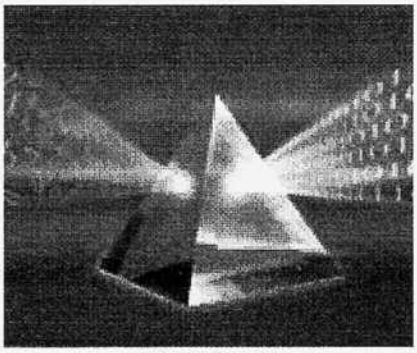
In the first half of this two-part article (1), we introduced the physical principle behind infrared (IR) absorptions and experimental setups used for investigating protein samples. This second part concludes by focusing on the use of IR spectroscopic data in formulation stability studies and for characterizing protein secondary structures and related changes (e.g., resulting from protein–excipient interactions). We also examine IR's use in screening protein formulations.

BAND ASSIGNMENTS

As the structural repeat units of proteins, the peptide bond exhibits a number of IR-active amide bands (Table 1). Nine characteristic bands are termed amide A, B, and I–VII. Current understanding of IR spectra of proteins with regards to those amide bands is largely based on normal coordinate analysis, which was pioneered by Miyazawa and associates in their milestone work on *N*-methylacetamide (2, 3) and subsequently extended to more complex systems.

The amide A (located at about 3400 cm^{-1}) and B (about 3090 cm^{-1}) bands originate from a Fermi resonance between the first overtone of amide II and the N–H stretching vibration (Figure 3 in Part 1 of this article). Amides I and II are the major bands in the protein IR spectrum. Amide I absorption originates from the C=0 stretching vibration (70–85%) of the amide group (coupled to in-phase bending of the N–H bond and stretching of the C–N bond), which gives rise to IR band(s) in the region between ~1600 and 1700 cm^{-1}. That vibrational mode is directly related to the protein "backbone" conformation. Amide II comes from the N–H bending (40–60%) and C–N stretching vibrations (18–40%) and is conformationally sensitive. Amides III and IV are very complex bands resulting from mixtures of several coordinate displacements. Out-of-plane motions are found in amides V, VI, and VII.

Because of technical and theoretical limitations, only amide bands I–III are used for investigating protein secondary structure (4–7). Of all the peptide amide modes, however, the most extensively used in such studies is amide I. Major factors responsible for conformational sensitivity include hydrogen bonding and couplings between transition dipoles (6, 7) (see Figure 2 in Part 1).



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Transition dipole coupling that leads to splitting of the amide I mode. The magnitude of such splitting depends on the orientation of and distance between interacting dipoles. Thus it provides information about the geometrical arrangements of peptide groups in a polypeptide chain. Exact frequencies of amide I and II absorptions are influenced by the strength of hydrogen bonds involving amide C=O and N–H groups, as depicted by the equation in Figure 2 of Part 1 (1, 6, 7). Each element of secondary structural conformation (e.g., alpha helix or beta sheet) is associated with a characteristic

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hydrogen bonding pattern between those groups. So each type of secondary structure gives rise to different frequencies at which amide bond vibrations occur, thus producing characteristic overall amide I and II vibrations (Figure 1). Such separation of subcomponent bands (Figure 2) for an overall amide I absorption forms the basis of protein structure analysis and quantification from vibrational spectroscopic data (**8–10**).

The relationship between amide I band position and type of secondary structure is best recognized by analyzing IR spectra of simple homopolypeptides that fold into well-defined and often homogeneous (purely alpha-helical or beta-sheet) structures. By contrast with such homopolypeptides, proteins usually fold into complex three-dimensional structures that include a variety of domains containing polypeptide segments folded into different types of secondary structures. Because each conformational entity contributes to the molecule's IR spectrum, observed amide I band contours are complex composites. They consist of many overlapping component bands that represent different structural elements, e.g., alpha helices, beta sheets, turns, and nonordered or irregular structures (Figure 2). A fundamental difficulty encountered in analyzing such composite band contours arises from the fact that the width of each contributing component band is usually greater than the separation between the maxima of adjacent peaks (Figure 2). So individual bands cannot be resolved and/or identified in the broad contours of experimentally measured spectra. Extraction of structural information encoded in those IR bands requires extensive mathematical analysis of experimental data.

In analyzing the amide I band, we must consider that some absorptions arising from buffer components may absorb between 1600 and 1700 cm^{-1}, thus perturbing analysis of the amide I protein absorptions. The most common interference is an absorption band of water in that region, but contributions of amino acid side chain absorptions must also be considered (**11**).

Other bands such as amide II have been used for the elucidation of structural information of proteins. Inclusion of that band with amide I has been reported by some authors using multivariate data analysis techniques to provide improved prediction accuracy (**4, 12**). Moreover, the amide II band has been used for monitoring hydrogen–deuterium exchange in proteins, providing information on subtle structural changes in protein secondary structure. Some authors have also used amide III band data to derive information with respect to protein secondary structure (**7, 13, 14**).

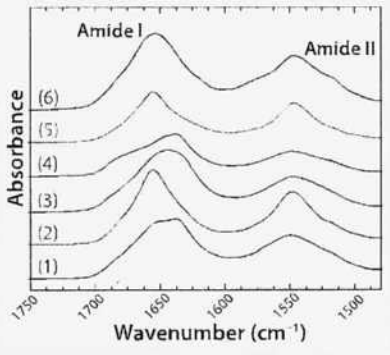
Figure 1 represents exemplarily different protein IR spectra obtained in transmission at protein concentrations of ~10 mg/mL in pure water at 25 °C. As can be deduced from the shape of the amide I absorption, secondary structural elements are different for these proteins. Hemoglobin shows a more or less symmetrical amide I absorption, whereas a broad amide I absorption is observed for alpha-lactalbumin and alcohol dehydrogenase. Such differences in overall amide I band shape come from different amounts of secondary structural elements in a protein structure.

Analysis and Applications

Within the 1600–1700 cm^{-1} IR spectral range, where the amide I absorption is detected, regions have been identified as being sensitive to particular secondary structural conformation. The "Band Assignments" box summarizes these structure sensitive regions within the amide I band. Hydrogen–deuterium exchange among amide hydrogen atoms causes a difference in band positions from those characteristically recorded in H_2O and D_2O.

Characterizing Protein Structure: Extraction of single-subcomponent bands is achieved using curve-fitting approaches to assign each subcomponent to a particular protein secondary structure. The principle of such procedures is to resolve an original protein structure into individual bands that fit its overall spectrum. To estimate the range and positions of discrete subcomponent absorption bands, band-narrowing techniques are applied. Based on this

Figure 1: FTIR spectra of various proteins in liquid formulation



[1] α-Lactalbumin
[2] Hemoglobin
[3] α-Chymotrypsin
[4] Alcohol dehydrogenase
[5] Human serum albumin
[6] α-1-Proteinase inhibitor

Table 1: Band assignments of the main infrared active vibrations of biomolecules

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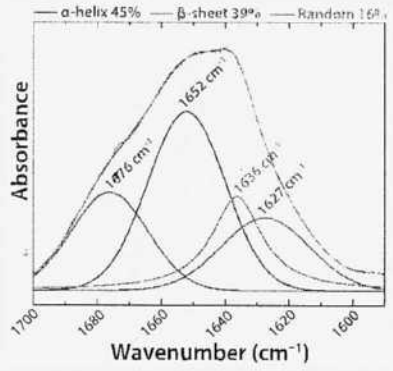
1080	Symmetric PO_2^- stretch
1047	C–OP stretch
980	Choline asymmetric stretch
625–770	Amide IV (mainly O=C-N deformation)
720	CH_2 rocking

Figure 2: An example of an amide I band with its underlying band subcomponents: alpha-helix (blue), beta-sheet (red), random coil (green)



procedure, secondary structure elements can be quantified.

One approach to extracting information on protein secondary structure from IR spectra is *Fourier self-deconvolution*, often referred as a "resolution enhancement" or "band narrowing" technique (**1, 15–17**). This procedure is based on an assumption that absorption bands are broadened (convoluted) in liquids by a certain function that causes a band overlap. A consequence is that the individual subcomponent bands cannot be distinguished in the overall amide I band. Fourier self-deconvolution decreases band widths, allowing separation of overlapping component bands that underlie a composite band's contour (**16**). This is also called deconvolution (**17**). The exact shape of the convolution function has not been determined. But it is assumed that Lorentzian and/or Gaussian functions are appropriate.

Two parameters are important for deconvolution: full width at half-height (FWHH) and a resolution enhancement factor. In most cases, experienced operators determine these two parameters. Their selection determines the number and peak maximum of resulting subcomponent bands. It is a critical operation that determines the quality of results for a curve fitting procedure.

Overlapping band separation can also be increased by calculating the second derivative (Figure 3) of an absorption spectrum, either in the frequency domain or through mathematical manipulations in the Fourier domain (**15, 18**). Note that spectral derivation does not preserve the relative intensities of absorption bands. They depend on the width of each absorption in an original spectrum, so narrow bands will be enhanced at the expense of broader bands. A distinct advantage of the Fourier self-deconvolution method is that it introduces less distortion. Particularly, it does not affect the integrated intensities (areas) of individual component bands. So the effect is not to increase instrumental resolution, but rather the extent to which individual component bands can be separated.

The degree of band narrowing (described by the resolution enhancement factor in Fourier deconvolution and the degree of derivation in derivative spectroscopy) is limited by a spectrum's signal/noise ratio. So analysts should avoid "overdeconvolution" or using higher derivatives, because noise will also be amplified and can be easily misinterpreted as a real band (**10**).

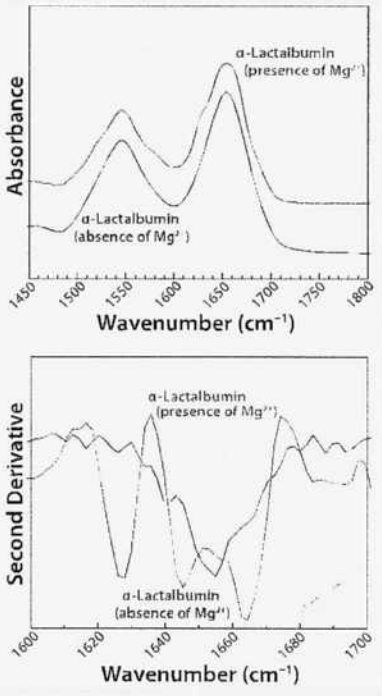
Another challenge can arise from the presence of atmospheric water vapor, which gives rise to narrow absorption bands especially in the region overlapping the main protein bands. Although the overlapping bands are often very weak in an original spectrum, their relative sharpness makes them disproportionally amplified upon either Fourier deconvolution or derivation. They may appear in a resolution-enhanced spectrum as artifacts that are indistinguishable from the real components of a protein band. This problem can be elegantly eliminated by technical solutions such as purging spectrometers with dry air. If necessary, residual water vapor absorption may be compensated for by spectral subtraction.

Figure 2 shows determination of secondary structural elements for a chemokine at a 1 mg/mL concentration in PBS buffer at pH 7.4 (H_2O, 25 °C). A Gaussian, Lorentzian, or mixed Gaussian–Lorentzian shaped curve is determined that best fits the original protein spectra (the black curve represents the original spectrum, and the magenta curve represents overall fit). To get initial parameters (band height, width, position, and baseline) for starting a fitting procedure for the individual subcomponent bands, Fourier self-deconvolution was used here. The best fit was obtained by a root mean-square analysis determining the optimal set of curve-fitting parameters, then the corresponding subcomponent band was determined. The area of each individual band is used to calculate its relative contribution to a particular protein secondary structure in relation to the overall area of the original spectrum.

In the example, the overall amide I band was fitted assuming just three secondary structural elements: alpha-helical, beta-sheet, and random coil structures. Analysis revealed that the protein structure is about 45% alpha-helical, 39% beta-sheet, and 16% random coil structures. Those results are in accordance with X-ray data. Our procedure assumes that the carbonyl molar absorption coefficient is equal for each individual structure.

Other possibilities for the determination of secondary protein structure are based on pattern

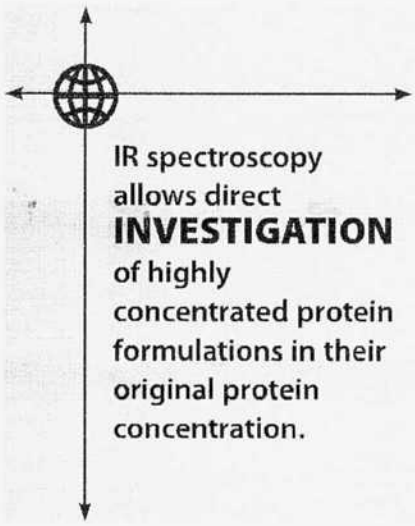
Figure 3: α-Lactalbumin in the respective presence and absence of magnesium ions; (TOP) IR spectra, (BOTTOM) second derivative of the amide I region



recognition. These methods use calibration matrices of IR spectra for proteins of known secondary structure (usually determined by X-ray crystallography). One matrix consists of target secondary structure fractions for proteins in a reference set; the other consists of their absorption data. The combined matrices serve as a calibration set. Based on that "training" set, a multivariate regression model is derived for predicting secondary structure elements of a "new" protein. The most widely used regression methods include factor analysis, principal component analysis, and singular value decomposition (17–22). Partial least-square methods and the methodology of factor analysis and multiple linear regressions are both routinely used, being implemented in commercially available software. In recent years, neural network analysis and two-dimensional correlation spectroscopy have become more important (23–26).

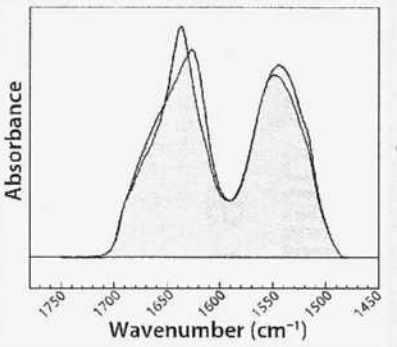
Testing Formulations: A number of excipients are known to bind to proteins or affect their stability. Protein–excipient interactions can easily be investigated using IR spectroscopy. For example, divalent magnesium cations interact with alpha-lactalbumin (Figure 3). Figure 3 TOP shows the original spectra (10 mg/mL, phosphate buffer, pH 6.2, 25 °C), and Figure 3 BOTTOM shows second-derivative spectra. The presence of magnesium cations induces a distortion of the amide I band, as illustrated by the appearance of a "shoulder" at about ~1630 cm^{-1}. It is clearer in the second derivative (Figure 3 BOTTOM). IR spectroscopic analysis of the amide I absorption revealed magnesium binding to the protein and inducing changes to its secondary structure.

In a number of cases during biopharmaceutical development, analysts may be interested only in recording differences between various protein formulations. The idea of such analysis is to compare an initial state of the protein product with its structures in a selected formulation — e.g., after being stressed by temperature or pH. Various approaches have been described, with "visual" comparison of IR spectra in the 1700–1200 cm^{-1} spectral region (amide I, II, and III absorptions) often used to identify spectral deviations. However, it is still unclear what constitutes "large" or "small" differences. In most approaches, correlation coefficients are calculated, which express the common features and similarities of the two compared spectra (27–29). A correlation coefficient for identical spectra can be set to 1, with the correlation coefficient at 0, when both spectra have nothing in common. The area overlap method implemented by several different groups uses the integrated difference between two area-normalized spectra (Figure 4). Each approach uses both raw and resolution-enhanced spectra.



Another useful method for detection of small changes in protein structure is difference spectroscopy, which involves subtracting a protein absorbance spectrum in one state from that of the same protein in another state. For example, the FTIR spectrum of a sample is obtained before and after being triggered by a particular effect that induces two different states of the protein (e.g., pH change, light, heat, or addition of an interaction partner). This approach reduces the complexity of interpreting conformational protein changes induced by the trigger, and the difference spectrum reflects only those groups that undergo a specific change

Figure 4: Example of an area overlap of two raw spectra. The area overlap of the two spectra (90 %) is indicated in yellow.



in their structure or environment (Figure 5).

Figure 5A shows the results from a FTIR–HATR experiment representing thermal-induced denaturation of an antibody at a concentration of 50 mg/mL. Up to a temperature of 60 °C, amide I changes are marginal (data not shown). Above 60 °C, however, clear changes are seen: The overall band intensity increases considerably. More important are changes in the band shape, indicating formation of intermolecular beta-sheet structures. Using the spectrum taken at 25 °C as a reference, derivative spectra could be calculated. These are plotted in Figure 5B, showing changes induced by thermal stress. The area of those changes can be calculated and plotted as a function of temperature.

Such protocols are used for fast screening of protein formulations as a function of temperature. The experiment is especially suited for analyzing highly concentrated protein formulations. Figure 6 shows denaturation curves for a 60-mg/mL protein solution at three different pH values. Denaturation temperature can be determined from the curve's sigmoidal shape. In the example, a formulation at pH 4.0 considerably increases thermally induced stability of the protein. Data are somewhat different when you compare the same formulations at much lower protein concentrations. IR spectroscopy thus allows direct investigation of highly concentrated protein formulations in their original protein concentration. Using highly sensitive calorimetric

Figure 5: (LEFT) Structural changes in the spectral region between 1600 and 1720 cm^{-1} representing the amide I absorption as a function of temperature; (RIGHT) difference spectroscopy (temperature dependence of the amide I absorption)

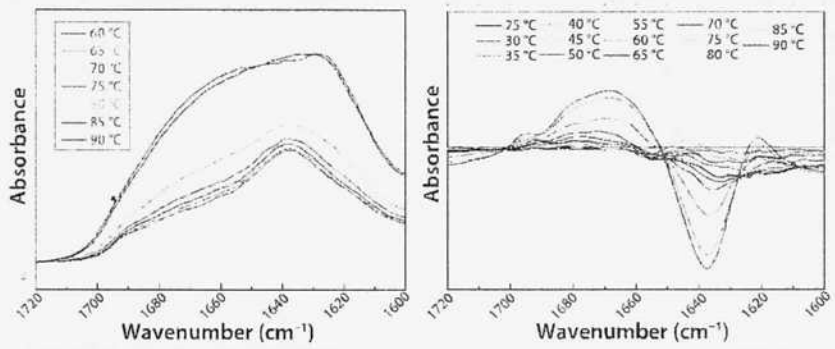
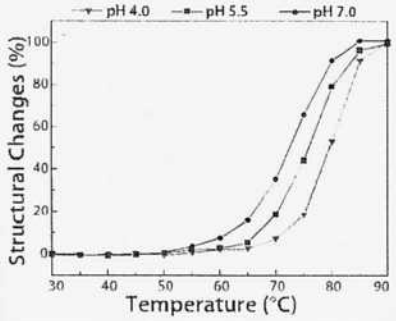


Figure 6: Formulation screening of a protein solution at 60 mg/mL in water at different pH values (temperature dependence of the amide I)



methods, protein concentration is usually limited to 0.1–10 mg/mL (depending on the protein involved).

IR spectroscopy is very helpful for a number of other applications of elucidating structural protein changes in formulation. Information concerning formation of protein aggregates can also be derived from FTIR measurements (30–33). Appearance of strong absorption bands below 1620 cm^{-1} often correlates with aggregation — usually associated with formation of strong beta-sheet structures. However, absence of such features in the amide I region does not indicate an absence of protein aggregates (34).

A Formulator's Cool Tool

Fourier-transform IR spectroscopy is a powerful technique for structural and conformational characterization of proteins because of its ability to test samples under different physical conditions: aqueous and nonaqueous solutions as well as dry samples.

Band Assignments

Band assignments of the subcomponent bands of the amide I absorption (data for a protein in H_2O):

1620–1640 cm^{-1}: Beta sheets

1640–1650 cm^{-1}: Nonordered structures

1650–1658 cm^{-1}: Alpha helices

1660–1680 cm^{-1}: Loops

1670–1695 cm^{-1}: Beta sheets

Measurements can be done for liquid formulations containing 1 to at least 100 mg/mL protein.

FTIR is well suited for studying protein stability in the development of protein formulations. It finds application for

- fast determination of protein secondary structure in solution or dry
- biopharmaceutical quality control
- biomolecular interactions (protein–ligand binding) monitoring
- protein determination and concentration measurements
- study of aggregation and fibrillation processes
- detection of conformational changes (e.g., due to altered pH or ionic strength)
- investigation of temperature and buffer influence
- formulation screening for highly concentrated protein formulations.

Acknowledgments

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Dec 02 2017 Page 214

One of the simplest ways to demonstrate uniqueness of a material is with cyclic voltammetry.

[-2V to +2V] is more than sufficient in most cases. It is also a very fast technique and very simple w/ a convergent solution.

Therefore our arsenal already includes

1. Open Circuit Potential (Relative Oxidation - Reduction Capacity)
2. Chronopotentiometry.
3. Cyclic Voltammetry. ~5 min per species
   Quick, Illustrative, Good for characteristic profile
4. Cyclic AC Voltammetry
   Excellent for species identification
5. Cyclic Normal Pulse Voltammetry
   Good for species identification, especially as backup.

I am now testing repeatability of cyclic voltammetry.

Here is what is happening:

The solutions are different from one another,
But only as a function of and as a point in time.

This looks to be very important. The behavior (and result) from the electrode appear to be a function of time.

This could mean either time a conditioning of electrode will be required to produce a useful solution.
This can be fine as long as it remains under control.

Notice you are also running solutions one direction, ie [2, -2] is the order of each cycle.

I can see that the solution is converging as a function of time.

Now we will reverse the direction from [-2, 2].
I am getting the same result.

The solution is converging but I am getting quite a few overloads. This likely means that the solution is too concentrated.

We have a problem here. With the passage of time all samples are converging toward the same solution.

Let's condition the electrodes and repeat the scrub.
On the initial run, all samples are highly different
@ that early point in time.

I am conditioning in dilute HCl w/ distilled water.
Chronopotentiometry settings are

$I = 10\ \mu A$ — I have changed to 1 mA.
$\Delta t = .01$ — I can visually see O_2 production
$t_{run} = 120\ sec$ — this way.

The conditioning of the electrodes does not seem to be sufficient to me. We still have a convergence of electrode behavior taking place.

Pretreatment? Conditioning prior to each run?

One of the first lessons is that you are not allowed excessive underloads or overloads!
This means the solution is either too strong or too weak to be in range.
It does not need to be strong @ all.

Ok, we have learned a lot here!

1. No excessive underloads or overloads allowed!
2. Concentrations must be in range to provide this.
3. OCP measurement IS REQUIRED!

In addition, you are seeing that the extended time of microwave digestion on the 1999 EPA filament may have depleted the sample. Or possibly because it is so old it is depleted.

Either way, IR, UV, NIR & cyclic voltammetry of the 1999 extended microwave digested sample is far less active than the alternative sample which is of more recent vintage. This is quite an eye opener.

And we see that hair and spider web are again much more closely related than the environmental filament is to either.

OCP must be incorporated into cyclic voltammetry & no excessive over or underloads.

You now have 3 very different but individually convergent cyclic voltammograms that are no longer dependent upon time.

A major success here now.

Dec 03 2017

It can be shown that the presence of dilute denatured alcohol can be shown with differential cyclic voltammetry. Plot on next page.

This continues to demonstrate the sensitivity and effectiveness of diff. cyclic voltammetry for detection, dependably in a spectrometric sense.

Uniqueness of solutions can be demonstrated by simply and clearly w/ this electrochemical method.

How they dilute MEK within denatured alcohol?

Denatured alcohol in H_2O is the most responsive of the trials that were conducted:

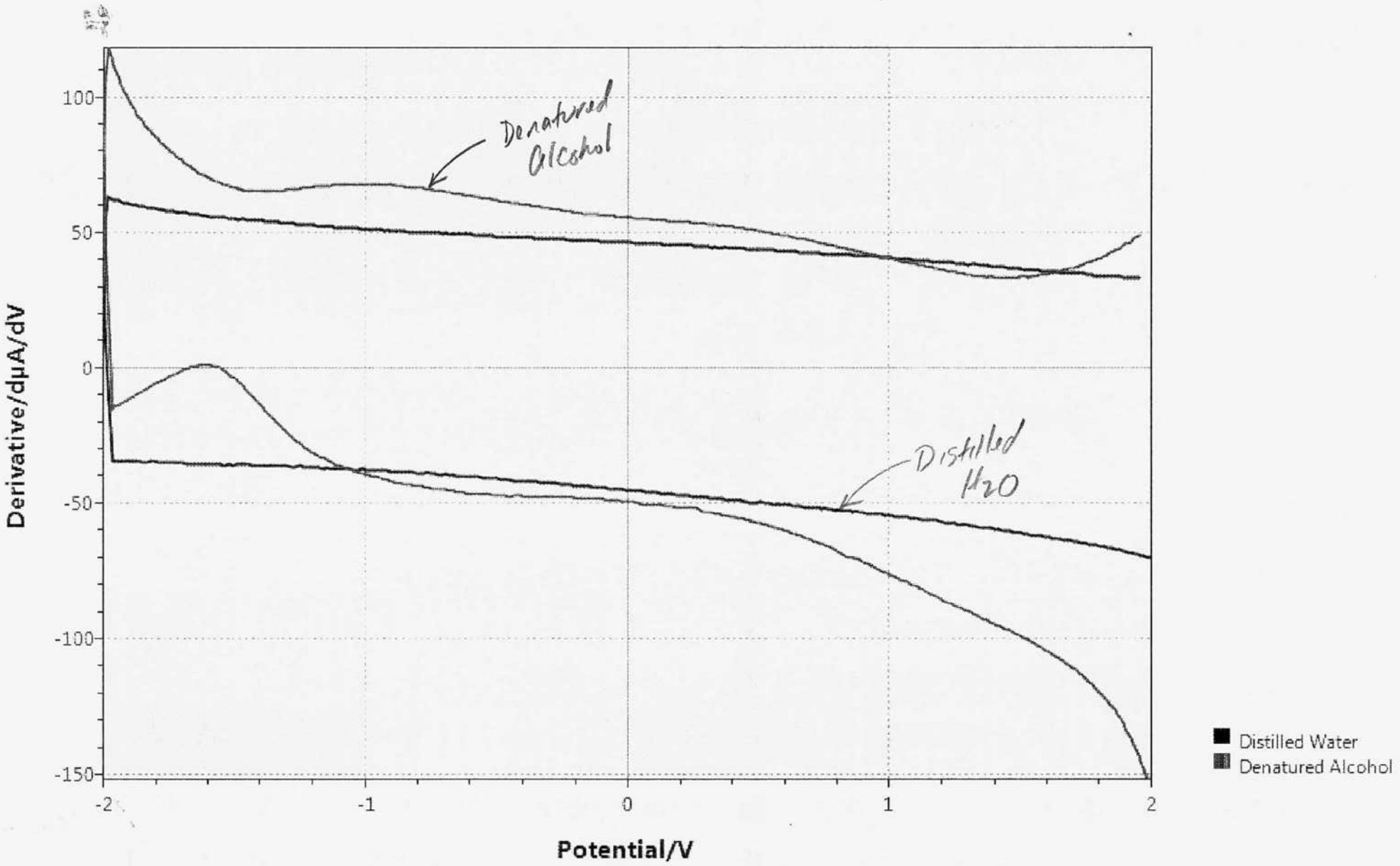
1. Distilled H_2O
2. Distilled H_2O w/ Den. Alcohol
3. Full Strength Den. Alcohol
4. MEK dilute in Den. Alcohol
5. MEK dilute in Distilled H_2O

but they all do have a detectable response in some fashion.

We see that elementation of over & underloads seems to be far more influential than excluding OCP but we will continue w/ the OCP Protocol. It's inclusion must be checked each time as it seems to have become unchecked.

Vit C is very responsive; it did not, however, double back on itself w/ multiple scans.

Denatured Alcohol vs Distilled Water - DIfferential Cyclic Voltammetry



Travel Project Proposals

We are not going to have infrared capability on the road. We should, however, have some options available with

1. electrochemistry
2. VIS spectrometry (Cary 4 & UV cuvettes) & optic probe!
3. a slight touch of NIR available
4. Hopefully some colorimetric tests (Cary dye & chemicals?)
5. ~~Reflectance spectrometry~~
6. Ultrasound
7. USB Microscope
8. ORP meter & pH
9. Urine tester

A Chemistry set will of course be invaluable.
The above does indeed seem to be your list of instrumentation

1. Working w/ blood, saliva & urine are very strong topics for research.

2. Optic probe on VIS spec provide for interesting topics.

3. The Legacy notebook are important to work with.

4. What is your focus in electrochemistry?
   1. Rainwater
   2. Body fluids
   3. Proteins available
   4. Water Quality
   5. Plant analysis? – No microwave, no torch
   6. EIS – why do it?
   7. Culture analysis

5. Virtual Chemistry Study?

If you go w/ Vertical Chemistry study, what book would you bring?

6. You have a series of Udemy courses.

7. You have fantastic water & soil testing kits to carry. Below the is too much and your own water test kit that you have developed.?

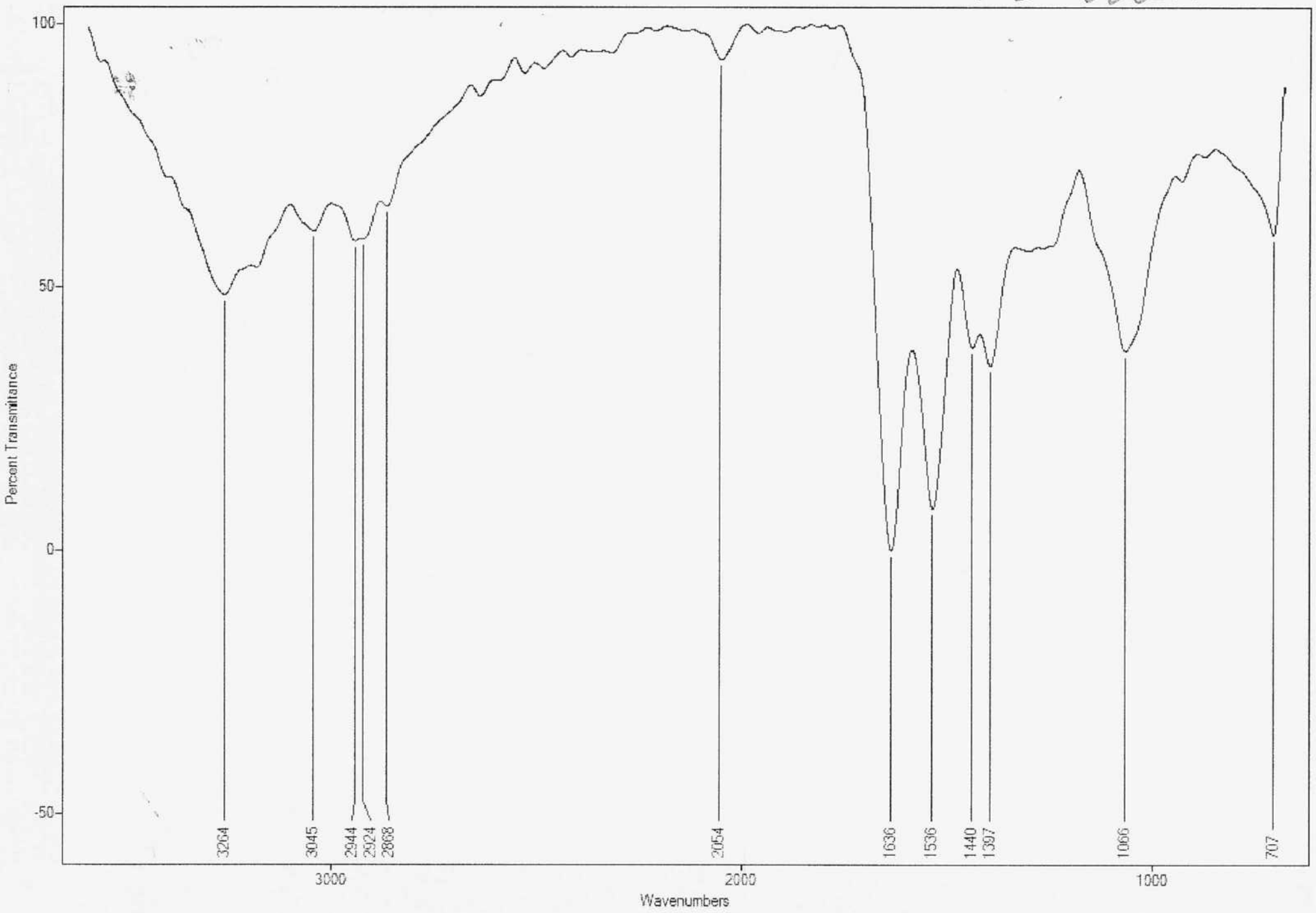
Here is where you run the risk of carrying too much.

Your proposed set of 1-6 is more than enough for the winter trip.

Carrying volumes 20 & 21 will be sufficient also for notes.

IR Saliva - CEC ATR

Saliva CEC ATR Dec 03 2017 - 01.JPEG



IR Blood CEC ATR

Blood CEC ATR Dec 03 2017 - 01.JPEG

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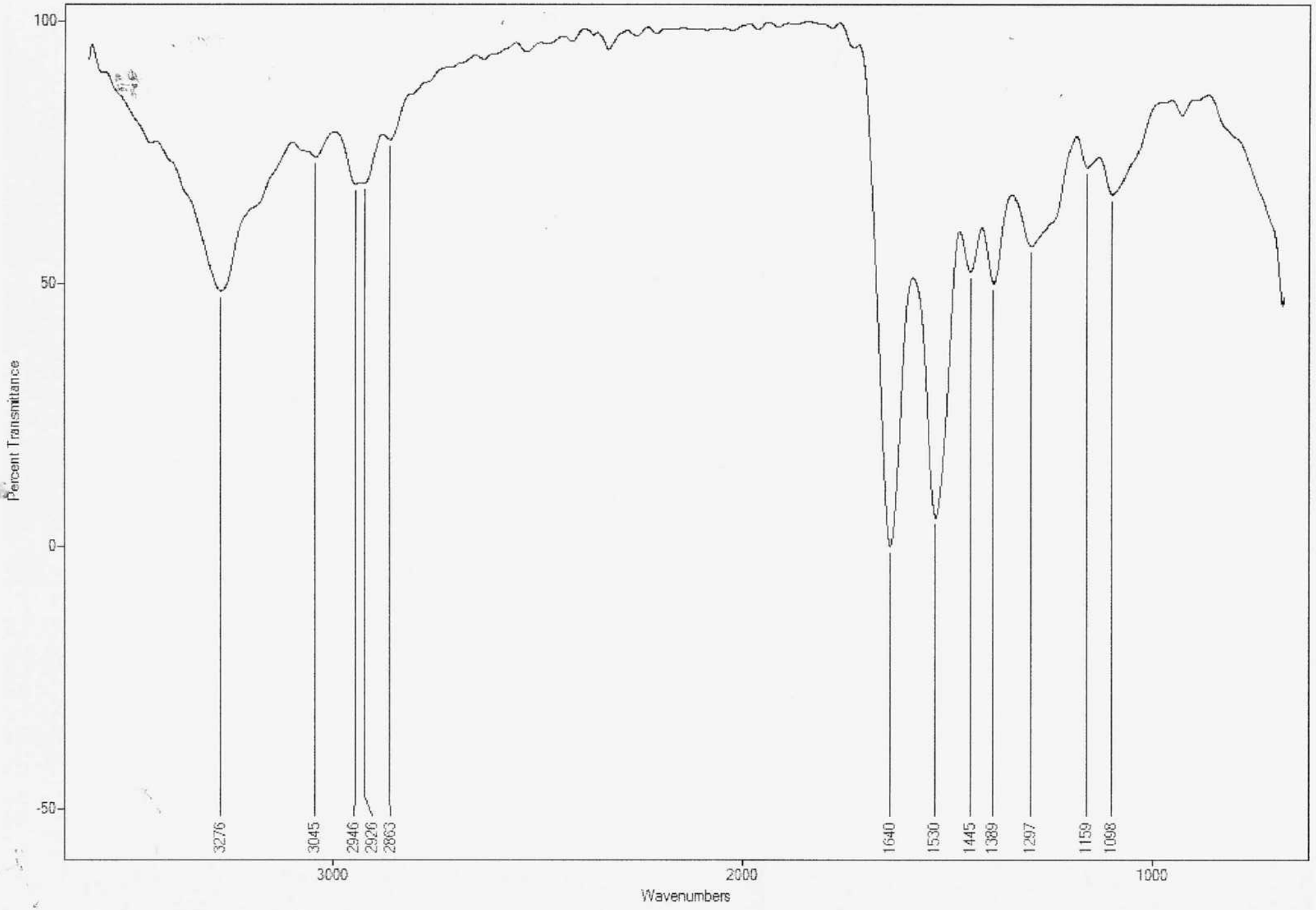

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A STUDY ON INFRARED SPECTROSCOPY OF HUMAN BLOOD

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Abstract: The paper reports IR spectroscopic data on human blood and its constituents. IR analysis has been made on whole blood, plasma and serum. The characteristic spectral bands pertaining to fibrinogen, hemoglobin, erythrocyte membrane lipids and other plasma proteins are identified. The paper explores the possibility of disease analysis by IR spectroscopy.
Keywords: FTIR spectroscopy; Human blood; Plasma; Blood Serum.

1. Introduction

In recent past, mid infrared and UV - Visible spectroscopic methods were efficiently used in the fields of biological sciences [1, 2]. Implementation of these techniques reduces time, resources and cuts cost. IR spectroscopy is emerging as a potential diagnostic tool in the medical and pharmacological fields to provide information about the different chemical structures of healthy and pathological tissues [3]. Blood being the chief circulatory medium of our body, reflects the physiological and pathological changes that take place in the tissues, which lead to the changes in the various plasma, serum and cellular constituents. In view of this, an attempt is made to analyse human blood spectroscopically in the IR range.

2. Materials and Methods

2.1. Sample collection

Collection and handling of a sample is an integral part of obtaining valid results. Here a disposable plastic syringe was used to collect venous blood. Blood samples were collected from healthy volunteers. Blood collection tubes with anticoagulant (EDTA- Ethylene Diamine Tetra Acetate) were inverted gently as soon after collection as possible to prevent clotting. The blood samples were brought to the laboratory in siliconized bottles, keeping them in ice cooled thermos. The samples were kept in refrigerator at 4^0C until used. Investigations were done within two to three hours after collection.

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2.2. Preparation of sample

First, spectral grade pure KBr powder was dried in an oven upto 60^0C for 24 hours. Then 1 gm powder was taken in an agate motor and was ground until it becomes fine powder. The ground powder was mixed with blood sample and transferred into the bore of a cylinder so that it was distributed across the polished face of lower plate. The polished face of the second plate towards the powder was inserted in to the bore by a plunger. The die assembly was connected to a vacuum pump and was kept under vacuum for approximately 2 min so as to remove air from the sample disk. The die was dismantled and the KBr disk was removed without touching its faces. Here, FTIR spectrometer of make *Bruker Optics* and model *Tensor 27* was used.

3. Results and Discussion

Fig. 1. presents FTIR spectrum of Human blood, which reveals a series of bands with different intensities and the spectral data is shown in Table 1. For the systematic analysis, IR spectrum is divided into three regions. Region I is from 4000 to 3000 cm^{-1}, concerned with water and hydroxyl group. This region is of considerable interest, because it reveals the nature of hydrogen bonding. Region II is 3000 to 1500 cm^{-1}, wherein bands for functional groups are observed. In this region, major IR absorption pertaining to fibrinogen occurs. Region III is 1500 – 200 cm^{-1}, which has significant importance in the context of biological minerals and their combinations.

The spectra of human blood indicate the presence of bands characteristics of water molecule and also of some functional groups concerned with proteins and lipids. The IR band at wave numbers 3294 cm^{-1} and 3065 cm^{-1} are related to Amide A and Amide B respectively. The dominating band at 1396 cm^{-1} may be originated due to the important protein of blood Fibrinogen. This band is related to the stretching C=O symmetric stretching vibrations of COO^-. A band around 2960 cm^{-1} is due to the -C-H asymmetric stretching of $-CH_3$ in Fatty acids, Phospholipids and Cholesterol esters. The band at 1106 cm^{-1} is related to HbO_2, exhibits $\nu_{(O2)}$ bond. The two most intensive bands are centered at 1652 cm^{-1} and 1547 cm^{-1} in the FTIR spectrum of human blood. They correspond to the Amide I and Amide II. Both bands are representative of secondary structures of proteins. Amide I peak arises from C=O hydrogen bonded stretching vibrations, and Amide II is attributed to C-N stretching; NH and CH_2 bending modes. Amide I and Amide II absorption bands are associated also with specific secondary sub- structures, such as α-helix, β-sheet, β-turn and random coil. The bands at

1307 cm^{-1} and 1248 cm^{-1} are related to Amide III bond components of proteins (C-N). The band at 1170 cm^{-1} corresponds to C-O-C asymmetric stretching vibrations of phospholipids. The bands at 1106 cm^{-1}, 1170 cm^{-1} and 1248 cm^{-1} are associated with triglycerides of human blood. The band at 2936 cm^{-1} is related to platelets due to -C-H symmetric stretching of $-CH_2$

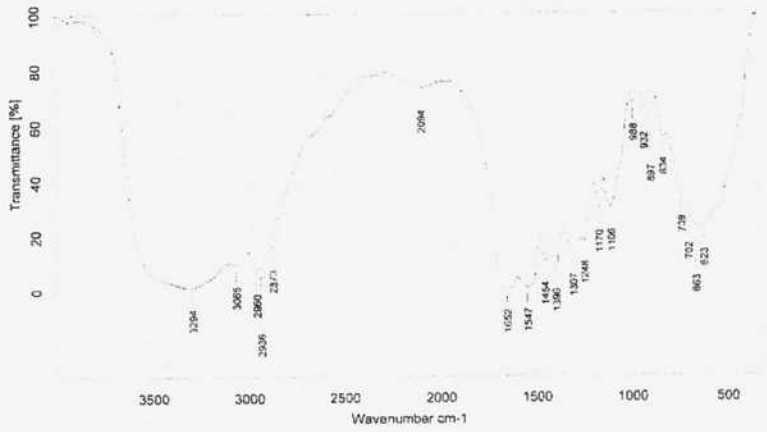

Fig. 1. FTIR spectra of human blood

Table 1- FTIR spectral data of human blood

Wave Number (cm^{-1})	*Characteristic vibrations of functional groups*
3294	Amide A, N-H stretching of proteins
3065	Amide B, N-H stretching
2960	-C-H asymmetric stretching of $-CH_3$ in **Fatty acids**, **Phospholipids**, **Cholesterol esters**
2936	-C-H symmetric stretching of $-CH_2$, **Platelets**
2873	-C-H symmetric stretching of $-CH_3$
2094	
1652	Amide I. – helical structures (C=O)
1547; 1454	Amide II Peak region – Protein (NH, C-N) CH_2 bend
1396	C=O symmetric stretching vibrations of COO^- **Fibrinogen**
1307, 1248	Amide III band components of proteins (C-N)
1170	C-O-C asymmetric stretching vibrations of phospholipids
1106	HbO_2 exhibits $\nu_{(O2)}$ band, **Oxy hemoglobin**
1106, 1170, 1248	Triglycerides

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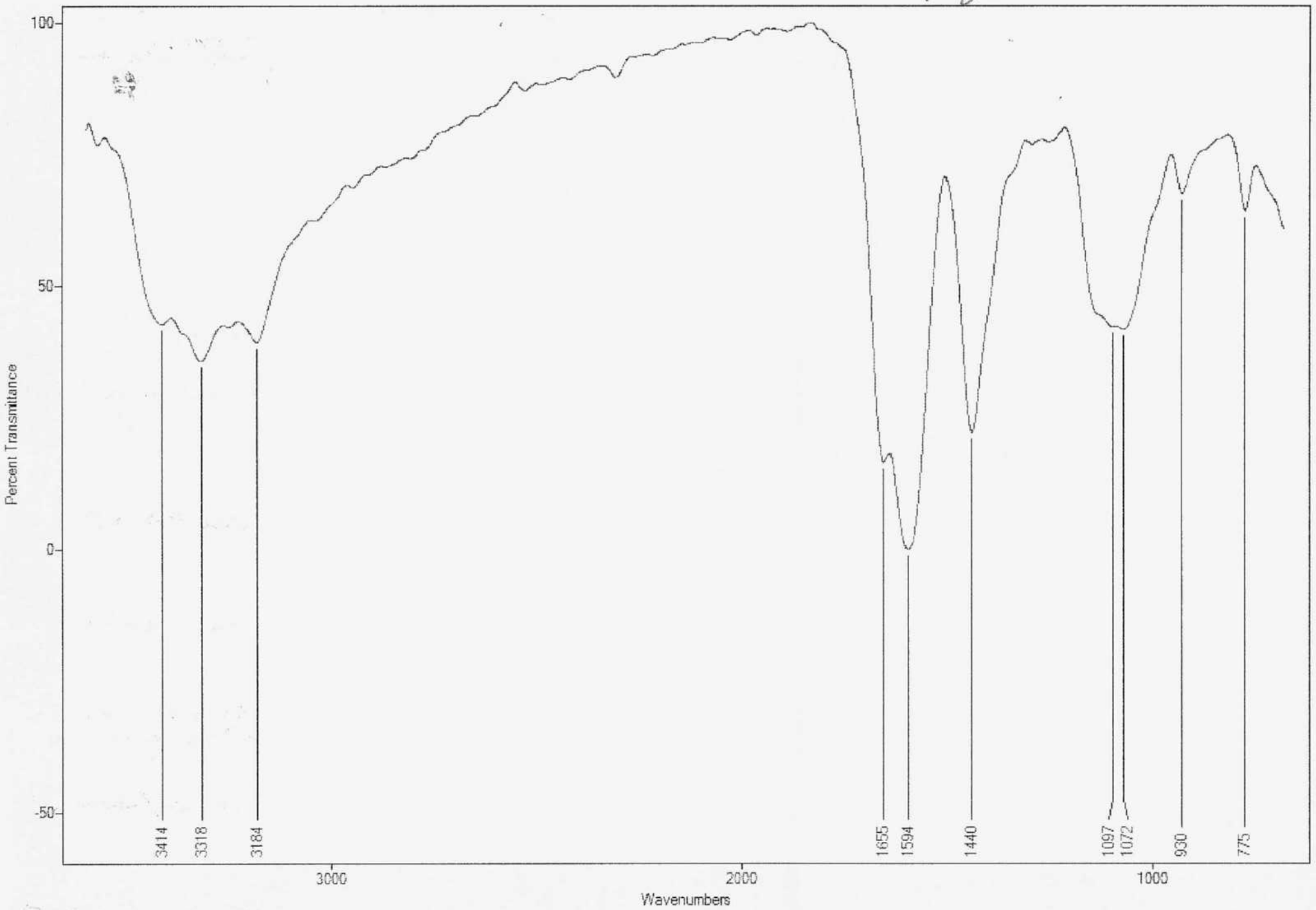
IR Urine CEC ATR

Urine CEC ATR Dec 03 2017 - 01.JPEG



Dec 05 2017

There are some refinements taken place w/ the OCP (open circuit potential) Cyclic voltammetry work (not AC). I find that it set more uniform results if the resolution of the scan is increased. It is also separating the curves and spikes much better.

I now have EStep set to .01 sec
and the scan rate dropped to 0.1 sec
and [2, -2]

OCP is set for Measure OCP
EVertex1 VS OCP
& tmax OCP = 10sec
Stability 0.1sec
] These simply may not be required.

Actually it is making a difference.

A longer tmax OCP stabilized the exact onset of the voltammogram.

You have actually learned how to avoid the crossover spike if the OCP after tmax OCP concludes is less than the start point (ie [-2]) you need to adjust tmax OCP and the Stability Criteria so that the voltammogram starts @ that point.
eg tmax OCP = 15sec produces a much better result.
Stability = .01 sec

OK you do not need OCP. You only need a light resolution to show the crossover property. Derivatives are highly unique

Lets look @ the saliva, blood & urine samples again under the higher resolution cyclic voltammetry. You do not seem to need OCP measurements; it always seem to converge nicely even w/ the up crossover.

Crossover does not seem to express an error, you do all one is up & one is down. It does seem to express a strong lack of reversibility however.

We can see that [redacted] urine samples differ quite a bit. [redacted]

The saliva samples are at a high level of agreement.

You achieve excellent convergence in all cases w/ 3 cycles.

The signatures of my blood, urine & saliva are remarkably similar @ the first derivative level.

In the second sample set the urine departs from the blood & saliva more so.

Saliva signature (4') is only moderately reasonably similar between the two individuals.

Urine is not very similar between the two individuals

Blood is only moderately similar between the two individuals.

I think the focus would shift to where the two curves most markedly differ.

Such differences occur in all paired sets between the two individuals, i.e.

1. Saliva differs with electrochemical segments, or ranges between the two individuals.
   4 major differences exist between the two individuals.

2. There three major differences in the urine between the two individuals.

3. Two major differences in the blood signature between the two individuals.

You would now progress toward species identification between the two individuals, w.r.t. Urine, blood & saliva. You would eventually learn likely learn of important differences between the two individuals.

I am now plugging away @ the thiocyanate issue. I have an excellent IR spectrum of sodium thiocyanate solution evaporated. Solution is weak but very good quality thiocyanate absorption peak.

I also have a thiocyanate peak from cigarette smoke placed into the gas cell. It took three tries and a good reference spectrum. Gain for gas cell now needs to be set to 10. How do you know that it is not an alkyne.

You notice that 2nd generation heating of the test tube is producing a better spectrum w/ respect to hydrocarbon production.

Trust is you do have an alkene here w/ thiacco smoke so I am not sure how you can prove the 2108 peak as a cyanide complex vs an alkene.

You must purge the cell w/ the aquarium pump when you are done.

While taking the same spectra you can double up the gas concentration if you are quick enough.

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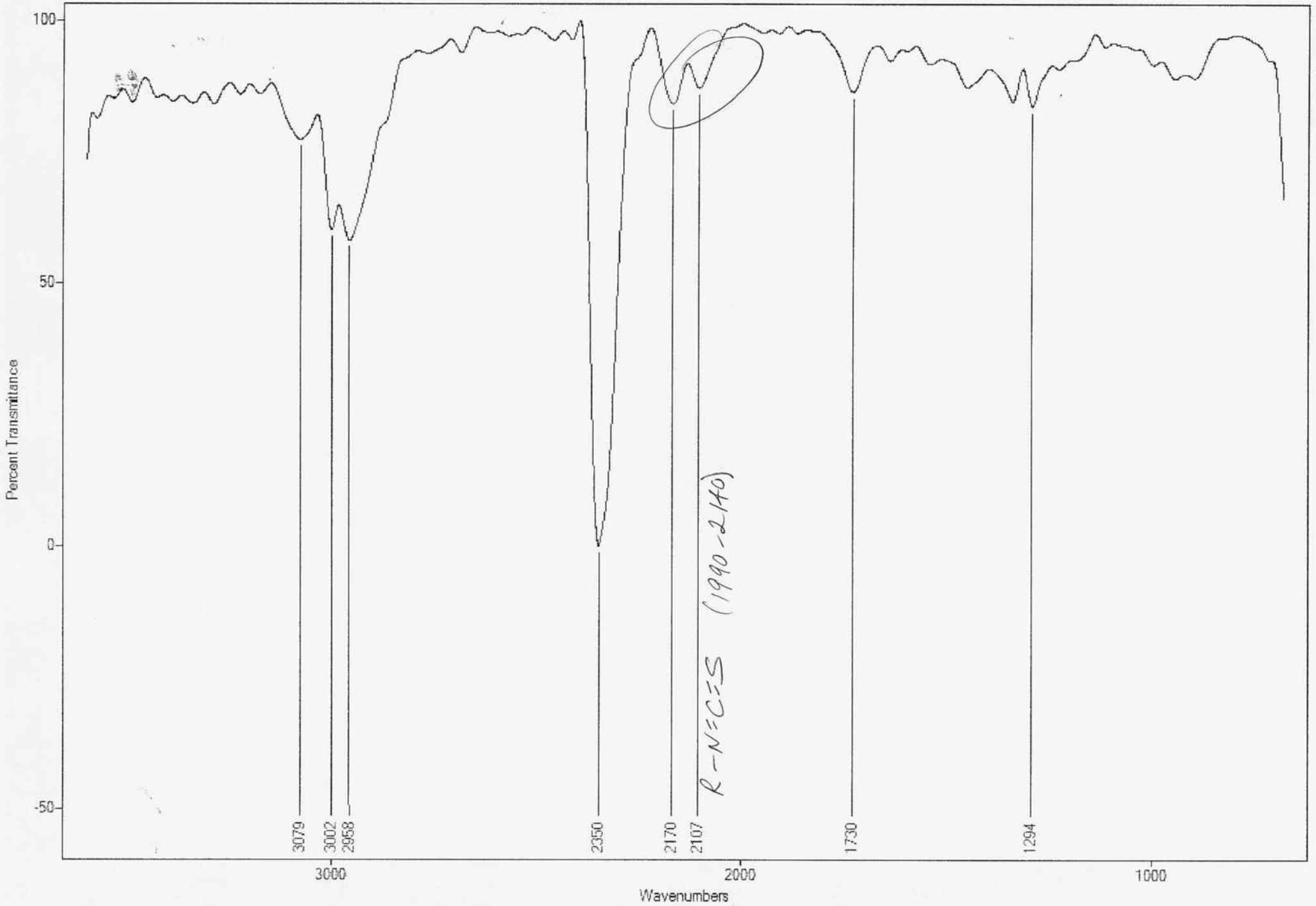
NO!

Cigarette Smoke - Gas Cell 2107: ~~Alkenes~~ or Cyanide Complex?

Alkynes, not alkenes are from 3310 - 3270 - You do not have.

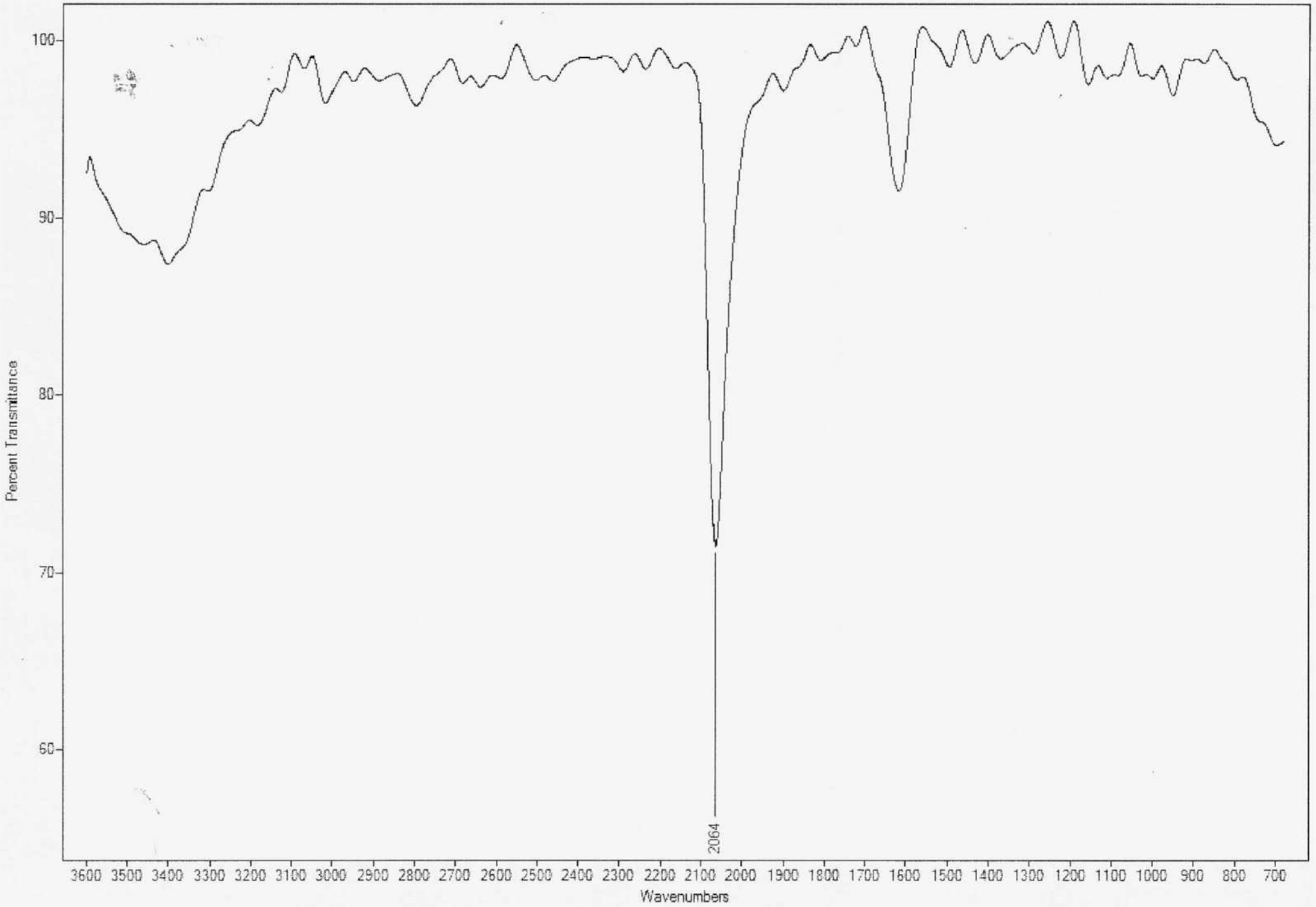
Cigarette Smoke Gas Cell Avg Dec 05 2017 - 04.JPEG



Sodium Thiocyanate Solution - Evaporated - ATR

Sodium Thiocyanate Solution Evaporated ATR Dec 04 2017 - 01.JPEG

Page 230A



Dec 06 2017 Page 232

We have an important colorimetric test that has now emerged for the thiocyanate ion SCN^-

It requires the Fe^{+3} ion. We have ferric nitrate available.

$Fe(NO_3)_3 \Rightarrow MW = 241.86$ gms/mole

Lets consider a 0.1M solution: $0.1(241.86) = 24.19$ gms/mole

$\frac{24.19 \text{ gms}}{1000 \text{ ml}} = \frac{X}{60 \text{ ml}}$ $X = 1.45$ gms.

Lets create this. Weigh boat: 3.20 gms
+ 1.45
4.65 gms

The colorimetric test appears to be quite sensitive.
We have detectable color usually w/ ~ 40 ul in ~ 5 ml H_2O of both 0.1M $Fe(NO_3)_3$ & Na-Thiocyanate each

$\frac{40 \text{ E-6 l}}{5\text{E-3 l}} = .008\%$ by volume

No of moles of sodium thiocyanate NaSCN :: MW = 81.072 gms/mole

40 ul of 0.1M NaSCN:

0.1 M NaSCN = $\frac{8.11 \text{ gms}}{1000 \text{ ml}}$

but we have 40 ul : $\frac{40\text{E-6 ml}}{1000 \text{ ml/l}}$ = ~~4E-8 liters~~ = 4E-5 liters

and $\frac{8.11 \text{ gms}}{\text{liter}} (4E\text{-}5 \text{ liters}) = 3.24E\text{-}4 \text{ gms}$ of NaSCN in pipette
$(= 0.32 \text{ mg})$

Now $\frac{3.24E\text{-}4 \text{ gms}}{5E\text{-}3 \text{ l}} = \frac{X}{1000 \text{ l}}$ $\quad X = \frac{6.48E\text{-}2 \text{ gms}}{1 \text{ liter}}$

and $\frac{6.48E\text{-}2 \text{ gms}}{81.072 \text{ gms/mole}} = 7.99E\text{-}4 \text{ M} \doteq 8.0E\text{-}4 \text{ M}$ solution

Therefore we see that we have easy visual identification of the SCN^- ion @ a ~ 8E-4 M Concentration of sodium thiocyanate. We can eventually test the limits of detection by both VIS spectrometry and hopefully by electrochemistry as well.

441 nm is claimed to be max absorbance, lets see what we have for the ~ 8E-4 M solution.
Actually the max looks to be below 400 nm.
Lets set range from 220 to 500 nm.

Max absorption is actually occurring @ ~ 231.5 nm. It increases continually & steadily from 500 to ~ 250 nm. Then we have a strong peak @ ~ 231.5 nm.
This suggests that the 441 nm point was actually quite arbitrary.

We do not have 230 nm capabilities on the road.
The range of the Pasco is from 380 - 950 nm.
The equipment was #400 + light probe = #500.
I think that you will need to choose 400 nm as your reference point. What about Brochem Cal analyzer?
It has filters @ 340 nm & 405 nm.

So in the long run, if you were to choose 405 nm you would be able to use both pasco and the Contrex Biochemical analyzer. This could be useful down the road.

Now let's see what max absorbance of a stronger solution is (more orange or red in visible).

Also let's compare $Fe(NO_3)_3$ by itself.

The absorbance @ 340 nm is ~3 times greater than @ 405 nm. It is interesting that the Contrex is actually working slightly in the UV range. Just @ the edge like the ThermoScientific model.

The stronger solution has a peak @ ~294 nm. And also a slight increase near 475 nm. This indeed approaches the range section

Now let's look @ $Fe(NO_3)_3$ by itself
I have taken 4 drops of $Fe(NO_3)_3$ & in 5 mL of H_2O.
This is therefore a reasonably strong concentration.

It actually starts to increase in absorbance right around 420 nm and it has high UV absorbance.

441 nm is actually not a bad choice. $Fe NO_3$ is actually quite low here, a reference can easily be subtracted if need be, and a strong SCN^- solution will absorb very nicely!

Contec has no real filter in the range. Their selection
340 405 510 546 578 620
therefore Contec offers no particular advantage here.

450 nm is not a bad choice. Let's adopt it.
If you have concern you can always subtract a reference.

Can we find thio now in saliva, urine, blood & hair?

On hair, I see that it is also producing a precipitate.
But even upon separation of the I still see increased absorbance @ 450 nm. Let's recalibrate w/ a focus of 400 to 500 nm.

		450 nm (Absorbance)
Scan 1	- Weak SCN Control	Ø.12
2	- $Fe(NO_3)$ alone (50 ul Ø.1 M)	Ø.08
3	- Hair, centrifuged 1 drop Ø.1 M $Fe(NO_3)_3$	Ø.2Ø, Ø.12
4	- Moderate - SCN - Control	Ø.38

Ok, now we have a clear pattern of absorbance in place.
It seems that our next need is to work on the concentration problem. This will be a standard Beer's Problem.

5	Urine Alone (400 ul in 5 ml)	Ø.04
6	Urine + (Fe^{+3}) (400 ul in 5 ml)	Ø.08

You therefore do have an indication of low levels (?) of thiocyanate in the urine.

Therefore, we clearly need to work out a regression Concentration.

Calibrate syringe as alternate pipette.

$\frac{66 \text{ drops}}{0.5 \text{ ml}} \quad \frac{1 \text{ drop}}{X} \quad X = .0076 \text{ ml}$

Therefore 1 drop from the syringe $\cong$ 7.5 ul

this is a useful alternative

You should have made a set of SCN standards but they are way too concentrated.

You chose 10, 30, 75, 150, 300, 600 ul of 0.1M NaSCN mixed w/ 100 ul .1M $Fe(NO_3)_3$ into 4 ml of H_2O each.

You drastically need to reduce the concentration.

Let's take the NaSCN & Considerably dilute it.
Lets take 100 ul of NaSCN and add it to 10 ml distilled H_2O. This ratio is: 100

We need to recover our info as quickly as possible

SCN^- Calibration - Regression - Concentration Model.

7 Tubes Tubes are 4 ml in Volume w/ Distilled Water

1-6 SCN 0.1M is diluted by a factor of 100.
It is therefore ~~0.01M~~ NaSCN 0.001M

Tube	0.01 M SCN	M	0.1 M $Fe(NO_3)_3$	Absorbance 450nm
1	10 ul	2.5E-6	100 ul	~~.091~~
2	30 ul	7.5E-6	100 ul	~~.05~~ .041
3	75 ul	1.88E-5	100 ul	.053
4	150 ul	3.75E-5	100 ul	.093
5	300 ul	7.5E-5	100 ul	.105
6	600 ul	1.5E-4	100 ul	.147
	0.1 M SCN			
7	50 ul = 5000 ul	1.25E-3	100 ul	.904
8	20 ul = 1000 ul	2.5E-4	100 ul	.430
~~9~~	~~2.5E-3~~			

Need something in between

lets dilute tube 8 by a factor of 2.

9* 10 ul this did not work. Not linear. ~~.44~~

Absorbance $\tilde{=}$ 1.7065E-4 ul + .080 $r^2 = .94$

Not bad

Urine

9 500 ul 100 ul ~~.116~~ .115

Must need a control urine urea is yellow.

10 500 ul None .079

The urea control matches the zero point exactly.

ul $\tilde{=}$ 5484.92(Abs) − 371.81 $r^2 = .94$

Urine ul = 259 ul

Simplified Ahead

We appear to be getting useful results.
Urine sample 1 produced a volume estimate of ~260 ml.
What is the concentration of SCN^- from this volume?

We have a .001 M solution of NaSCN
MW of NaSCN is 81.072 gms/mole

a .001 M solution is

$\frac{.081 \text{ gms}}{100 \text{ ml}} \cdot 260 \text{ ml} = 2.106E-2 \text{ gms}$ in 4 ml of solution + Urine

but our dilution ratio in the urine is $\frac{4 \text{ ml}}{0.5 \text{ ml}} = 8$

Therefore our actual concentration in the urine is

$\frac{8(2.106E-2 \text{ gms})}{4 \text{ ml of urine}} = \frac{.0042 \text{ gms}}{1 \text{ ml}} \cong \frac{4.2 \text{ gms}}{\text{liter}}$

$= \frac{4.2 \text{ mg}}{\text{ml}}$ That seems high to me.

Assume urine sample is 200 ml
Then means 0.84 gms per urine sample.
This seems high to me.

Now remember this would be the estimate for NaSCN.
But the SCN portion of this molecule is only
71.7% of the total.

Simplified Ahead

Therefore the actual concentration estimate for SCN^- for Urine sample 1 is

$$.711\left(\frac{4.2\text{gms}}{\text{liter}}\right) = \frac{3.01\text{gms}}{\text{liter}} \doteq \frac{3.0\text{gms}}{\text{liter}}$$

1st estimate of SCN^-

What are known values?

I find a molar concentration for saliva.
A high number for saliva is 4E-3M
A low number for saliva is 1E-3

A 1M solution of NaSCN contains

$$.711\left(\frac{81.072\text{gms}}{1000\text{ml}}\right) = \frac{58.1\text{gms}}{\text{liter}}$$ We estimate 3 gms

$$\frac{3\text{gms}}{58.1\text{gms}}(1M) = .052M = 5.2E\text{-}2M$$ This would be a factor of ~10 greater than saliva ??.

This would sound like a problem

In blood, an average is found to be ~ $\frac{54\text{ mg}}{\text{liter}}$ This is a very small number.

Urine was too variable to record

Another source gives 0-100 mg/liter
We are off by a factor of ~ 60 too high.

Simplified Ahead

Urine sample 2 shows an absorbance of 0.200. ($\bar{x}$ of 2 runs)
The same sample shows an absorbance peak of ~2½
that of sample 1 w.r.t. thiocyanate @ 2055 cm^{-1}.

The model has predicts a concentration of

ul = 5484.92(.200) − 311.84 = 725 ul

Peak Ht .6cm 2.0

$\frac{725 \text{ ul (sample 2)}}{260 \text{ ul sample 1}}$ = 2.8 times. factor ÷ $\frac{2.0}{.6}$ = 3.3 factor

Lets measure abs:

This means that our concentration estimates correlate very well w/ the infrared plot relative differences and vice versa (not VI SUL !).

This suggests that there truly is a difference between the two samples of the magnitude.

You need to check the magnitude of your numbers. But the relative ratio looks good.

Candidates:

1. Hair extract
2. CDB Protein
3. Blood
4. Saliva
5. Air Filter

Simplified Ahead

Sample No 1 on hair does not show any evidence of a color reaction.

We should be able to calibrate our concentration very easily now.

~~Take 4 ml H_2O.~~ Dilute NaSCN
Add 100 to 1 thiocyanate solution.
ie Take 10 ml distilled water and 100 ul of 0.1 NaSCN

Now take 4 ml of H_2O
Add 50 ml $Fe(NO_3)_3$ OK for 1 ~~test~~.

Must still too strong.
Dilute by 10 ml again. This will be 200 to 1.
& Use 100 ul dilute NaSCN
and 100 ul $Fe(NO_3)_3$
Final:
100 to 1 0.1 M NaSCN
100 ul of 100 to 1
100 ul of ~~FeSCN~~ $Fe(NO_3)_3$ 0.1M

Absorbance = 0.139
This is a perfect solution
This should offer a good reference point.
100 to 1 makes our stock solution a .001M NaSCN.
We take 100 ul of this and place in 4 ml H_2O.
This means the test tube has $\frac{4 \text{ ml}}{\cancel{100}\ 0.1 \text{ ml}}$ = 40 dilution ratio.
So the test tube is $\frac{.001 \text{ M}}{40}$ = 2.5E-5 M NaSCN.

Simplified Ahead

Next, we know that the solution has an absorbance of 0.139 ⇨ 2.5E-5 SCN

Idea is a 1 pt. Calculation.

The means

$V1 = 5484.92(0.139) - 371.01 = 390.6$

The says than an absorbance.

So.

~~Abs~~ Conc	Abs
0	.08
2.5E-5 ~~.139~~	.139

Use This: Simplified Now. Quite Reasonable Numbers.

OK, our problem can now be radically simplified. We actually do have concentrations already available

Conc (NaSCN)	Absorbance
7.5E-6M	.041
1.00E-5	.053
3.75E-5	.093
7.5E-5	.105
1.5E-4	.147
2.5E-4	.430
1.25E-3	.904

$Abs \cong 682.6 \cdot Molarity_{NaSCN} + .080 \qquad r^2 = 0.94$

$Molarity_{NaSCN} \cong 1.371E\text{-}3\,(Abs) - 9.29E\text{-}5 \qquad r^2 = .94$

	Abs	$Molarity_{NaSCN}$	Dilution Ratio	Actual Molarity NaSCN
Urine #1	.115	6.486E-5	(4/.5) = 8	5.181E-4
Urine #2	.200	1.013E-4	(4/.5) = 8	1.450E-3

$(5.181E\text{-}4M)\left(81.07 \frac{gms}{liter\text{-}mole}\right) \cong .042 \frac{gms}{liter} = \frac{42\,mg}{liter}$ $\quad 71.7\%\ SCN \cong 30\,mg/liter$

$(1.450E\text{-}3M)\left(81.07 \frac{gms}{liter}\right) = .118 \frac{gms}{liter} = \frac{118\,mg}{liter} \cong 85\,mg/liter$

These numbers seem to be perfectly in range.

Protein Absorbance = 0.137

Wgt of Protein 45.52
- 45.13
.39 gms
dissolved in ~ 4.0 ml H_2O
100 µl $Fe(NO_3)_3$ used.

Molarity = 1.371E-3 (Abs) − 9.29E-5 = 9.493E-5M

But this is dissolved in ~ 4.0 ml H_2O
Mass of protein is 0.39 gms
Assume density of protein is 1.
This ratio is 12.56
so Actual Molarity is estimated as (9.493E-5)/(12.56) = 1.193E-3M

$\approx$ 1.193E-3M $\left(\frac{81.01 \text{ gms}}{\text{mole}}\right)$ = $\frac{.097 \text{ gms}}{\text{liter}}$ = $\frac{97 \text{ mg}}{\text{liter}}$

Dec 01 2017

It appears that a method (colorimetric) has been established to determine the existence and concentration of the thiocyanate ion.

The method can and should already be applied to:

1. Urine
2. saliva
3. blood
4. hair
5. air filter extract
6. CO2 protein.
7. Rainwater

Absorbance of the air filter extract = 0.146
4 ml H_2O / 100 ul ethanol air filter extract, 100 ul $Fe(NO_3)_3$.

This leads to:

Molarity $\approx$ 1.371E-3 (0.146) - 9.29E-5 = 1.073E-4 M

but the dilution ratio of the sample is $\frac{4\text{ml}}{0.1\text{ml}}$ = 40

Therefore the concentration of the HEPA air filter extracts:

40 (1.037E-4 M) = 4.148E-3 M

leads to

$$\left(\frac{4.148E\text{-}3\ M}{1\ M}\right)\left(\frac{81.07\text{gms}}{\text{liter}}\right) = \frac{0.336\text{gms}}{\text{liter}} = \frac{336\text{mg}}{\text{liter}}$$

This is a high concentration. $SCN^- = (.717)\frac{336\text{mg}}{\text{liter}} \approx \frac{241\text{mg}}{\text{liter}}$.

IR should detect this.

We have run the IR plot on the same sample. It is interesting to note that the IR plot does not show the presence of the SCN^- functional groups. That is interesting. It will be beneficial to see if the finding can be confirmed independently through electrochemistry.

The reading colorimetrically is quite strong and is not doubted. Questions on species, solubility, etc.

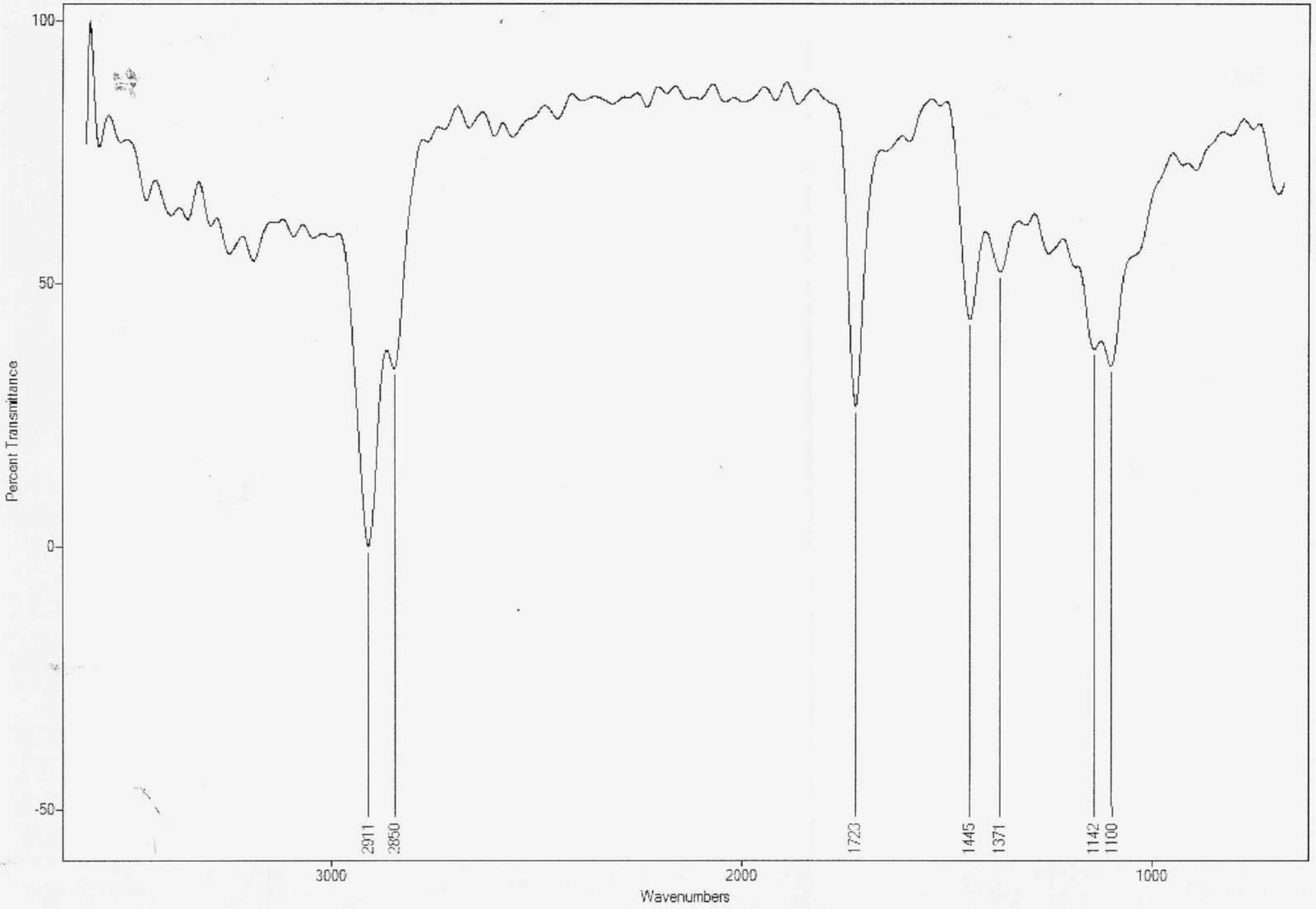
Next point of interest. The IR actual best match seems to be w/ one of the hair extractions. This is also somewhat surprising.

Remember that you have a method of separating this compound into two separate components, and this is important. This was AC completed w/ liquid Chromatography.

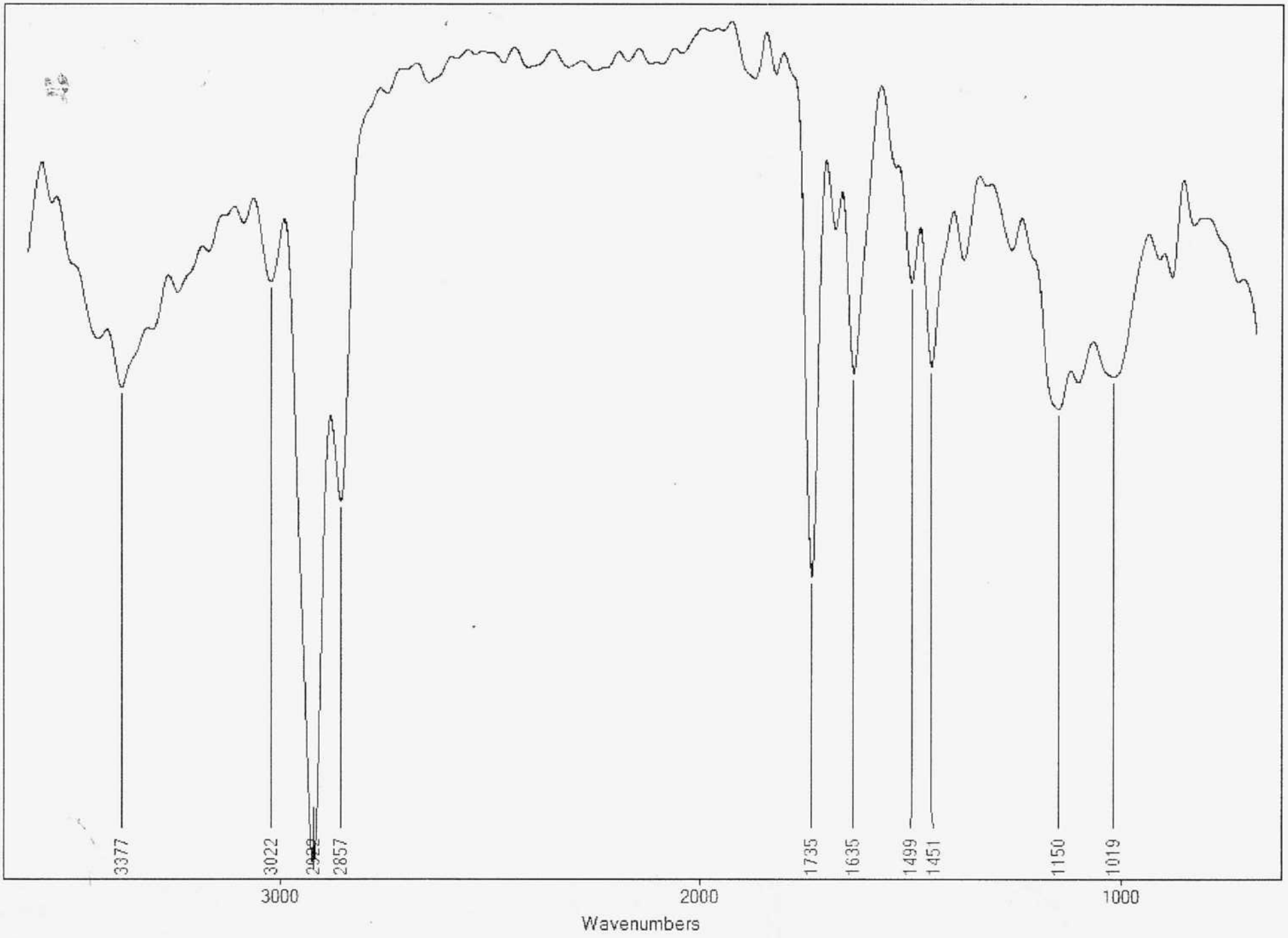
You are now examining seven different sample types and they all have a high probability of sharing common COB elements (eg, thiocyanates)

Lets recover the date range of the LC work.

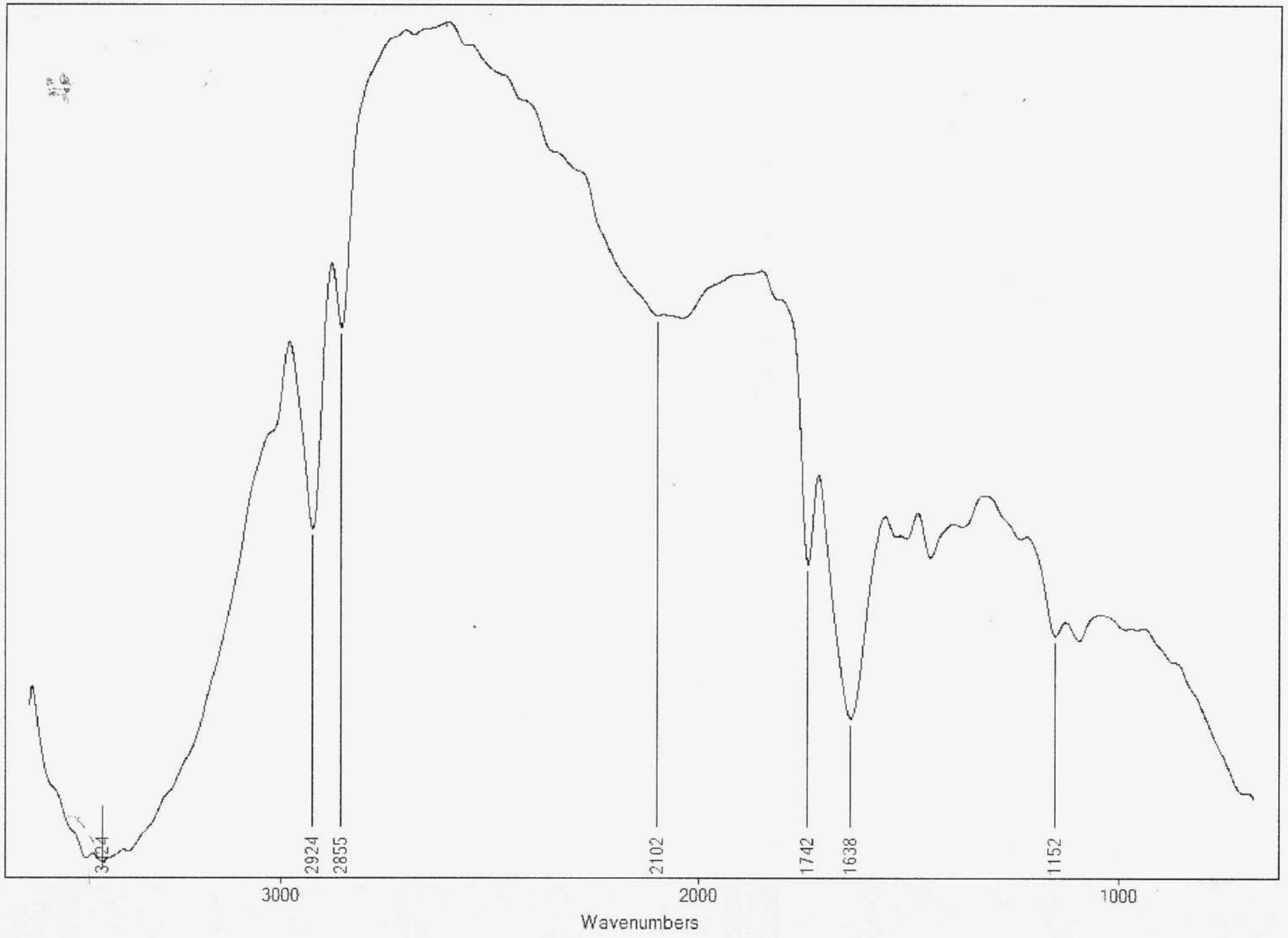
HEPA Air Filter Extract Soaked Ethanol ATR Dec 07 2016 - 02.JPEG



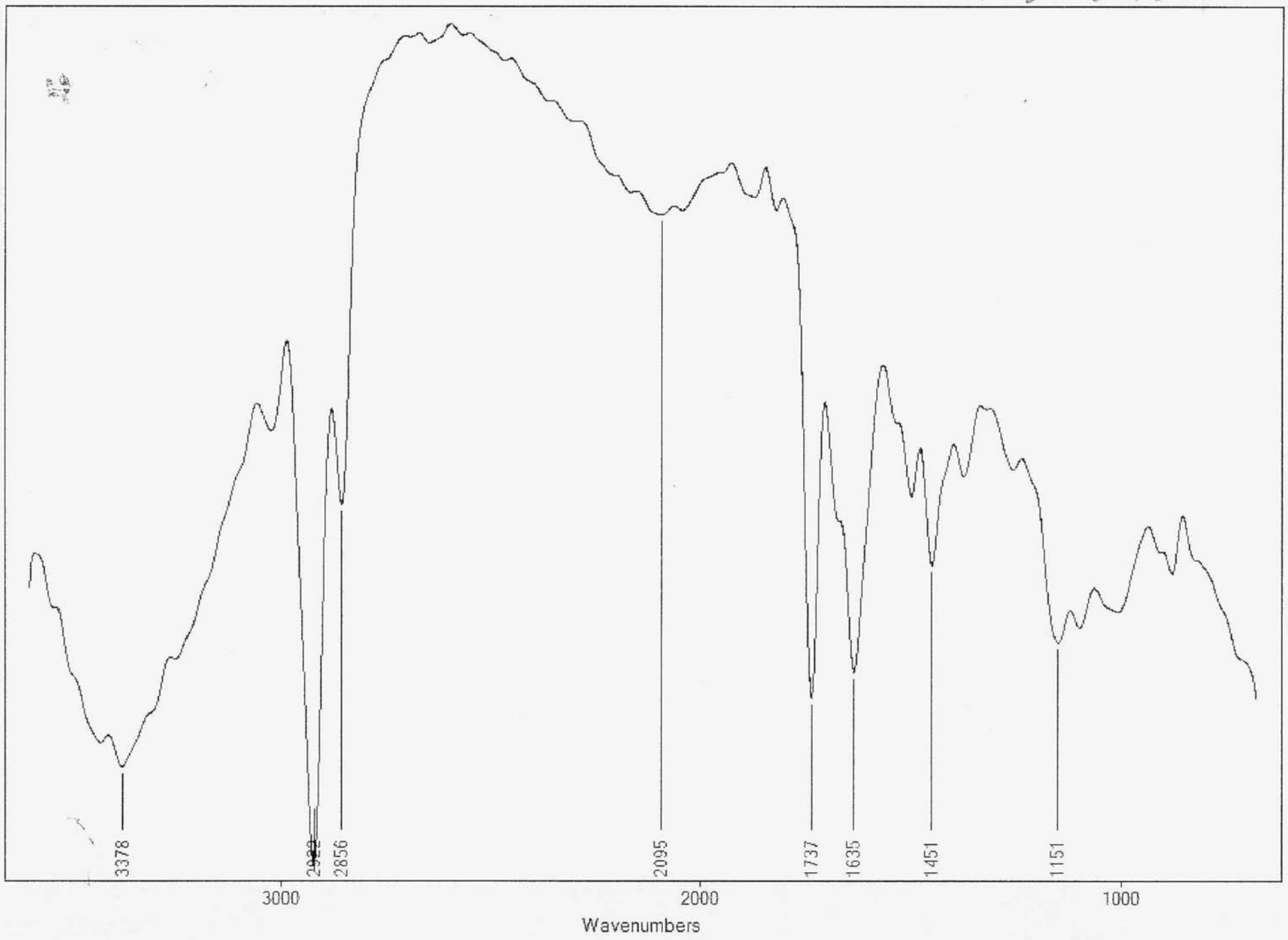
HEPA Filter LC Component 01 Sep 24 2017 - 01.JPEG



HEPA Filter LC Component 02 KCl Sep 24 2017 - 01.JPEG



HEPA Filter LC AVERAGE OF COMPONENTS 01 AND 02 Sep 24 2017 - 03.JPEG



The plot on the thiocyanate assay is getting quite thick. The compound is definitely showing up across a wide spectrum of samples. We will have to take them one @ a time.

The colorimetric test is going to be especially valuable. We have found SCN in a recent saliva sample direct ATR.

& Thiocyanate is a thyroid inhibitor, amongst other toxicities. This is the drug focus of the investigation.

We will have to take one sample type @ a time. Recall the list:

1. Air Filter
2. Rainwater
3. CDB Secreted Protein
4. Urine
5. Blood
6. Saliva
7. Hair.
8. Skin 9. Env Filament?

We will keep cross Checking that sample types colorimetrically and via instrument protocols

The air filter extract is already interesting. We have strong success colorimetrically. However, direct by IR fails. However, if you look @ your well documented Liquid column chromatography work of Sep 24 2017 Component #2 (shown on enclosed IR plots) we see that we have it very strongly. Remember that Component #2 is the Protein component, and that it was separated with the use of NaOH in the liquid chromatography column. (LC).

So this is a good example of where a particular method or protocol applied to a sample type make all the difference in the isolation, appearance & separation of a component. Direct IR of the HEPA ethanol extract does not reveal the characteristics of component #2 (protein) until it is first separated out by LC under its own independent protocol.

This shows you what you are up against. Hopefully you can recall your methods for all sample types.

The goal here will be to confirm the existence of the SCN component in all sample type by at least 2 independent methods.

(We must be cautious w/ IR w/ acids, bases, oxidizers, etc)

We have now:

~~(one sample)~~

1. ~~Urine by IR and colorimetric~~
2. HEPA air filter by IR and colorimetric.

(IR is in combination w/ LC producing component #2)

2. Urine by colorimetric, Does not seem to appear in IR ?? Electrochemical ?
3. Saliva by IR — UV has absorption 240 to 220.

Can we get urine by IR? (It does not seem so)

4. COB Protein Colorimetric & IR Jun 14 2017 by Distillation (matches HEPA filter - LC Extraction IR Sep 24 2017)

Dec 08 2007

Let us look @ electrochemistry of NaSCN.

I have learned something very helpful towards improving the stability and repeatability of AC Voltammetry runs:

1. Let the electrodes sit for 1 minute between runs. (Maybe this can be included as a post processing step)

2. Tap the electrodes prior to the run to disperse the bubbles that form upon the electrode during a run.

This gives smoother and more repeatable curves. Process is to average the last 3 coinciding curves and then to examine the derivative for both peaks and zero crossings (slope breaks).

Good data seems to be collected here.

AC Voltammetry: NaSCN

NaSCN produces:

Peaks	Zero Crossings
-2.41	-2.57
-2.10	
+.86	
+1.92	
+1.26	

There are no repeats in the set. Looking for SCN, Na

2.41 No match?
-2.10 O_3, O_2, H (2.07)
+.86 NH_4, NO_3 (.88) OH^-, H_2O (.88) NO_3, N_2O_4 (-.85)
+1.92 N_2 (-1.87) S_2O_8, SO_4 (2.00)
+1.26 H_2O, OH^- (-1.22) O_2, H_2O (1.22) O_3, H_2O (1.24) N_2O (1.27) NH_4 (1.27)
-2.57 N_2 (2.65)

We disregard O, H.
Remaining elements are N (nitrogen indication) n = 5
S n = 1
It is reasonable to presume that Carbon exists.
We therefore do find evidence of CNS compound electrochemically w/ the analysis of sodium thiocyanate. We did not find Na.

We will look for the peaks set up on a further analysis, such as urine.
Eg .86, 1.92, 1.26 & 2.57 (mean)
or
.88, .85, 1.87, 2.00, 1.27, 2.65 actual
N N N S N N

Also wait 1 min & disperse bubbles for NPV as well.

You are getting overloads. Consider dilution.
Diluting the solution to 1/3 of original did indeed eliminate the overloads.

As a redundant method, cyclic normal pulse voltammetry (NPV) produce the following results:

Derivative Analysis:

−1.96	weak	SO_4 (2.00)
−1.36	weak	N_2H_5 (1.42)
−2.49		No Match?
2.86		Na (2.71) ?

A weaker level of corroboration, as expected. But we nevertheless have some further indication of N & S, and possible Na but uncertain on that.

All being said, electrochemistry does indicate the more probable existence of N & S in the compound. Carbon can not actually be inferred but may be considered.

N
S
C reasonably considered
Na possibly considered.

Therefore we are on track as much as ever could be expected. Now the question is, how does a urine sample behave?

Urine has many components w/in it. This can be confusing.

What we learn next is that the cola development, at least in urine, develops more intensely w/ time. Our urine sample which measured @ Absorbance = 0.200 24 hrs later now measures @ 0.377 @ 450 nm.

This is a significant increase. The cola development all the more confirms the presence of SCN^- in the urine sample (#2).

For urine sample #2 using AC Voltammetry:

Peaks (Slope Breaks)	Zero Crossings (Maximums, Minimums)
-2.27 H_2 (2.25)	-1.62 N_2O, NO (1.59) Al (1.66)
	+1.88 FeO_4, Fe^{3+} (1.90) N_2 (-1.87)
	+2.26 H_2 (2.25)

That is about it. Candidate elements are

N	(2)	Our most likely element is nitrogen.
Al	(1)	Could signify ammonia, protein, SCN^-
Fe	(1)	Further testing would be required.

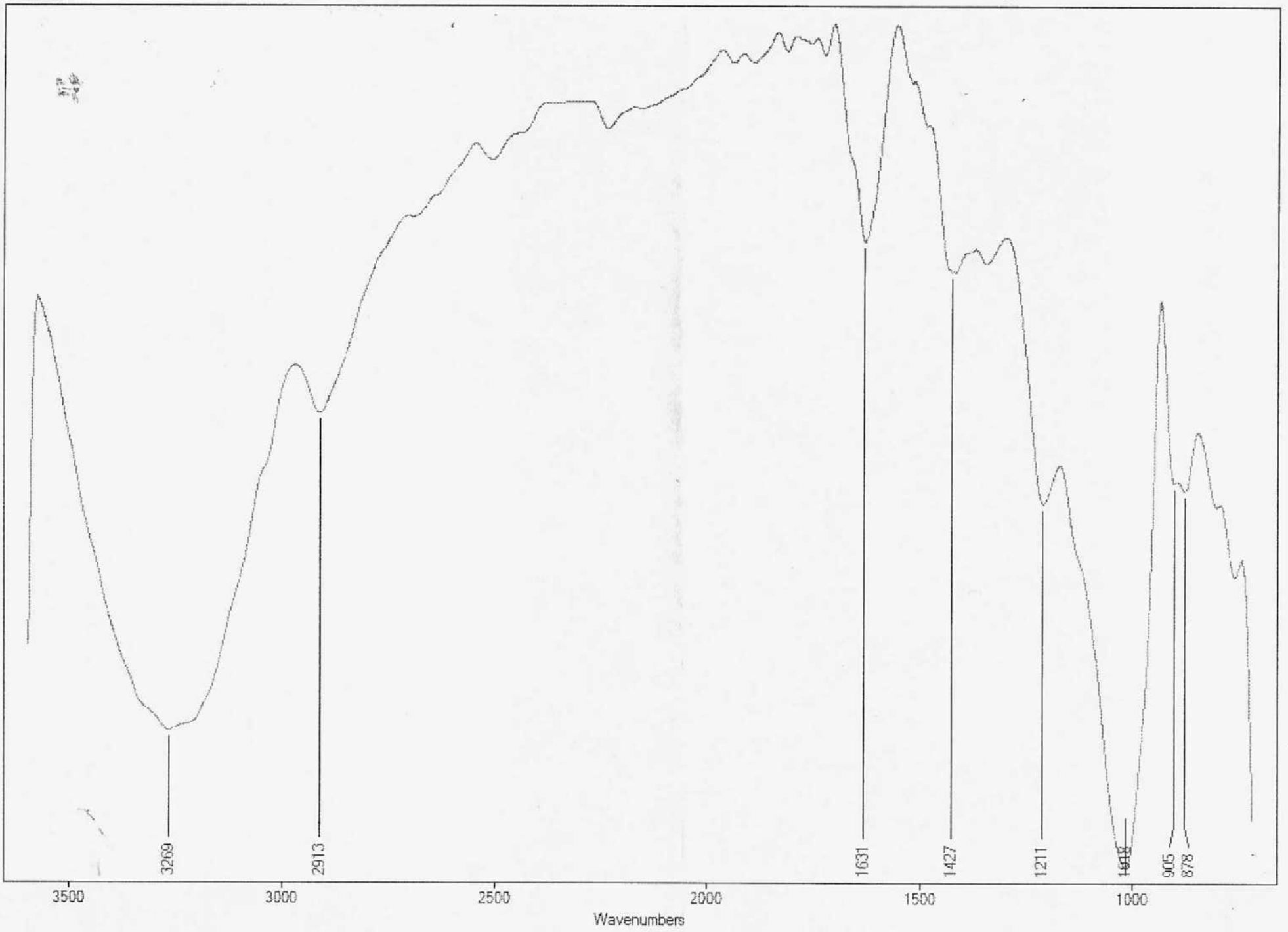
It is giving a lot of error and noise now between 1900 & ~~1600~~ 1300 Cm^{-1}. This is really a problem. I am not sure what is causing it. It is causing derivative peaks to be lost.

We have learned some IR tricks today.

1. You can remove ATR cradle to get a blank of air if you need to. Scale it by a factor of 1/2 and it will be very close to actual ATR and fully usable. This way you do not lose a sample.

2. A Hamming smoothing window is your superior. It only take one iteration @ ~ 60 to get a smooth plot. Truncate it when you are finished.

CDB Reconstituted - Evaporated Protein Watch Glass Dec 08 2017 - 04.JPEG



CDB Secreted - Evaporated Average Dec 08 2017 - 03.JPEG

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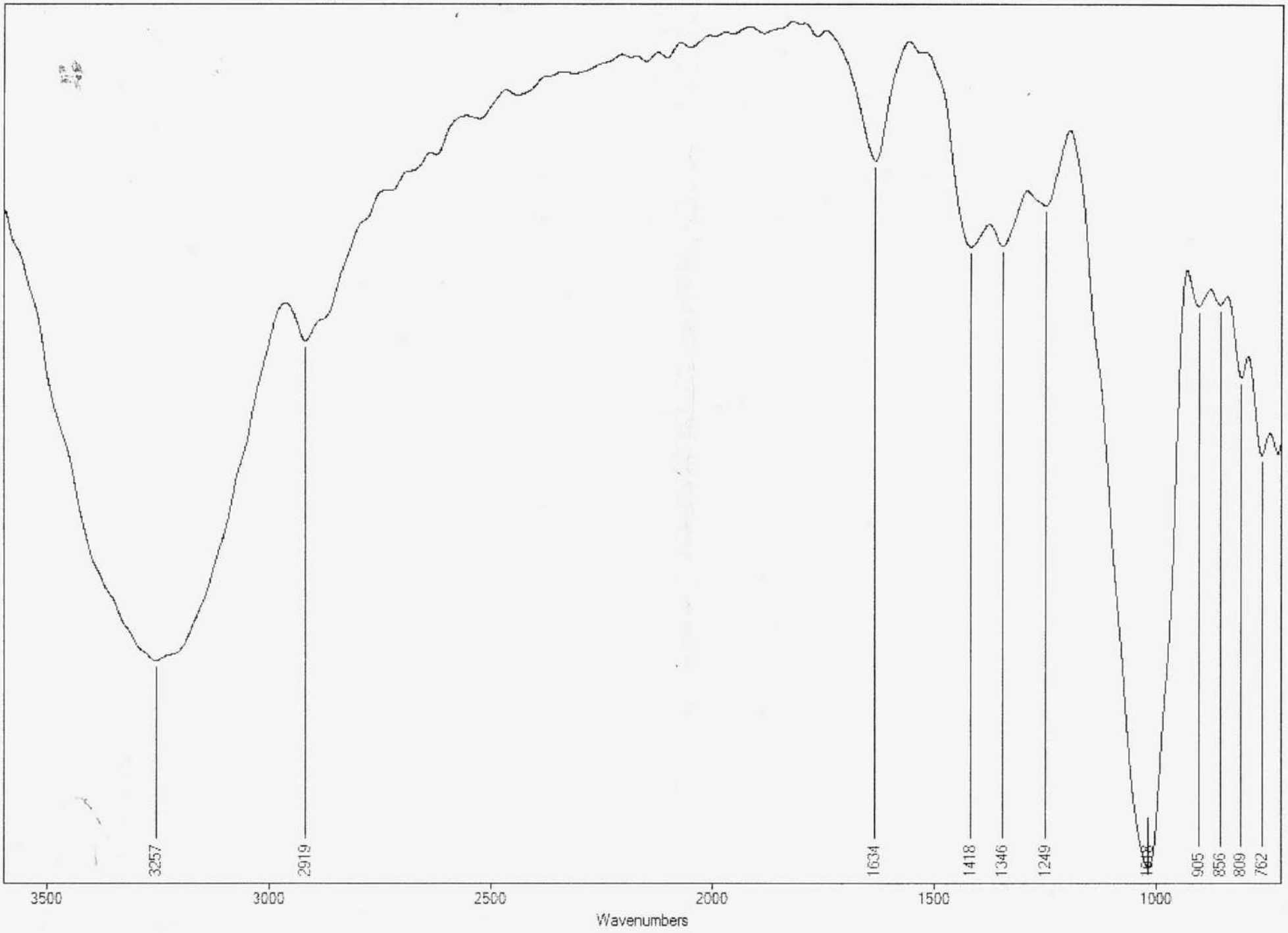


Dec 09 2017

CDB Viscous Protein (Reconstituted) Differential AC Voltammetry Analyses. Use only absolute value to rank

We have the following peaks (no zero crossings)

Broad	−2.25	H_2 (2.25)
Strong	−2.31	Mg (−2.31) Al (−2.33) Al (−2.31)
minor	−.03	NO_3, NO_2 (+.01) H_2 (Ø.00) Fe (+.04) O_2 (+.07)
Weak	+1.04	NO, N_2O_4 (+1.04,) NO (N_2O_4) (1.01) HNO_2 (Ø.99) NO_3 (Ø.96)
Moderate	+1.45	N_2H_5 (1.42) Cl (1.48, 1.47, 1.45)
Weak	+1.56	NO (1.59) Cl (1.61, 1.43) Mn (1.51, 1.56)
Moderate	+1.90	N_2 (1.87), Fe (1.90)

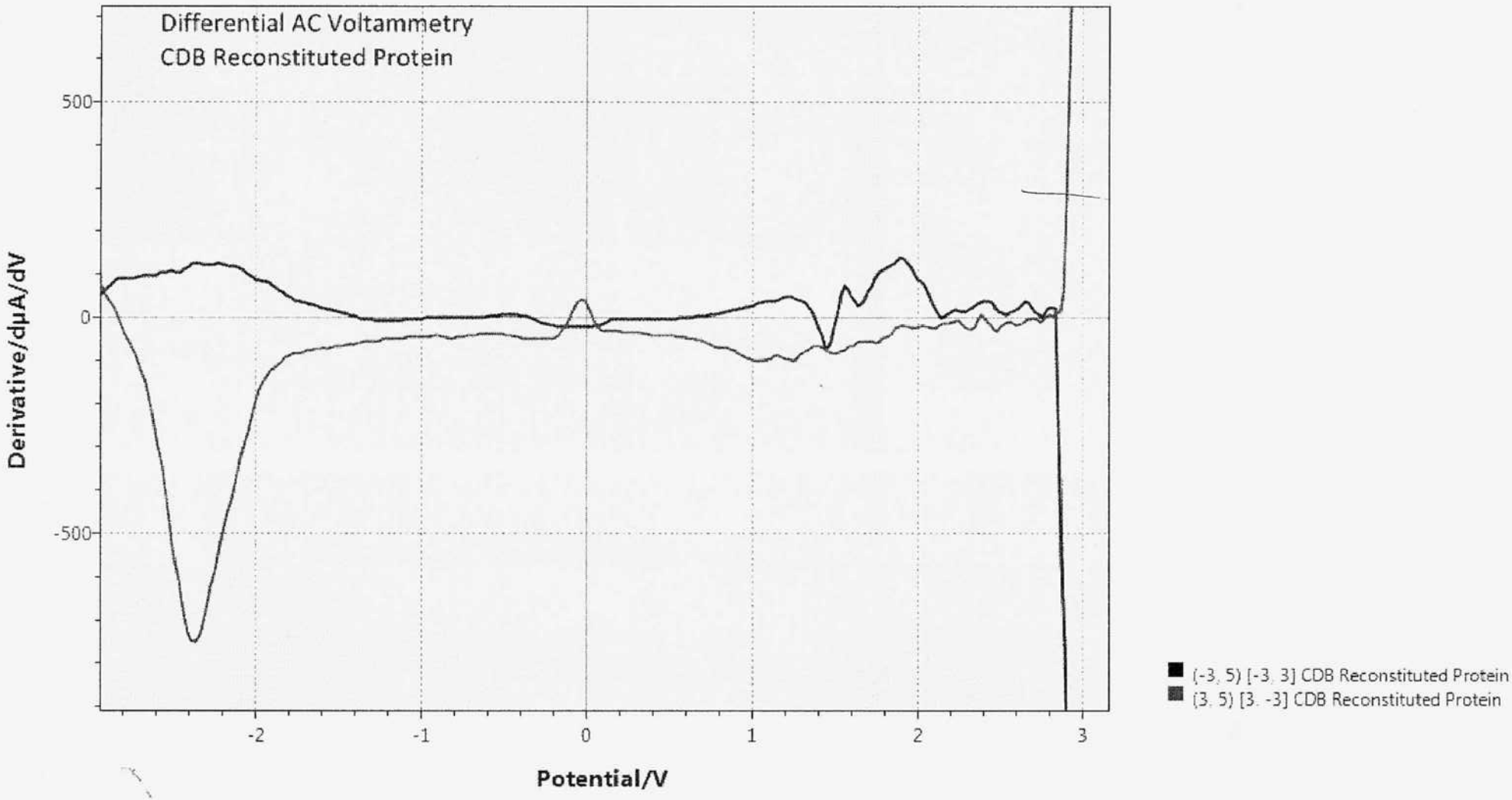
Elements of interest are.

Mg (1)
Al (2)
N (8)
Fe (2)
Cl (5)
Mn (2)

Given that the primary elements known to be available through the culture medium are Fe, N, C, S

We therefore accept Fe & N as being detectable through Cyclic AC Voltammetry.
It is called an organometallic protein w/ thiocyanate element.
IR & NIR analyses can be added to the analyses.

CDB Differential AC Voltammetry.jpg



The protein structure, to some degree can be assessed from:

1. electrochemistry	Fe, N
2. thiocyanate colorimetric	SCN
3. IR	
4. NIR	Ar OH, Ar CH
5. Water solubility.	OH

We also have our list of known attributes, OK, posted on next page.

The protein acts like a shellac when it is evaporated on the ATR plate; quite transparent as a film.

We need to extract the Chromatogram of pyrolysis to estimate a minimum carbon number.

We know that pyrolysis was responsible for a severe skin reaction on my neck that lasted for more than 2 weeks. This can never be repeated unless it occurs in a controlled environment.

CDB Visceral Protein Characterization

Morgellons:

Unique Protein Isolated & Characterized

by
Clifford E Carnicom
Aug 13 2017
Edited Oct 01 2017

Note: Carnicom Institute is not offering any medical advice or diagnosis with the presentation of this information. CI is acting solely as an independent research entity that is providing the results of extended observation and analysis of unusual biological conditions that are evident. Each individual must work with their own health professional to establish any appropriate course of action and any health related comments in this paper are solely for informational purposes.

A protein generated by the microorganism associated with the Morgellons condition (tentatively classified in past research as a "cross-domain bacteria", i.e., CDB) has been isolated and characterized in several ways. There is little doubt that this protein is at the heart of the physiological and biochemical changes that occur within the body by those affected. Related research has been conducted with success for some time, however, the recent work represents a different and separate approach from previous accomplishments. Proteins are at the crux of biochemistry and biological research, and they have great importance in relation to biological structure. There are usually numerous applications (beyond health aspects alone) that develop with the advent of a new or isolated protein, and it is expected that the current work can eventually follow this suit.

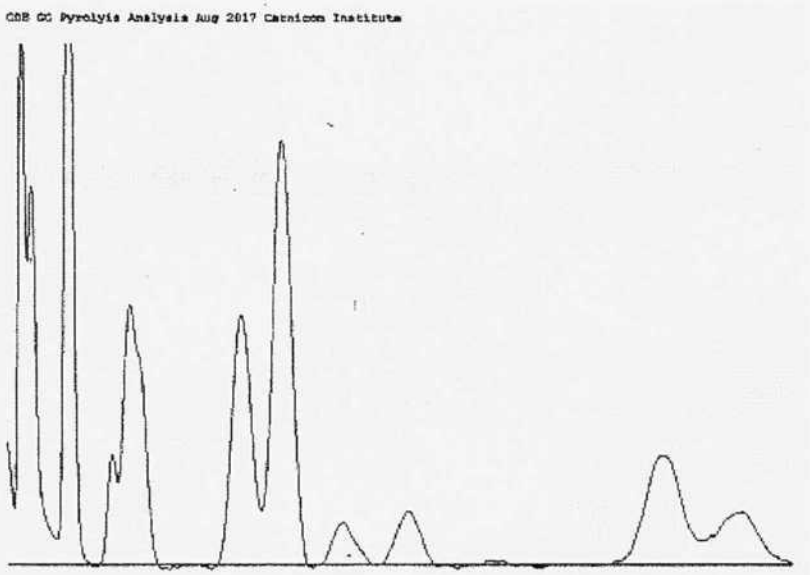
Only the general nature of the protein will be described at this point. The protein is organometallic in nature, highly water soluble, and strongly acidic. Additional resources of significance and support from the health communities will be required to develop the series of discoveries into tangible benefits.

Some of the methods that been employed to define the unique nature and characteristics of the protein include:

1. The molecular weight of the protein has been estimated with laboratory methods.
2. The solubility and polarity of the protein has been assessed.
3. Pyrolysis with gas chromatography (GC) has been applied to the protein to examine its thermal decomposition into various subcomponents.
4. Headspace methods have been used to examine the nature and volatility of gaseous metabolism of the microorganism.
5. Infrared (IR) analysis has been used to identify the primary functional groups of the protein, along with the analysis of various GC trapped

components.

6. Ultraviolet (UV) analysis of the protein has been conducted.
7. Candidate amino acid composition, at least to a partial extent, has been established.
8. The pH of the protein has been measured.
9. The isolectric point of the protein has been determined via titration.
10. Precipitation methods for the protein have been developed.
11. A metallic nature of the protein has been verified.
12. The index of refraction for the protein has been determined by measurement.
13. A concentration-dilution model for the protein has been developed based upon the index of refraction.
14. The polarimetric nature of the protein has been examined.
15. The electrical conductivity of the protein as a function of concentration and dilution has been determined.
16. The Oxidation Reduction Potential (OPR) of the dilute protein has been measured.
17. A colorimetric test for the existence of the protein has been established.
18. Initial molecular models proposals have been established for some of the simpler components of the headspace-pyrolysis components with GC – IR coupling.
19. Initial anticipated impacts upon physiology, i.e., absorption levels, are under investigation.
20. The Bradford reagent identification test for protein identification has been applied via visible light spectroscopy.



GC Pyrolysis Chromatogram of Numerous Components of CDB Isolated Protein
(significant hydrocarbon structure is identified within)

The isolation and characterization of this particular protein and its properties are of importance and uniqueness in the research related to the Morgellons condition. The attributes identified are numerous and specific to the microorganism that has been extensively identified, examined and

researched. The uniqueness of the protein is essentially guaranteed. The method of development of the protein also represents a distinct and recent advance in the history of CI research, and it is hoped at some point that the work will be placed to the advantage and benefit of the public.

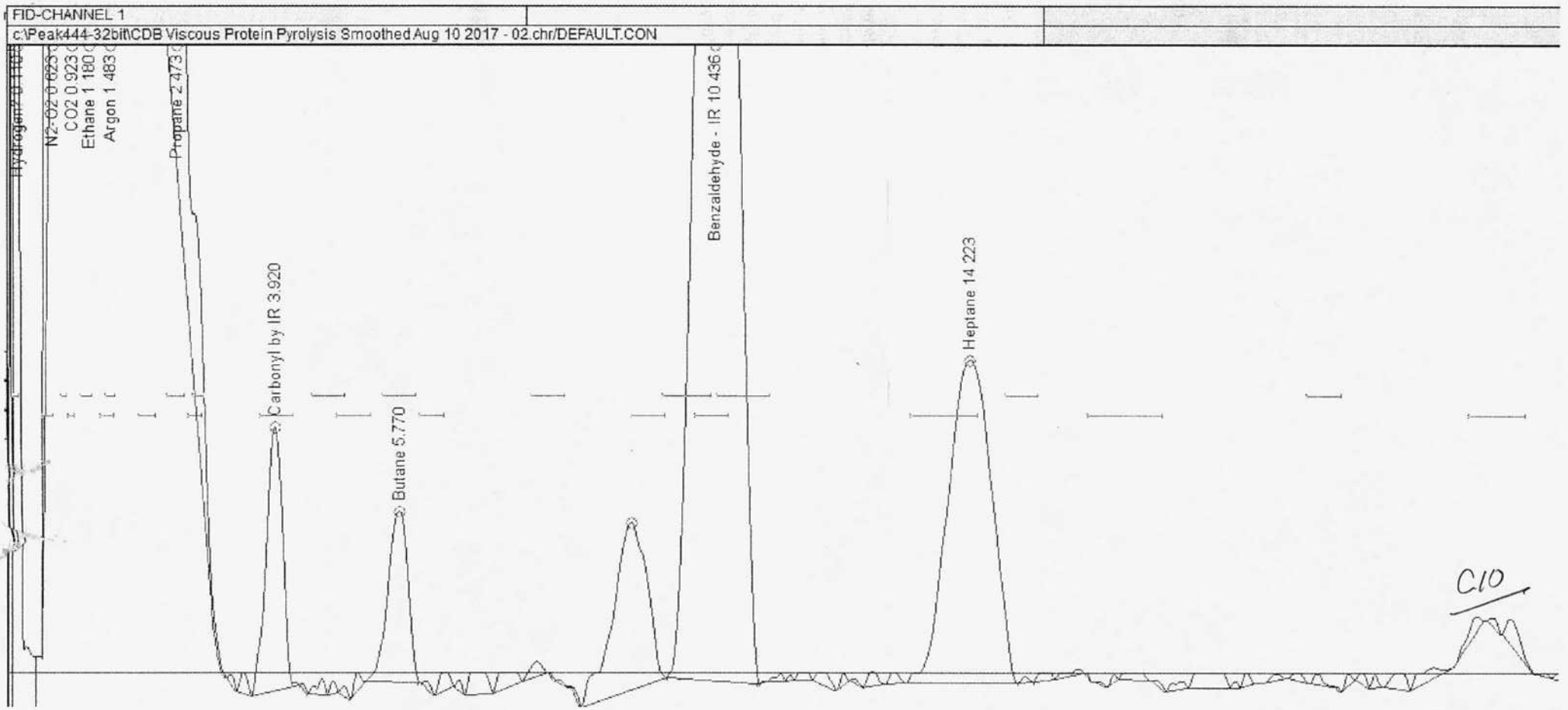
Clifford E Carnicom
Aug 11 2017
Edited Oct 01 2017

Born Clifford Bruce Stewart
Jan 19 1953

CDB Protein Pyrolysis - Gas Chromatography
Serious Health Effects Can Result from Exposure to Gases

$C_3, C_4, C_6, C_7, C_{10} \ni \Sigma \geq C_{30}$
SCN, ArOH, ArCH, Fe, N

CDB Pyrolysis Gas Chromatography



I have once again conducted a thiocyanate colorimetric test on saliva for sample #2.

[redacted]

Circumstances:
4 ml H_2O
200 ul saliva
1 drop 1M HCl
reagent is 100 ul 0.1M $Fe(NO_3)_3$.

Concentration determination:
Molarity ≅ 1.371E-3 [redacted] − 9.29E-5 = [redacted] M

Therefore [redacted] M $\left(\frac{4\text{ ml}}{0.1\text{ ml}}\right)\left(\frac{81.072\text{ gms}}{\text{liter}}\right)$ = [redacted] = [redacted] mg

When we did our calibration run we used urine. Realize we obtained an absorbance of 0.200 but we used 500 ul of urine. Here we used only 100 ul. We obtained a concentration of 85 mg SCN^- in the urine sample. [redacted]. This would be one reason the IR plot shows the presence elevated. We will repeat IR and also compare to individual #1
Conclusion: Saliva levels are much higher but even urine appear to be elevated for individual #2.

The colorimetric test fails to show a distinction in concentration during this run. Both samples measure absorbance @ 0.19 & therefore depict the same concentration colorimetrically:

$$\frac{1.68\text{E-}4\text{ M}}{1\text{ M}}\left(\frac{4\text{ ml}}{.2\text{ ml}}\right)\frac{81.072\text{ gms}}{\text{l}} = .272\frac{\text{gms}}{\text{liter}} = 272\frac{\text{mg}}{\text{liter}}$$

"Aromatic Isothiocyanates"

$R-N=C=S$

The same as the ~~issue~~ Thiocyanates - broad level distribution

[redacted]

Saliva [redacted] ATR Dec 09 2017 - 01.JPEG

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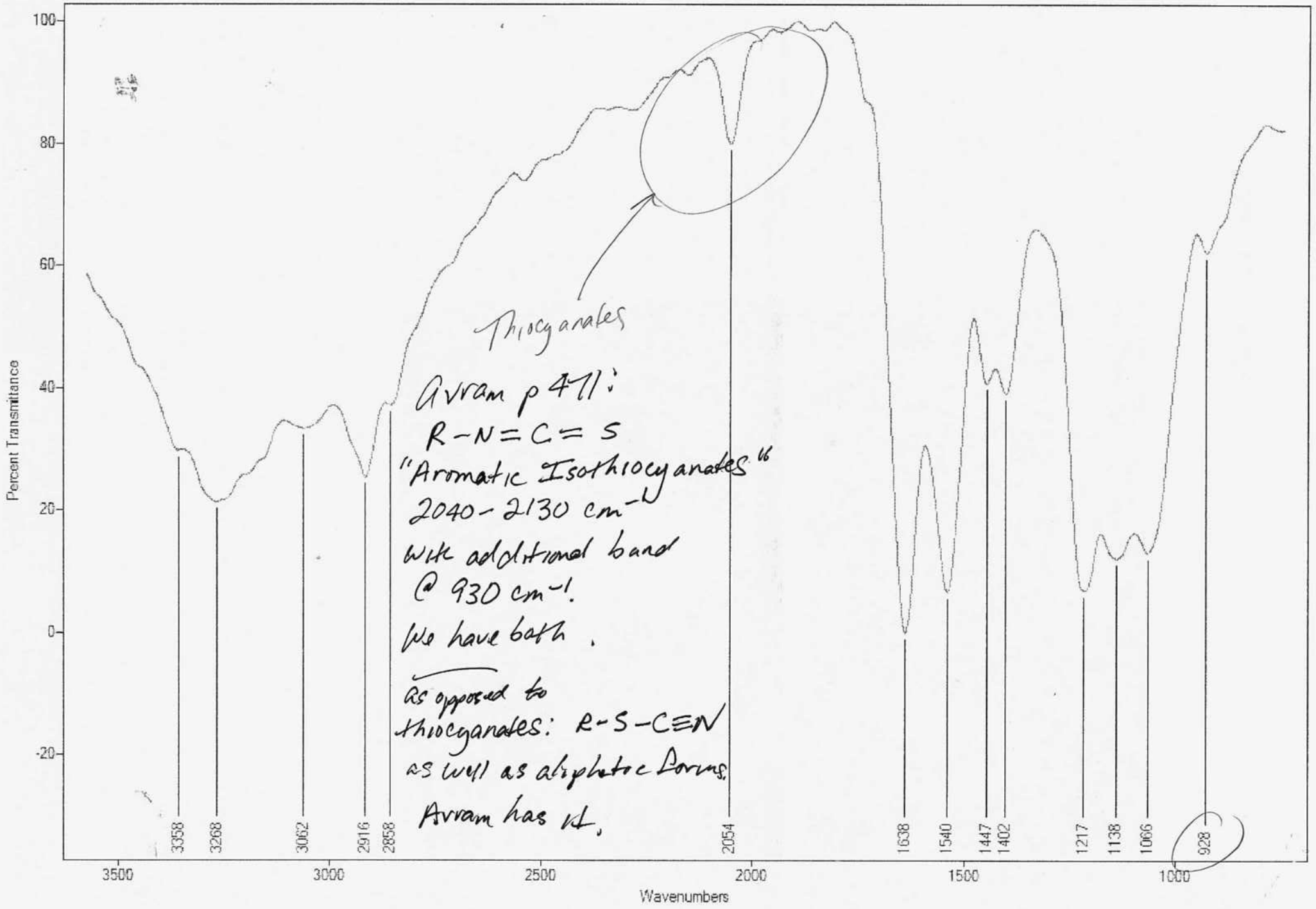
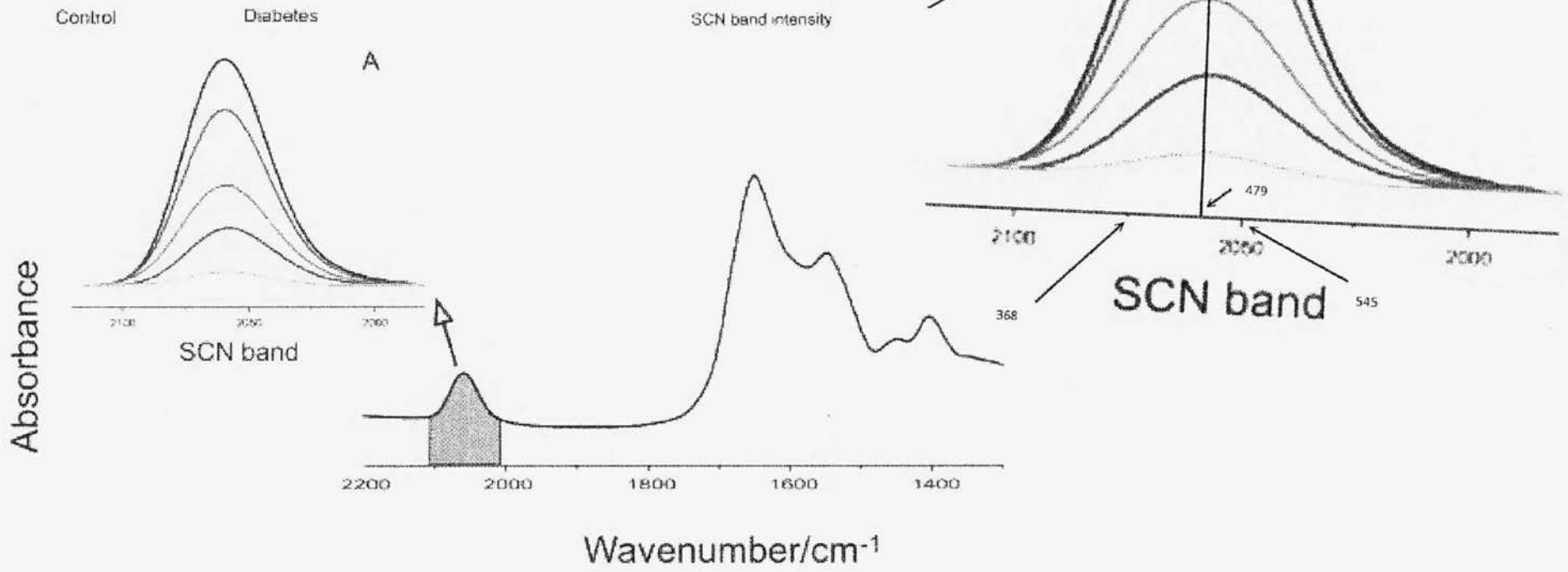


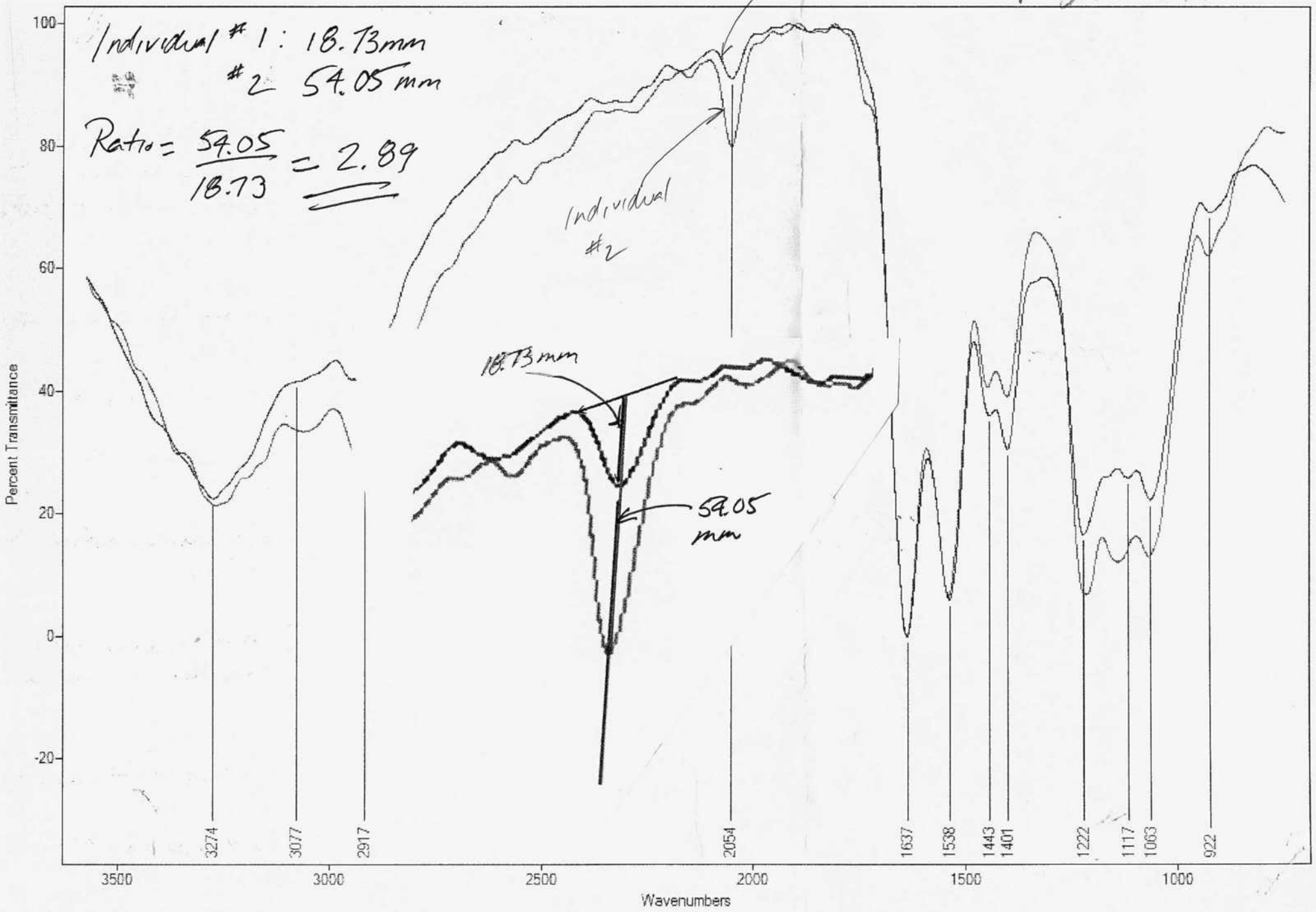
Thiocyanate IR absoprtion in IR



Thiocyanate and glucose signatures in the IR spectra of saliva samples. (A) Representative thiocyanate band intensities in the IR spectra of saliva from diabetes (n = 2) and control subjects (n = 2) chosen to highlight that clear differences in salivary thiocyanate signals are readily apparent in saliva; (B) The histogram represents the integrated area (mean, s.e. SCN^- content) in subjects with diabetes (red bar) and healthy controls (blue bar); and (C) the correlation plot revealing the association between SCN- band intensity and glucose concentration in

It is anticipated that Sample #2 has a thiocyanate concentration ~ 3 times greater than sample #1.

Saliva Comparison Thiocyanate ATR Dec 09 2017 - 01.JPEG



Dec 10 2017 Page 265

We are loaded on the road room.

—

Apparently not so in the lab anymore. The ingrained instrument is now completely out of commission. I am fairly certain that the detector detecta Lan failed. There is a $1400 part.

We are entirely out of commission w/ IR now until we raise about $2000 to get the instrument repaired. Since we are leaving for a while, there is no hope of this occurring until at least Feb-Mar.

So back to some old ways w/ qualitative chemistry again and it can never be the same.

—

On another note, the digested hair samples (10M NaOH, microwave, low power, 30 min) require neutralization after the process, as the ~~color alone of~~ pH alone affects the color of solution. It is quite dark after microwave retrieval, neutralization lightens the color considerably.

At this time, there is no indication of isothiocyanates existing w/in the alkaline digested samples. There is no color change w/ the addition of Fe^{+3}.

We see large amounts during pyrolysis but this is not the same. I also did not see it in either urine or blood so you may be restricted to environmental sample occurrence or saliva.

=

You also have not found a way to detect no thiocyanate electrochemically as of yet.

But the colorimetric test is quite reliable all by itself.

—

It, I electrochemical analysis of the COB protein says that we have an iron - sulfur protein.

Amide I
Amide II
R-O-S(=O)-O-R' sulfate
O

iron
Ar OH, Ar CH

* This says to me that we have an iron sulfur protein as component in the COB protein secreted.

" Iron - sulfur clusters belong to the most ancient cofactors in life".

Ferredoxin cluster is involved in bacterial nitrogen fixation, which we know we have.

Ferredoxins act as " capacitors" that can change the oxidation state of iron between +2 & +3.

There are bacterial type ferredoxins Fe_4S_4

Ferredoxins are acidic, low molecular weight, soluble iron-sulfur proteins that mediate electron transfer

4Fe4S & 3Fe-4S are typically found in bacteria.

Ferredoxins are any of a group of red-brown proteins containing iron and sulfur acting as an electron carrier during photosynthesis, nitrogen fixation, & oxidation reduction reactions.

This is obviously very important.

We have a core issue identified here.

* The ferredoxin is what is disrupting the iron oxidation in the blood.

Isothiocyanates are interfering w/ the thyroid.

Dec 11, 2014

Lets start piecing together some of the structure that is likely to be present in the CDB protein.

Lets start by learning about the amide bands. First, I am noticing some very mixed messages.

~~Two~~ Three sources say that the Amide A band is in the region of 3400 - 3500, then two of those same sources go on to list Amide A in a tabular sense @ about 3250-3300 and then the other @ 3294.

I have 3269 which matches the tabular forms so I can only point out the contradiction @ this point.

—

Now that we know that we state that

1. The amide bands (A, B, I–VI) result from the peptide group, the structural unit of a protein.
2. We also know that amides are central to peptide formation so lets see how that process develops

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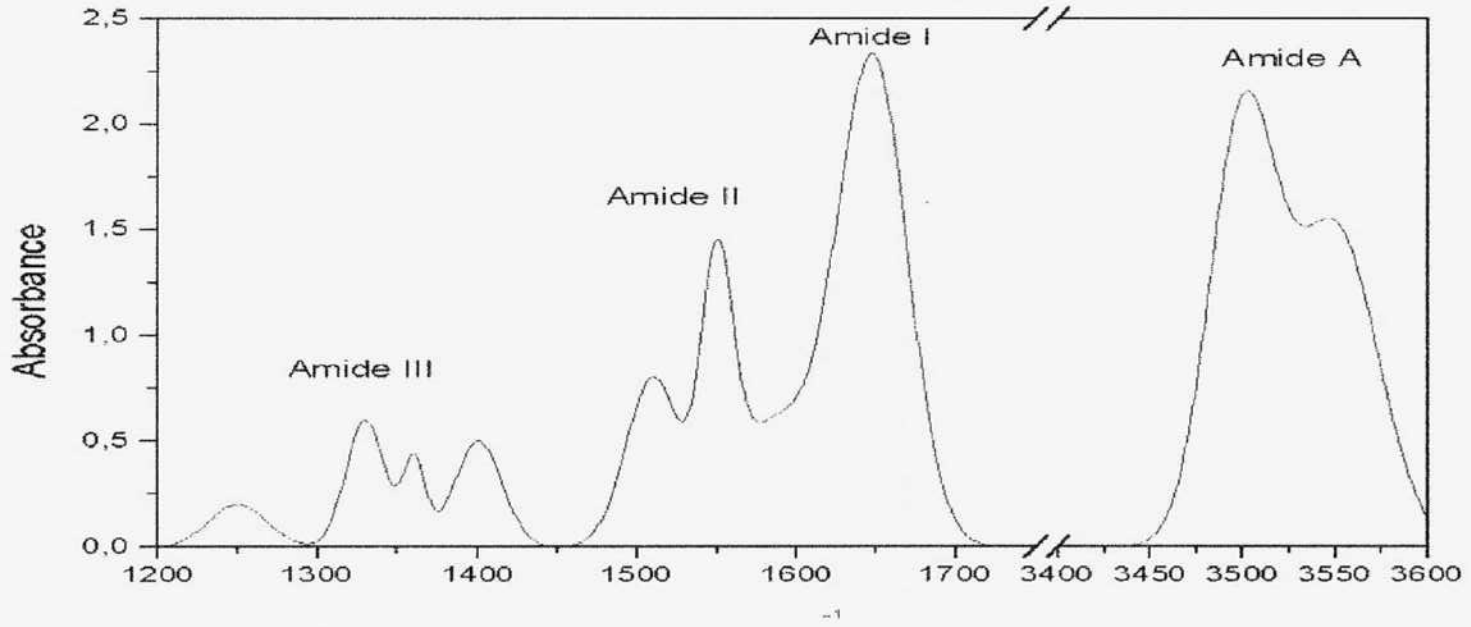
Amide Vibrations

3294 in blood paper (India)

3065 in blood paper (India)

Amide vibrations

The peptide group the structural repeat unit of proteins gives up to 9 characteristic bands named amide A, B, I, II ... VII. The amide A band (about 3500 cm^{-1}) and amide B (about 3100 cm^{-1}) originate from a Fermi resonance between the first overtone of amide II and and the N-H stretching vibration. Amide I and amide II bands are two major bands of the protein infrared spectrum. The amide I band (between 1600 and 1700 cm^{-1}) is mainly associated with the C=O stretching vibration (70-85%) and is directly related to the backbone conformation. Amide II results from the N-H bending vibration (40-60%) and from the C-N stretching vibration (18-40%). This band is conformationally sensitive. Amide III and IV are very complex bands resulting from a mixture of several coordinate displacements. The out-of-plane motions are found in amide V, VI and VIII.



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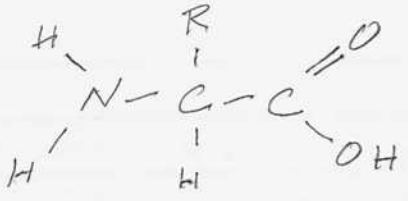
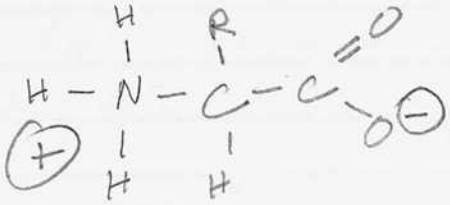
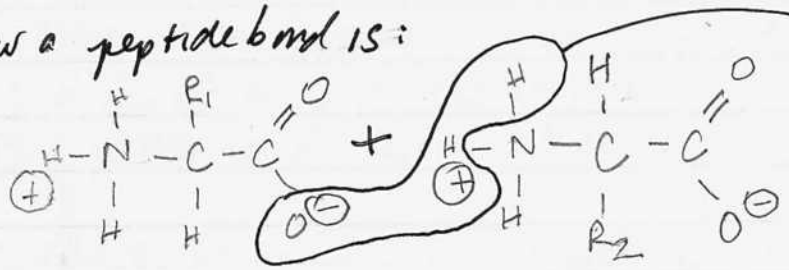
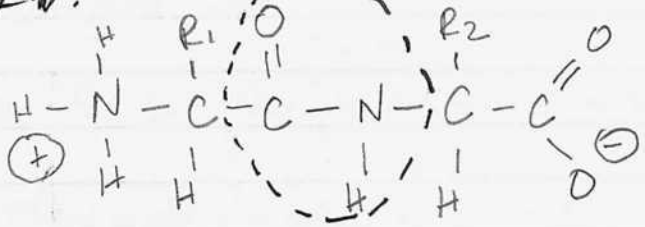
Amino acid is 

or an alternative is 

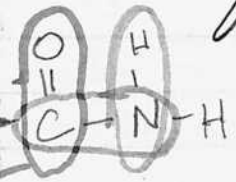
Notice the hydrogen has displaced itself.

Now a peptide bond is: 

This goes to water H_2O

leads to: 

This is a peptide bond.

Now look @ the definition of an amide: 

What is happening is that the amino acids are LINKED by amide (FUNCTIONAL) groups.

Amide I comes mainly from C=O (~70-85%)

Amide II comes from N-H (~40-60%) and C-N (~18-40%)

Amide A and B resonate from Amide II

Amide III & IV get a lot more complicated.

Now we see the connection. Basically there is a lack of a lot of infrared activity based upon the existence of a certain functional group type.

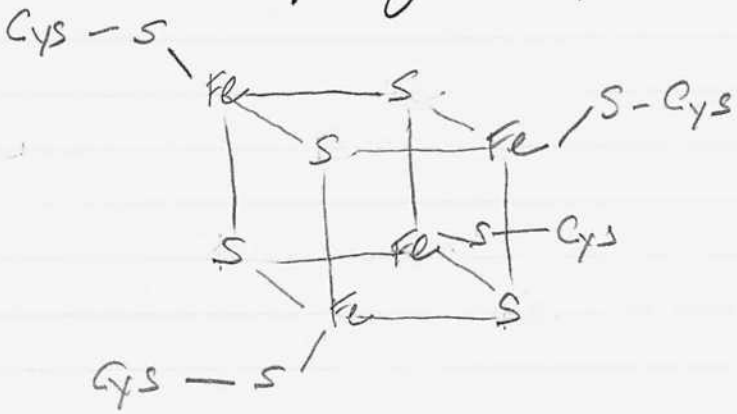
We now know know that we have a protein (i.e. a peptide bond) in the structure.

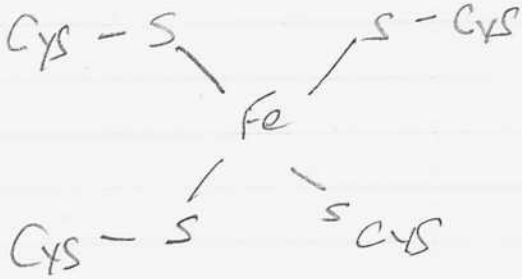
What else do we have that we know?

All signs are that we have a ferredoxin-type. What is that likely structure?

There is what is known as "bacterial type ferredoxins". They have the structure Fe_4S_4.

Then is an example of an F_4S_4





Low potential & high potential ferredoxins

Joins with: